DOWN THE ROAD

Air Pollution Exposure, and Child's Neuropsychological and Neurobiological Development

Małgorzata Joanna Lubczyńska

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Air Pollution Exposure, and Child's Neuropsychological and Neurobiological Development

Blootstelling aan luchtverontreininging, en neuropsychologische en neurobiologische ontwikkeling van kinderen

Exposición a la contaminación del aire, y desarrollo neuropsicológico y neurobiológico de niños

THESIS

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by

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~ to dla ciebie tatko ~

CONTENT

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, objectives, results (5 original research articles), a general discussion, conclusions, and a summary in English and in Dutch. The thesis is focused on the associations between fetal and childhood exposures 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 Barcelona, 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

GENERAL INTRODUCTION

GENERAL 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).

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.

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.

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 $\mu g/m^3$, 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 10 μm and 2.5 μm (coarse particles or PM_{COARSE}), 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 $\mu g/m^3$ for 24h averages, and to 40 $\mu g/m^3$ for annual averages. The AQGs by WHO set the current annual average concentration limits to 20 $\mu g/m^3$. 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 $\mu g/m^3$ whereas the limits specified by the WHO equal 10 $\mu g/m^3$. 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]pyrene (B[a]P).

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.

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).

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

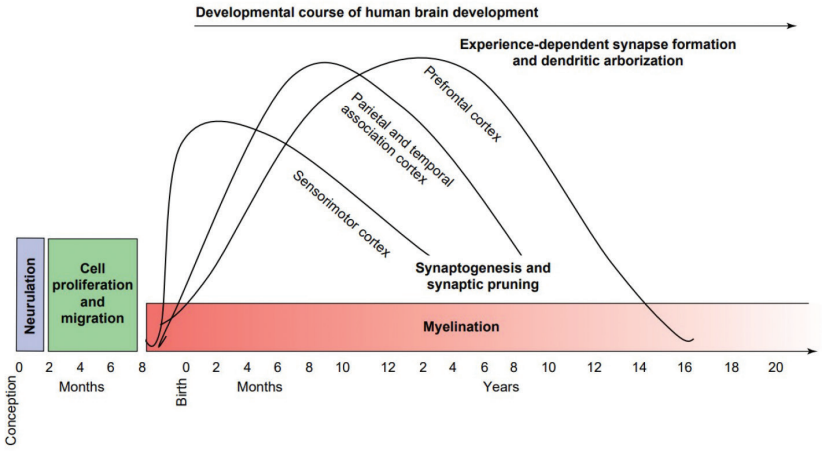

Figure 1: Course of human brain development (12)

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.

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 grey 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.

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.

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).

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).

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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OBJECTIVES

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

RESULTS

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: Exposure to air pollution during pregnancy and childhood, and white matter microstructure in preadolescents

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

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

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Short running title: Elemental particulate composition and child's cognitive and psychomotor function

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ABSTRACT

Background: Little is known about developmental neurotoxicity of particulate matter composition. We aimed to investigate associations between exposures to elemental composition of outdoor $PM_{2.5}$ at birth and cognitive and psychomotor functions in childhood.

Methods: We analyzed data from 4 European population-based birth cohorts in the Netherlands, Germany, Italy and Spain, with recruitment in 2000-2006. Elemental composition of $PM_{2.5}$ measurements were performed in each region in 2008-2011 and land use regression models were used to predict concentrations at participants' residential addresses at birth. We selected 8 elements (copper, iron, potassium, nickel, sulfur, silicon, vanadium and zinc) and used principal component analysis to combine elements from the same sources. Cognitive (general, verbal, and non-verbal) and psychomotor (fine and gross) functions were assessed between 1 and 9 years of age. Adjusted cohort-specific effect estimates were combined using random-effects meta-analysis.

Results: 7,246 children were included in this analysis. Single element analysis resulted in negative association between estimated airborne iron and fine motor function (-1.27 points [95% CI -2.48 to -0.06] per 100 ng/m^3 increase of iron). Association between the motorized traffic component, derived from principal component analysis, and fine motor function was not significant (-0.29 points [95% CI -0.64 to 0.06] per unit increase). None of the elements were associated with gross motor function or cognitive function, although the latter estimates were predominantly negative.

