Air pollution exposure, and child's neuropsychological and neurobiological development

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~ to dla ciebie tatko ~

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PREFACE

This joint PhD thesis was written between 2015 and 2019 at Barcelona Institute for Global health (ISGlobal), formerly the Centre for Research in Environmental Epidemiology (CREAL), and at Erasmus University Medical Center (EMC). It was supervised by Prof. Mònica Guxens and by Prof. Henning Tiemeier. This work comprises a compilation of the scientific publications co-authored by the PhD candidate according to the procedures of the Biomedicine PhD program of the Department of Experimental and Health Sciences of University Pompeu Fabra, and of the PhD program in Health Sciences organized by the Netherlands Institute for Health Sciences of Erasmus University of Rotterdam. The research presented in this thesis has been funded by Instituto de Salud Carlos III and co-funded by Health Effects Institute, grant number R-82811201.

The thesis includes an abstract in English and in Spanish, a general introduction, the objectives, the results (5 original research articles), a general discussion, and conclusions. The thesis is focused on the associations between fetal and childhood exposure to various air pollutants and child's brain development. The scientific papers included in this thesis are based on air pollution data from the European Study of Cohorts for Air Pollution Effects (ESCAPE), Transport related Air Pollution and Health impacts – Integrated Methodologies for Assessing Particulate Matter (TRANSPHORM), and Measurements of Ultrafine particles and Soot in Cities (MUSiC) projects, as well as on data from various European prospective birth cohorts.

As a part of the joint PhD training, the candidate did two scientific stays in Erasmus University Medical Center (Department of Child and Adolescent Psychiatry), totaling a period of one year. During those stays, the candidate actively participated in data collection for the Generation R cohort. In Barclona, the candidate participated in data collection for INMA-Sabadell cohort.

ABSTRACT

Air pollution is a major public health concern, leading to worldwide morbidity and premature mortality. In the recent years, exposure to air pollution has also been linked to neurological and neuropsychological diseases, with fetuses and children identified as some of the most vulnerable populations. However, the evidence to date is still too limited to draw definitive conclusions. This thesis aimed to fill some of the existing knowledge gaps regarding the associations between fetal and childhood exposure to various air pollutants ubiquitous in urban areas, with neurological and neuropsychological alterations in children. To this aim, we used air pollution data collected within ESCAPE, TRANSPHORM, and MUSiC projects, and our study population consisted of children from various European prospective birth cohorts, with data available on the outcome of interest, as well as on child and parental socioeconomic, and life-style characteristics. Our results reinforced the notion that exposure to air pollution in the early years of life is harmful for children's neurodevelopment.

RESUMEN

La contaminación del aire es un problema importante de salud pública que provoca morbilidad y mortalidad prematura en todo el mundo. En los últimos años, la exposición a la contaminación del aire también se ha relacionado con enfermedades neurológicas y neuropsicológicas, siendo los fetos y niños identificados como algunas de las poblaciones más vulnerables. Sin embargo, la evidencia es todavía demasiado limitada para extraer conclusiones definitivas. El objetivo de esta tesis fue completar algunas de las lagunas de conocimiento existentes sobre las relaciones entre la exposición durante la vida fetal y la infancia a diversos contaminantes del aire en áreas urbanas, con alteraciones neurológicas y neuropsicológicas en niños. Para este objetivo, utilizamos los datos de contaminación del aire recogidos dentro de proyectos ESCAPE, TRANSPHORM, y MUSiC, y nuestra población de estudio consistió en niños de varias cohortes de nacimientos europeos, con datos disponibles sobre el resultado de salud de interés, así como en aspectos socioeconómicos y las características de estilo de vida de los niños y sus padres. Nuestros resultados reforzaron la noción de que la exposición a la contaminación del aire en los primeros años de vida es perjudicial para el desarrollo neurológico de los niños.

ABBREVIATIONS

ASD	Autism Spectrum Disorder
AQG	Air Quality Guidelines
B[a]P	benzo[a]pyrene
DTI	diffusion tensor imaging
EPA	Environmental Protection Agency
EU	European Union
HPA	hypothalamic-pituitary-adrenal (axis)
MRI	magnetic resonance imaging
NO_2	nitrogen dioxide
NO_X	nitrogen oxides
PM	particulate matter
PM_{10}	particulate matter, aerodynamic diameter ≤ 10 μm
$PM_{2.5}$	particulate matter, aerodynamic diameter ≤ 2.5 μm
$PM_{0.1}$	particulate matter, aerodynamic diameter ≤ 0.1 μm
PM_{COARSE}	particulate matter, difference between PM_{10} and $PM_{2.5}$
PAHs	polycyclic aromatic hydrocarbons
UFP	ultra-fine particles
US	United States
WHO	World Health Organization

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1. INTRODUCTION

Environmental pollution - contamination of air, water and soil by external substances - is a worldwide problem. Not only is environmental pollution contributing to deterioration of the environment and to climate change, but it is also dire for human health. The Lancet Commission on Pollution and Health reported in 2015 that environmental pollution was accountable for approximately 9 million premature deaths in 2015, equaling 16% of all premature deaths worldwide (1). For comparison, smoking was accountable for approximately 12%, while alcohol and drug use together were accountable for 5% of total premature deaths worldwide in 2015. Moreover, the Lancet Commission observed that pollution, in particular outdoor air pollution, is continuously worsening in most countries. The main reasons for the global increases in air pollution are, amongst other, uncontrolled urbanization and the growing use of petroleum-powered motor vehicles. In this thesis, we focus on one specific type of environmental pollution, namely outdoor air pollution, which will be called air pollution henceforth. Worldwide deaths in 2015 attributable to air pollution made up more than 70% of the total deaths due to environmental pollution, resulting in 6.5 million premature deaths (1).

1.1. Air pollution

Air pollution is a term indicating the presence of substances in the atmosphere that are harmful to the environment and to human health. While the levels of air pollution are slowly declining in high-income countries following years of air pollution combat initiatives together with advances in knowledge and technology, the levels are still on the rise in middle-income and low-income countries. While clearly proven to be untrue, air pollution is still often seen as an unfortunate, yet inevitable side effect of economical growth, a belief that impedes global mitigation of air pollution (1). Hence, the global levels are still on the rise, together with all the thereto related adverse consequences, many of which are still not well comprehended or possibly even unknown.

1.1.1. Sources of air pollution

The sources of air pollution can be divided into two main categories: natural sources and man-made sources. Natural sources include, among others, releases from volcanic eruptions, dust storms and volatile organic compound emissions from vegetation. Man-made sources include, but are not limited to, burning of fossil fuels, agriculture, industrial operations, and waste treatment (2). Considering the variety and the diverse nature of the sources,

it is not surprising that air pollution profiles differ based on location and time. Regarding the spatial variability of air pollution, the profile strongly depends on land use. Cities are generally characterized by high levels of air pollution originating from burning of fossil fuels, while agricultural areas can have large concentrations of methane, emitted during livestock management (2). The profile of air pollution is also indicative of the economic status of a region. High-income countries are mainly characterized by pollution from fossil fuel burning and are currently seeing a reduction in concentration levels, middle-income countries are experiencing an increase in pollution from fossil fuel burning, and low-income countries are mostly polluted by biomass and coal burning practices (1). The level of pollution is also strongly time-dependent, as generally the sources intensify during the day. In this thesis, we focus on a specific air pollution profile, namely one representative of urban areas in Europe. This profile is determined by burning of fossil fuels by motorized vehicles.

1.1.2. Composition of air pollution

Air pollution is mainly composed of gasses and tiny solid particles known as particulate matter. The environmental protection agency from the United States of America (US-EPA) designated six major air pollutants as criteria pollutants, namely carbon monoxide, nitrogen oxides, sulfur dioxide, ozone, particulate matter, and lead, suggesting that the overall quality of the air can be determined by the concentration levels of these six pollutants (3). In this thesis, we centered the attention on nitrogen oxides and particulate matter, as these pollutants: i) have motorized traffic as one of the main sources in urban areas in Europe, ii) are documented to be harmful to human health, and iii) have been well-measured over the years.

Nitrogen oxides

Nitrogen oxides (NO_X) refer to a group of seven gasses that are composed of nitrogen and oxygen molecules. The two most ubiquitous gasses of the group are nitrogen monoxide (NO) and nitrogen dioxide (NO_2), and henceforward NO_X will signify a combination of NO and NO_2. While NO is generally not considered to be dangerous to human health at concentrations commonly occurring in the air, NO_2 is classified as hazardous. NO_X is formed from the reaction of nitrogen and oxygen during combustion (3). Therefore, in areas heavy on traffic, which is driven by combustion of fossil fuels, the ambient concentrations of NO_X, and thus also NO_2, can be substantial. NO_2 is a highly reactive reddish-brown gas, and chronic exposure to NO_2 has been linked to many adverse health effects (4). Due to its harmfulness, NO_2 is included in the air quality standards legislations developed by the European Union (EU) (5). The maximum hourly concentration permissible equals 200 $\mu g/m^3$, and the maximum

concentration averages over one year period are not to exceed 40 μg/m^3, the latter equaling the standards set in the air quality guidelines (AQGs) by the World Health Organization (WHO). According to the Air Quality report published in 2018 by European Environment Agency (EEA), in a recent three-year period (2014, 2015 and 2016), approximately 7% of the urban population within the 28 EU Member States (EU-28) lived in areas with annual NO_2 pollution concentrations above the set annual standard (5).

Particulate matter

Particulate matter (PM), also referred to as particles or particulates, are solid and/or liquid matter of microscopic size dispersed in the atmosphere (3). While there are naturally occurring particulates in the air originating from salt spray, dust storms, volcanic eruptions and other natural sources, large quantity of particles currently present in the atmosphere originates from human activities, such as fossil fuel combustion and biomass burning (3). Hereafter, any mention of PM refers to particulates from anthropogenic sources, unless otherwise specified. PM is considered to be one of the most harmful types of air pollution, due to its potential to infiltrate into human organs and blood stream, potentially causing permanent damage and even death (6). The ability of the particles to penetrate into the organs and the blood stream largely depends on the size of the particles. Public health researchers are primarily interested in PM of microscopic and nanoscopic size as the penetration potential increases with decreasing size (7). PM is commonly subdivided into the following categories: PM with aerodynamic diameter of less than 10 μm (PM_{10}), between 10μm and 2.5 μm (coarse particles or PMcoarse), less than 2.5 μm (fine particles or $PM_{2.5}$), and PM with aerodynamic diameter of less than 0.1 μm (ultra-fine particles (UFPs), nano-particles or $PM_{0.1}$). The current EU legislations for the maximum concentrations of PM_{10} are set to 50 μg/m^3 for 24h averages, and to 40 μg/m^3 for annual averages. The AQGs by WHO set the current annual average concentration limits to 20 μg/m^3. Between 2014 and 2016, 13% to 19% of EU-28 urban population was exposed to PM_{10} levels exceeding the 24h maximum values legislated by the EU, while 42% to 52% were exposed to annual PM_{10} concentrations exceeding the commissioned maximum levels by the WHO (5). The maximum annual concentration guidelines for $PM_{2.5}$ differ between EU and WHO as well. The limits set by EU equal 25 μg/m^3 whereas the limits specified by the WHO equal 10 μg/m^3. From the population living in urban areas of EU-28 between 2014 and 2016, 6 to 8% of the population was exposed to $PM_{2.5}$ levels above the EU legislated limits, and 74 to 85% was exposed to $PM_{2.5}$ levels above the WHO limits (5). While UFPs are presumed to have the most harmful implications for human health due to their nanoscopic scale and therefore high potential of penetration into the organs and the blood stream, there are currently no legislations related to the maximum concentrations permissible.

Composition of particulate matter

Particulates are composed of solid and/or liquid matter and the exact profile of their composition depends largely on the source. Generally, the most common components of PM are sulfates, nitrates, ammonia, sodium chloride, black carbon, mineral dust and water (8). Black carbon is also known as soot, and results from an incomplete combustion of hydrocarbons. Commonly, air pollution monitoring campaigns measure light absorbance of PM as a proxy for black carbon. Also several trace components have repeatedly been found in particulates of all sizes. These include, but are not limited to, (heavy) metals such as copper, iron, lead, mercury and zinc, organic carbon, and polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyreen (B[a]P).

1.2. Air pollution and human health

Both short-term and long-term air pollution exposure can prompt health implications, the majority of which are of cardiovascular and respiratory origin (1). While likely less prevalent and less well understood to date, exposure to air pollution can also have implications for the central nervous system, resulting in brain damage and thereto related disorders (9). Air pollution is being considered a silent epidemic, with precise mortality and morbidity tolls difficult to pinpoint, and with many of the conditions attributable to the exposure not yet included in the estimates. Therefore, it is expected that with growing knowledge and evidence, the global burden of disease from air pollution will increase profoundly.

1.3. Early life formation of the brain

Fetuses, newborns, and children are particularly vulnerable to the harmful influences of air pollution, as their defense mechanisms and immune systems are still in development. Additionally, lower dosages of toxins can cause harm, compared to dosages harmful to adults due to smaller body size, as they inhale more air than adults per unit of body weight (9). Moreover, children tend to breathe faster than adults, increasing the inhaled dosages of pollutants. The developmental period is characterized by numerous vital and often fragile processes that are taking place, crucial for a proper development, and disruption of any of these processes by external stressors, such as air pollution, might lead to irreversible alterations that manifest in later life (10). Many studies to date have linked maternal exposure to air pollution during pregnancy and child's exposure in early life to adverse health outcomes in childhood, such as increased risk for low birth weight, lung damage and compromised lung growth, higher risk of development of asthma, and many more (11). Associations between maternal exposure to air

pollution during pregnancy and exposure during early years of life and neurodevelopmental disorders, are also increasingly being documented and are at the center of interest in this thesis.

Neurodevelopment is characterized by many vital and often highly fragile processes such as neurulation, cell proliferation and migration, myelination, and synaptic pruning (Figure 1) (12).

In addition to a healthy genesis and formation, the various areas and components of the developing brain need to be correctly interrelated among one another to allow for fundamentally proper functioning of this highly complex organ (13). Most of these processes start during embryonic life and continue throughout childhood, making the fetal life and childhood a period of high vulnerability to external stressors. Human brain at birth weighs approximately one fourth of its adult weight, and irregular increases of mass follow throughout childhood (14,15).

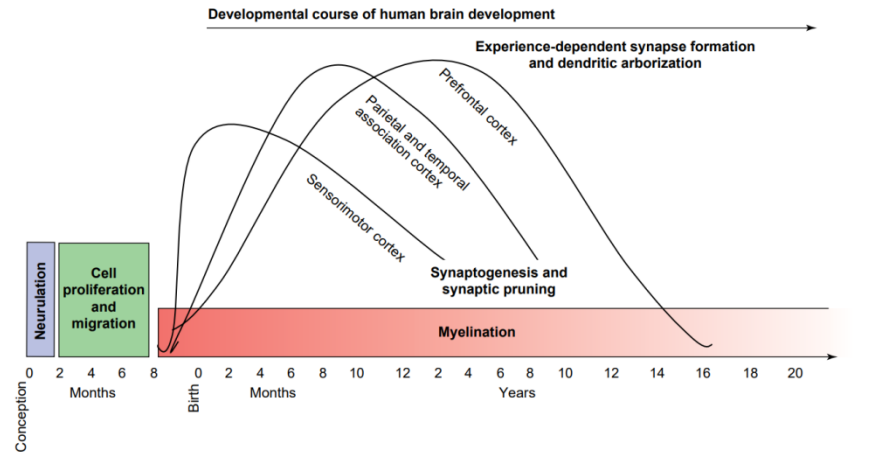

Figure 1: Developmental course of human brain development (12)

During fetal period, brain development is mainly centered on neurogenesis, neuron migration and neuron differentiation (14). Neurons are interconnected nerve cells responsible for information processing in the brain. Most of the neurons are produced by midpoint of the gestational period and most of the production happens in the ventricular zone (neuron production). From there, the majority of the neurons migrate to different areas of the developing cortex, depending on the functions to perform (neuron migration). Different layers and areas of the cortex require different sort of neurons, therefore different types of neurons need to be formed (neuron differentiation). The neurons then develop axons and dendrites, to integrate into the information processing networks, also called neural networks. Axons are the main channels for sending signals from neurons, and dendrites are responsible for the reception of input from other neurons. Except for neurogenesis, which is completed during fetal life, the other

processes continue after birth throughout the postnatal period. In the last stages of the fetal period, another process is initiated, namely myelination, which is among the most important processes for optimal brain development. Myelination is responsible for coating of the neuronal axons with a fatty layer, and this process starts on average 28 weeks after conception and continues throughout childhood and adolescence. It is essential for efficient functioning of the brain through quick and healthy neural communication. Generally, due to myelination the brain weight increases from approximately 400 grams at birth to 1,100 grams at 36 months, with continued growth throughout childhood and adolescence, albeit at a slower pace (14,15).

The increasing size of the brain is correlated with increasing complexity, which corresponds to enhanced complexity in behavioral, cognitive and motor functions during the development of the brain. There are also two inverse processes taking place during fetal life and childhood, crucial for healthy functioning of the brain (14). Apoptosis - nonpathological and controlled death of cells - peaks during the fetal period, while synaptic exuberance and pruning - overproduction of neural connections succeeded by their systematic elimination - occurs mainly in the postnatal period (14). While the exact relationship between neurobiological development of the brain and neuropsychological development of children is not yet fully deciphered, it is clear that proper neurobiological development underlies a healthy neuropsychological development.

1.4. Neurobiological assessment

Magnetic Resonance Imaging (MRI) is a non-invasive and safe method to obtain an in vivo peek into human brain. The method uses potent magnetic fields, magnetic field gradients, and radio waves to create images of the organs of interest. The number of epidemiological studies using MRI to assess neurodevelopment is rapidly growing, nevertheless many questions still remain unanswered. Neuroimaging can be broadly divided into two main categories, namely structural imaging and functional imaging. In this thesis only structural imaging techniques are considered, specifically structural T1 imaging and diffusion tensor imaging (DTI) techniques. Structural T1 imaging allows for visualization of gray and white matter structures in the brain through contrast differences induced by different T1 relaxation times of tissue types (16). For example, the relaxation time of grey matter is higher than the relaxation time of white matter, which makes grey matter appear darker as compared to white matter on a T1 scan, thereby allowing for visual differentiation between the two.

DTI is a method to study the microstructure of the white matter, also referred to as a study of white matter integrity. It measures water diffusion profile in the white matter quantifying the overall directionality and the

magnitude of water diffusion within brain tissue (17). Myelination is responsible for increases in relative white matter volume and for water diffusion changes within white matter tracts, thus DTI can give insight into the condition of myelin, a process crucial to healthy brain development (14,17). As healthy brain development underlies a healthy neuropsychological development, the use of MRI is considered to be a helpful tool to assist in understanding of neuropsychological characteristics by studying neurobiological properties.

1.5. Neuropsychological assessment

A child's cognitive and psychomotor function, and behavioral and emotional problems can be evaluated from very early age on using validated and age appropriate neuropsychological questionnaires and tests. These tools are very useful for detection, but unlike MRI, they cannot provide insight into biological characteristics, thereby limiting their potential to help to understand the possible mechanisms behind air pollution related alterations in the brain.

1.6. Air pollution, neuropsychological and neurobiological development

It has been long inferred, and recently proved by identification of nanoparticles in human brain samples, that particulate matter can penetrate into the brain (18). The most plausible pathways are via systemic circulation through the blood brain barrier or through olfactory bulb after inhalation (9).

1.6.1. Possible biological mechanisms

Once penetrated into the brain, inflammation, oxidative stress, an imbalance between antioxidants and oxidants in favor of the latter, and chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, are the most likely potential mechanisms through which air pollution can cause damage (9,19). This theory has support from a number of experimental studies in animals. In one study, brains of dogs from a highly polluted area were compared to brains of dogs from a less polluted area, and indeed markers of inflammation were detected in several brain regions in the brains of the highly exposed dogs (20). Experimental studies in mice and other rodents confirm these observations and demonstrate a causal relationship; animals exposed to higher levels of air pollution show higher levels of pro-inflammatory agents, microglia activation, and markers of oxidative stress in the brain, as compared to lower exposed controls (21). Other experimental studies have demonstrated that brief exposure to particulate matter rapidly

activated the HPA axis which is part of the stress response system of the body. Chronic exposure to air pollution could lead to chronic activation and dysfunction of the HPA axis (19). A study carried out on post-mortem children supports the hypothesis that the mechanisms observed in experimental studies presumably apply to human as well. In this study, brains of children with accidental deaths from high and low polluted areas were compared and the findings revealed that the brains of the highly exposed children showed alterations known to reflect indicators for Alzheimer's disease, namely the presence of hyperphosphorylated tau (HPτ) and $A\beta_{42}$ diffuse plaques, as compared to their lower exposed peers (22).

1.6.2. Existing body of evidence

Epidemiological studies investigating the possible association between exposure to air pollution and child's brain development are emerging. In a review from 2016, 31 published studies were identified that examined the relationship between pre- or postnatal exposure to air pollution and neuropsychological development assessed with the use of various test batteries (23). The main collective conclusion was that an association exists between pre- or postnatal exposure to air pollution, particularly PAH, PM, and NO_X, with compromised neuropsychological development of children, manifested mainly through a lower intelligence quotient in highly exposed children. Another review, published in 2016, examined the existing body of evidence for the relationship between exposure to air pollution in early life and autism spectrum disorder (ASD) (24). ASD is an overarching term for a group of neurodevelopmental conditions with a spectrum of specific behaviors, generally characterized by impaired social interaction and communication, together with obsessions, repetitive behaviors and repetitive movements, and narrow interests. The main conclusion was that there is evidence, although limited, for an association between exposure to air pollution early in life and diagnosis of ASD. The associations with prenatal exposure to PM and diagnosis of ASD provided the most solid evidence. Other studies have found some indication, although inconclusive, for an association between exposure to air pollution during fetal life and behavioral and emotional problems in childhood, manifested through depressive and anxiety symptoms and aggressive symptoms (25–29). The results of studies on the association between exposure to air pollution and prevalence of attention deficit (hyperactivity) disorder have also not been conclusive to date (30). Recently, several groups studied the relationship between exposure to air pollution during fetal life and childhood with neurobiological development assessed with the help of MRI scans. The use of MRI could aid the understanding of the mechanisms behind the relationship between air pollution exposure and neurodevelopment, but the number of studies carried out to date is still too limited to draw definitive conclusions. The majority of the existing studies using MRI focused on white matter and found associations between exposure to air pollution during fetal life and

childhood, and alterations in the structure of white matter, as well as in white matter integrity, which was assessed in one study only (31).
This recent increase in the number of studies looking into the relationship of air pollution with neuropsychological and neurobiological development, is leading to a growing body of evidence for the association between air pollution exposure and compromised neurodevelopment. However, there are still many unanswered questions remaining. For example, most studies analyzed only few main pollutants, without examining their composition, or without trying to disentangle various mixtures. This gap prohibits the identification of the most toxic components, or the understanding of simultaneous exposures. Also, existing studies are mainly addressing either prenatal or postnatal exposures, rather than both, while the association between air pollution exposure and compromised neurodevelopment might be present in both periods. In this thesis, we confront these gaps and expand the current body of evidence, thereby partially filling the existing gap in knowledge.