Conclusion: Our results suggest that iron, a highly prevalent element in motorized traffic pollution, may be a neurotoxic compound. This raises concern given the ubiquity of motorized traffic air pollution

INTRODUCTION

Air pollution is a serious threat to human health. The potential effects of air pollution on human brain is an active area of research (Block et al., 2012). Particulate matter (PM), highly prevalent in traffic related air pollution, could reach the brain and other organs by translocation to the systemic circulation following a deposition in the pulmonary region after inhalation (Block et al., 2012). The brain of a fetus could be reached via an indirect path as the placenta and the blood-brain barrier grant only a partial protection against entry of environmental toxicants to which the mother is exposed. As the brain is in the process of development and the detoxification mechanisms are relatively immature, the potential adverse effects of exposure to air pollution during pregnancy are of particular concern (Block et al., 2012; Grandjean and Landrigan, 2014).

Although the precise biological mechanisms are yet to be clarified, there is some evidence for a negative association between pre- and postnatal exposure to outdoor PM and children's cognition, psychomotor development, and behavioral problems (Guxens and Sunyer, 2012; Guxens et al., 2014, 2015; Suades-González et al., 2015). It has been hypothesized that traffic-related PM might be neurotoxic mainly through some of its components such as polycyclic aromatic hydrocarbons (PAHs), black carbon, and trace elements, potentially leading to increased oxidative stress and increased activation of brain microglia, the primary regulators of neuroinflammation (Block et al., 2012). Studies focusing on PAHs found negative association with children's cognition and behavioral problems (Edwards et al., 2010; Lovasi et al., 2014; Perera et al., 2006, 2009, 2013; Wang et al., 2010). Moreover, a recent study using magnetic resonance imaging found preliminary evidence for reduction in the white matter surface of the left hemisphere of the brain in childhood with increased prenatal concentrations of PAHs, associated with slower information processing speed (Peterson et al., 2015). Studies with focus on pre- and postnatal exposure to black carbon also found a negative association with cognitive and/or psychomotor development (Chiu et al., 2013; Suglia et al., 2008), although these findings were inconsistent.

To date, developmental neurotoxicity has been documented for only a small number of existing trace elements (Grandjean and Landrigan, 2014). Studies addressing the association between pre- and/or postnatal exposure to trace elements in outdoor air and children's brain development are very limited in number. The few existing studies have linked higher levels of several airborne elements including arsenic, cadmium, chromium, lead, manganese, mercury, nickel, selenium and vanadium, to elevated prevalence of autism spectrum disorder (Lam et al., 2016). Additionally, the only study to date that focused on airborne elements and cognition, found evidence for a negative association between childhood exposure at schools to airborne elements originating from motorized traffic sources and specific cognitive functions in school aged children (Basagaña et al., 2016). However, for many elements, sparse evidence of neurotoxicity is possibly a consequence of limited amount of research addressing the topic rather than absence of an association (Grandjean and Herz, 2015).

Therefore, the aim of this study was to analyze the association between exposure at birth to a set of elements measured in outdoor PM with aerodynamic diameter of less than 2.5 micrometers ($PM_{2.5}$) and cognitive and psychomotor function in childhood using data from four European cohorts. The elemental components examined in this study were copper, iron, potassium, nickel, sulfur, silicon, vanadium and zinc, selected based on their reflection of major anthropogenic emission sources. This study builds on a previous epidemiological study that investigated the association between air pollution and neuropsychological development in 6 European cohorts (Guxens et al., 2014). In that study, the authors found a negative association between prenatal exposure to NO_2 and PM - latter borderline significant - and psychomotor function in childhood. The cohorts included in the current study are a subset of the cohorts studied previously due to the availability of elemental composition data. Also, in the current study we used additional neuropsychological domains and some of the tests included, were carried out at older ages.

METHODS

Population and Study Design

This study is part of the ESCAPE (European Study of Cohorts for Air Pollution Effects; www.escapeproject.eu) project. The aim of the project was to investigate the association between exposure to outdoor air pollution and health within prospective cohort studies. In the current study, we included 4 European population-based birth cohorts: GENERATION R (The Netherlands) (Jaddoe et al., 2012), DUISBURG (Germany) (Wilhelm et al., 2008), GASPII (Italy) (Porta et al., 2007), and INMA-Sabadell (Spain) (Guxens et al., 2012), a selection based on the availability of elemental composition of $PM_{2.5}$ and neuropsychological data. Mother-child pairs were recruited between 2000 and 2006. A total of 7,246 children aged between 1 and 9 years was included in this analysis and had data on exposures and at least one of the neuropsychological outcomes (Table 1). Local authorized Institutional Review Boards granted the ethical approval for the studies and all participants provided signed informed consent.

Exposure to Elemental Composition of Outdoor $PM_{2.5}$

The exposure of each participant to the elemental composition of $PM_{2.5}$ was estimated using standardized procedure based on land use regression (LUR) methodology (de Hoogh et al., 2013). The locations of the measuring stations were based on the specific characteristics of each study area including a large diversity of potential sources of air pollution variability, and were selected in a manner to maximise the representativeness of the residential addresses of the cohort participants (Eeftens et al., 2012). We focused on fine particles rather than coarse, due to their higher potential to translocate to the systemic circulation because of the smaller size (Phalen et al. 2010). $PM_{2.5}$ concentrations in outdoor air were measured at 40 sites in the Netherlands/Belgium and Catalunya, and 20 sites in Ruhr area and Rome three times over a year (in summer, winter, and an intermediate season) during a two-week period each time to capture seasonal variations (Eeftens et al., 2012). The campaigns took place between 2008 and 2011. The filters were sent to Cooper

Table 1. Description of the Participating Birth Cohort Studies

Origin (city/area)	Setting			Elemental Components	Cognitive function					Psychomotor function				
	Cohort name	Pregnancy period	No. of participants at baseline	LUR models available	Test	Domain	Age (years)	Evaluator	No.[a]	Test	Domain	Age (years)	Evaluator	No.[a]
Dutch (Rotterdam)	Generation R	2001-2005	8737	Cu, Fe, K, Ni, S, Si, V, Zn	MCDI	verbal	1.5	Parents	**4397**	MIDI	FM, GrM	1	Parents	**4704**
					SON-R	non-verbal	6	Trained staff	**4580**					
German (Ruhr area)	Duisburg	2000-2003	232	Cu, Fe, Ni, S, Si, V, Zn	BSID II	GC	1	Psychologist	**186**	N/A				
					BSID II	GC	2	Psychologist	**178**					
					HAWIK-IV	GC, verbal, non-verbal	8-10	Psychologist	**95**					
Italian (Rome)	GASPII	2003-2004	719	Cu, Fe, K, Ni, S, Si, V, Zn	DDST II	verbal	1.5	Parents	**546**	DDST II	FM, GrM	1.5	Pediatrician	**546**
					WISC-III	GC, verbal, non-verbal	7	Psychologist	**450**	DDST II	FM, GrM	4	Parents	**551**
Spanish (Sabadell)	INMA-Sabadell	2004 -2006	740	Cu, Fe, K, Ni, S, Si, V, Zn	BSID I	GC	1.5	Psychologist	**519**	MSCA	FM, GrM	4	Psychologist	**439**
					MSCA	GC, verbal, non-verbal	4	Psychologist	**439**					

BSID, Bayley Scales of Infant Development (I-first edition, II-second edition); DDST II, Denver Developmental Screening Test II; FM, Fine motor; GC, General cognition; GrM, Gross motor; HAWIK-IV, Hamburg Wechsler Intelligenztest für Kinder - IV; MCDI, McArthur Communicative Development Inventory; MIDI, Minnesota Infant Development Inventory; MSCA, McCarthy Scales of Children's Abilities; N/A, not available; SON-R, De Snijders-Oomen Niet-verbale Intelligentietest-Revisie; WISC, Wechsler Intelligence Scale for Children

[a]Number of subjects with airborne elemental components of $PM_{2.5}$ and cognitive/psychomotor function data available