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2. OBJECTIVES

The objective of this thesis was to assess the relationship between fetal and childhood exposure to air pollution, and neuropsychological and neurobiological development in children and preadolescents.

The specific objectives were:

- To assess the relationship between exposure to elemental composition of outdoor $PM_{2.5}$ at birth and cognitive and psychomotor function in childhood in four European birth cohorts

- To assess the relationship between prenatal and postnatal exposure to air pollution and emotional and aggressive symptoms in children from 8 European birth cohorts

- To assess the relationship between air pollution exposure during fetal life, brain morphology, and cognitive function in school-age children

- To assess the relationship between air pollution exposure during fetal life and childhood, and brain morphology in preadolescents

- To assess the relationship between fetal and childhood exposures to air pollution and white matter microstructure in preadolescents

3. RESULTS

In this section, the following five scientific papers are presented:

Paper I: Exposure to elemental composition of outdoor $PM_{2.5}$ at birth and cognitive and psychomotor function in childhood in four European birth cohorts

Paper II: Prenatal and postnatal exposure to air pollution and emotional and aggressive symptoms in children from 8 European birth cohorts

Paper III: Air pollution exposure during fetal life, brain morphology, and cognitive function in school-age children

Paper IV: Air pollution exposure during fetal life and childhood, and brain morphology in preadolescents

Paper V: Fetal and childhood exposures to air pollution and white matter microstructure in preadolescents

3.1. Paper I

Exposure to elemental composition of outdoor $PM_{2.5}$ at birth and cognitive and psychomotor function in childhood in four European birth cohorts

Małgorzata J. Lubczyńska, Jordi Sunyer, Henning Tiemeier, Daniela Porta, Monika Kasper-Sonnenberg, Vincent W.V. Jaddoe, Xavier Basagaña, Albert Dalmau, Francesco Forastiere, Jürgen Wittsiepe, Barbara Hoffmann, Mark Nieuwenhuijsen, Gerard Hoek, Kees de Hoogh, Bert Brunekreef, Mònica Guxens

Published in: Environment International 2017, 109: 170 - 180

Lubczyńska MJ, Sunyer J, Tiemeier H, Porta D, Kasper-Sonnenberg M, Jaddoe VWV, et al. Exposure to elemental composition of outdoor PM 2.5 at birth and cognitive and psychomotor function in childhood in four European birth cohorts. Environment international. 2017;109:170–80. DOI: 10.1016/j.envint.2017.09.015

3.2. Paper II

Prenatal and postnatal exposure to air pollution and emotional and aggressive symptoms in children from 8 European birth cohorts

Ainhoa Jorcano, Małgorzata J. Lubczyńska, Livia Pierotti, Hicran Altug, Ferran Ballester, Giulia Cesaroni; Hanan El Marroun, Ana Fernandez, Carmen Freire, Wojciech Hanke, Gerard Hoek, Jesús Ibarluzea, Carmen Iñiguez, Pauline W. Jansen, Johanna Lepeule, Iana Markevych, Kinga Polańska, Daniela Porta, Tamara Schikowski, Remy Slama, Marie Standl, Adonina Tardon, Tanja G.M Vrijkotte, Andrea von Berg, Henning Tiemeier, Jordi Sunyer, Mònica Guxens

Published in: Environment International 2019, 131

Jorcano A, Lubczyńska MJ, Pierotti L, Altug H, Ballester F, Cesaroni G, et al. Prenatal and postnatal exposure to air pollution and emotional and aggressive symptoms in children from 8 European birth cohorts. Environment international. 2019;131:104927. DOI: 10.1016/ j.envint.2019.104927

3.3. Paper III

Air pollution exposure during fetal life, brain morphology, and cognitive function in school-age children

Mònica Guxens; Małgorzata J. Lubczyńska; Ryan L. Muetzel; Albert Dalmau-Bueno; Vincent W.V. Jaddoe; Gerard Hoek; Aad van der Lugt; Frank C. Verhulst; Tonya White; Bert Brunekreef; Henning Tiemeier; Hanan El Marroun

Published in: Biological Psychiatry 2018, 84: 295–303

Guxens M, Lubczyńska MJ, Muetzel RL, Dalmau-Bueno A, Jaddoe VW., Hoek G, et al. Air Pollution Exposure During Fetal Life, Brain Morphology, and Cognitive Function in School-Age Children. Biological psychiatry (1969). 2018;84(4):295–303. DOI: 10.1016/j.biopsych.2018.01.016

3.4. Paper IV

Air pollution exposure during fetal life and childhood, and brain morphology in preadolescents

Małgorzata J. Lubczyńska, Ryan L. Muetzel, Hanan El Marroun, Gerard Hoek, Ingeborg Kooter, Manon Hillegers, Meike W. Vernooij, Tonya White, Henning Tiemeier, Mònica Guxens

Submitted to Environmental Research

Air pollution exposure during fetal life and childhood and brain morphology in preadolescents

Malgorzata J. Lubczyńska, Ryan L. Muetzel, Hanan El Marroun, Gerard Hoek, Ingeborg Kooter, Manon Hillegers, Meike W. Vernooij, Tonya White, Henning Tiemeier, Mònica Guxens

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Conflicts of interest: none

Abstract

Background. Studies investigating the relationship between exposure to air pollution and brain development using magnetic resonance images are emerging. However, most studies on air pollution exposure, brain morphology, and cognitive function in school-age children, have focused only on prenatal exposures, and have included a limited selection of pollutants. Here, we aim to expand the current knowledge by assessing the relationship between exposure during fetal life as well as childhood to a wider selection of air pollutants, with brain morphology in a large population of preadolescents.

Methods. We used data from 3,133 preadolescents from a birth cohort from Rotterdam, the Netherlands (enrollment: 2002-2006). Concentrations of nitrogen oxides, fine, coarse, and ultrafine particles, and composition of fine particles were estimated at each home address that the participants have resided in during fetal life and entire childhood, using land use regression models. Structural brain images were obtained when the participants were 9-12 years old. We assessed the relationships between air pollution exposure and cortical and subcortical brain volumes and ventricular volume, as well as surface-based morphometric data, adjusting for child and parental socioeconomic, and life-style characteristics.

Results. We found associations between fetal and childhood exposures to air pollution with larger global and sub-cortical volumes, smaller volume of corpus callosum (e.g. -45.3mm^3 of the anterior part of the corpus callosum [95%CI -76.3 to -14.3] per 1,000 units/m^3 increase in oxidative potential of fine particles during fetal life, and -23.4mm^3 [95%CI -38.9 to -7.9] for each μg/m^3 increase in organic carbon during childhood), and thinner cortex. Higher exposure to air pollution during childhood was also associated with larger cortical surface area. The associations with fetal exposure to air pollution were predominantly observed in girls.

Conclusion. Higher fetal and childhood exposure to air pollution was associated with brain morphology in preadolescents of 9-12 years old, with associations with mainly fetal exposures being predominantly observed in girls. These sex-specific associations could possibly be explained by different stage of pubertal maturation.

Introduction

The evidence for effects of air pollution on health is accumulating (1,2). The fetal life and childhood are particularly vulnerable periods with respect to the harmful influences of air pollution, as the defense mechanisms and immunities of fetuses, newborns, and young children are not yet fully developed, while lower dosages of toxins can cause harm, as compared to harmful dosages to adults (3,4). The early years of life are characterized by numerous vital and often fragile developmental processes crucial for a proper development of the body. Healthy brain development is dependent on a sequence of such processes including neuronal genesis, synaptic pruning and myelination, and disruption of any of these processes by external stressors might lead to irreversible alterations that could manifest in later life in neurological or psychiatric disorders (5). Air pollution is considered such potential stressor, and has been associated with brain tissue inflammation, oxidative stress, and chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis (3,6).

Recently, magnetic resonance imaging (MRI) has been employed to investigate the effects of air pollution on brain development. MRI is a non-invasive method that does not utilize ionizing radiation while permitting an in vivo glimpse into brain (micro)structure, function, blood flow, and metabolite concentrations, making this method appropriate for research in children. Previous studies using MRI mostly found associations between exposure to air pollution during fetal life and childhood, and alterations in white matter (microstructure) in preadolescence (7–10). To date, only one study from our group found evidence for an association between exposure to air pollution during fetal life and structural alteration of cerebral cortex (11). In that study, we showed that higher exposure to fine particles during pregnancy was associated with thinner cortices in school-age children of 6 to 10 years old. This association partially mediated the relationship between exposure to fine particles during pregnancy and cognitive impairment manifested by weakened inhibitory control. In the current study, we build on this previous work including a four times larger population of slightly older children between 9 to 12 years. Moreover, we include air pollution exposure during fetal life as well as during childhood from a wider selection of air pollutants, including components of particulate matter and its oxidative potential, as well as ultrafine particles, highly ubiquitous in urban settings, thereby increasing the comprehensiveness of the study.

Thus, the aim of this study was to examine the association between fetal and childhood exposures to a large number of air pollutants with brain morphology in preadolescents aged 9-12 years evaluated with the use of magnetic resonance imaging. Moreover, we also examined whether the associations differed between girls and boys. Our hypothesis was that higher exposure to air pollution is associated with brain morphology, and that such

associations might differ between girls and boys due to their different stage of pubertal maturation at ages between 9 and 12 years.

Methods

Population and Study Design

This study is embedded in the Generation R Study, a population-based birth cohort from fetal life onwards, based in Rotterdam, the Netherlands (12). A total of 8,879 pregnant women were enrolled and children were born between April 2002 and January 2006. Additionally, 899 women were recruited shortly after the birth of their child. When the children were between the ages of 9 and 12 years, they were invited to participate in an MRI session (n=8,548) (13). In total, 3,992 mothers and their children agreed to participate and consented in writing (13). From this total, 3,133 children were from a singleton pregnancy, had good quality imaging scans and data on air pollution, and were included in this analysis. The Medical Ethics Committee of the Erasmus Medical Centre in Rotterdam, The Netherlands, granted ethical approval for the study.

Exposure to Traffic Related Air Pollution

Air pollution concentrations were assigned to all home addresses of each participant during fetal and childhood periods, with fetal life stretching from conception to birth, and childhood stretching from birth until the MRI session, using a standardized procedure described elsewhere (14–17). Briefly, within the ESCAPE (European Study of Cohorts for Air Pollution Effects) and TRANSPHORM (Transport related Air Pollution and Health impacts – Integrated Methodologies for Assessing Particulate Matter) projects, three two-week measurements of nitrogen oxides (NO_X, NO_2) were performed in the warm, cold, and intermediate seasons between February 2009 and February 2010 at 80 sites spread across the Netherlands and Belgium (18). Additionally, at 40 of those sites particulate matter (PM) measurements were carried out (19). Specifically, PM with aerodynamic diameter less than 10μm (PM_{10}), less than 2.5μm ($PM_{2.5}$), absorbance of $PM_{2.5}$ fraction ($PM_{2.5}$absorbance), and composition of $PM_{2.5}$ consisting of polycyclic aromatic hydrocarbons (PAHs), benzo[a]pyrene (B[a]P), organic carbon (OC), copper (Cu), iron (Fe), potassium (K), silicon (Si), zinc (Zn), and oxidative potential of $PM_{2.5}$ (OP) were measured (14,15,17). PM mass between 10μm and 2.5μm (PM_{COARSE}) was calculated by subtracting $PM_{2.5}$ from PM_{10}. The evaluation of OP was performed using two acellular methods: dithiothreitol (OP_{DTT}) and electron spin resonance (OP_{ESR}) (17). Another campaign within the MUSiC (Measurements of Ultrafine particles and Soot in Cities) project measuring PM with aerodynamic diameter less than 0.1μm, known as ultra-fine particles (UFP), was held in 2013 at 80 sites in Rotterdam (16). The concentration of UFP was monitored in real time for 30 minutes at each site in three different seasons. For each pollutant, the

results of all measurements were averaged to obtain one annual mean concentration for each pollutant after correcting them for temporal variability. This temporal correction was done by first calculating the difference between the concentration for a specific sampling period and the annual average at a continuous reference monitoring site, and then subtracting that difference from each measurement. Next, a variety of potential land use predictors was assigned to each monitoring site and linear regression modeling was applied to determine which combination of predictors explained the levels of the pollutants most accurately, resulting in land use regression models (14–17,19,20). Land use predictors were then also assigned to each address that the participants have lived at during the period of interest, i.e. since conception until the MRI session, and the land use regression models were applied to predict air pollution levels at each of these addresses. Taking into account the time spent at each address and weighting the pollution levels accordingly, we then obtained a single, mean air pollution concentration of each pollutant for each participant for the fetal period (i.e. since conception until birth) and for the childhood period (i.e. since birth until the MRI session). For those participants that were recruited shortly after birth, we considered the address at birth as representative for the pregnancy period. As no historical data was available for the majority of the pollutants under study to perform back- and forward extrapolation of the concentrations to match the exact periods of interest, we assumed that the spatial contrast remained constant over time as it was shown in previous studies (21).

Structural Magnetic Resonance Imaging

To familiarize the participating children with magnetic resonance environment, each child underwent a mock scanning session prior to the actual MRI session (22). The scans were performed on a 3 Tesla General Electric scanner (GE, MR750W, Milwaukee, USA) using an 8-channel receive-only head coil. The structural T1 images were obtained using the following sequence parameters: TR = 8.77ms; TE = 3.4ms; TI = 600ms; Flip Angle = 10°; FOV = 220mm x 220mm; acquisition matrix = 220 x 220; slice thickness = 1mm; number of slices = 230; voxel size = 1mm x 1mm x 1mm; and ARC Acceleration = 2. The obtained T1 images were then processed with FreeSurfer analysis suite, version 6.0, and global metrics of cortical and subcortical volumes were extracted, along with surface-based morphometric data. Global metrics of cortical and subcortical volumes included total brain volume, cerebellum, cortical and sub-cortical gray matter volumes including thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens, cerebral and cerebellar white matter volumes, corpus callosum, and total ventricular volume comprising fourth ventricle, septum pellucidum, and lateral ventricles. Surface-based morphometric data represented the cortical thickness at each vertex

(~160,000) and was acquired by computing the shortest distance between white matter and pial surface. Additionally, surface area of the cortex at each vertex was obtained from the morphometric data by calculating the average area of the triangles touching the specific vertex. The preprocessing, correction, and assessment of the quality of the images are described in detail elsewhere (23).

Potential confounding variables

Potential confounding variables were defined based on the scientific literature on the association between air pollution exposure and brain development (12). Maternal and paternal educational level, monthly household income, maternal and paternal country of birth, maternal and paternal age at intake, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, parity, marital status, and maternal and paternal psychological distress (using the Brief Symptom Inventory) were collected by questionnaires during pregnancy. Maternal and paternal weight and height were measured or self-reported in the 1st trimester of pregnancy in the research center and the pre-pregnancy body mass index was calculated. Maternal intelligence quotient was assessed at child's age of 6 years with the Ravens Advanced Progressive Matrices Test, set I. Child's genetic ancestry was estimated based on the genome-wide single-nucleotide polymorphism data from whole blood at birth, and 4 principal components of ancestry were included here to better correct for population stratification (24,25). Child's sex was obtained from the hospital records and child's age was calculated from the date of scanning session.

Statistical Analyses

Missing data

We applied multiple imputation of missing values using chained equations to impute missing potential confounding variables among all participants with available data on the exposure and the outcome. We obtained 25 completed datasets that we analyzed using standard procedures for multiple imputation. The percentage of missing values was below 30% except for paternal education level, paternal psychological distress, and child genetics ancestry which had 36%, 39%, and 35% of missing values respectively. Distributions in imputed dataset were very similar to those observed (data not shown).

Non-response analysis

Children included in the analysis (n=3,133) were more likely to have parents from a higher socioeconomic position compared to children that were not included (n=6,477) (Supplementary Table 1). We used inverse probability weighting to correct for selection bias that potentially arises when only population with available exposure and outcome data, here thus of higher socioeconomic position, is included as compared to a full initial cohort

recruited at pregnancy (26). Briefly, we used information available for all participants at recruitment to predict the probability of participation in the study, and used the inverse of those probabilities as weights in the analyses so that results would be representative for the initial population of the cohort.

Main analyses

In this study, we first used a global approach wherein we examined the relationships between exposure to each pollutant during fetal life and childhood independently, with the volumes of cortical and subcortical structures and total ventricular volume, at 9 to12 years of age. We used linear regression models to assess the relationships and adjusted them for potential confounding variables described in previous section. Additionally, we adjusted subcortical volumes, cerebellar, and total ventricular volume, for intracranial volume to ascertain relativity to the head size. Total brain volume, total grey matter volume, cortical gray matter volume, subcortical grey matter volume, and cerebral white matter volume were not adjusted for intracranial volume due to high correlations with the latter (between $r = 0.81$ and $r = 0.93$). We first performed single pollutant analyses wherein each pollutant was studied separately. Next, we ran multi-pollutant analyses using the exposome-wide association study (ExWAS) approach (27). Briefly, we corrected all the analyses for the number of tests with respect to the multiplicity of included pollutants using the effective number of independent tests correction (28). The effective number of independent tests correction equaled 7.42 for fetal exposure models and 8.28 for childhood exposure models. Next, we tested the effect modification of the associations by sex by adding interaction terms into the models. We screened for interaction terms with a $p<0.05$ and subsequently performed stratified analyses to examine the influence of each sex separately on the main results.

Secondly, in order to examine different cortical morphological metrics, and to determine whether global effects are being driven by localized associations in the brain, we analyzed exposure to each pollutant during fetal life and childhood separately with regional differences in cortical morphology using a surface-based vertex-wise approach. We adjusted the analyses for potential confounding variables described in previous section. As the vertices per hemisphere are numerous (~160,000), the analyses were corrected for multiple testing using built-in Monte Carlo null- Z simulations with 10,000 iterations (Cluster forming threshold = 0.001) (29). We first performed single pollutant analyses wherein each pollutant was studied separately. Next, we ran multi-pollutant analyses using the ExWAS approach (27), wherein we corrected all the analyses for the number of tests with respect to the exposure using the effective number of independent tests correction (28). The effective number of independent tests equaled 7.42 for fetal exposure models and 8.28 for childhood exposure models. To combine the fetal and

childhood air pollution exposures, we analyzed simultaneously those exposures that showed associations with a specific outcome after correction for multiple testing, using multiple linear regression model.

The analyses were carried out with R (version 3.4.2: R Core Team (2017)) using an in-house R package (https://github.com/slamballais/QDECR), and with STATA (version 14.0; StataCorporation, College Station, TX).

Results

Participant characteristics are shown in Table 1. Median air pollution exposure levels during fetal life of the participants were 34.1 μg/m³ for NO_2 and 16.8 μg/m³ for $PM_{2.5,}$ and 32.4 μg/m³ for NO_2 and 16.7 μg/m³ for $PM_{2.5}$ during childhood (Table 2). Correlations between the exposures in the two periods were generally moderate, ranging between 0.48 for NO_2 and 0.68 for B[a]P. Correlations between the concentrations of different pollutants in a given period varied considerably depending on the pollutant (Supplementary Figures 1 and 2). Mothers with a higher level of education, a higher monthly household income, and nulliparous at the index pregnancy, were more likely to be exposed to higher levels of NO_2 during pregnancy (data not shown). These associations were however not consistent between the different pollutants (data not shown).

In the global approach we studied the associations between exposure to each pollutant separately during fetal life and childhood with global volumes of cortical and subcortical structures, and total ventricular volume. We found that higher exposure to several air pollutants was associated with larger volumes of total brain, total grey matter, subcortical grey matter, cerebral cortex, caudate, putamen, amygdala, nucleus accumbens, cerebellar cortex, and cerebellar white matter, while associated with lower volumes of thalamus and corpus callosum (Table 3). After applying the multi-pollutant analysis, higher fetal exposure to OP_{ESR} remained associated with smaller volume of the anterior part of corpus callosum (-45.3 mm³ [95%CI -76.3 to -14.3] for each unit/m³ increase of OP_{ESR}). Higher childhood exposure to PM_{10} and PM_{COARSE} also remained associated with larger volume of the putamen (470.3 mm³ [95%CI 172.5 to 768.2] for each 10 μg/m³ increase of PM_{10}, and (357.8 mm³ [95%CI 142.4 to 573.1] for each 5 μg/m³ increase of PM_{COARSE}), while higher childhood exposure to OC remained associated with a smaller volume of the anterior part of the corpus callosum (-23.4 mm³ [95%CI -38.9 to -7.9] for each 1 ng/m³ increase of OC). When we simultaneously assessed the fetal exposure to OP_{ESR} and the childhood exposure to OC with the volume of the anterior part of the corpus callosum, both associations remained (data not shown). We then stratified by sex those associations that showed a p-value for interaction <0.05 and we observed that the results with mainly fetal exposures were driven predominantly by

associations found in girls, while the results with childhood exposures were observed in both sexes (Supplementary Table 2 and 3). We also observed associations between exposure to several pollutants during fetal life with larger volumes in girls that were not observed when both sexes were analyzed together (Supplementary Table 2).

In the analyses wherein we analyzed exposure to each pollutant during fetal life and childhood separately with regional differences in cortical morphology using a surface-based vertex-wise approach, we found that overall, higher fetal exposures were related to smaller cortical thickness (Table 4 and Figure 1). Higher childhood exposures were also related to thinner cortex, as well as to a larger cortical surface area. After applying the multi-pollutant analysis, only higher childhood exposure to elemental Zn remained associated with larger cortical surface area in the precentral gyrus of the right hemisphere and in the pericalcarine region of the left hemisphere (240.0 mm^2 and 289.0 mm^2 respectively, for each 10 ng/m^3 increase in elemental Zn).