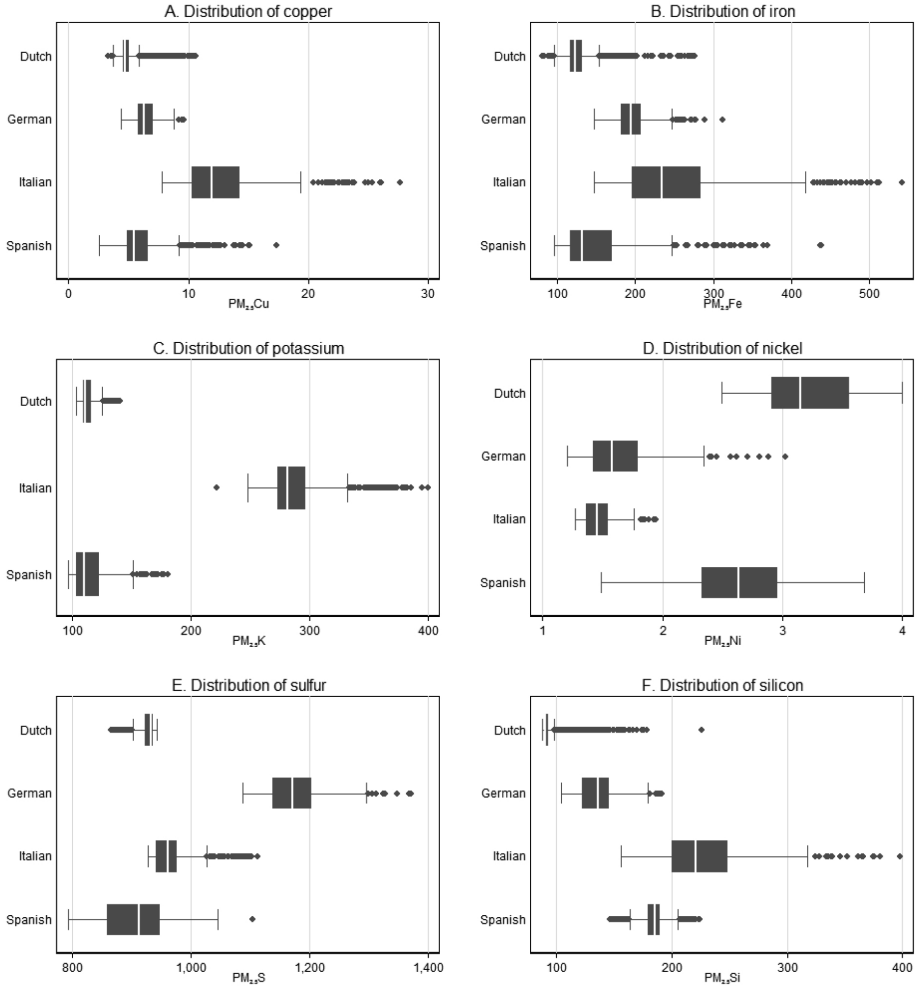


Figure 1. Distribution of $PM_{2.5}$ elemental composition levels in ng/m^3 (copper (A), iron (B), potassium (C), nickel (D), sulfur (E), silicon (F), vanadium (G) and zinc (H)), $PM_{2.5}$ mass in µg/m^3 (I) and motorized traffic pollution scores (J) in the participating cohorts.

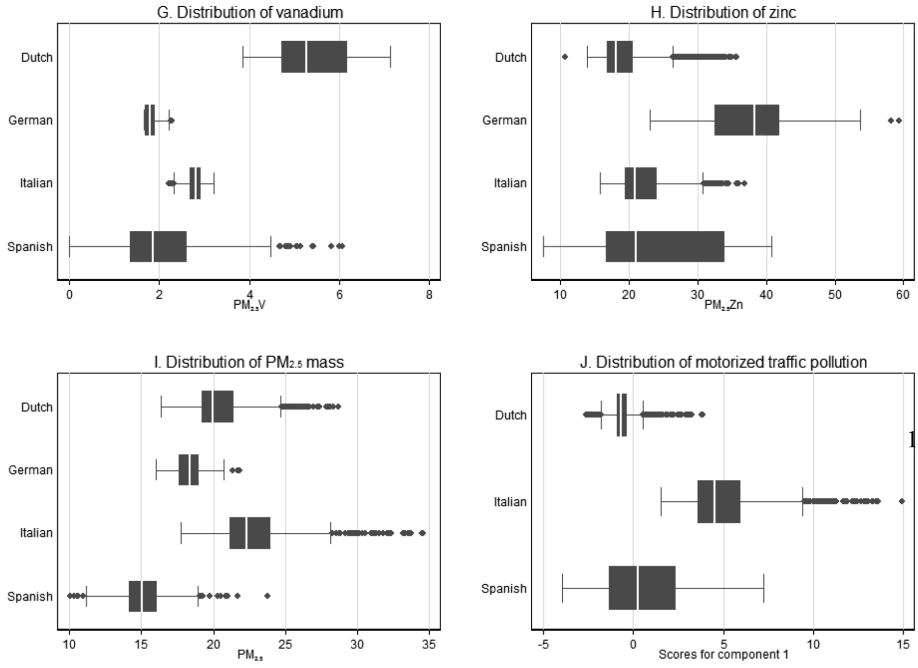


Figure 1. (Continued)

Environmental Services (Portland, Oregon, USA) to analyze their elemental composition using X-Ray Fluorescence (XRF) (de Hoogh et al., 2013; Tsai et al., 2015). The results of the three measurements were then averaged, adjusting for temporal trends using data from a continuous reference site, resulting in one mean annual concentration for each element identified in the composition of $PM_{2.5}$.

Following previous ESCAPE studies on elemental components (de Hoogh et al., 2013; Pedersen et al., 2016; Wang et al., 2014) we selected 8 elements based on their reflection of major anthropogenic emission sources and on data availability determined by (i) the coefficient of variation aquired from duplicate samples, (ii) the percentage of samples in which the element was detected and (iii) the availability of relevant georgraphical data needed as predictor variables in the LUR models. Copper (Cu), iron (Fe) and zinc (Zn) reflect brake linings, tire wear (Zn), and industrial (smelter) emissions (Fe, Zn), silicon (Si) and potassium (K) reflect crustal materials and biomass burning (K) and fossil fuel combustion is reflected by nickel (Ni), vanadium (V) and sulfur (S) (Viana et al., 2008).

Following a previous study on birth outcomes (Pedersen et al., 2016), concentrations of the selected elements were assigned at each participants' home address at birth to obtain an estimation of the pregnancy exposure using mean annual area-specific LUR model

estimates based on 2008-2011 data (Table 1). Fixed increments per elemental component were applied to facilitate comparability. The model predictors and a description of model performances are reported elsewhere (de Hoogh et al., 2013). Due to insufficient data quality, LUR models of potassium could not be developed for the German cohort (Table 1). Next, we pooled the exposure data of participants from the cohorts together and applied principal component analysis (PCA) to the estimated elemental concentrations at the residential addresses, in order to combine elements from the same sources into one score. Oblique promax rotations were allowed. Since the levels of potassium could not be estimated for the German cohort, that cohort was not included in the pooled PCA.

Cognitive and Psychomotor Function

Neuropsychological tests used to assess the cognitive and psychomotor function of children were administered by psychologists, pediatricians or trained research staff, or by questionnaires answered by the parents, and differed between the cohorts (Table 1). For each cohort, the tests and questionnaires that measured each neuropsychological function in a similar way and derived in comparable score distribution, were selected. Cognitive function scales measured general, verbal, and/or non-verbal cognitive functions and psychomotor function scales measured fine and gross motor functions (Table 1). To homogenize the scales, we converted all raw scores into standard deviation units using the z-score (z-score is calculated as the raw score minus the sample mean, divided by the standard deviation) and standardized them to a mean of 100 and a standard deviation of 15 (new score = 100 + (15 × z)) (Guxens et al., 2014). For each domain, higher scores corresponded to better neuropsychological function.

Potential confounding variables

Available potential confounding variables were defined a priori based on direct acyclic graph (DAG) (Figure, Supplementary Material 1) and selected as similarly as possible across the cohorts. Maternal information included age at delivery (continuous in years), height (continuous in centimeters), pre-pregnancy body mass index (continuous in kg/m^2), smoking during pregnancy (yes or no), alcohol consumption during pregnancy (yes or no), marital status (monoparental household: yes or no) and parity (0, 1, ≥2). Parental information included educational level (low, medium, high) and country of birth (country of the cohort or foreign country). Maternal height and pre-pregnancy weight were obtained at the enrollment in the study, or self-reported in the first trimester of the pregnancy, at birth or two weeks after birth of the child. The other variables were collected through questionnaires either during pregnancy or at birth. For education level, standardization of cohort-specific categories was applied to create a common variable (Guxens et al., 2014). Child's age at the time of the cognitive and psychomotor function assessment, and the evaluator for the assessment, were also recorded.

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 exposure and at least one outcome variable (Table, Supplementary Material 2). We obtained

25 completed datasets that we analyzed using standard procedures for multiple imputation (Spratt et al., 2010; Sterne et al., 2009). Children with available exposure and outcome data (n=7,246) were more likely to have parents with higher socioeconomic status compared to those recruited initially in the cohorts but without available data on exposure and outcome (n=3,182) (Tables, Supplementary Material 3 and 4). We used inverse probability weighting (IPW) to correct for loss to follow-up, i.e. to account for selection bias that potentially arises when only population with available exposure and outcome data, and here thus with relatively higher socioeconomic status, is included as compared to a full initial cohort recruited at pregnancy (Weisskopf et al., 2015; Weuve et al., 2012). 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 populations of the cohorts. The variables used to create the weights are described in Table, Supplementary Material 5.