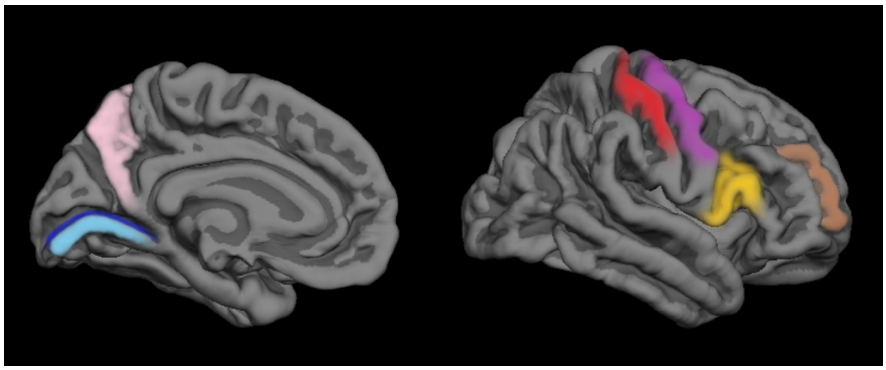

Figure 1. Medial view of the left hemisphere (on the left) and lateral view of the right hemisphere (on the right) of the brain
Exposure to air pollution was association with alterations in the highlighted areas (based on results shown in Table 4): red, postcentral gyrus, purple, precentral gyrus; yellow, pars triangularis; brown, rostral middle frontal gyrus; light blue, lingual gyrus; dark blue, pericalcarine cortex; and pink, precuneus

Table 1. Participant characteristics

Participant characteristics	Distribution		
	Percentage	Mean	(SD)
Child's sex (boy vs. girl)	50.0		
Maternal education level			
Primary education or lower	6.4		
Secondary education	40.6		
Higher education	53.0		
Paternal education level			
Primary education or lower	5.1		
Secondary education	39.3		
Higher education	55.6		
Monthly household income at intake			
<900€	7.6		
900€ - 1.600€	13.8		
1.600€ - 2.200€	14.4		
>2.200€	64.2		
Maternal country of birth			
The Netherlands	57.6		
Other Western	8.5		
Non-Western	33.9		
Paternal country of birth			
The Netherlands	67.8		
Other Western	5.8		
Non-Western	26.3		
Family status at intake			
Married	50.1		
Living together	39.0		
No partner	10.9		
Maternal parity (nulli vs. multiparous)	58.1		
Maternal smoking use during pregnancy			
Never	77.7		
Smoking use until pregnancy known	8.8		
Continued smoking use during pregnancy	13.5		
Maternal alcohol use during pregnancy			
Never	41.8		
Alcohol use until pregnancy know	14.5		
Continued alcohol use during pregnancy	43.7		
Maternal age at intake (years)		31.1	(4.9)
Paternal age at intake (years)		33.5	(5.4)
Maternal pre-pregnancy body mass index (kg/m2)		23.4	(4.1)
Paternal body mass index at intake (kg/m2)		25.3	(3.6)
Maternal height (cm)		168.1	(7.3)
Paternal height (cm)		182.4	(7.6)
Maternal overall psychological distress during pregnancy		0.3	(0.3)
Paternal overall psychological distress during pregnancy		0.1	(0.2)
Maternal intelligence quotient score		97.7	(14.7)

Values are percentages for categorical variables and mean (standard deviation) for continuous variables.

Table 2: Air pollution exposure levels during fetal life and during childhood, and Spearman's correlations between the exposures at the two time periods

	Fetal life			Childhood			Spearman's
Pollutant	p25	p50	p75	p25	p50	p75	Correlation
NO_X	40.9	46.4	57.9	38.4	43.1	51.8	0.55
NO_2	31.9	34.1	36.6	29.4	32.4	35.1	0.48
PM_{10}	26.0	26.7	27.9	25.6	26.3	27.2	0.52
PM_{COARSE}	9.2	10.1	10.6	8.6	9.5	10.3	0.56
$PM_{2.5}$	16.6	16.8	17.2	16.5	16.7	17.0	0.59
$PM_{25}abs$	1.5	1.6	1.8	1.4	1.5	1.7	0.53
ΣPAH	0.8	0.9	1.1	0.8	0.9	1.1	0.67
B[a]P	0.1	0.1	0.1	0.1	0.1	0.1	0.68
OC	1.5	1.8	2.0	1.4	1.7	1.9	0.59
Cu	4.5	4.6	5.0	4.2	4.5	4.9	0.54
Fe	114.2	119.8	129.2	106.5	116.4	124.9	0.53
K	108.6	110.6	114.5	108.2	110.2	113.4	0.60
Si	87.9	88.8	90.8	87.6	88.7	90.6	0.62
Zn	17.6	18.9	21.1	17.4	18.7	20.8	0.55
OP_{DTT}	1.3	1.3	1.4	1.2	1.3	1.4	0.58
OP_{ESR}	1001.4	1037.0	1101.3	965.4	1016.5	1073.6	0.58
UFP	9506.0	10044.6	10944.7	8420.0	9646.4	10391.2	0.51

B[a]P, benzo[a]pyrene; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; OC, organic carbon; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter with different aerodynamic diameters: less than 10μm (PM_{10}); between 10μm and 2.5μm (PMcoarse); less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; Si, elemental silicon; UFP, ultra-fine particles; Zn, elemental zinc.

Table 3. Adjusted associations (only p<0.05 shown) of exposure during fetal life and childhood to various air pollutants with cortical and subcortical brain volumes and total ventricular volume in preadolescents at 9-12 years old

		fetal life				childhood			
Brain region	pollutant	Coef.	95% CI		p-value	Coef.	95% CI		p-value
Total brain	NO_2	9447.8	1518.4	17377.1	0.020	---	---	---	---
	PM_{COARSE}	21902.2	2814.2	40990.2	0.025	---	---	---	---
	UFP	20985.2	572.4	41398.0	0.044	---	---	---	---
Total grey matter	NO_2	6115.6	1507.3	10723.8	0.010	---	---	---	---
	PM_{COARSE}	12814.2	1725.4	23902.9	0.024	---	---	---	---
Subcortical grey matter	NO_X	262.2	24.7	499.7	0.031	---	---	---	---
	NO_2	488.6	132.7	844.4	0.007	---	---	---	---
	PM_{10}	1210.0	152.2	2267.9	0.025	1487.7	250.7	2724.7	0.019
	PM_{COARSE}	1116.1	260.6	1971.6	0.011	---	---	---	---
	$PM_{2.5}$	---	---	---	---	1859.7	10.9	3708.6	0.049
	$PM_{2.5abs}$	592.7	12.3	1173.0	0.045	---	---	---	---
Cerebral cortex	NO_2	4314.4	360.0	8268.9	0.033	---	---	---	---
Thalamus	PAH	---	---	---	---	-164.2	-302.1	-26.2	0.020
	B[a]P	---	---	---	---	-170.1	-311.0	-29.3	0.018
	OP_{ESR}	-225.8	-439.2	-12.4	0.038	---	---	---	---
Caudate	OP_{DTT}	---	---	---	---	388.7	82.2	695.3	0.013
Putamen	NO_X	61.6	4.2	118.9	0.036	87.2	22.9	151.4	0.008
	NO_2	---	---	---	---	84.2	7.3	161.1	0.032
	PM_{10}	259.3	3.4	515.2	0.047	**470.3**	**172.5**	**768.2**	**0.002**
	PM_{COARSE}	217.7	11.1	424.3	0.039	**357.8**	**142.4**	**573.1**	**0.001**
	$PM_{2.5}$	401.7	62.1	741.3	0.021	620.6	175.4	1065.8	0.007
	$PM_{2.5abs}$	---	---	---	---	175.9	17.9	333.8	0.029
Putamen	PAH	126.2	4.4	247.9	0.042	---	---	---	---
Amygdala	Si	80.9	3.6	158.2	0.040	91.6	12.3	171.0	0.024

Nucleus accumbens	NO_X	---	---	---	---	12.3	1.3	;	23.4	0.029
	NO_2	15.2	0.4	; 30.0	0.044	14.9	1.6	;	28.1	0.028
	PM_{10}	---	---	---	---	70.5	19.2	;	121.8	0.007
	$PM_{2.5}$abs	---	---	---	---	27.6	0.4	;	54.9	0.047
	K	---	---	---	---	72.5	13.6	;	131.5	0.016
	Zn	---	---	---	---	20.7	4.1	;	37.2	0.015
Cerebellar cortex	PM_{10}	2397.6	88.5	; 4706.7	0.042	---	---		---	---
	PAH	---	---	---	---	1365.7	21.3	;	2710.1	0.047
	B[a]P	---	---	---	---	1383.7	10.8	;	2756.6	0.048
	K	2839.8	191.4	; 5488.1	0.036	---	---		---	---
	Zn	970.5	39.2	; 1901.7	0.041	---	---		---	---
Cerebellar white matter	PM_{10}	---	---	---	---	777.3	19.9	;	1534.7	0.044
	$PM_{2.5}$	---	---	---	---	1282.3	152.2	;	2412.3	0.026
Posterior part of the corpus callosum	NO_2	-11.7	-23.3	; -0.1	0.048	---	---		---	---
	OP_{ESR}	-32.6	-63.6	; -1.6	0.039	---	---		---	---
Mid-posterior part of the corpus callosum	OC	-12.8	-23.1	; -2.4	0.016	---	---		---	---
	OP_{ESR}	-21.1	-41.9	; -0.2	0.048	---	---		---	---
Mid-anterior part of the corpus callosum	OP_{DTT}	---	---	---	---	-64.4	-121.5	;	-7.3	0.027
Anterior part of the corpus callosum	NO_2	-13.4	-25.0	; -1.8	0.024	---	---		---	---
	$PM_{2.5}$	---	---	---	---	-68.5	-128.8	;	-8.3	0.026
	$PM_{2.5}$abs	-19.0	-38.0	; -0.1	0.048					
	OC	---	---	---	---	**-23.4**	**-38.9**	;	**-7.9**	**0.003**
	Cu	-37.3	-69.2	; -5.4	0.022	-44.7	-81.2	;	-8.2	0.017
	Fe	-29.7	-57.4	; -1.9	0.036	---	---		---	---
	OP_{ESR}	**-45.3**	**-76.3**	; **-14.3**	**0.005**	-42.1	-74.6	;	-9.6	0.012

Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}); between 10µm and 2.5µm (PMcoarse); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe,

elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficient and 95% CI were estimated through linear regression analysis calculated per increments of: 20 $\mu g/m^3$ for NO_X; 10 $\mu g/m^3$ for NO_2; 10 $\mu g/m^3$ for PM_{10}; 5 $\mu g/m^3$ for PM_{coarse}; 5 $\mu g/m^3$ for $PM_{2.5}$; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1 ng/m^3 for PAHs; 0.1 ng/m^3 for B[a]P; 1 $\mu g/m^3$ for OC; 5 ng/m^3 for Cu in $PM_{2.5}$; 100 ng/m^3 for Fe in $PM_{2.5}$; 50 ng/m^3 for K in $PM_{2.5}$; 100 ng/m^3 for Si in $PM_{2.5}$; 10 ng/m^3 for Zn in $PM_{2.5}$; 1 nmol DTT/min/m^3 for OP_{DTT}; 1,000 arbitrary units/m^3 for OP_{ESR}; and 10,000 particles/cm^3 for UFP. Models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session. Subcortical brain volumes were additionally adjusted for intracranial volume. In bold: associations that remain after effective number of tests correction

Table 3. Adjusted associations (only p<0.025 shown) of exposure during fetal life and childhood to various air pollutants with cortical thickness and cortical surface area in preadolescents at 9-12 years old

		Cortical thickness					
		Fetal life			Childhood		
Brain region	Pollutant	Coef.	Size (mm)	p-value	Coef.	Size (mm)	p-value
Postcentral gyrus - RH	OC	-0.06	163.8	0.024	---	---	---
	UFP	---	---	---	0.06	183.0	0.015
Rrostral middle frontal gyrus - RH	$PM_{2.5}$abs	-0.07	173.8	0.019	---	---	---
	Cu	-0.12	197.8	0.011	---	---	---
Lingual gyrus- LH	OP_{DTT}	---	---	---	-0.15	176.6	0.019

		Cortical surface area					
		Fetal life			Childhood		
Brain region	Pollutant	Coef.	Size (mm^2)	p-value	Coef.	Size (mm^2)	p-value
Precentral gyrus- RH	Zn	---	---	---	**0.01**	**240.0**	**0.001**
	OP_{ESR}	---	---	---	0.02	156.6	0.019
Postcentral gyrus- RH	K	---	---	---	0.04	206.3	0.004
Precuneus - LH	Zn	---	---	---	0.02	177.4	0.010
Pericalcarine cortex - LH	Zn	---	---	---	**0.02**	**289.0**	**0.001**
Pars triangularis - RH	PM_{COARSE}	---	---	---	-0.10	179.7	0.011

Coef, coefficient; LH/RH, left or right hemisphere; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: between 10µm and 2.5µm (PMcoarse); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; OC, organic carbon; Cu, elemental copper; K, elemental potassium; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin

resonance); UFP, ultra-fine particles. Coefficient and 95% CI were estimated through linear regression analysis calculated per increments of: 10 μg/m^3 for NO_2; 5 μg/m^3 for PM_{coarse}; 10^{-5}m^{-1} for $PM_{2.5}$abs; 1 μg/m^3 for OC; 5 ng/m^3 for Cu in $PM_{2.5}$; 50 ng/m^3 for K in $PM_{2.5}$; 10 ng/m^3 for Zn in $PM_{2.5}$; 1 nmol DTT/min/m^3 for OP_{DTT}; 1,000 arbitrary units/m^3 for OP_{ESR}; and 10,000 particles/cm^3 for UFP. Models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session.
In bold: associations that remain after effective number of tests correction.

Discussion

In this study, we observed associations between exposure to a number of highly ubiquitous air pollutants in two periods that are characterized by dynamic and fragile brain development, namely fetal life and childhood, and brain morphology in preadolescents at 9 to12 years old. Overall, higher exposure to air pollution during fetal life and childhood was associated with larger global and subcortical volumes, smaller volume of corpus callosum, and thinner cortex. Moreover, higher exposure to air pollution during childhood was also associated with larger cortical surface area. The associations with fetal exposure to air pollution were predominantly observed in girls. After the multi-pollutant analysis, only few associations remained.

Higher exposure to various air pollutants both during fetal life and childhood was related to smaller cortical thickness, although these associations did not survive the multi-pollutant analysis. Nevertheless, these findings are notably in line with our previous study, with 387 children participating in both studies, wherein we studied the relationship between exposure to fewer air pollutants, including nitrogen oxides, coarse particles, and fine particles, exclusively during fetal life, with brain morphology in school-age children of 6-10 years that were oversampled based on several maternal characteristics, such as depression, cannabis use, and smoking, and on behavior problems of the children (11). There, we found that exposure to fine particles during fetal life was associated with a thinner cortex in several brain regions. The identified regions in the current study showed a clear overlap with the regions identified in our previous study, being located in the anterior and middle regions of the right hemisphere and in the posterior region of the left hemisphere. However, most of the pollutants that showed associations in the single pollutant analysis in the current study, such as organic carbon and elemental Cu, have not been studied previously, impeding direct comparisons between the two studies.

Unlike in our previous study, in the current study we found associations between exposure to various air pollutants during fetal life and measures of cortical and subcortical volumes, but none remained in the multi-pollutant analysis. After stratifying by sex, we showed consistent associations with larger volumes in several grey matter structures of the brain predominantly driven by results observed in girls, and several remained in the multi-pollutant analysis. A possible explanation for the discrepancy between the results observed in girls and boys, is that air pollution can have influence on estrogen receptor genes and estrogen receptor signaling (30,31), thereby evoking different responses between the two sexes, such as earlier brain developmental processes in girls compared to boys. Also, girls usually enter the puberty between the ages of 10 and 14 years, and boys between the ages of 12 and 16 years, thus the alterations related to air pollution exposure

observed in girls might be observed in boys at a slightly later age. Higher childhood exposures to various air pollutants were also generally associated with larger volumes of grey matter structures of the brain, but only the associations between exposure to PM_{10} and coarse particles with larger volume of the putamen remained in the multi-pollutant analysis. After stratifying by sex, we observed that associations between childhood exposures, and measures of cortical and subcortical volumes were driven by results observed in both girls and boys.

We also observed larger surface area in the precentral gyrus of the right hemisphere and in the pericalcarine region of the left hemisphere, in particular to exposure to elemental Zn in the multi-pollutant analysis. While thinner cortex is generally considered to be a marker of impaired cortical structure, being often associated with neuropsychological disorders such as depression or schizophrenia (32,33), the clinical long-term implications of a larger volume of various structures of the brain and larger surface area of the cortex in children at that age range are unclear. Although in the first years of life increase in brain volume is generally associated with healthy development, some patterns of brain maturation that take place between childhood and adolescence involve dynamic changes in both grey and white matter, with grey matter volume showing decreases and white matter volume showing increases (34). Therefore, higher volume and surface area of different grey matter structures could be a sign of a delayed maturation of the brain, or inadequate synaptic pruning, rather than healthy development in preadolescents between 9 and 12 years, although many of the differences in brain structures observed at this age range could likely be of transient nature (35). Repeated assessments of neuroimaging across childhood and adolescence will allow for this to be investigated further.

We also observed a smaller volume of corpus callosum, which is the largest white matter commissure in the human brain, in relation to higher fetal exposure to oxidative potential of fine particles, a quantification of the potentiality of fine particles to induce oxidative stress, in the multi-pollutant analysis. Oxidative stress, together with inflammation, and chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, are the most likely potential mechanisms through which air pollutants can cause damage to the brain (3,6). Oxidative stress occurs when free radicals exceed antioxidant defense mechanisms forming an imbalance between antioxidants and oxidants in favor of the latter. The brain is an organ with a high oxygen depletion rate that primarily comprises lipids, with white matter being richer in lipids than grey matter. Lipids are easily oxidized, and moreover, the brain lacks solid defenses of antioxidants, making it vulnerable to lesions induced by oxidative stress (36). Oxidative stress is highly involved in the process of brain aging, in neurodegenerative diseases, and in other adverse neurological and neuropsychological adversities (37). The results also suggested that

higher childhood exposure to organic carbon was associated with a smaller volume of the corpus callosum in the multi-pollutant analysis. Organic carbon, together with black carbon, is formed by incomplete combustion. During incomplete combustion of fossil fuels such as oil and coal, the proportion of organic carbon to black carbon tends to be small, while during incomplete combustion of biomass fuels such as wood, the proportion of the former is much larger (38). In line with our results, a study by Peterson et al. (9) found an association between higher exposure to polycyclic aromatic hydrocarbons during the third trimester of pregnancy and lower white matter surface, in children from 7 to 9 years old. Another study comparing brain morphology of children living in highly polluted areas of Mexico City versus children living in less polluted areas, found an association between higher exposure to air pollution with lower white matter volumes and an increased number of white matter hyperintensities (8,10). Overall, our current findings add to the growing body of evidence of an association between exposure to air pollution and white matter alterations. Further studies examining the clinical implications of such alterations in childhood, and later in life, are warranted.

The strengths of the current study are: i) large sample size with good quality imaging data; ii) standardized and validated air pollution assessment modeled to the individual level of each participant both during fetal life and during childhood, taking into account changes of residence of the participants during both periods; iii) large number of pollutants, increasing the comprehensiveness of the study; iv) use of advanced statistical methods including inverse probability weighting to reduce possible selection and attrition bias in the study; v) two independent, complementing approaches to quantify brain structures, namely global measures and surface-based vertex-wise method, and vi) adjustment for various socioeconomic and lifestyle variables that are known to be potentially associated with air pollution exposure and brain structure in children.

Several limitations should also be considered. First, sampling campaigns were carried out when the children were between 3.5 and 9 years old and historical pollution data from routine monitoring stations in the study areas was not available for all the pollutants to extrapolate the levels to the specific fetal life and childhood periods for each child. We therefore assumed that the contrast of concentrations of the pollutants remained spatially stable over time. This assumption is based on existing studies wherein spatial stability of the contrast over time for periods stretching from 8 to 18 years is demonstrated for different traffic related air pollutants (21). Nevertheless, we cannot discard the possibility of misclassification, which is more likely to occur in the fetal exposure estimates than the childhood exposure estimates, as the sampling campaigns were carried out when children were between 3.5 and 9 years old. Second, despite the extensive adjustment for potential

confounding variables performed in this study, the results might still be influenced by residual confounding by other relevant, yet unavailable or not inferred, potential confounding variables. Third, we observed that children with data on exposure and outcome were more likely to have parents with higher socioeconomic status as compared to children that did not have that data available, which could lead to a potential selection bias. To minimize this possible bias, we used multiple imputation followed by inverse probability weighting. Nevertheless, it is possible that we have missed associated variables which would have an important effect on the results. Finally, our study is based on a single measurement of the brain structural morphology in preadolescence. Repeated neuroimaging assessments across childhood and adolescence would give insight into trajectories of brain development, and could help to understand the developmental alterations related to air pollution exposure over time. Further studies adopting such approach are needed to better understand the relationship between exposure to air pollution and long-lasting alteration in brain morphology.

In summary, we found associations between higher fetal and childhood exposure to various pollutants, with larger global and subcortical volumes, smaller volume of corpus callosum, and thinner cortex in preadolescents. Moreover, higher exposure to air pollution during childhood also showed associations with larger cortical surface area. The observed associations involved exposure to air pollution during both key developmental periods, namely fetal life and childhood, demonstrating the importance of examination of both periods in future studies. The included pollutants are all highly ubiquitous in urban areas, thereby exposing a large number of children to the associated potential health risks. Since this is the first study to find relationships between fetal and childhood exposures to air pollution with larger volumes of various grey matter structures, as well as a smaller volume of corpus callosum, and the interpretation of the results is equivocal, more studies are warranted to confirm our findings.