After visual inspection for linearity, we used linear regression models to analyze the relationships of each single element and PCA component with each neuropsychological function. Additionally, we performed the analyses with prenatal $PM_{2.5}$ and NO_2 levels and each neuropsychological function to make the comparison with the previous study (Guxens et al., 2014) straightforward. Concentrations of the pollutants were introduced as continuous variables and were not transformed. When the age of a child was not linearly related with cognitive or psychomotor function scale, we used the best transformation of age found using fractional polynomials (Royston et al., 1999). The models were adjusted for all potential confounding variables described in the previous sub-chapter.

We carried out a two-steps analysis. First, associations were analyzed separately for each cohort. Second, cohort-specific effect estimates were combined in a meta-analysis. Because the data originated from four different regions with divergent characteristics, we decided to use a conservative approach selecting a priori random effect meta-analysis method thereby also adding to the homogeneity and comparability of the analyses. We used Cochran Q test and I^2 statistic to indicate total variability in the estimates that is attributable to between-cohort heterogeneity (Higgins and Thompson, 2002). When the same outcome was measured at multiple ages in a cohort, the score at the oldest age was taken into account in the meta-analysis. Exception was made for the general cognitive function in the German cohort wherein the second oldest age was selected due to substantially larger sample size compared to the sample size of the oldest age (Table 1). Finally, to test the sensitivity of the results, we repeated the meta-analyses including younger ages among the cohorts where the outcomes were measured at different ages, as well as including the oldest age for the German cohort. All statistical hypothesis tests were two-tailed with significance level set at $p<0.05$ and were carried out using STATA (version 14.0; StataCorporation, College Station, TX).

Table 2. Distribution of Parental Characteristics

Cohort Study	Cohort Country	No.	Maternal Education Level		Paternal Education Level			Maternal Country of Origin	Paternal Country of Origin	Maternal Age at Delivery
			Low	Medium	Low	Medium	High	Foreign	Foreign	(Years)
Generation R	The Netherlands	5911	9.3	41.9	9.6	41.8	48.6	44.3	42.1	31.0 (5.0)
Duisburg	Germany	190	2.0	37.9	24.6	24.2	51.3	13.2	18.4	31.2 (4.7)
GASPII	Italy	614	13.6	50.7	1.7	6.5	31.7	3.8	2.4	33.4 (4.4)
INMA-Sabadell	Spain	531	27.4	41.5	35.9	42.7	21.4	10.2	11.4	31.7 (4.2)

Cohort Study	Cohort Country	No.	Maternal Pre-Pregnancy Body Mass Index	Maternal Height	Maternal Alcohol During Pregnancy	Maternal Smoking During Pregnancy	Parity	Marital Status
			(kg/m²)	(cm)	(yes)	(yes)	Nulliparous	Monoparental
Generation R	The Netherlands	5911	22.6 (20.8 to 25.2)	168.0 (7.4)	42.5	14.3	57.1	11.2
Duisburg	Germany	190	22.9 (20.8 to 25.7)	167.6 (6.2)	11.5	22.6	56.8	2.7
GASPII	Italy	614	21.3 (19.8 to 23.7)	164.8 (5.8)	35.6	11.3	5.8	0.5
INMA-Sabadell	Spain	531	22.7 (21.0 to 25.4)	162.4 (6.0)	21.1	29.4	57.3	1.1

Values are percentages for the categorical variables, mean (standard deviation) for the continuous normally distributed variables, and median (interquartile range) for the continuous non-normally distributed variables

Table 3. Fully adjusted combined associations between exposure to elemental components and the identified pollution source at birth and general, verbal, and non-verbal cognitive function

	General cognitive function			Verbal cognitive function			Non-verbal cognitive function		
		Test for			Test for			Test for	
	Coef. (95% CI)	Heterogeneity, P	I	Coef. (95% CI)	Heterogeneity, P	I	Coef. (95% CI)	Heterogeneity, P	I
Copper (Cu)	-1.72 (-5.18 to 1.74)	0.066	63.3	-0.28 (-1.60 to 1.04)	0.683	0.0	-1.02 (-2.35 to 0.30)	0.397	0.0
Iron (Fe)	-1.30 (-3.33 to 0.73)	0.191	39.7	-0.28 (-1.48 to 0.92)	0.810	0.0	-1.09 (-2.30 to 0.13)	0.706	0.0
Potassium (K)	-0.88 (-2.83 to 1.07)	0.311	2.7	0.01 (-1.63 to 1.65)	0.888	0.0	-0.65 (-2.79 to 1.48)	0.217	34.6
Nickel (Ni)	-2.04 (-4.69 to 0.61)	0.788	0.0	-0.22 (-1.30 to 0.87)	0.611	0.0	-0.17 (-1.46 to 1.11)	0.362	6.3
Sulfur (S)	-2.44 (-5.94 to 1.06)	0.750	0.0	-2.80 (-5.99 to 0.39)	0.731	0.0	-0.45 (-3.72 to 2.82)	0.375	3.5
Silicon (Si)	-2.57 (-5.61 to 0.47)	0.777	0.0	-1.18 (-3.35 to 1.00)	0.878	0.0	-0.73 (-2.95 to 1.49)	0.859	0.0
Vanadium (V)	-4.65 (-12.21 to 2.92)	0.210	35.9	-0.24 (-1.21 to 0.73)	0.425	0.0	-1.05 (-4.53 to 2.42)	0.023	68.6
Zinc (Zn)	-0.68 (-1.91 to 0.56)	0.494	0.0	-0.05 (-0.92 to 0.81)	0.912	0.0	0.10 (-0.73 to 0.94)	0.725	0.0
Motorized traffic[a]	-0.26 (-0.66 to 0.15)	0.419	0.0	-0.16 (-0.51 to 0.19)	0.671	0.0	-0.19 (-0.55 to 0.16)	0.661	0.0

[a]Motorized traffic component was acquired using the principle component analysis (PCA). See Supplementary Table 6 for detailed configuration of the component.

Coefficient and 95% CI were estimated by random-effects meta-analysis by cohort. Models were adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing per increments of 5 ng/m^3 for Cu $PM_{2.5}$; 100 ng/m^3 for Fe $PM_{2.5}$; 50 ng/m^3 for K $PM_{2.5}$; 1 ng/m^3 for Ni $PM_{2.5}$; 200 ng/m^3 for S $PM_{2.5}$; 100 ng/m^3 for Si $PM_{2.5}$; 2 ng/m^3 for V $PM_{2.5}$; and 10 ng/m^3 for Zn $PM_{2.5}$

Table 4. Fully adjusted combined associations between exposure to elemental components and the identified pollution source at birth, and fine and gross motor function

	Fine motor function			Gross motor function		
	Coef. (95% CI)	Test for Heterogeneity, P	I	Coef. (95% CI)	Test for Heterogeneity, P	I
Copper (Cu)	-0.95 (-2.27 to 0.37)	0.816	0.0	0.29 (-2.13 to 2.70)	0.053	66.0
Iron (Fe)	-1.27 (-2.48 to -0.06)	0.784	0.0	-0.08 (-1.91 to 1.75)	0.111	54.5
Potassium (K)	-1.11 (-2.71 to 0.49)	0.386	0.0	1.02 (-0.60 to 2.64)	0.356	3.2
Nickel (Ni)	-1.12 (-5.93 to 3.69)	0.003	83.0	1.85 (-0.78 to 4.48)	0.145	48.3
Sulfur (S)	-1.03 (-4.36 to 2.29)	0.606	0.0	1.76 (-1.58 to 5.10)	0.774	0.0
Silicon (Si)	-1.53 (-3.63 to 0.58)	0.976	0.0	-1.62 (-3.66 to 0.41)	0.457	0.0
Vanadium (V)	-0.86 (-4.95 to 3.22)	0.003	82.3	-0.21 (-4.15 to 3.72)	0.007	79.6
Zinc (Zn)	-0.35 (-1.22 to 0.51)	0.652	0.0	0.59 (-0.58 to 1.75)	0.221	33.8
Motorized traffic[a]	-0.29 (-0.64 to 0.06)	0.879	0.0	0.10 (-0.41 to 0.61)	0.122	52.5

[a]Motorized traffic component was acquired using the principle component analysis (PCA). See Supplementary Table 6 for detailed configuration of the component.
Coefficient and 95% CI were estimated by random-effects meta-analysis by cohort. Models were adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing per increments of 5ng/m^3 for Cu $PM_{2.5}$; 100ng/m^3 for Fe $PM_{2.5}$; 50ng/m^3 for K $PM_{2.5}$; 1ng/m^3 for Ni $PM_{2.5}$; 200ng/m^3 for S $PM_{2.5}$; 100ng/m^3 for Si $PM_{2.5}$; 2ng/m^3 for V $PM_{2.5}$; and 10ng/m^3 for Zn $PM_{2.5}$.