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Paper IV – Supplementary Material

Air pollution exposure during fetal life and childhood, and brain morphology in preadolescents

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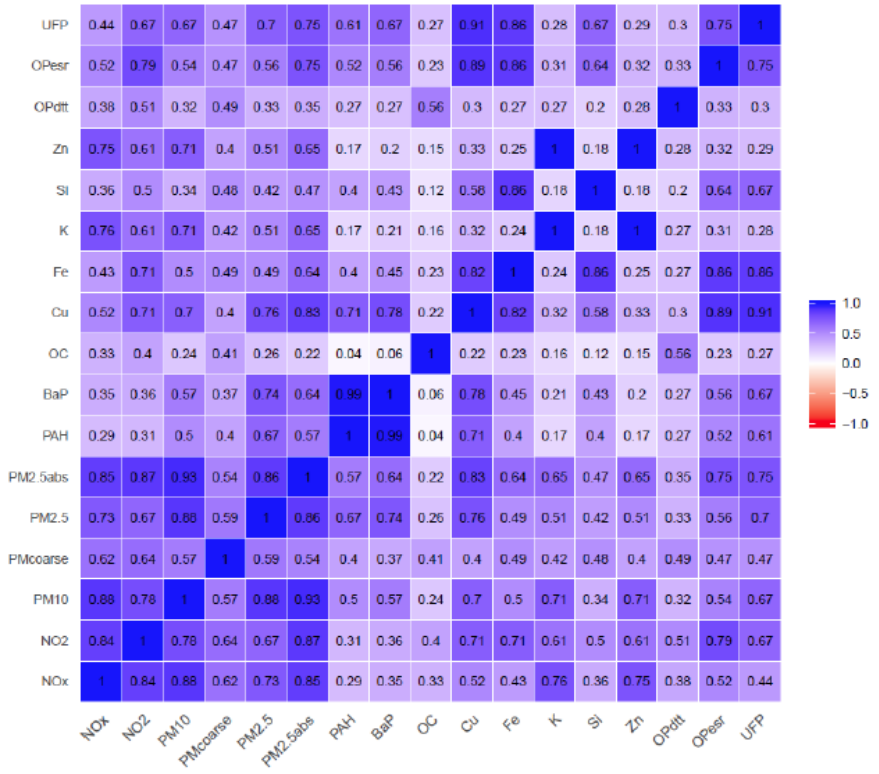
Supplementary Table 1: Comparison of participant characteristics between included and non-included subjects in the study among the 9,610 eligible subjects

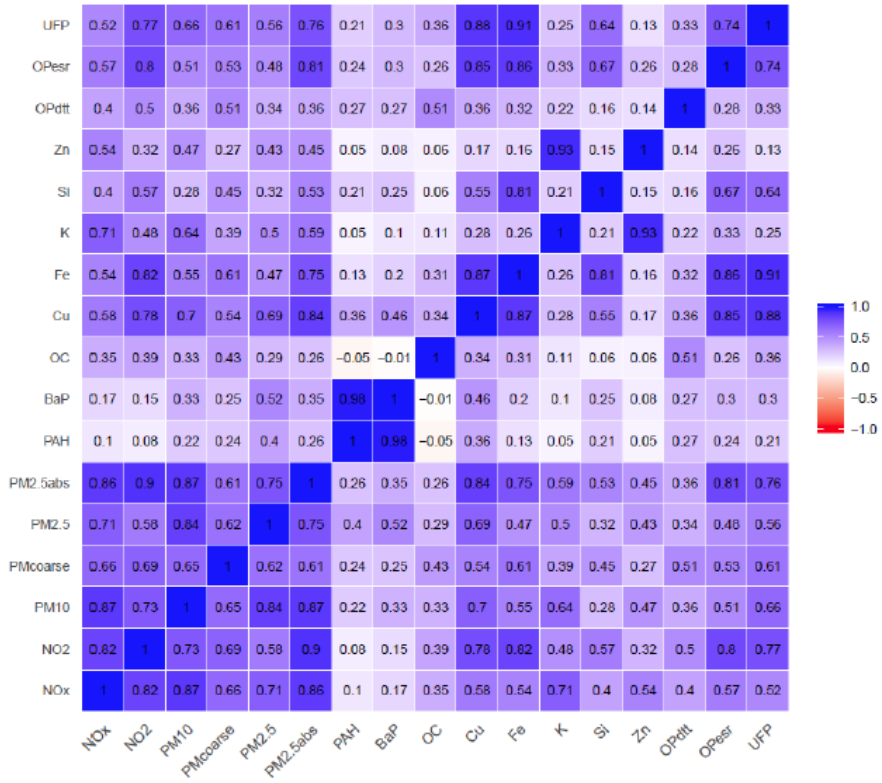
	Distribution		
Participant characteristics	Included (n = 3133)	Not included (n = 6477)	p-value
Maternal education level			<.001
Primary education or lower	6.4	13.8	
Secondary education	40.6	48.8	
Higher education	53.0	37.4	
Paternal education level			<.001
Primary education or lower	5.1	10.3	
Secondary education	39.3	42.2	
Higher education	55.6	47.5	
Monthly household income			<.001
<900€	7.6	15.4	
900€ - 1.600€	13.8	20.9	
1.600€ - 2.200€	14.4	15.3	
>2.200€	64.2	48.5	
Maternal country of birth			<.001
The Netherlands	57.6	46.0	
Other Western	8.5	8.7	
Non-Western	33.9	45.4	
Paternal country of birth			<.001
The Netherlands	67.8	57.7	
Other Western	5.8	7.4	
Non-Western	26.3	34.9	
Family status			<.001

Married	50.1	49.7	
Living together	39.0	34.0	
No partner	10.9	16.3	
Maternal parity (nulli vs. multiparous)	58.1	53.8	<.001
Maternal smoking use during pregnancy			<.001
Never	77.7	71.3	
Smoking use until pregnancy known	8.8	8.4	
Continued smoking use during pregnancy	13.5	20.3	
Maternal alcohol use during pregnancy			<.001
Never	41.8	53.8	
Alcohol use until pregnancy know	14.5	13.2	
Continued alcohol use during pregnancy	43.7	33.0	
Maternal age (years)	31.1 (4.9)	29.3 (5.5)	<.001
Paternal age (years)	33.5 (5.4)	32.3 (5.9)	<.001
Maternal pre-pregnancy body mass index (kg/m2)	23.4 (4.1)	23.8 (4.5)	0.003
Paternal body mass index (kg/m2)	25.3 (3.6)	25.3 (3.4)	0.444
Maternal height (cm)	168.1 (7.3)	166.6 (7.4)	<.001
Paternal height (cm)	182.4 (7.6)	181.1 (8.0)	<.001
Maternal overall psychological distress	0.3 (0.3)	0.3 (0.4)	<.001
Paternal overall psychological distress	0.1 (0.2)	0.2 (0.3)	<.001
Maternal intelligence quotient score	97.7 (14.7)	94.0 (15.7)	<.001

Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.
χ^2 test for categorical variables and t-student test for continuous variables



Supplementary Figure 1. Correlations between the pollutants during fetal life
Abbreviations: B[a]P, benzo[a]pyrene; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; OC, organic carbon; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter with different aerodynamic diameters: less than 10μm (PM_{10}); between 10μm and 2.5μm (PM_{COARSE}); less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; Si, elemental silicon; UFP, ultra-fine particles; Zn, elemental zinc.



Supplementary Figure 2. Correlations between the pollutants during childhood

Abbreviations: B[a]P, benzo[a]pyrene; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; OC, organic carbon; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}); between 10µm and 2.5µm (PM_{COARSE}); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; Si, elemental silicon; UFP, ultra-fine particles; Zn, elemental zinc.

Supplementary Table 2. Adjusted associations (only p<0.05 shown) of exposure during fetal life to various air pollutants with cortical and subcortical brain volumes and total ventricular volume in preadolescents at 9-12 years old, stratified by sex.

		girls				boys			
brain region	pollutant	estimate	min95		max95	p	estimate	min95	max95	p
Total brain	NO_2	**18503.6**	**7358.5**	;	**29648.8**	**0.001**	---	---	---	---
	PM_{10}	**49886.0**	**15703.5**	;	**84068.5**	**0.005**	---	---	---	---
	PM_{COARSE}	**45615.0**	**18370.7**	;	**72859.4**	**0.001**	---	---	---	---
	$PM_{2.5}$	52991.5	8265.6	;	97717.5	0.021	---	---	---	---
	Fe	**45465.7**	**16446.9**	;	**74484.5**	**0.002**	---	---	---	---
	K	46935.1	7979.0	;	85891.2	0.019	---	---	---	---
	Si	45356.1	10179.6	;	80532.6	0.012	---	---	---	---
	Zn	16788.7	3049.4	;	30528.0	0.017	---	---	---	---
	OP_{ESR}	32963.0	2585.7	;	63340.3	0.034	---	---	---	---
	UFP	**47734.9**	**18177.9**	;	**77291.9**	**0.002**	---	---	---	---
Total grey matter	NO_X	**6253.5**	**1856.9**	;	**10650.1**	**0.006**	---	---	---	---
	NO_2	**11214.2**	**4725.4**	;	**17702.9**	**0.001**	---	---	---	---
	PM_{10}	**29060.2**	**9140.9**	;	**48979.6**	**0.005**	---	---	---	---
	PM_{COARSE}	**27151.6**	**11302.8**	;	**43000.5**	**0.001**	---	---	---	---
	Fe	**24877.7**	**7952.8**	;	**41802.6**	**0.004**	---	---	---	---
	K	29351.9	6685.9	;	52018.0	0.012	---	---	---	---

Total grey matter	Si	22983.5	2454.6	;	43512.4	0.029	---	---	---	---
	Zn	10445.2	2451.3	;	18439.0	0.011	---	---	---	---
	OP_{ESR}	19068.0	1367.1	;	36769.0	0.035	---	---	---	---
	UFP	23152.3	5908.7	;	40396.0	0.009	---	---	---	---
Subcortical grey matter	Fe	1669.4	387.2	;	2951.5	0.011	---	---	---	---
	Si	2022.8	471.1	;	3574.4	0.011	---	---	---	---
Cerebral cortex	NO_2	**8129.8**	**2566.4**	;	**13693.2**	**0.005**	---	---	---	---
	PM_{10}	21542.6	4467.2	;	38618.0	0.014	---	---	---	---
	PM_{COARSE}	**20476.7**	**6891.9**	;	**34061.5**	**0.003**	---	---	---	---
	Fe	18206.5	3710.4	;	32702.6	0.014	---	---	---	---
	K	20047.1	605.6	;	39488.7	0.043	---	---	---	---
	Zn	7214.8	357.9	;	14071.7	0.039	---	---	---	---
Cerebral white matter	NO_2	6778.7	1624.2	;	11933.1	0.010	---	---	---	---
	PM_{COARSE}	**17738.8**	**5125.3**	;	**30352.2**	**0.006**	---	---	---	---
	$PM_{2.5}$	26553.2	5941.6	;	47164.9	0.012	---	---	---	---
	Cu	15312.2	781.6	;	29842.8	0.039	---	---	---	---
	Fe	**19394.1**	**6012.8**	;	**32775.5**	**0.005**	---	---	---	---
	Si	20949.6	4744.5	;	37154.7	0.012	---	---	---	---
	UFP	**23534.5**	**9914.4**	;	**37154.6**	**0.001**	---	---	---	---

Cerebellar cortex	Si	3371.6	37.7	; 6705.5	0.048	-3019.4	-5991.0	; -47.8	0.046
Total ventricle	PM_{10}	-2384.7	-4577.0	; -192.4	0.033	---	---	---	---
Mid-posterior part of corpus callosum	Si	---	---	---	---	-33.8	-64.3	; -3.2	0.031

Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}); between 10µm and 2.5µm (PM_{COARSE}); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficient and 95% CI were estimated through linear regression analysis calculated per increments of: 20 µg/m³ for NO_X; 10 µg/m³ for NO_2; 10 µg/m³ for PM_{10}; 5 µg/m³ for PM_{COARSE}; 5 µg/m³ for $PM_{2.5}$; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1 ng/m³ for PAHs; 0.1 ng/m³ for B[a]P; 1 µg/m³ for OC; 5 ng/m³ for Cu in $PM_{2.5}$; 100 ng/m³ for Fe in $PM_{2.5}$; 50 ng/m³ for K in $PM_{2.5}$; 100 ng/m³ for Si in $PM_{2.5}$; 10 ng/m³ for Zn in $PM_{2.5}$; 1 nmol DTT/min/m³ for OP_{DTT}; 1,000 arbitrary units/m³ for OP_{ESR}; and 10,000 particles/cm³ for UFP. Models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session. Subcortical brain volumes were additionally adjusted for intracranial volume.

In bold: associations that remained after effective number of tests correction.

Supplementary Table 3. Adjusted associations (only p<0.05 shown) of exposure during childhood to various air pollutants with cortical and subcortical brain volumes and total ventricular volume in preadolescents at 9-12 years old, stratified by sex.

		girls				boys			
brain region	pollutant	estimate	min95		max95	p	estimate	min95		max95	p
Total brain	Zn	**18622.3**	**5819.7**	;	**31424.9**	**0.005**	---	---		---	---
Total grey matter	Zn	**11287.2**	**3832.9**	;	**18741.4**	**0.003**	---	---		---	---
Subcortical grey matter	OC	---	---		---	---	926.8	238.6	;	1615.1	0.009
Cerebral cortex	Zn	8997.7	2615.2	;	15380.3	0.006	---	---		---	---
Caudate	OC	-160.2	-291.5	;	-29.0	0.017	---	---		---	---
Nucleus accumbens	K	**134.6**	**51.2**	;	**218.1**	**0.002**	---	---		---	---
Total ventricle	NO_X	-624.3	-1161.4	;	-87.2	0.023	---	---		---	---
	NO_2	-708.2	-1331.3	;	-85.2	0.026	---	---		---	---
	$PM_{2.5}abs$	-1384.5	-2715.6	;	-53.4	0.042	---	---		---	---
Mid-posterior part of corpus callosum	PAH	---	---		---	---	-20.5	-40.1	;	-0.8	0.041

Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10μm (PM_{10}); between 10μm and 2.5μm (PM_{COARSE}); less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficient and 95% CI were estimated through linear regression analysis calculated per increments of: 20 $\mu g/m^3$ for NO_X; 10 $\mu g/m^3$ for NO_2; 10 $\mu g/m^3$ for PM_{10}; 5 $\mu g/m^3$ for PM_{COARSE}; 5 $\mu g/m^3$ for $PM_{2.5}$; $10^{-5} m^{-1}$ for $PM_{2.5}$abs; 1 ng/m^3 for PAHs; 0.1 ng/m^3 for B[a]P; 1 $\mu g/m^3$ for OC; 5 ng/m^3 for Cu in $PM_{2.5}$; 100 ng/m^3 for Fe in $PM_{2.5}$; 50 ng/m^3 for K in $PM_{2.5}$; 100 ng/m^3 for Si in $PM_{2.5}$; 10 ng/m^3 for Zn in $PM_{2.5}$; 1 nmol DTT/min/m^3 for OP_{DTT}; 1,000 arbitrary units/m^3 for OP_{ESR}; and 10,000 particles/cm^3 for UFP. Models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session. Subcortical brain volumes were additionally adjusted for intracranial volume.

3.5. Paper V

Fetal and childhood exposures to air pollution and white matter microstructure in preadolescents

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Conflict of interest: None reported

Abstract

Background. Air pollution has been related to brain structural alterations, but a relationship with white matter development is unclear.

Objectives. We aimed to assess whether fetal and childhood exposures to air pollution are related to white matter microstructure in preadolescents.

Methods. We used data of 2,954 children from Generation R Study, a population-based birth cohort in Rotterdam, The Netherlands (2002-2006). Concentrations of 17 air pollutants including nitrogen oxides, particulate matter (PM), and components of PM were estimated at participant's homes during fetal life and childhood using land-use regression models. Diffusion tensor images were obtained at 9-12 years, and white matter microstructure measures (fractional anisotropy (FA) and mean diffusivity (MD)) were computed. We performed linear regression adjusting for socioeconomic and life-style characteristics. We ran single pollutant analyses followed by multi-pollutant analyses using Deletion/Substitution/Addition algorithm.

Results. In the single pollutant analyses, higher exposure to several air pollutants during fetal life and childhood was associated with lower FA and higher MD. In the multi-pollutant analyses, fetal exposure to fine particles remained associated with FA (-0.71 [95%CI -1.26, -0.16] per 5μg/m^3 fine particles increase) and fetal exposure to elemental silicon remained associated with MD (0.06 [95%CI 0.01, 0.11] per 100ng/m^3 silicon increase). Regarding childhood exposures, nitrogen oxides remained associated with FA (-0.14 [95%CI -0.23, -0.04] per 20μg/m^3 nitrogen oxides increase), while elemental zinc and oxidative potential of PM remained associated with MD (0.03 [95%CI 0.01, 0.04] per 10ng/m^3 zinc increase and 0.07 [95%CI 0.00, 0.44] per 1nmol DTT/min/m^3 oxidative potential increase). When fetal and childhood exposures were mutually adjusted, the associations of fetal exposure to silicon and childhood exposure to zinc with global MD remained.

Discussion. Fetal and childhood exposure to air pollutants from tailpipe and non-tailpipe emissions from road traffic were associated with lower FA and higher MD in white matter of preadolescents.

Introduction

The evidence for the harmful effects of air pollution on human health is increasing (Beelen et al. 2014; Chen et al. 2017; Kaufman et al. 2016; Pedersen et al. 2013; Raaschou-Nielsen et al. 2013). Animal studies focusing on the association between exposure to air pollution and brain are leading to growing documentation of a relationship with neuroinflammation and oxidative stress (Block et al. 2012). Due to relatively immature detoxification mechanisms of fetuses and infants, as well as due to the many developmental processes taking place during the fetal life and childhood, direct and indirect exposures to air pollution during these developmental periods could lead to alterations in the brain even at relatively low levels of exposure (Block et al. 2012; Grandjean et al. 2014).

To date, most epidemiological studies have used neuropsychological instruments to assess the relationship between exposure to air pollution and child's neurodevelopment, demonstrating relationships between higher exposures and lower cognitive performance, impaired motor function, and more behavioral problems (Suades-González et al. 2015). However, these studies provide limited understanding of potential structural and functional brain alterations that underlie these associations. The use of magnetic resonance imaging (MRI) allows for identification of such alterations and the limited number of existing studies using MRI, found evidence for associations between exposure to air pollution during fetal life or childhood, and white- and grey matter abnormalities, generally indicating a decrease in white and grey matter mass with higher exposure to air pollution (Calderón-Garcidueñas et al. 2008; Calderón-Garcidueñas et al. 2011; Guxens et al. 2018; Mortamais et al. 2017; Peterson et al. 2015; Pujol et al. 2016a; Pujol et al. 2016b). The use of diffusion tensor imaging, utilized to quantify white matter microstructure, has been limited to a single study, which showed that airborne elemental copper was associated with alterations in white matter microstructure adjacent to caudate nucleus (Pujol et al. 2016b). Unlike anatomical imaging, which is used to measure the grey and white matter structure of the brain, diffusion tensor imaging measures the magnitude and the directionality of water diffusion within the white matter. These microstructural properties measured by diffusion tensor imaging, allow detection of subtle alterations in white matter which may not be observable with conventional anatomical imaging, and which may reveal characteristics typifying healthy brain development (Schmithorst et al. 2010), as well as characteristics that could be indicative of various psychiatric disorders (White et al. 2008). The diffusion profile of white matter can be expressed with the use of two common scalar values: fractional anisotropy which indicates the overall directionality of water diffusion, and mean diffusivity which describes the magnitude of water diffusion within brain tissue. One of the most important processes for optimal brain development is myelination, essential for efficient functioning of the brain through quick and healthy neural

communication (Tilborg et al. 2018). Myelination starts on average 28 weeks after conception and continues throughout adolescence, and is responsible for increases in relative white matter volume and for water diffusion changes within white matter tracts (Tilborg et al. 2018), which can be examined using diffusion tensor imaging. Moreover, diffusion tensor images reveal information about the density of axonal fiber packing in the brain, another measure that is indicative of white matter integrity (Dimond et al. 2019).

Existing studies on the relationship between exposure to air pollution and neurodevelopment assessed using MRI, analyzed a relatively narrow number of air pollutants thereby limiting the opportunity to disentangle which pollutants are most harmful. This becomes relevant when different pollutants reflect different sources of exposure, such as tail-pipe emissions, brake linings, or tire wear markers. Additionally, to our knowledge, the existing studies have either focused on exposure during fetal life or childhood, but not both. As myelination is a process that occurs across these both developmental periods (Tilborg et al. 2018), understanding whether the timing of exposure to air pollution has a distinct and negative impact on neurodevelopment, is crucial. Also, regarding exposure assessment during childhood, the existing studies that analyzed the relationship between childhood exposures and neurodevelopment assessed using MRI, either looked at exposures measured using urinary metabolites, or exposures measured at schools, which likely reflect different sources of pollution and/or different exposure conditions. Therefore, we aimed to analyze the associations between fetal and childhood residential exposures to a wide range of air pollutants with white matter microstructure in preadolescents. Our hypothesis was that higher exposure to air pollution is associated with lower fractional anisotropy and higher mean diffusivity of white matter, generally associated with impaired neurodevelopment.

Methods

Population and Study Design

This study is embedded in the Generation R Study, a population-based birth cohort from fetal life onwards, based in the urban area of Rotterdam, the Netherlands (Kooijman et al. 2016). A total of 8,879 pregnant women were enrolled and children were born between April 2002 and January 2006. Additionally, 899 women were recruited shortly after the birth of their child, also falling within the period between April 2002 and January 2006. When the children were between 9 and 12 years old, they were invited to participate in an MRI session (n=8,548) (White et al. 2018). In total, 3,992 mothers and their children complied with the invite and consented in writing (White et al. 2018). From this total, 2,954 children were from a singleton pregnancy, had good quality imaging scans and data on air pollution, and were included in this analysis. Mothers of the included participants were more likely to have a

higher level of education, higher intelligence quotient, higher household income, and to be Dutch compared to the mothers of children who came to the MRI assessment but were excluded from the final sample (data not shown). The Medical Ethics Committee of the Erasmus Medical Centre in Rotterdam, The Netherlands, granted ethical approval for the study.

Exposure to Air Pollution

Air pollution concentrations were estimated for all reported home addresses of each participant during the fetal and childhood periods following a standardized procedure (Guxens et al. 2018; de Hoogh et al. 2013; Jedynska et al. 2014; Montagne et al. 2015; Yang et al. 2015). In brief, within the ESCAPE (European Study of Cohorts for Air Pollution Effects) and TRANSPHORM (Transport related Air Pollution and Health impacts – Integrated Methodologies for Assessing Particulate Matter) projects, three two-week measurements of nitrogen oxides (NO_X, NO_2) were performed in the warm, cold and intermediate seasons between February 2009 and February 2010 at 80 sites spread across the Netherlands and Belgium (Montagne et al. 2015). Additionally, at 40 of those sites particulate matter (PM) with aerodynamic diameter less than 10μm (PM_{10}), between 10μm and 2.5μm (PMcoarse), less than 2.5μm ($PM_{2.5}$), absorbance of $PM_{2.5}$ fraction ($PM_{2.5}$absorbance), and composition of $PM_{2.5}$ consisting of polycyclic aromatic hydrocarbons (PAHs), benzo[a]pyrene (B[a]P), organic carbon (OC), copper (Cu), iron (Fe), potassium (K), silicon (Si), zinc (Zn), and oxidative potential of $PM_{2.5}$ (OP) measurements were carried out (de Hoogh et al. 2013; Jedynska et al. 2014; Yang et al. 2015). The OP was evaluated using two acellular methods: dithiothreitol (OP_{DTT}) and electron spin resonance (OP_{ESR}) (Yang et al. 2015). Another campaign within the MUSiC (Measurements of Ultrafine particles and Soot in Cities) project measuring PM with aerodynamic diameter less than 0.1μm (ultra-fine particles (UFP)) was held in 2013 at 80 sites in Rotterdam (Montagne et al. 2015). The number concentrations of UFP were measured in real time for 30 minutes at each site in three different seasons. For each pollutant, the results of the measurements were averaged, adjusting for temporal trends using data from a continuous reference site, resulting in one annual mean concentration for each pollutant.

A variety of potential land use predictors, such as proximity to the nearest road, traffic intensity on the nearest road, and population density, was then assigned to each monitoring site and linear regression modeling was applied to determine which combination of predictors explained the levels of the pollutants most accurately, resulting in land use regression models (de Hoogh et al. 2013; Jedynska et al. 2014; Montagne et al. 2015; Yang et al. 2015). In this study, we only focused on pollutants whose land use regression models included at least one traffic predictor. Next, these land use regression models were applied to each address that the participants have lived at

during the period of interest, i.e. since conception until the MRI session. Taking into account the time spent at each address and weighting the pollution levels accordingly, we then obtained a single, mean air pollution concentration of each pollutant for each participant for the fetal period (i.e. since conception until birth) and for the childhood period (i.e. since birth until the MRI session). For those participants that were recruited shortly after birth (n=310) we considered the address at birth as representative for the pregnancy period. As no historical data was available for majority of the pollutants under study to perform back- and forward extrapolation of the concentrations to match the exact periods of interest, we assumed that the spatial contrast remained constant over time as it has been previously shown for periods up to 18 years (Eeftens et al. 2011; Gulliver et al. 2013).

Diffusion Tensor Imaging

Image Acquisition

To familiarize participants with the magnetic resonance environment and therefore reduce the possibility of failure to complete the scanning session, each child underwent half an hour mock scanning session prior to the actual MRI (White et al. 2018). To limit the movement of the head, the participating children were accommodated utmost, by providing them with a thorough explanation before the scanning session, the possibility to watch a movie or listen to music during the session, and by placement of cushions around the head to fixate the head in a comfortable way. The scans were performed on a 3 Tesla General Electric scanner (GE, MR750W, Milwaukee, WI) using an 8-channel receive-only head coil. Diffusion tensor imaging data were obtained using an axial spin echo with 35-direction echo planar imaging sequence (TR = 12.500 ms, TE = 72 ms, field of view = 240 mm x 240 mm, Acquisition Matrix = 120 x 120, slice thickness = 2 mm, number of slices = 65, Asset Acceleration Factor = 2, b = 900 s/mm2).

Image pre-processing

The pre-processing was performed with the use of the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al. 2012). First non-brain tissue was removed and then the images were rectified for artifacts induced by eddy currents, and for translations or rotations that potentially arose due to minor movement of the head during the scanning session. The B-table was then rotated based on the rotations calculated and applied to the diffusion data during the eddy-current correction step. Next, using RESTORE approach from the Camino diffusion MRI toolkit (Cook et al. 2006) a diffusion tensor was fitted at each voxel, followed by the computation of fractional anisotropy (FA) and mean diffusivity (MD).

Probabilistic tractography

To establish connectivity distributions for several large fibre bundles, the automated FSL plugin AutoPtx (de Groot et al. 2015) was used to perform probabilistic white matter fiber tractography on the scans of each participant. This package includes a set of pre-defined seed, target and exclusion masks for a number of large white matter tracts. After a nonlinear registration of the FA map of each participant to the FMRIB58 FA map, these pre-defined seed, target and exclusion masks are warped back to each participant's native space. The FSL Bayesian Estimation of Diffusion Parameters Obtained Using Sampling Techniques (BEDPOSTx) along with the FSL ProbtrackX were used, taking into account two fiber orientations, to conduct probabilistic fiber tractography (Behrens et al. 2003; Behrens et al. 2007). The amount of successful seed-to-target attempts from the identified connectivity distributions were used to normalize the connectivity distributions, followed by introduction of a threshold to eliminate voxels that were implausible to belong to the true distribution. By weighting voxels based on the connectivity distribution, with voxels with higher probability of being part of the true distribution receiving higher weight, average FA and MD values were assessed for each white matter tract.

DTI quality assurance

For automatic assessment of slice-wise variation and properties of artifacts in each diffusion-weighted volume, the DTIPrep tool (https://www.nitrc.org/projects/dtiprep/) was used. Next, maps of sum-of-squares error (SSE) from the calculations of diffusion tensor were studied for signals characteristic of artifacts. Each SSE map was classified by a value from 0 to 3, with 0 indicating no artifacts, 1 indicating mild artifacts, 2 indicating moderate artifacts, and 3 indicating severe artifacts. If the automated QC or the SSE map inspection was poor, these cases were excluded from analyses. Finally, an examination of accuracy with respect to the nonlinear registration of the scans to standard space was performed, to ensure seed and target masks for tractography were properly aligned to native space.

Construction of Global DTI metrics

In order to estimate a 'global' estimate of FA and MD, which may better capture associations which have relatively small effect sizes which spatially are wide-spread in the brain, we ran a confirmatory factor analysis on scalar metrics from 12 commonly-defined white matter tracts: cingulum bundle, cortico-spinal tract, inferior longitudinal fasciculus, superior longitudinal fasciculus, uncinate fasciculus (one per hemisphere), forceps minor and forceps major (interhemispheric). The confirmatory factor analysis essentially generates a weighted average of all 12 tracts based on the factor loadings. For FA and MD, a separate (though structured identically) factor analysis was run to produce a factor score (global metric of FA and MD) (Muetzel et

al. 2018). Global metrics are factors scores from a confirmatory factor analysis (i.e., standardized scores centered on 0, and ranging from roughly -5 to 5 for FA, and -0.5 to 0.5 for MD) and thus do not conform to the standard values typically seen with DTI (e.g., FA ranging from 0 to 1). All FA values from specific tracts are presented on the proper scale (e.g., for FA from 0 to 1). For the MD values from specific tracts, a scaling factor of 10^9 was used. FA indicates the tendency for preferential water diffusion in white matter tracts, which is lower in white matter with certain features (e.g. white matter tracts in which the comprising axons are less densely packed, and the directionality of the water diffusion is not uniformly directed as compared to well organized tracts). MD describes the magnitude of average water diffusion in all directions within brain tissue, with higher values generally occurring in white matter tracts that show a less well organized structure.

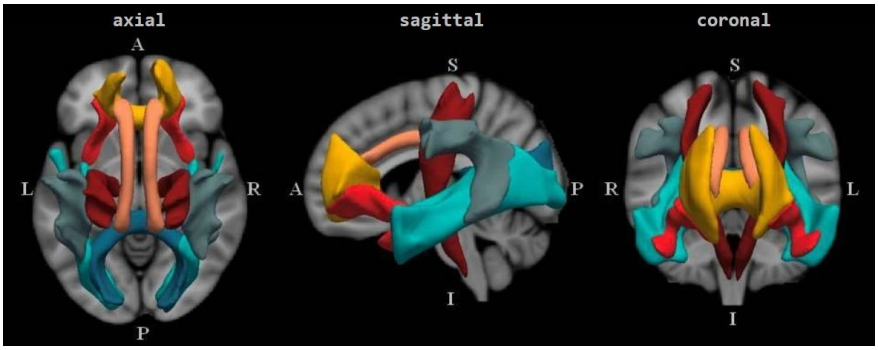

Figure1. Group average representations of the tracts in standard coordinate space. Dark red, cingulum bundle; blue, forceps major; yellow, forceps minor; turquoise, inferior longitudinal fasciculus; gray, superior longitudinal fasciculus; red, uncinate fasciculus. R, right; L, left; A, anterior; P, posterior; I, inferior; S, superior.

Potential confounding variables

Potential confounding variables were defined based on scientific literature and on availability of data within the Generation R cohort (Guxens et al. 2018). Maternal and paternal educational level, monthly household income, maternal and paternal country of birth, maternal and paternal age at enrollment in the cohort, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, parity, marital status, and maternal and paternal psychological distress (using Brief Symptom Inventory) were collected by questionnaires during pregnancy. Maternal and paternal weight and height were measured or self-reported in the 1st trimester of pregnancy and pre-pregnancy body mass index was calculated. Maternal intelligence quotient was assessed at child's age of 6 years with Ravens Advanced Progressive Matrices Test, set I. Child's genetic ancestry was estimated based on the genome-wide single-nucleotide polymorphism data from whole blood

at birth, and 4 principal components of ancestry were included here to better correct for population stratification. Child's sex was obtained from hospital records at birth and child's age was collected at the scanning session.

Statistical Analyses

We applied multiple imputation of missing values using chained equations to impute missing potential confounding variables among all participants with available data on the exposure and the outcome. We obtained 25 completed datasets that we analyzed using standard procedures for multiple imputation. Children included in the analysis (n=2,954) were more likely to have parents from a higher socioeconomic position compared to children that were not included (n=6,656) (Table 1). To correct for selection bias that potentially arises when only population with available exposure and outcome data is included as compared to a full initial cohort recruited at pregnancy we used inverse probability weighting (Weisskopf et al. 2015; Weuve et al. 2012). In brief, we used information available for all participants at recruitment to predict the probability of participation in the current study, and used the inverse of those probabilities as weights in the analyses so that results would be representative for the initial populations of the cohorts. The variables used to create the weights, as well as the distribution of the obtained weights, can be found in Figure S1.

After visual inspection of the distributions, we used linear regression models to analyze the relationships between concentrations of air pollutants first during fetal life and then during childhood, and white matter microstructure metrics. We first performed single pollutant analyses wherein each pollutant was studied separately. Next, we ran multi-pollutant analyses using the Deletion/Substitution/Addition algorithm which has shown relatively good performance with reference to a compromise between sensitivity and false discovery proportion compared to other similar methods (Agier et al. 2016). Briefly, the Deletion/Substitution/Addition algorithm is an iterative selection method, which selects the variables that are most predictive of the outcome by cross-validation, taking into account the correlation matrix of the variables, and simultaneously correcting for multiple testing. This algorithm allows three steps at each performed iteration, namely 1) deletion: removal of a variable, 2) substitution: replacement of one variable with another one, and 3) addition: insertion of a variable to the pending model. The exploration for the optimal model, with optimal model representing a combination of variables with the smallest value of root-mean-square deviation, begins with the intercept model and continues with the deletion, substitution, and addition process to identify the optimal combination of variables. To assure the adjustment for all potential confounding variables in each model, we fixated the potential confounders, allowing only the air pollution exposures to participate in the selection process. When two or more pollutants showed a correlation of 0.90 or more, we only included the

pollutant which land use regression model showed a better performance based on the R^2 of the model. As the Deletion/Substitution/Addition algorithm is based on a cross-validation process which is subject to random variations, we ran each model 200 times selecting the final model based on frequency of occurrence (at least 10%). We performed two separate analyses using the Deletion/Substitution/Addition algorithm: one including only the fetal life air pollution exposures; and the second one including only the childhood air pollution exposures. To combine the fetal life and childhood air pollution exposures, we analyzed simultaneously those exposures that were selected by the Deletion/Substitution/Addition algorithm, and that remained associated after mutual adjustment in cases where more than one pollutant was selected per time period, using linear regression model.

Additionally, the pollutants that remained associated with global white matter microstructure metrics in the multi-pollutant approach were also analyzed for their relationship with FA and MD in twelve separate white matter tracts (Figure 1) applying false discovery rate correction. If more than one pollutant showed association with white matter microstructure in a specific tract, those pollutants were also analyzed simultaneously in one model.

As we considered the address at birth as representative for the pregnancy period for those participants that were recruited shortly after birth (n=310), and as their mothers were slightly older and from a higher socioeconomic position as compared to mothers recruited during pregnancy (data not shown), we repeated the fetal life analyses excluding the children from mothers recruited shortly after birth, to test the sensitivity of the results.

Finally, to quantify the measurement error in the air pollution assessment, thereby quantifying the uncertainty in the exposure-outcome associations, we used a bootstrap method that decomposes the error into two components - the classical-like component and the Berkson-like component (Szpiro et al. 2011). By simulating the exposure based on the actual measurements and introducing different levels of variability related to the parameters of the land use regression model, the estimation of the bias in the health effect estimates was quantified as the difference between the empirical means obtained assuming various levels of variability in the parameters, and no variability.

All models were carried out with the imputed datasets (except for the measurement error calculations which were carried out with one imputed dataset), were corrected for a potential selection bias using inverse probability weighting, and were adjusted for potential confounding variables described in the section above. Statistical analyses were carried out using STATA (version 14.0; StataCorporation, College Station, TX) and R (version 3.4.2; R Core Team (2017)).

Table 1. Participant characteristics and comparison between included and non-included subjects in the study among the 9,610 eligible subjects

	Distribution		
Participant characteristics	Included (n=2,954)	Not included (n=6,656)	p-value
Maternal education level			<.001
Primary education or lower	176 (6.5%)	775 (13.6%)	
Secondary education	1,092 (40.1%)	2,784 (48.8%)	
Higher education	1,453 (53.4%)	2,148 (37.6%)	
Paternal education level			<.001
Primary education or lower	92 (4.9%)	335 (10.2%)	
Secondary education	700 (37.6%)	1,420 (43.1%)	
Higher education	1,069 (57.4%)	1,542 (46.8%)	
Monthly household income at at intake			<.001
<900€	172 (7.5%)	658 (15.2%)	
900€ - 1,600€	319 (13.8%)	891 (20.6%)	
1,600€ - 2,200€	329 (14.3%)	663 (15.3%)	
>2,200€	1,486 (64.4%)	2,110 (48.8%)	
Maternal country of birth			<.001
The Netherlands	1,702 (58.7%)	2,766 (45.8%)	
Other Western	252 (8.7%)	516 (8.5%)	
Non-Western	944 (32.6%)	2,761 (45.7%)	
Paternal country of birth			<.001
The Netherlands	1,419 (69.5%)	2,207 (57.2%)	
Other Western	120 (5.9%)	283 (7.3%)	
Non-Western	502 (24.6%)	1,368 (35.5%)	
Family status at intake			<.001
Married	1,394 (51.5%)	2,808 (49.1%)	
Living together	1,023 (37.8%)	1,989 (34.7%)	
No partner	292 (10.8%)	928 (16.2%)	
Maternal parity (nulli vs. multiparous)	1,630 (57.2%)	3,473 (54.3%)	<.001
Maternal smoking use during pregnancy			<.001
Never	2,004 (78.2%)	3,956 (71.3%)	
Smoking use until pregnancy known	222 (8.7%)	470 (8.5%)	
Continued smoking use during pregnancy	338 (13.2%)	1,123 (20.2%)	
Maternal alcohol use during pregnancy			<.001
Never	973 (41.7%)	2,773 (53.4%)	
Alcohol use until pregnancy know	335 (14.4%)	691 (13.3%)	
Continued alcohol use during pregnancy	1,023 (43.9%)	1,728 (33.3%)	
Maternal age at intake (years)	31.2 (4.8)	29.3 (5.5)	<.001
Paternal age at intake (years)	33.5 (5.3)	32.3 (5.9)	<.001
Maternal pre-pregnancy body mass index (kg/m^2)	23.4 (4.0)	23.8 (4.5)	0.003
Paternal body mass index (kg/m^2)	25.2 (3.3)	25.4 (3.6)	0.141
Maternal height (cm)	168.1 (7.4)	166.7 (7.4)	<.001
Paternal height (cm)	182.6 (7.7)	181.1 (8.0)	<.001
Maternal psychological distress during pregnancy	0.3 (0.3)	0.3 (0.4)	<.001
Paternal psychological distress during pregnancy	0.1 (0.2)	0.2 (0.3)	<.001
Maternal intelligence quotient score	97.9 (14.7)	94.0 (15.7)	<.001

Values are counts (percentages) for the categorical variables and mean (standard deviation) for the continuous variables.
χ^2 test for categorical variables and t-student test for continuous variables

Results

Participant characteristics are shown in Table 1. Mean air pollution exposure levels during fetal life of the participants were 35.1μg/m^3 for NO_2 and 16.5μg/m^3 for $PM_{2.5}$, and 32.8μg/m^3 for NO_2 and 16.4μg/m^3 for $PM_{2.5}$ during childhood (Table 2). Correlations between the exposures in the two periods of interest were generally moderate, ranging between 0.40 for NO_2 and 0.63 for OC (Table 2). Mothers with a higher level of education, a higher monthly household income, and nulliparous were more likely to be exposed to higher levels of NO_2 during pregnancy. These associations were however not consistent between the different pollutants (Tables S1 and S2). Correlations between the concentrations of pollutants also varied considerably depending on the pollutant (Figures S2 and S3). Based on the correlations, we excluded PM_{10}, B[a]P, K, and UFP from the multi-pollutant analysis as they showed correlations higher than 0.90 with $PM_{2.5}$absorbance, PAHs, Zn, and Cu respectively, but had a poorer performing land use regression model (with exception of B[a]P which was excluded as it is one of the components of PAHs).

In the single pollutant analysis, higher levels of NO_X, PM_{10}, $PM_{2.5}$, and $PM_{2.5}$absorbance during fetal life were associated with lower global FA. Higher levels of NO_X, NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5}$absorbance, Cu, Fe, Si, OP_{ESR}, and UFP during fetal life showed associations with higher global MD (Table 3). In the multi-pollutant analysis, $PM_{2.5}$ exposure during fetal life remained associated with global FA (0.71 lower global FA [95% CI: -1.26 to -0.16] per 5 μg/m^3 increase of $PM_{2.5}$) (Table 4). We also observed an association between a simultaneous exposure to $PM_{2.5}$ and PAHs during fetal life, and global FA, showing lower values of global FA with higher levels of $PM_{2.5}$ and higher values of global FA with higher levels of PAHs. Fetal life exposure to Si remained associated with global MD in the multi-pollutant analysis (0.06 higher global MD [95%CI 0.01 to 0.11] per 100 ng/m^3 increase of Si).

Regarding air pollution exposure during childhood, higher levels of NO_X, NO_2, $PM_{2.5}$absorbance, OC, and K were associated with lower global FA. Higher levels of NO_X, NO_2, PM_{10}, PMcoarse, $PM_{2.5}$, $PM_{2.5}$absorbance, K, Si, Zn, and OP_{DTT} showed associations with higher global MD (Table 3). In the multi-pollutant analysis, exposure to NO_X during childhood remained associated with global FA (0.14 lower global FA [95% CI: -0.23 to -0.04] per 20 μg/m^3 increase of NO_X) while Zn and OP_{DTT} remained associated with global MD (0.03 higher global MD [95% CI: 0.01 to 0.04] per 10 ng/m^3 increase in Zn, and 0.07 higher MD [95%CI 0.00, 0.44] per 1 nmol DTT/min/m^3 increase in OP_{DTT}) (Table 4). Exclusion of children with mothers recruited shortly after the pregnancy, did not lead to notable changes in the results (Table S3).

When fetal life and childhood exposures were analyzed simultaneously, they no longer showed associations with global FA (Table S4). However, the associations between fetal life exposure to Si and childhood exposure to Zn, and global MD remained after mutual adjustment.

In analyses wherein the twelve white matter tracts were studied separately, no association between exposure to air pollution and FA was found after correction for multiple testing (Tables S5 and S6). However, higher exposure to Si during fetal life remained associated with higher MD in cingulate gyrus part of cingulum and in superior longitudinal fasciculus of the left hemisphere, and in forceps minor. Regarding air pollution exposures during childhood, higher exposure to Zn remained associated with higher MD in six tracts: uncinate fasciculus tract of the right hemisphere; cingulate gyrus part of cingulum of both hemispheres; superior longitudinal fasciculus of both hemispheres; and forceps minor (Table S6). When we analyzed the relationship between fetal life exposure to Si and childhood exposure to Zn simultaneously and MD in the three tracts that were associated with both pollutants in the previous step, all the associations remained (Table S4).

The quantification of the measurement error in the air pollution assessment using a bootstrap method suggested that the measurement error introduced in our study did not bias the health effect estimates substantially (Table S7).

Table 2. Air pollution exposure levels during fetal life and during childhood, and Pearson's correlations between the exposures at the two time periods

	Fetal life				Childhood				
Pollutant	Mean	p25	p50	p75	Mean	p25	p50	p75	Correlation
NO_X	51.1	40.9	46.6	58.2	47.0	38.4	43.1	52.1	0.55
NO_2	34.7	31.9	34.2	36.7	32.6	29.4	32.5	35.1	0.47
PM_{10}	27.1	26.0	26.7	28.0	26.6	25.7	26.3	27.2	0.52
PM_{coarse}	9.9	9.2	10.1	10.6	9.5	8.6	9.5	10.3	0.56
$PM_{2.5}$	17.0	16.6	16.8	17.2	16.8	16.5	16.7	17.1	0.61
$PM_{2.5}$abs	1.7	1.5	1.6	1.8	1.6	1.4	1.5	1.7	0.53
PAHs	1.0	0.8	0.9	1.1	1.0	0.8	0.9	1.1	0.66
B[a]P	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.67
OC	1.7	1.5	1.8	2.0	1.6	1.4	1.7	1.9	0.60
Cu	4.9	4.5	4.6	5.0	4.6	4.2	4.5	4.8	0.53
Fe	123.4	114.1	119.8	129.1	116.8	106.6	116.5	124.4	0.52
K	113.0	108.5	110.5	114.8	112.1	108.1	110.2	113.4	0.61
Si	93.0	87.9	88.8	90.5	91.6	87.6	88.6	90.4	0.60
Zn	20.2	17.6	18.8	21.3	20.0	17.4	18.7	20.8	0.55
OP_{DTT}	1.3	1.3	1.3	1.4	1.3	1.2	1.3	1.4	0.59
OP_{ESR}	1079.4	1000.7	1036.6	1100.1	1037.9	964.7	1014.7	1072.2	0.57
UFP	10330.3	9509.9	10058.5	10926.3	9547.1	8446.0	9644.8	10385.0	0.49

NO_X, nitrogen oxides in µg/m^3; NO_2, nitrogen dioxide in µg/m^3; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}) in µg/m^3; between 10µm and 2.5µm (PMcoarse) in µg/m^3; less than 2.5µm ($PM_{2.5}$) in µg/m^3; $PM_{2.5}$absorbance, absorbance of $PM_{2.5}$ filters in $10^{-5}m^{-1}$; PAHs, polycyclic aromatic hydrocarbons in ng/m^3; B[a]P, benzo[a]pyrene in ng/m^3; OC, organic carbon in ng/m^3; Cu, elemental copper in ng/m^3; Fe, elemental iron in ng/m^3; K, elemental potassium in ng/m^3; Si, elemental silicon in ng/m^3; Zn, elemental zinc in ng/m^3; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol in nmol DTT/min/m^3 and OP_{ESR} – electron spin resonance in arbitrary units/m^3); UFP, ultra-fine particles in particles/cm^3.

Table 3. Adjusted associations between exposure during fetal life and childhood to single air pollutants and global fractional anisotropy, and global mean diffusivity at 9-12y

	Global fractional anisotropy							
	Fetal life				Childhood			
Pollutant	Coef.	95% CI		p-value	Coef.	95% CI		p-value
NO_X	**-0.11**	**-0.20**	**-0.02**	**0.018**	**-0.14**	**-0.23**	**-0.04**	**0.007**
NO_2	-0.11	-0.25 ;	0.03	0.109	**-0.13**	**-0.25**	**-0.01**	**0.029**
PM_{10}	**-0.49**	**-0.90**	**-0.08**	**0.018**	-0.45	-0.91 ;	0.01	0.056
PM_{coarse}	-0.05	-0.37 ;	0.27	0.757	-0.29	-0.63 ;	0.04	0.086
$PM_{2.5}$	**-0.71**	**-1.26**	**-0.16**	**0.012**	-0.46	-1.14 ;	0.21	0.179
$PM_{2.5}$abs	**-0.29**	**-0.51**	**-0.07**	**0.012**	**-0.27**	**-0.51**	**-0.02**	**0.032**
PAHs	0.01	-0.19 ;	0.21	0.952	0.15	-0.09 ;	0.38	0.216
B[a]P	-0.06	-0.24 ;	0.13	0.563	0.11	-0.14 ;	0.35	0.382
OC	-0.12	-0.29 ;	0.05	0.175	**-0.20**	**-0.38**	**-0.03**	**0.024**
Cu	-0.32	-0.71 ;	0.06	0.097	-0.22	-0.65 ;	0.21	0.323
Fe	-0.20	-0.54 ;	0.14	0.247	-0.22	-0.53 ;	0.09	0.156
K	-0.38	-0.84 ;	0.08	0.103	**-0.53**	**-1.03**	**-0.03**	**0.039**
Si	-0.28	-0.70 ;	0.15	0.198	-0.24	-0.66 ;	0.19	0.277
Zn	-0.12	-0.28 ;	0.04	0.130	-0.13	-0.27 ;	0.02	0.098
OP_{DTT}	0.21	-0.34 ;	0.75	0.448	-0.14	-0.69 ;	0.42	0.622
OP_{ESR}	-0.19	-0.55 ;	0.17	0.299	-0.21	-0.57 ;	0.16	0.259
UFP	-0.26	-0.63 ;	0.11	0.173	-0.21	-0.56 ;	0.15	0.250

Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}); between 10µm and 2.5µm (PMcoarse); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$absorbance, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficient and 95% CI were estimated through linear regression analysis and the models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session was also collected per increments of: 20 µg/m³ for NO_X; 10 µg/m³ for NO_2; 10 µg/m³ for PM_{10}; 5 µg/m³ for PM_{coarse}; 5 µg/m³ for $PM_{2.5}$; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1 ng/m³ for PAHs; 0.1 ng/m³ for B[a]P; 1 µg/m³ for OC; 5 ng/m³ for Cu in $PM_{2.5}$; 100 ng/m³ for Fe in $PM_{2.5}$; 50 ng/m³ for K in $PM_{2.5}$; 100 ng/m³ for Si in $PM_{2.5}$; 10 ng/m³ for Zn in $PM_{2.5}$; 1 nmol DTT/min/m³ for OP_{DTT}; 1,000 arbitrary units/m³ for OP_{ESR}; and 10,000 particles/cm³ for UFP.

Table 3 (continuation). Adjusted associations between exposure during fetal life and childhood to single air pollutants and global fractional anisotropy, and global mean diffusivity at 9-12y

	Global mean diffusivity							
	Fetal life				Childhood			
Pollutant	Coef.	95% CI		p-value	Coef.	95% CI		p-value
NO_X	**0.01**	**0.00** ;	**0.02**	**0.050**	**0.02**	**0.01** ;	**0.03**	**0.005**
NO_2	**0.02**	**0.00** ;	**0.04**	**0.021**	**0.02**	**0.00** ;	**0.03**	**0.011**
PM_{10}	**0.05**	**0.00** ;	**0.10**	**0.042**	**0.07**	**0.01** ;	**0.12**	**0.027**
PM_{coarse}	0.03	-0.01 ;	0.07	0.186	**0.04**	**0.00** ;	**0.09**	**0.038**
$PM_{2.5}$	**0.09**	**0.02** ;	**0.15**	**0.014**	**0.11**	**0.03** ;	**0.20**	**0.010**
$PM_{2.5}$abs	**0.04**	**0.01** ;	**0.06**	**0.012**	**0.04**	**0.01** ;	**0.07**	**0.009**
PAHs	0.01	-0.01 ;	0.04	0.259	0.01	-0.02 ;	0.04	0.477
B[a]P	0.02	-0.01 ;	0.04	0.149	0.01	-0.02 ;	0.04	0.342
OC	0.02	-0.01 ;	0.04	0.153	0.02	0.00 ;	0.04	0.088
Cu	**0.05**	**0.01** ;	**0.10**	**0.030**	0.03	-0.02 ;	0.09	0.221
Fe	**0.05**	**0.01** ;	**0.09**	**0.018**	0.03	-0.01 ;	0.07	0.102
K	0.04	-0.02 ;	0.09	0.185	**0.09**	**0.03** ;	**0.15**	**0.006**
Si	**0.07**	**0.02** ;	**0.12**	**0.010**	**0.05**	**0.00** ;	**0.11**	**0.047**
Zn	0.01	-0.01 ;	0.03	0.195	**0.03**	**0.01** ;	**0.05**	**0.003**
OP_{DTT}	0.06	-0.01 ;	0.13	0.069	**0.09**	**0.02** ;	**0.16**	**0.016**
OP_{ESR}	**0.04**	**0.00** ;	**0.09**	**0.047**	0.04	0.00 ;	0.09	0.080
UFP	**0.05**	**0.01** ;	**0.10**	**0.023**	0.03	-0.01 ;	0.08	0.127

Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}); between 10µm and 2.5µm (PMcoarse); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$absorbance, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficient and 95% CI were estimated through linear regression analysis and the models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session was also collected per increments of: 20 µg/m^3 for NO_X; 10 µg/m^3 for NO_2; 10 µg/m^3 for PM_{10}; 5 µg/m^3 for PM_{coarse}; 5 µg/m^3 for $PM_{2.5}$; 10^{-5}m^{-1} for $PM_{2.5}$abs; 1 ng/m^3 for PAHs; 0.1 ng/m^3 for B[a]P; 1 µg/m^3 for OC; 5 ng/m^3 for Cu in $PM_{2.5}$; 100 ng/m^3 for Fe in $PM_{2.5}$; 50 ng/m^3 for K in $PM_{2.5}$; 100 ng/m^3 for Si in $PM_{2.5}$; 10 ng/m^3 for Zn in $PM_{2.5}$; 1 nmol DTT/min/m^3 for OP_{DTT}; 1,000 arbitrary units/m^3 for OP_{ESR}; and 10,000 particles/cm^3 for UFP.

Table 4. Results of the multi-pollutant analyses between: fetal life $PM_{2.5}$, PAHs, and OP_{DTT} exposures and global fractional anisotropy; childhood NO_X, OP_{DTT} and OC exposures, and global fractional anisotropy; fetal life Si, and OP_{DTT} exposures and global mean diffusivity; and childhood Zn, OP_{DTT} and Si exposures and global mean diffusivity.

Fetal life exposures and global fractional anisotropy						
Pollutants selected by DSA algorithm	% of runs	Pollutant	Coef.	95% CI		p-value
$PM_{2.5}$ + PAHs + OP_{DTT}	24.5	$PM_{2.5}$	-1.49	-2.25	; -0.73	<0.001
		PAHs	0.33	0.06	; 0.59	0.017
		OP_{DTT}	0.50	-0.07	; 1.07	0.087
$PM_{2.5}$ + PAHs	20	$PM_{2.5}$	-1.32	-2.06	; -0.58	<0.001
		PAHs	0.33	0.06	; 0.60	0.017
$PM_{2.5}$	13	$PM_{2.5}$	-0.71	-1.26	; -0.16	0.012

Childhood exposures and global fractional anisotropy						
Pollutants selected by DSA algorithm	% of runs	Pollutant	Coef.	95% CI		p-value
NO_X	22.5	NO_X	-0.14	-0.23	; -0.04	0.007
NO_X + OP_{DTT} + OC	10.5	NO_X	-0.13	-0.24	; -0.03	0.015
		OP_{DTT}	0.46	-0.19	; 1.11	0.163
		OC	-0.19	-0.40	; 0.01	0.059

Fetal life exposures and global mean diffusivity						
Pollutants selected by DSA algorithm	% of runs	Pollutant	Coef.	95% CI		p-value
Si + OP_{DTT}	13.5	Si	0.06	0.01	; 0.11	0.018
		OP_{DTT}	0.05	-0.02	; 0.11	0.171

Childhood exposures and global mean diffusivity						
Pollutants selected by DSA algorithm	% of runs	Pollutant	Coef.	95% CI		p-value
Zn + OP_{DTT}	46.5	Zn	0.03	0.01	; 0.04	0.005
		OP_{DTT}	0.07	0.00	; 0.14	0.046
Zn + OP_{DTT} + Si	23	Zn	0.02	0.01	; 0.04	0.008
		OP_{DTT}	0.06	-0.01	; 0.13	0.078
		Si	0.04	-0.02	; 0.09	0.183

Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; OC, organic carbon; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol); PAHs, polycyclic aromatic hydrocarbons; $PM_{2.5}$, particulate matter with diameter of less than 2.5μm; Si, elemental silicon; Zn, elemental zinc.

Discussion

We observed associations between exposure to air pollutants in two critical periods of brain development, fetal life and childhood, and white matter microstructure in preadolescents aged 9-12 years. The multi-pollutant analysis narrowed these results down to associations between exposure to $PM_{2.5}$ and elemental Si during fetal life, and exposure to nitrogen oxides and elemental Zn during childhood, and white matter microstructure. When fetal life and childhood exposures were analyzed simultaneously, associations of fetal life exposure to Si and childhood exposure to Zn remained. Higher exposures to pollutants were related to lower FA and higher MD, generally considered as indicators for atypical white matter microstructure and previously associated with psychiatric and neurological disorders (White et al. 2008, Aoki et al. 2017; van Ewijk et al. 2012).

Among the exposures during fetal life that showed associations with white matter microstructure, exposure to $PM_{2.5}$ remained associated with global FA in the multi-pollutant analysis, with higher levels of $PM_{2.5}$ related to lower global FA. Exposure to $PM_{2.5}$ is one of the main human health concerns, with associated health effects including those in neurological and neuropsychological domains, among many others (Beelen et al. 2014; Block et al. 2012; Chen et al. 2017; Kaufman et al. 2016; Pedersen et al. 2013; Raaschou-Nielsen et al. 2013). The multi-pollutant analysis also identified the combination of fetal life exposures to $PM_{2.5}$ and PAHs in relationship with global FA, however, PAHs did not show an association in the single pollutant models. Moreover, the two pollutants showed a correlation of 0.66 which possibly suggests that these results were driven by collinearity, thereby demonstrating the difficulty of a multi-pollutant approach in highly correlated settings.

Fetal life exposure to Si remained associated with global MD in the multi-pollutant analysis, with higher levels of Si related to higher global MD. These results were also reflected in three white matter tracts, location-wise moderately in accordance with findings of our previous study wherein we found association between higher levels of air pollution during pregnancy and thinner cerebral cortex in precuneus and rostral middle frontal regions in children of 6-10 years old (Guxens et al. 2018). Si has not been documented as a potential neurotoxicant to date. However, it can be considered as a marker for resuspended road dust (Viana et al. 2008), and therefore our findings likely reflect exposure to high traffic in general, rather than Si explicitly.

In analyses of exposures to air pollution during childhood, the association between higher levels of NO_X and lower global FA remained in the multi-pollutant analysis. In Europe, the predominant source of NO_X gasses in the air is an incomplete combustion of hydrocarbons originating mainly from

diesel fuel (Cyrys et al. 2003). Exposure to diesel exhaust has been linked to numerous adverse health effects, such as increased the risk of neuroinflamation (Block et al. 2012). Results of the multi-pollutant analysis also suggested a robust association between higher childhood exposure to Zn, a marker for brake linings and tire wear (Viana et al. 2008), and higher global MD. The association between childhood exposure to Zn and higher global MD was further supported by identification of six white matter tracts including association and callosal tracts and tracts of the limbic system, wherein higher exposure to Zn showed associations with higher MD. Regarding the location in the brain, these results are in accordance with the findings of our previous study (Guxens et al. 2018). Zn is a vital trace element for proper brain development processes and brain functions later in life (Gower-Winter et al. 2012), however, its accumulation in the brain can cause excitotoxicity, oxidative stress, and impairment of the generation of cellular energy (Gower-Winter et al. 2012). We also observed an association between childhood exposure to higher oxidative potential of $PM_{2.5}$, a measure to quantify the potentiality of $PM_{2.5}$ to induce oxidative stress, and higher global MD. Oxidative stress, together with inflammation, and chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, are the most likely mechanisms through which air pollutants can cause damage to the brain (Block et al. 2012; Thomson 2013).

To date, the only epidemiological study investigating the associations between air pollution and white matter microstructure found that exposure to higher levels of Cu at schools was associated with higher FA in regions adjacent to caudate nucleus in children aged 8-12 years (Pujol et al. 2016b). Similar to Zn, Cu reflects brake linings (Viana et al. 2008). In our study, we did not find an association between fetal or childhood exposure to Cu and FA. The discrepancies in the results between the study of Pujol et al. and our study might be attributable to differences in exposure assessment with respect to location and timing (school levels at 8-10 years vs. residential levels during fetal life and from birth until 9-12 years), different Cu concentrations (8.7 ng/m^3 vs. 4.7 ng/m^3), or differences in sample size (263 vs. 2,954 children).

Our study has a number of considerable strengths: i) large sample size for a population based neuroimaging study in an urban setting; ii) use of advanced statistical methods including inverse probability weighting to reduce possible selection and attrition bias in the study; iii) adjustment for various socioeconomic and lifestyle variables that are known to be potentially associated with air pollution exposure and brain structure in children; iv) standardized and validated air pollution assessment in two key developmental periods with insufficiently large measurement error to bias the health effect estimates, and v) large number of simultaneously assessed pollutants in an advanced multi-pollutant approach. Correlations between

the exposures during fetal life and during childhood were only moderate, allowing us to disentangle associations in these two periods.
There are also several limitations in our study. Sampling campaigns were carried out when children were between 3.5 and 9 years old and historical pollution data the study areas was not available for all the pollutants to extrapolate the levels to the specific periods of interest. We therefore assumed that the concentrations of the pollutants remained spatially stable over time based on previous research supporting stability of spatial contrast in air pollution for periods up to 18 years (Eeftens et al. 2011; Gulliver et al. 2013). Another limitation of this study is the high correlation between some of the pollutants. We used an advanced variable selection technique that has demonstrated better results than alternative methods in settings comparable to ours (Agier et al. 2016). Nevertheless, we still obtained some implausible results. Further methodological research is still needed to unequivocally identify specific pollutants of a complex mixture, particularly if they are derived from the same source. Also, despite the careful and comprehensive selection of potential confounding variables, we cannot discard the possibility of residual confounding of other variables that we either did not consider, or we considered but were unable to include due to poor measurement or lack of measurement, like for example a perfect control for socioeconomic status. Residual confounding could introduce bias and thereby lead to incorrect estimates of the main associations (Weisskopf et al. 2018).

Finally, lower FA and higher MD have generally been associated with impaired neurodevelopment, and have been related to psychiatric and neurological disorders, such as autism spectrum disorder and attention deficit hyperactivity disorder (Aoki et al. 2017; van Ewijk et al. 2012). However, the brain is a highly complicated organ, which undergoes many developmental processes, many of which take place simultaneously, and healthy progression of such processes can sometimes have opposing characteristics (Di Martino et al. 2014). Therefore, our results should be interpreted with caution.

In summary, we found an association between higher exposure to air pollutants representative of brake linings, tire wear, and tailpipe emissions originating mainly from combustion of diesel, with lower FA and higher MD of white matter in preadolescents. To put our results into context, the observed changes in FA and MD could be translated to a developmental delay in white matter microstructure of 0.5 up to 2 months based on previously reported findings, showing typical average changes of the magnitudes of FA and MD in healthy developing children of 4 to 11 years old (Krogsrud et al. 2016). The observed associations involved exposure to air pollution during both key developmental periods, namely fetal life and childhood, demonstrating the importance of examination of both periods in

future studies. All pollutants showing associations have traffic as their main source, and are therefore highly ubiquitous in urban settings, putting a very large portion of children at risk. Based on our results, the current direction towards innovative solutions for cleaner energy vehicles, are strongly supported by the authors. However, these measures might not be completely adequate to mitigate health problems attributable to traffic related air pollution as we also observed associations with elemental zinc which is a marker for brake linings and tire wear. Further studies are warranted to confirm these results.

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Paper V – Supplementary Material

Air pollution exposure during fetal life and childhood, and brain morphology in preadolescents

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Figure S1: Description of the obtained inverse probability weights (IPW)

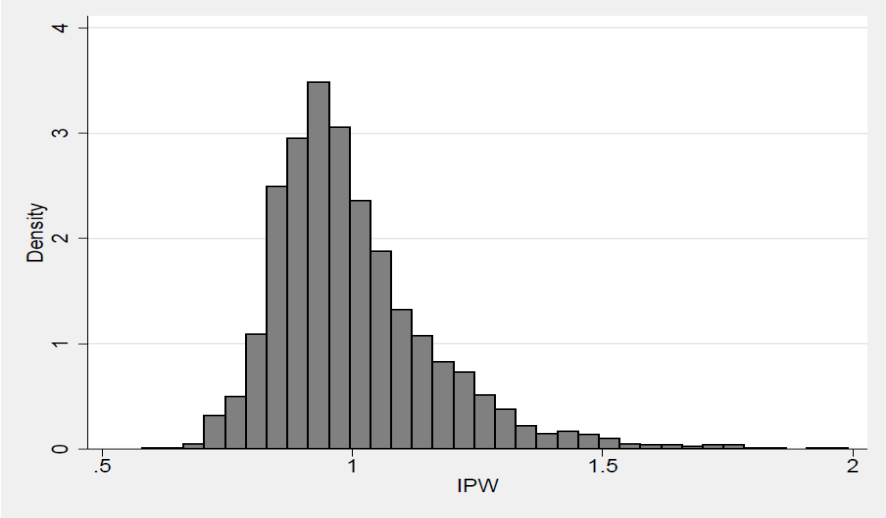

Figure S1: Distribution of the final inverse probability weights. The predictors used for the initial calculation of the weights were parental age, participation of the partner in the study, parental ethnicity, child's ethnicity, parental education, marital status, household income, intake period (prenatal vs. postnatal), parity, maternal weight, parental body mass index, maternal height, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy gestational birth weight, parental psychological distress, maternal intelligence quotient, child's gender, and child's genetic ancestry. The variables selected ($p<0.2$) were maternal age, participation of the partner in the study, parental ethnicity, child's ethnicity, parental education, intake period (prenatal vs. postnatal), parity, maternal weight, maternal smoking during pregnancy, maternal intelligence quotient, child's gender, and child's genetic ancestry. Then, to reduce the influence of extreme values, we used the most significant variables ($p<0.001$), i.e. maternal age, maternal education, parity, maternal weight, maternal smoking during pregnancy, maternal IQ, and child's genetic ancestry, to calculate the final weights.

Table S1. Exposure levels to NO_X and $PM_{2.5}$ during fetal life by participant characteristics

Participant characteristics	n	NO_X	p-value	$PM_{2.5}$	p-value
Maternal education level			<.001		0.010
Primary education or lower	176	47.7 (10.9)		16.4 (0.3)	
Secondary education	1,092	51.0 (17.7)		16.5 (0.5)	
Higher education	1,453	52.4 (16.6)		16.5 (0.5)	
Paternal education level			0.466		0.713
Primary education or lower	92	50.4 (16.7)		16.5 (0.4)	
Secondary education	700	52.8 (19.6)		16.5 (0.5)	
Higher education	1,069	52.3 (17.2)		16.5 (0.5)	
Monthly household income at intake			<.001		0.487
<900€	172	47.8 (12.7)		16.5 (0.4)	
900€ - 1,600€	319	49.0 (13.6)		16.5 (0.4)	
1,600€ - 2,200€	329	53.7 (15.7)		16.5 (0.5)	
>2,200€	1,486	52.4 (17.2)		16.5 (0.5)	
Maternal country of birth			<.001		0.134
The Netherlands	1,702	52.2 (18.2)		16.5 (0.5)	
Other Western	252	52.1 (15.6)		16.5 (0.5)	
Non-Western	944	49.7 (13.4)		16.5 (0.4)	
Paternal country of birth			0.005		0.175
The Netherlands	1,419	52.9 (18.9)		16.5 (0.5)	
Other Western	120	52.8 (15.9)		16.5 (0.5)	
Non-Western	502	50.0 (14.1)		16.5 (0.4)	
Family status at intake			<.001		0.002
Married	1,394	50.5 (14.7)		16.5 (0.4)	
Living together	1,023	53.5 (19.9)		16.5 (0.6)	
No partner	292	49.1 (13.3)		16.5 (0.4)	
Maternal parity			<.001		<.001
nulliparous	1,630	52.8 (18.2)		16.5 (0.5)	
1 child	883	49.8 (14.2)		16.5 (0.4)	
2 or more children	338	49.2 (13.4)		16.4 (0.4)	
Maternal smoking use during pregnancy			0.502		0.659
Never	2,004	51.6 (17.3)		16.5 (0.5)	
Smoking use until pregnancy known	222	50.2 (14.3)		16.5 (0.5)	
Continued smoking use during pregnancy	338	51.6 (16.1)		16.5 (0.5)	
Maternal alcohol use during pregnancy			0.131		0.007
Never	973	50.8 (17.2)		16.5 (0.4)	
Alcohol use until pregnancy know	335	52.7 (16.0)		16.6 (0.6)	
Continued alcohol use during pregnancy	1,023	52.0 (17.3)		16.5 (0.5)	
Maternal age at intake (years)	2,954	0.04	0.052	-0.03	0.113
Paternal age at intake (years)	2,077	-0.01	0.631	-0.02	0.266
Maternal pre-pregnancy body mass index (kg/m^2)	2,181	0.00	0.944	-0.02	0.265
Paternal body mass index (kg/m^2)	2,070	-0.04	0.054	-0.03	0.203
Maternal height (cm)	2,638	0.05	0.008	0.04	0.027
Paternal height (cm)	2,074	0.00	0.968	0.06	0.011
Maternal psychological distress during pregnancy	2,237	0.00	0.850	0.00	0.847
Paternal psychological distress during pregnancy	1,785	-0.05	0.047	-0.01	0.561
Maternal intelligence quotient score	2,688	0.04	0.021	0.05	0.009

Abbreviations: NOx, nitrogen oxide; $PM_{2.5}$, particulate matter with diameter of <2.5µm.

Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

Table S2. Exposure levels to Si and Zn during fetal life by participant characteristics

Participant characteristics	n	Si	p-value	Zn	p-value
Maternal education level			0.032		0.007
Primary education or lower	176	92.3 (10.1)		19.3 (2.9)	
Secondary education	1,092	92.2 (13.3)		20.3 (4.4)	
Higher education	1,453	93.8 (17.7)		20.3 (4.1)	
Paternal education level			0.601		0.111
Primary education or lower	92	92.1 (8.8)		19.5 (3.8)	
Secondary education	700	93.6 (19.3)		20.5 (4.7)	
Higher education	1,069	94.0 (17.9)		20.4 (4.1)	
Monthly household income at enrollment			0.735		<.001
<900€	172	93.2 (14.0)		19.3 (3.7)	
900€ - 1,600€	319	92.4 (14.0)		19.4 (3.7)	
1,600€ - 2,200€	329	93.3 (15.9)		20.6 (4.4)	
>2,200€	1,486	93.5 (17.4)		20.6 (4.4)	
Maternal country of birth			0.469		<.001
The Netherlands	1,702	93.1 (16.7)		20.4 (4.3)	
Other Western	252	93.4 (19.2)		20.4 (4.5)	
Non-Western	944	92.4 (10.9)		19.7 (3.8)	
Paternal country of birth			0.245		0.006
The Netherlands	1,419	93.7 (17.9)		20.6 (4.4)	
Other Western	120	95.5 (26.6)		20.0 (3.6)	
Non-Western	502	92.7 (12.6)		19.9 (4.1)	
Family status at enrollment			0.108		<.001
Married	1,394	92.4 (13.3)		20.1 (4.2)	
Living together	1,023	93.6 (18.2)		20.6 (4.3)	
No partner	292	93.8 (16.5)		19.5 (3.4)	
Maternal parity			0.041		<.001
nulliparous	1,630	93.6 (17.6)		20.5 (4.3)	
1 child	883	92.5 (13.8)		19.9 (4.0)	
2 or more children	338	91.5 (9.7)		19.7 (3.9)	
Maternal smoking use during pregnancy			0.261		0.860
Never	2,004	93.2 (16.7)		20.2 (4.2)	
Smoking use until pregnancy known	222	93.4 (13.0)		20.1 (4.0)	
Continued smoking use during pregnancy	338	91.7 (11.0)		20.3 (4.7)	
Maternal alcohol use during pregnancy			0.223		0.026
Never	973	92.6 (14.4)		20.1 (4.2)	
Alcohol use until pregnancy know	335	93.5 (19.4)		20.8 (4.6)	
Continued alcohol use during pregnancy	1,023	93.9 (16.9)		20.2 (4.0)	
Maternal age at enrollment (years)	2,954	-0.03	0.161	0.05	0.003
Paternal age at enrollment (years)	2,077	-0.01	0.762	0.01	0.815
Maternal pre-pregnancy body mass index (kg/m^2)	2,181	-0.02	0.476	-0.02	0.444
Paternal body mass index (kg/m^2)	2,070	-0.03	0.245	-0.01	0.516
Maternal height (cm)	2,638	0.04	0.062	0.06	0.003
Paternal height (cm)	2,074	-0.01	0.808	-0.04	0.054
Maternal psychological distress during pregnancy	2,237	0.01	0.587	-0.02	0.375
Paternal psychological distress during pregnancy	1,785	0.00	0.904	-0.03	0.143
Maternal intelligence quotient score	2,688	0.02	0.322	0.05	0.017

Abbreviations: Si, elemental silicon; Zn, elemental zinc.
Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

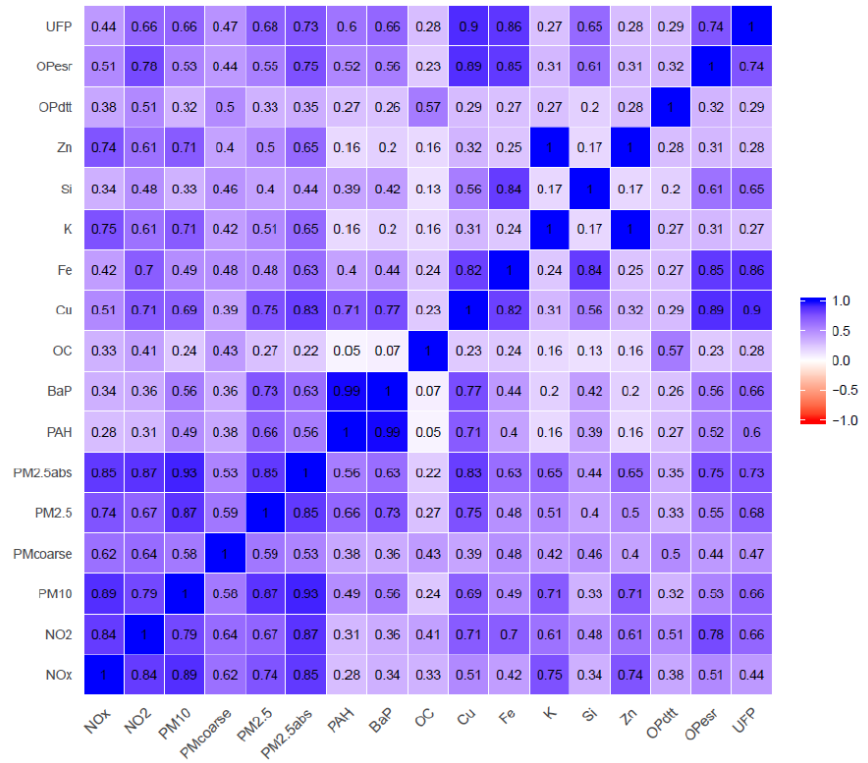

Figure S2. Correlations between levels of the pollutants during fetal life

Abbreviations: NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10µm (PM_{10}); between 10µm and 2.5µm (PMcoarse); less than 2.5µm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles.

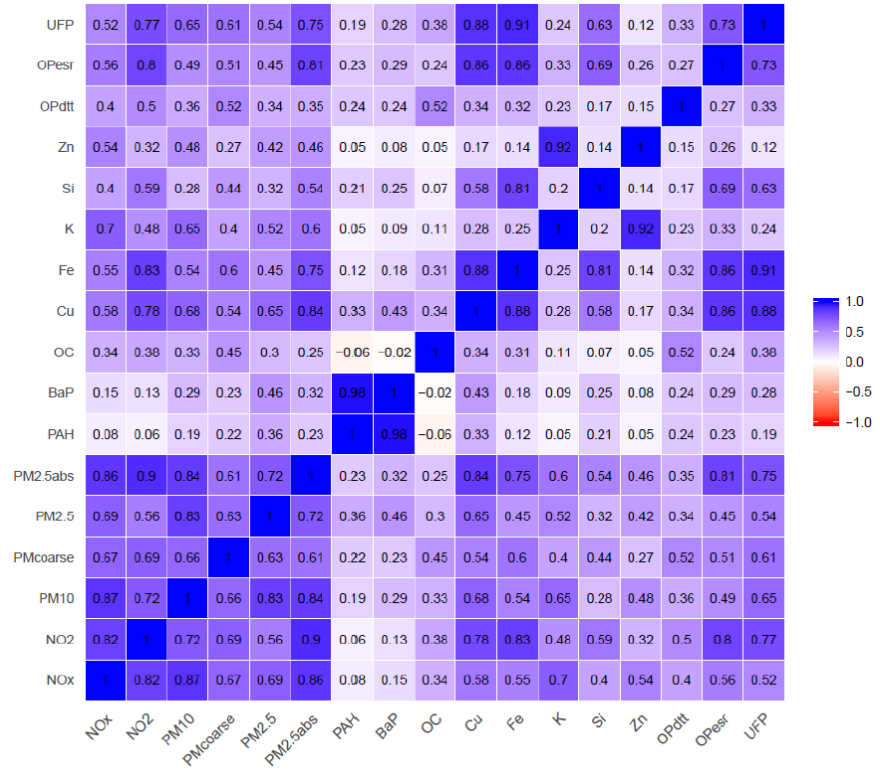

Figure S3. Correlations between levels of the pollutants during childhood

Abbreviations: NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10μm (PM_{10}); between 10μm and 2.5μm (PMcoarse); less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters; PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; OC, organic carbon; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles.

Table S3. Adjusted associations between exposure during fetal life to air pollutants selected by the multi-pollutant model and global fractional anisotropy, and global mean diffusivity at 9-12y, excluding participants of mothers recruited after birth (n=310)

Fetal life exposures and global fractional anisotropy						
Pollutants selected by DSA algorithm	Pollutant	Coef.	95% CI			p-value
$PM_{2.5}$ + PAHs + OP_{DTT}	$PM_{2.5}$	-1.53	-2.34	;	-0.73	<0.001
	PAHs	0.33	0.05	;	0.61	0.022
	OP_{DTT}	0.52	-0.08	;	1.12	0.091
$PM_{2.5}$ + PAHs	$PM_{2.5}$	-1.36	-2.14	;	-0.58	0.001
	PAHs	0.33	0.05	;	0.62	0.020
$PM_{2.5}$	$PM_{2.5}$	-0.73	-1.29	;	-0.16	0.012

Fetal life exposures and global mean diffusivity						
Pollutants selected by DSA algorithm	Pollutant	Coef.	95% CI			p-value
Si + OP_{DTT}	Si	0.05	0.00	;	0.11	0.049
	OP_{DTT}	0.06	-0.02	;	0.13	0.129

Abbreviations: Coef, coefficient; CI, confidence intervals; DSA, Deletion/Substitution/Addition; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol); PAHs, polycyclic aromatic hydrocarbons; $PM_{2.5}$, particulate matter with diameter of less than 2.5µm; Si, elemental silicon.

Table S4. Results of adjusted analyses wherein fetal life exposures and childhood exposures were introduced simultaneously: fetal life exposure to $PM_{2.5}$absorbance and childhood exposure to NO_X, and global fractional anisotropy, and fetal life exposure to Si and childhood exposure to Zn, and global mean diffusivity, and mean diffusivity in three white matter tracts

Fetal life and childhood exposures combined, and global fractional anisotropy

Pollutant	Period	Coef.	95% CI	p-value
$PM_{2.5}$	fetal life	-0.48	-1.07 ; 0.10	0.105
NOx	childhood	-0.10	-0.21 ; 0.00	0.053

Fetal life and childhood exposures combined, and global mean diffusivity

Pollutant	Period	Coef.	95% CI	p-value
Si	fetal life	0.06	0.01 ; 0.11	0.024
Zn	childhood	0.02	0.01 ; 0.04	0.009
OP_{DTT}	childhood	0.06	-0.01 ; 0.13	0.069

Fetal life and childhood exposures combined, and mean diffusivity in cingulate gyrus part of cingulum of the left hemisphere

Pollutant	Period	Coef.	95% CI	p-value
Si	fetal life	0.0082	0.0015 ; 0.0151	0.017
Zn	childhood	0.0041	0.0017 ; 0.0065	0.001

Fetal life and childhood exposures combined, and mean diffusivity in superior longitudinal fasciculus of the left hemisphere

Pollutant	Period	Coef.	95% CI	p-value
Si	fetal life	0.0083	0.0030 ; 0.0137	0.002
Zn	childhood	0.0023	0.0004 ; 0.0042	0.016

Fetal life and childhood exposures combined, and mean diffusivity in forceps minor

Pollutant	Period	Coef.	95% CI	p-value
Si	fetal life	0.0158	0.0085 ; 0.0232	>0.001
Zn	childhood	0.0041	0.0015 ; 0.0067	0.002

Abbreviations: Coef., coefficient; CI, confidence intervals; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol); NO_X, nitrogen oxides; $PM_{2.5}$, particulate matter with diameter of <2.5μm; Si, elemental silicon; Zn, elemental zinc.

Table S5. Adjusted associations between exposure during fetal life to $PM_{2.5}$, and during childhood to NO_X, with fractional anisotropy in twelve white matter tracts at 9-12y

Fractional anisotropy	Fetal life exposure to $PM_{2.5}$			
	Coef.	95% CI		p-value
uncinate fasciculus left hemisphere	-0.00892	-0.01808	0.00023	0.056
uncinate fasciculus right hemisphere	-0.00467	-0.01283	0.00349	0.258
cingulate gyrus part of cingulum left hemisphere	-0.00987	-0.02302	0.00328	0.140
cingulate gyrus part of cingulum right hemisphere	-0.00622	-0.01810	0.00566	0.301
superior longitudinal fasciculus left hemisphere	-0.00585	-0.01260	0.00090	0.089
superior longitudinal fasciculus right hemisphere	-0.00878	-0.01601	-0.00154	0.018
forceps minor	-0.01276	-0.02261	-0.00291	0.012
forceps major	-0.00297	-0.01350	0.00757	0.578
inferior longitudinal fasciculus left hemisphere	-0.00286	-0.00946	0.00373	0.390
inferior longitudinal fasciculus right hemisphere	-0.00199	-0.00880	0.00483	0.564
corticospinal tract left hemisphere	-0.00754	-0.01389	-0.00119	0.020
corticospinal tract right hemisphere	-0.00800	-0.01440	-0.00161	0.015

Fractional anisotropy	Childhood exposure to NO_X			
	Coef.	95% CI		p-value
uncinate fasciculus left hemisphere	-0.00184	-0.00346	-0.00022	0.027
uncinate fasciculus right hemisphere	-0.00165	-0.00309	-0.00021	0.026
cingulate gyrus part of cingulum left hemisphere	-0.00175	-0.00408	0.00057	0.137
cingulate gyrus part of cingulum right hemisphere	0.00015	-0.00195	0.00226	0.886
superior longitudinal fasciculus left hemisphere	-0.00062	-0.00182	0.00057	0.302
superior longitudinal fasciculus right hemisphere	-0.00165	-0.00293	-0.00037	0.012
forceps minor	-0.00173	-0.00348	0.00001	0.052
forceps major	-0.00025	-0.00211	0.00162	0.794
inferior longitudinal fasciculus left hemisphere	-0.00102	-0.00219	0.00015	0.086
inferior longitudinal fasciculus right hemisphere	-0.00145	-0.00266	-0.00024	0.019
corticospinal tract left hemisphere	-0.00129	-0.00241	-0.00017	0.024
corticospinal tract right hemisphere	-0.00070	-0.00183	0.00043	0.223

Abbreviations: Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; $PM_{2.5}$, particulate matter with diameter of <2.5μm.
*Significance (p-value < 0.050) remained after false discovery rate correction for multiple testing was applied

Table S6. Adjusted associations between exposure during fetal life to Si, and during childhood to Zn and OP_{DTT}, with mean diffusivity in twelve white matter tracts at 9-12y

Mean diffusivity**	Fetal life exposure to Si			
	Coef.	95% CI		p-value
uncinate fasciculus left hemisphere	0.00259	-0.00258	0.00775	0.323
uncinate fasciculus right hemisphere	0.00359	-0.00153	0.00872	0.167
cingulate gyrus part of cingulum left hemisphere	0.00934	0.00249	0.01619	0.008*
cingulate gyrus part of cingulum right hemisphere	0.00761	0.00089	0.01434	0.027
superior longitudinal fasciculus left hemisphere	0.00892	0.00350	0.01434	0.002*
superior longitudinal fasciculus right hemisphere	0.00610	-0.00008	0.01229	0.053
forceps minor	0.01690	0.00945	0.02435	<.001*
forceps major	-0.00089	-0.01650	0.01472	0.910
inferior longitudinal fasciculus left hemisphere	0.00699	0.00034	0.01364	0.040
inferior longitudinal fasciculus right hemisphere	0.00680	-0.00072	0.01432	0.076
corticospinal tract left hemisphere	0.00207	-0.00861	0.01275	0.697
corticospinal tract right hemisphere	-0.00102	-0.01111	0.00907	0.840

Mean diffusivity**	Childhood exposure to Zn			
	Coef.	95% CI		p-value
uncinate fasciculus left hemisphere	0.00184	0.00004	0.00365	0.046
uncinate fasciculus right hemisphere	0.00242	0.00063	0.00421	0.009*
cingulate gyrus part of cingulum left hemisphere	0.00439	0.00199	0.00679	<.001*
cingulate gyrus part of cingulum right hemisphere	0.00359	0.00123	0.00594	0.003*
superior longitudinal fasciculus left hemisphere	0.00256	0.00066	0.00445	0.009*
superior longitudinal fasciculus right hemisphere	0.00305	0.00089	0.00522	0.006*
forceps minor	0.00460	0.00199	0.00721	0.001*
forceps major	0.00030	-0.00518	0.00577	0.915
inferior longitudinal fasciculus left hemisphere	0.00248	0.00015	0.00481	0.037
inferior longitudinal fasciculus right hemisphere	0.00286	0.00022	0.00549	0.034
corticospinal tract left hemisphere	0.00101	-0.00272	0.00475	0.586
corticospinal tract right hemisphere	0.00247	-0.00105	0.00600	0.165

Mean diffusivity**	Childhood exposure to OP_{DTT}			
	Coef.	95% CI		p-value
uncinate fasciculus left hemisphere	0.00383	-0.00295	0.01060	0.265
uncinate fasciculus right hemisphere	0.00245	-0.00428	0.00917	0.472
cingulate gyrus part of cingulum left hemisphere	0.01008	0.00111	0.01905	0.028
cingulate gyrus part of cingulum right hemisphere	0.00737	-0.00144	0.01617	0.100
superior longitudinal fasciculus left hemisphere	0.00292	-0.00417	0.01001	0.414
superior longitudinal fasciculus right hemisphere	0.00526	-0.00283	0.01335	0.199
forceps minor	0.00799	-0.00176	0.01773	0.107
forceps major	0.00803	-0.01236	0.02843	0.437
inferior longitudinal fasciculus left hemisphere	0.00745	-0.00127	0.01616	0.093
inferior longitudinal fasciculus right hemisphere	0.00558	-0.00428	0.01544	0.264
corticospinal tract left hemisphere	0.00597	-0.00796	0.01989	0.391
corticospinal tract right hemisphere	0.00209	-0.01115	0.01532	0.753

Abbreviations: Coef, coefficient; CI, confidence intervals; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol); Si, elemental silicon; Zn, elemental zinc.

*Significance (p-value < 0.050) remained after false discovery rate correction for multiple testing was applied

**Values were multiplied by 10^9

Table S7. Results of the adjusted associations between exposure during fetal life and childhood to single air pollutants and global fractional anisotropy, and global mean diffusivity at 9-12y, with and without accounting for measurement error

Fractional anisotropy

	Fetal life						Childhood					
	original results			with measurement error			original results			with measurement error		
pollutant	95% CI		stderr	95% CI		stderr	95% CI		stderr	95% CI		stderr
NO_X	-0.20	-0.02	0.045	-0.19	-0.01	0.046	-0.23	-0.04	0.049	-0.23	-0.04	0.050
NO_2	-0.25	0.03	0.068	-0.24	0.02	0.068	-0.25	-0.01	0.059	-0.25	-0.02	0.060
PM_{10}	-0.90	-0.08	0.205	-0.99	-0.03	0.245	-0.91	0.01	0.232	-1.12	-0.04	0.275
PM_{coarse}	-0.37	0.27	0.161	-0.38	0.26	0.164	-0.63	0.04	0.169	-0.65	0.00	0.167
PM_{25}	-1.26	-0.16	0.277	-0.99	-0.11	0.224	-1.14	0.21	0.340	-1.06	0.14	0.306
PM_{25}abs	-0.51	-0.07	0.113	-0.51	-0.05	0.119	-0.51	-0.02	0.122	-0.51	-0.03	0.122
Cu	-0.32	-0.71	0.193	-0.69	0.05	0.189	-0.65	0.21	0.217	-0.66	0.18	0.215
Fe	-0.20	-0.54	0.172	-0.55	0.14	0.175	-0.53	0.09	0.156	-0.55	0.06	0.155
K	-0.38	-0.84	0.230	-0.97	0.25	0.310	-1.03	-0.03	0.253	-0.70	0.17	0.221
Si	-0.28	-0.70	0.214	-0.73	0.15	0.225	-0.66	0.19	0.216	-0.34	0.06	0.102
Zn	-0.12	-0.28	0.081	-0.48	0.20	0.174	-0.27	0.02	0.075	-0.18	0.00	0.047

Mean diffusivity

	Fetal life						Childhood					
	original results			with measurement error			original results			with measurement error		
pollutant	95% CI		stderr	95% CI		stderr	95% CI		stderr	95% CI		stderr
NO_X	0.00	0.02	0.005	0.00	0.02	0.006	0.01	0.03	0.006	0.01	0.03	0.007
NO_2	0.00	0.04	0.008	0.00	0.04	0.009	0.00	0.03	0.007	0.01	0.03	0.006
PM_{10}	0.00	0.10	0.025	0.00	0.11	0.030	0.01	0.12	0.028	0.01	0.14	0.032
PM_{coarse}	-0.01	0.07	0.020	-0.02	0.06	0.019	0.00	0.09	0.021	0.00	0.08	0.021
PM_{25}	0.02	0.15	0.034	0.01	0.12	0.028	0.03	0.20	0.041	0.02	0.18	0.040
PM_{25}abs	0.01	0.06	0.014	0.01	0.06	0.015	0.01	0.07	0.015	0.01	0.07	0.015
Cu	0.01	0.10	0.023	0.01	0.10	0.024	-0.02	0.09	0.026	-0.02	0.09	0.027
Fe	0.01	0.09	0.021	0.01	0.09	0.021	-0.01	0.07	0.019	0.00	0.07	0.018
K	-0.02	0.09	0.028	-0.04	0.12	0.042	0.03	0.15	0.031	0.01	0.17	0.042
Si	0.02	0.12	0.026	0.01	0.13	0.031	0.00	0.11	0.026	0.00	0.11	0.027
Zn	-0.01	0.03	0.010	-0.02	0.06	0.022	0.01	0.05	0.009	0.01	0.06	0.013

Coef, coefficient; CI, confidence intervals; stderr, standard error; NO_X, nitrogen oxides; NO_2, nitrogen dioxide; PM, particulate matter with different aerodynamic diameters: less than 10μm (PM_{10}); between 10μm and 2.5μm (PMcoarse); less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$absorbance, absorbance of $PM_{2.5}$ filters; Cu, elemental copper; Fe, elemental iron; K, elemental potassium; Si, elemental silicon; Zn, elemental zinc. Coefficient and 95% CI were estimated through linear regression analysis and the models were adjusted for parental educational levels, household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal alcohol consumption during pregnancy, maternal parity, marital status, and parental psychiatric symptoms, parental heights and body mass indices, maternal intelligence quotient, child's genetic ancestry, child's gender and child's age at the scanning session was also collected per increments of: 20 $\mu g/m^3$ for NO_X; 10 $\mu g/m^3$ for NO_2; 10 $\mu g/m^3$ for PM_{10}; 5 $\mu g/m^3$ for PM_{coarse}; 5 $\mu g/m^3$ for $PM_{2.5}$; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 5 ng/m^3 for Cu in $PM_{2.5}$; 100 ng/m^3 for Fe in $PM_{2.5}$; 50 ng/m^3 for K in $PM_{2.5}$; 100 ng/m^3 for Si in $PM_{2.5}$; 10 ng/m^3 for Zn in $PM_{2.5}$.

4. GENERAL DISCUSSION

4.1. Rationale

Exposure to outdoor air pollution is increasingly being recognized as an important risk factor for neuropsychological disorders. These adverse neuropsychological outcomes could influence the health status of individuals, including the impairment of cognitive development, and an increased risk of development of various mental disorders, such as autism spectrum disorder (1,2). Understanding the associations between early life exposure to outdoor air pollution, as well as understanding the biological mechanisms underlying such associations, is crucial, yet insufficient to date. The work performed in this thesis was conducted with the main aim to reduce this existing gap in knowledge. In this chapter, I will present the main findings of this thesis, together with methodological considerations that need to be addressed, and discussion about the implications of this research for public health and policy making. I will end with several ideas and recommendations for future directions.

4.2. Main findings

4.2.1. Exposure to air pollution and neuropsychological development

A growing body of evidence suggests that exposure to air pollution during early years of life is associated with compromised neuropsychological development (1,2). However, due to the novelty of this academic discipline, and thus often a limited number of published studies, several neuropsychological domains are still understudied, or the body of evidence is still not sufficiently large to be considered unequivocal. Therefore, one of the objectives of this thesis was to expand the current knowledge on the associations between exposure to air pollution and certain neuropsychological domains in children. To this aim, Paper I of this thesis was a follow-up on a previous epidemiological study by Guxens et al (3) that investigated the association between air pollution and neuropsychological development in 6 European cohorts. In that study, the authors found a negative association between prenatal exposure to NO_2 and PM and psychomotor function in childhood. In Paper I, we looked in-depth into specific components of $PM_{2.5}$, as single pollutants as well as combined into latent variables depending on their source, and their associations with cognitive and psychomotor function in children from 4 European birth cohorts. PM comprises numerous components, many of which have been considered neurotoxic and attributed to various adverse health effects. In our

study, we found a negative association between exposure to airborne iron at birth, an element highly prevalent in motorized traffic air pollution, and fine motor function, assessed in children of 1 to 9 years of age. Gross motor function and cognitive function were not significantly associated with exposures at birth to any of the elemental components of PM, although the effect estimates of the latter were predominantly negative. In Paper II, we looked at a different domain of neuropsychological development, namely the emotional and behavioral domain. While a number of studies that have been carried out to date generally found an association between exposure to air pollution with autism spectrum disorder (1), and little to no association with attention-deficit/hyperactivity disorder, other areas of emotional and behavioral domain are understudied to date (4–9). Therefore, we assessed whether prenatal and postnatal exposure to air pollution was related to depressive and anxiety symptoms, and aggressive symptoms in children from 8 European birth cohorts. While studies in adults generally report positive associations between exposure to air pollution and the odds of emotional problems, including depression and anxiety (10–13), we did not find similar results in children of 7 to 11 years old. It is plausible that the development of emotional and behavioral problems related to air pollution exposure emerges only later in life and that our study population was therefore too young to already have developed such symptoms.

4.2.2. Exposure to air pollution and neurobiological development

Although studies assessing the relationship between exposure to air pollution and neuropsychological development are valuable to recognize the possible harmful influences of exposure on the outcome, they provide limited to no understanding of potential structural and functional brain alterations that could underlie these associations. A number of studies started using MRI to assess these underlying alterations and found evidence for relationships between higher exposure to air pollution during fetal life or childhood, and white- and grey matter abnormalities (14–19). However, these studies are very limited in sample size, resulting in an insufficiently large body of evidence to be considered unequivocal. With Papers III, IV, and V, we aimed to increase the current body of evidence on the association between exposure to air pollution during early years of life and neurobiological development, thereby decreasing the existing gap in knowledge. In Paper III, we examined the relationship between exposure to air pollution during fetal life and brain morphological alterations in children of 6 to 8 years of age in a subset of a population-based birth cohort from Rotterdam, the Netherlands. We found that exposure during pregnancy to fine particulate matter was associated with a thinner cortex in various regions of the brain. Moreover, the thinner cortex in the precuneus and the rostral middle frontal regions, partially mediated the association between exposure to fine particles and

impaired inhibitory control. In Paper IV, we followed these results up by increasing the population size fourfold, assessing exposures during fetal life as well as during childhood, and including a larger number of pollutants, making the study more comprehensive. Moreover, unlike the study population from Paper III, the population from Paper IV was not oversampled for certain maternal characteristics and was therefore more likely to be representative of the general population. Also, the study population from Paper IV was approximately 4 years older than study population from Paper III. We found that a higher fetal and childhood exposure to pollutants representative of traffic related sources was associated with attenuated cortical thickness and larger cortical and subcortical volumes in preadolescents of 9 to 12 years old. Also, higher fetal and childhood exposure to air pollution was related to lower volume of the corpus callosum, which is the largest white matter structure in the brain. Moreover, higher exposure to air pollution during childhood was also associated with larger cortical pial surface area. The associations with fetal exposure to air pollution were predominantly observed in girls. The areas of the alterations in the cortex, corresponded to the areas identified in Paper III. While a thinner cortex is generally associated with neuropsychological disorders such as depression or schizophrenia (20,21), it is not entirely clear whether larger cortical volume and area, and larger volumes of other structures of the brain, such as corpus callosum, are positive or negative at this specific age. Although it might be indicative of a healthy development, it might as well be a sign of a delayed maturation of the brain. We then looked in-depth into the association between fetal life and childhood exposures to air pollution and white matter microstructure in the same large population of preadolescents, and reported the results in Paper V. We found that higher exposure to pollutants representative of brake linings, tire wear, and tailpipe emissions originating mainly from combustion of diesel (22), was associated with alterations in white matter microstructure of preadolescents between 9 and 12 years old, namely with lower fractional anisotropy and higher mean diffusivity. Generally, normal white matter microstructure development is characterized by gradually increasing fractional anisotropy and decreasing mean diffusivity (23), and the alterations related to air pollution exposure observed in our study could be translated to a developmental delay of the white matter microstructure of 0.5 up to almost 2 months.

4.3. Methodological considerations

All the papers presented in this thesis were based on prospective population-based birth cohorts with a follow-up from fetal life onwards. In Papers I and II, data from multiple cohorts were included and meta-analyzed, providing increased statistical power to detect potential, relatively small associations which individual studies might not have been able to identify due to insufficient power, and higher representativeness of the general population

(24). However, only one of the cohorts comprised an imaging study, and therefore the subsequent four papers included in this thesis were based only on data from that one cohort (25). The prospective nature of birth cohorts allows for an adequate assessment of the relationship between early life exposures and the related long term health effects, making prospective birth cohorts a highly valuable study design in environmental epidemiology. Nevertheless, the studies presented in this thesis also encounter several limitations, mainly with reference to exposure and outcome assessments, to confounding, and multiple testing. Each of these limitations will be discussed separately successively.

4.3.1. Exposure assessment

Exposure misclassification

Epidemiological studies require accurate data on exposure to correctly assess the relationships between the exposure and the health outcomes of interest. In studies addressing health problems associated with exposure to air pollution, the exposure is often modeled to represent personal levels of participating population based on central measurements, while personal monitoring of air pollution would be a more precise method to assess individual levels of exposure (26). However, in cohort studies including a large number of participants, the use of personal monitors would be highly labor-intensive and very expensive (26). Furthermore, while more accurate, data from personal monitors are usually less representative as indicator of long-term exposure in comparison to the estimations at individual level assessed using appropriate models, since they are only carried out for short periods of time. Additionally, personal measurements are likely to have a fairly inaccurate reflectance of outdoor exposures because of the time spent indoors exposed to indoor sources. Nevertheless, modeled exposure is more likely to be prone to misclassification. In this thesis, air pollution was modeled to the individual level of home addresses of each participant using land use regression models based on validated measurements (27–31). Sampling campaigns were carried out when children were between 0 and 10 years old and historical pollution data of the study areas was not available for all the pollutants to extrapolate the levels to the specific periods of interest. We therefore assumed that the spatial contrast of air pollution remained stable over time. This assumption was based on previous research supporting stability of spatial contrast in air pollution for periods up to 18 years (32,33). Nevertheless, this assumption could lead to misclassification of the exposure. Misclassification could also arise if participants changed addresses and this change was not documented and therefore not accounted for in our analyses. Another source of misclassification could emerge if the total outdoor air pollution exposure of a participant would be completely

different from the residential exposure. For example, if the work of a participating mother was located in a traffic dense area and she was therefore exposed to high levels of air pollution during pregnancy, but she lived in an area with low exposure levels, her assigned modeled exposure levels would not represent her actual exposure well. However, there is no reason to assume that the potential misclassification in our studies is differential, as differential misclassification occurs when the frequency of the misclassification is related to the outcome. With non-differential misclassification the similarity in exposure levels between the participants increases, thereby resulting in a possible underestimation or dilution of the true strength of the association rather than overestimation (34).

Measurement error

Another limitation related to the exposure assessment is the possibility of introduction of measurement error in the air pollution estimates (35). Measurement error is introduced when the modeled exposures are different from the actual, measured exposures, and comprises classical-like and Berkson-like error. Classical-like error arises from the uncertainty related to the selection of the parameters of the exposure estimation model, in our case the land use regression model, and it may bias the health effect estimates. Also, it could potentially inflate the standard error of the health effect estimates. The Berkson-like error arises from the smoothing of the exposure surface. While it causes little to no bias in the measurements, it is likely to inflate the standard error of the health effect estimates (36). Measurement error is a very common limitation in air pollution epidemiology, nevertheless only recently researchers have started to occasionally address this issue in their studies. We attempted to investigate to what extent the measurement error is affecting our obtained health effect estimates. For that purpose, in Paper V, we took advantage of the availability of the actual measurements of air pollution to quantify the error in the land use regression models used to estimate individual levels of exposure of the study participants, thereby quantifying the uncertainty in the exposure-outcome association. We used a bootstrap method that decomposes the error into two components - the classical-like component and the Berkson-like component (36). Then, by simulating the exposure based on the actual measurements and introducing different levels of variability of the parameters of the land use regression model, the estimation of the bias in the health effect estimates was quantified as the difference between the empirical means obtained assuming various levels of variability in the parameters, and no variability. The results suggested that the measurement error introduced in our studies did not bias the health effect estimates substantially.

Multi-pollutant analysis

While the understanding of the health effects of exposure to an isolated pollutant is necessary and important, it is also clear that such a scenario does not reflect actual outdoor conditions. Rather, humans are exposed to a mixture of pollutants, highlighting the importance of multi-pollutant analysis. Such analysis leads to another methodological consideration that needs to be addressed, namely the number of pollutants analyzed and the correlations between them. The land use regression models used in this thesis were developed using land use predictors related mainly to traffic, such as distance to major roads and number of vehicles per time unit (27–31). Therefore, to a large degree, the modeled exposure estimates represent pollutants with traffic as their main source of origin, resulting in high to occasionally very high correlations between the pollutants. This hinders the ability to disentangle specific pollutants of a complex mixture. Moreover, high correlations between pollutants increase the likelihood of collinearity when analyzed simultaneously. Collinearity has the tendency to increase the variance of one or more estimated regression coefficients, which might result in regression coefficients switching sign (37). In order to overcome this limitation, we took three different approaches. In Paper I, where we analyzed only the elemental composition of the fine particles without restriction to the elemental components originating from traffic, we used latent variables to analyze the associations with health outcomes of interest. Using principle components analysis, we grouped the elemental components according to their most likely source. The strength of such approach is that the dimensionality of the data decreases, reducing the possibility of type I error. Also, by grouping highly correlated pollutants together into one latent variable, the issue of high correlations and possible collinearity is being taken care of. The limitation, however, is that grouping several pollutants together into one latent variable makes it impossible to identify individual pollutants that might be responsible for associations with health outcomes. Our second approach to deal with high correlations between the pollutants, was the introduction of a method used in exposome-wide association studies (ExWAS) in Paper IV. In the ExWAS method, the pollutants are examined one by one, followed by a correction for the number of analyses performed, to reduce the likelihood of making inferences based on chance findings (38). By studying the pollutants one by one instead of (partially) simultanously, the issue of collinearity is non-existent. The third approach to overcome the limitations related to high correlations between the pollutants was the introduction of a multi-pollutant model in Paper V. Several different methods exist to study multiple pollutants simultaneously, and we selected the Deletion/Substitution/Addition algorithm based on a relatively good performance regarding a trade-off between sensitivity and false discovery proportion as compared to other methods (38). It is an iterative selection method, which selects the exposures that are most predictive of the outcome

by cross-validation, taking into account the correlation matrix of air pollutants, and simultaneously correcting for multiple testing. While the model seemed stable and overall provided reasonable results, the high correlations still led to some implausibilities. Further methodological research is needed to unequivocally identify specific pollutants of a complex mixture, particularly if they are derived from the same source.

4.3.2. Outcome assessment

Heterogeneity in neuropsychological tests

While the inclusion of multiple cohorts increases sample size and the representativeness of the study population to the general population, several limitations could also arise related to such approach. Since the cohorts are conducted independent from one another, their protocols are often not streamlined, generating discrepancies between assessments, collected variables, and adapted timelines. Such discrepancies are responsible for heterogeneity in the collected data, which could increase the errors in the final estimates. In our analyses, the main discrepancy was found in the health outcome data, namely in the neuropsychological tests used. Mainly in Paper I, but to some extent also in Paper II, the cohorts used different tests to assess child's neuropsychological development. We aimed to minimize this heterogeneity by carefully selecting those tests, or parts of the tests, that represent similar neuropsychological domains across the cohorts, thereby adding to their comparability. Moreover, in Paper I, we aimed to increase the comparability between the tests by standardizing the various test scores to mean of 100 and a standard deviation of 15. In Paper II, we used validated cut-off points to identify children with borderline and/or clinical symptoms for the two tests included, and stratified the analyses by test as sensitivity analyses.

Single time point data

One of the limitations of this thesis related to the outcome assessment is the lack of repeated measures of the outcome data, being available only at one time point. Having repeated measurements of the outcome data, makes it possible to analyze changes in the outcome related to the exposure over time, therefore increasing the feasibility of causal inference (39). Unfortunately repeated measurements of the outcome data were not available for the studies included in this thesis. However, we aimed to establish a temporal relationship between exposure and outcome by modeling the exposure data to represent exposures during pregnancy, as well as during childhood prior to the outcome assessment. Nevertheless, such approach is insufficient to infer causality as the dynamic processes related to the outcome of interest cannot be modeled. This depicts the importance of

future studies to look at repeated measurement to better understand the associations between exposure and outcome.

What is good and what is bad?

Another limitation related to the outcome is the uncertainty related to the interpretation of the directionality of the results in Paper IV. In summary, we observed an association between higher fetal and childhood exposure to pollutants, with thinner cortex, larger cortical and subcortical volumes, and lower volume of the corpus callosum in preadolescents. Moreover, higher exposure to air pollution during childhood also showed associations with larger cortical pial surface area. While thinner cortex is generally considered to be detrimental, having been associated with neuropsychological disorders such as depression or schizophrenia (20,21), it is unclear whether larger cortical volume and area, and larger volumes of other structures of the brain, are beneficial or also detrimental. On the one hand, a study analyzing whether polygenic susceptibility for psychiatric disorders and cognitive traits was related to brain morphological measurements in children drawn from the same cohort as our study population, found that polygenic scores for intelligence and educational attainment showed a positive association with total brain volume (40). On the other hand however, while seemingly exceptional, a large body of evidence suggests that larger brain volume during childhood is associated with autism spectrum disorder (41). As some patterns of brain maturation that take place between childhood and adolescence involve dynamic changes in both grey and white matter (42), it is difficult to disentangle which increases and decreases are beneficial and which are detrimental at the age of 9 to 12 years. It is important to note however, that it is highly implausible that exposure to air pollution would be beneficial for brain health, and therefore, any spurious results are more likely related to methodological constraints and the imperfect nature of epidemiological studies.

4.3.3. Confounding

Prospective nature of birth cohorts allows for the collection of rich database on potential confounding variables, including various child and parental socioeconomic, and life-style characteristics, making it possible to adjust the final models accordingly. Despite the availability of many potential confounding variables in our studies, methodological considerations concerning this subject matter are two-fold in this thesis. The first consideration that needs to be addressed, relates to Papers I and II. In those two papers, we analyzed several cohorts simultaneously, leading to heterogeneity in the data. To minimize this heterogeneity, we included only potential confounding variables that were available in all the participating cohorts, thereby increasing the comparability between the cohorts. The

second consideration relates back to the first consideration, but also applies to the remaining three papers presented in this thesis. Namely, despite the careful and comprehensive selection of potential confounding variables, we cannot discard the possibility of residual confounding of other variables that we either did not consider, or we considered but were unable to include due to poor measurement or lack of measurement, like for example a perfect control for socioeconomic status. Residual confounding could introduce bias and thereby lead to incorrect estimates of the main associations, as well as further hinder causal inference (43).

4.3.4. Multiple testing

Correction for multiple testing is a topic of an ongoing debate in environmental epidemiology, generally dividing the scientific community into two groups; those in favor of correcting for multiple testing and those opposing it. On the one hand, the inclusion of multiple tests in a study increases the likelihood of type I error, meaning that the possibility increases that the obtained significant results are in fact chance findings (44). On the other hand, too strict of a correction might increase the likelihood of type II error, which means that the actual significant results are being discarded as insignificant based on the correction, which might limit the comprehensiveness and potentiality of the findings, especially in exploratory research. Therefore, this debate boils down to being between Scylla and Charybdis. Our approach in this thesis was to acknowledge both evils, and present the results transparently with and without corrections, or address this limitation when appropriate.

4.4. Implications for public health and for policy making

The studies presented in this thesis suggest that fetal and childhood exposures to air pollution play an adverse role in brain development, and the observed relationships prevailed even at levels of exposure well below the EU legislations for the maximum concentrations, such as fine particulate matter exposure levels in the majority of our study population(45). Taking into account the current ubiquity of air pollution worldwide, one can only conclude that the implications for public health are not to be overlooked. In the previous section, we provided some insight into the methodological aspects that studies on air pollution epidemiology could seek to address and possibly improve, mainly concerning the refinement of exposure and outcome assessment. In the current section we present implications of our findings for public health and for policy making.

4.4.1. Implications for public health

Our study on the relationship between exposure at birth to elemental components of fine particulate matter with cognitive and psychomotor

functions in childhood, suggested a lower fine motor function related to higher exposure to airborne iron. Although such decrease seems rather small and negligible on individual level, on population level it would increase the number of children performing below average. Compromised fine motor skills could have negative influence on child's academic performance, physical activity, and other aspects of life (46,47). When we studied the relationship between exposure to air pollution with depressive and anxiety symptoms, and aggressive symptoms in children, our results did not suggest an association. In our next study, we found an association between fine particles exposure during fetal life and impairment in inhibitory control in school-age children which was partially mediated by thinner cortex in several brain areas. Inhibitory control regulates self-discipline and is key to temptation resistance and impulse control, and its impairment has been related to addictions and attention deficit hyperactivity disorder, among other behavioral disorders (48). We also identified positive associations between air pollution exposure and cortical and sub-cortical brain volumes, and larger cortical pial surface area, and a negative association with the volume of corpus callosum. While the interpretation of the directionality of our findings is equivocal due to dynamic changes in both grey and white matter involved in the process of brain maturation that takes place between childhood and adolescence (42), merely the concept that exposure to air pollution during fetal life and childhood has an influence on the morphology of the developing brain, is concerning. Finally, we also observed an association between higher fetal and childhood exposure to air pollution and alterations in white matter microstructure in preadolescents, with the differences being translatable to a developmental delay in white matter microstructure of 0.5 up to 2 months. White matter microstructure is a quantifiable marker for the state of myelination - one of the most important processes for optimal brain development (49). Moreover, such alterations in white matter microstructure have been associated with psychiatric and neurological disorders, such as autism spectrum disorder and attention deficit hyperactivity disorder (50,51). In general, the findings of the studies presented in this thesis, suggest that exposure to higher levels of air pollution during fetal life and childhood is associated with various neurodevelopmental alterations. Although air pollution exposure is of involuntary nature, unlike for example first-hand smoking, individual choices can have an impact on personal exposure. For instance, while air pollution is highly ubiquitous and Geoffrey Rose's Prevention Paradox – stating that prevention strategies should mainly focus on general population rather than on individuals – clearly holds true, the exposure is not equally distributed in space, and people living closer to traffic dense areas are at higher risk of being exposed to higher levels of air pollution. Also, individual choices that contribute to air pollution, such as use of cars instead of public or active transportation, could have an impact on personal exposure in the long run. On individual level,

such contribution versus mitigation choices might seem too small to make a difference, but little by little, a little becomes a lot.

4.4.2. Implications for policy making

The results presented in this thesis clearly suggest that exposure to air pollution during fetal life and childhood is associated with alterations in developmental processes of the brain. The identified associations were often observed with air pollution levels below the EU legislations for the maximum concentrations (45). Taking into account the ubiquity of air pollution and the involuntary nature of this exposure, these results clearly show that policy makers should consider lowering the current legislated standards, and above all strive to lower the current levels of air pollution. While we acknowledge that the disentanglement of specific pollutants was very challenging in our study setting due to the complex mixture of air pollution, and that the majority of our conclusions refer to air pollution in general, we did identify pollutants originating specifically from brake linings, tire wear, and tailpipe emissions from combustion of diesel in some of the observed associations. These findings indicate that although the current direction towards innovative solutions for cleaner energy vehicles is a step in the right direction, these measures might not be completely adequate to mitigate health problems attributable to traffic related air pollution as we also observed associations markers for brake linings and tire wear.

4.5. Future research directions

Although after a decade of research, enough scientific evidence is available to infer that exposure to air pollution has a compromising impact on human brain, several gaps in knowledge still exist. One of such gaps is the lack of studies in adolescents. Adolescence is a period of big changes in human body, undergoing rapid hormonal changes and all thereto related transformations. The discrepancy between the results on the relationship of air pollution with depression and anxiety between studies in children and in adults, clearly demonstrates the need for studies in adolescents to better understand the topic. Another recommendation for directions of future research is the inclusion of repeated outcome measurements, as majority of the work in this field to date is of cross-sectional nature. Repeated measurements allow for assessment of brain trajectories over time, thereby increasing the possibility of causal inference. Next, since the brain undergoes many dynamic processes during development, often of highly varying time-scales (Figure 1) (52), analyzing the mean of the exposure over a long period of time, hinders the detection of specific windows of vulnerability. Therefore, studying such potential temporal windows of vulnerability might provide a better understanding as to which developmental processes are susceptible to alterations related to air pollution exposure. We are currently working on this study and expect to publish the results in the near future.

Relating to exposure assessment, my recommendation would be to focus on improving the measurements and modeling of ultrafine particulates, by increasing the duration of the measurement campaigns and by increasing the number of monitoring networks, as ultrafine particles have the highest potential of penetrating into the brain due to their nanoscopic size (53). I would also recommend expanding the study areas. Currently, most of the research on the relationship between air pollution exposure and neurodevelopment has been performed in Europe and the US. It would be very informative and interesting to learn whether the identified relationship also holds true in other parts of the world, where air pollution levels and composition, as well as human susceptibility, might be different from Europe or the US. Finally, I would recommend the inclusion of ozone. Evidence from numerous epidemiological studies from the US suggests that ozone atmospheric pollution is a risk factor for neurodegenerative diseases (54–56).

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5. CONCLUSIONS

The main conclusions of this thesis are:

- Higher exposure to air pollution at birth, and in particular to airborne iron, an element of fine particulate matter highly prevalent in motorized traffic air pollution, was associated with lower fine motor function in children from 1 to 9 years old.
- Prenatal and postnatal exposure to various air pollutants did not show any association with emotional and aggressive symptoms in children from 7 to 11 years old.
- Exposure to fine particulate matter during fetal life was associated with thinner cortex, as well as with an impairment of inhibitory control in children from 6 to 8 years old.
- Thinner cortex in the precuneus and rostral middle frontal regions partially mediated the relationship between exposure to fine particulate matter with compromised inhibitory control in children from 6 to 8 years old.
- Fetal and childhood exposure to various air pollutants representative of traffic related sources, was associated with attenuated cortical thickness, larger cortical and subcortical volumes, lower volume of corpus callosum, and larger cortical pial surface area in preadolescents from 9 to 12 years old.
- The relationship between fetal exposure to various air pollutants and larger cortical and subcortical volumes in preadolescents from 9 to 12 years old, was mainly observed in girls.
- Fetal and childhood exposure to pollutants representative of brake linings, tire wear, and tailpipe emissions, showed associations with alterations in white matter microstructure in preadolescents from 9 to 12 years old.