RESULTS

Parental characteristics of the study population are shown in Table 2. The percentage of higher-educated mothers was highest in the Dutch cohort while the percentage of higher-educated fathers was highest in the German cohort. The highest percentage of both - lower educated mothers and lower educated fathers - was in the Spanish cohort. The highest percentage of mothers consuming alcohol during pregnancy was in the Dutch cohort whereas the highest percentage of mothers smoking during pregnancy was in the Spanish cohort. The proportion of parents that were born in a country different than that of the study and the percentage of single parent households was highest in the Dutch cohort.

Cohort specific concentration levels of each element are shown in Figure 1. Correlations between the modelled concentrations of the pollutants varied considerably depending on the pollutant and the region (Table, Supplementary Material 6). The PCA resulted in identification of two principal components with a combined R^2 of 78% and a low correlation of <0.20. Component 1 was loaded primarily with copper, iron and sulfur suggesting a reflectance of motorized traffic pollution. Component 2 was comprised predominantly of positive loadings of nickel and vanadium and negative loadings of silicon and potassium, making the conceptualization of component 2 highly ambiguous (Table, Supplementary

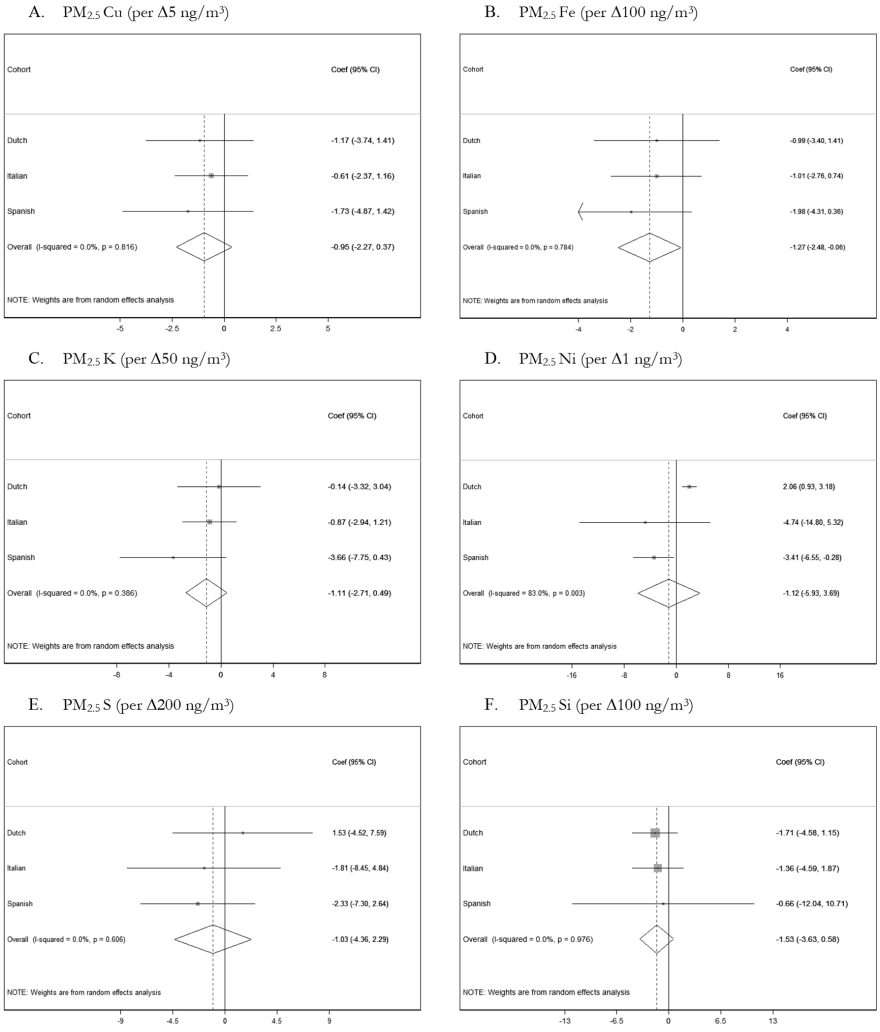
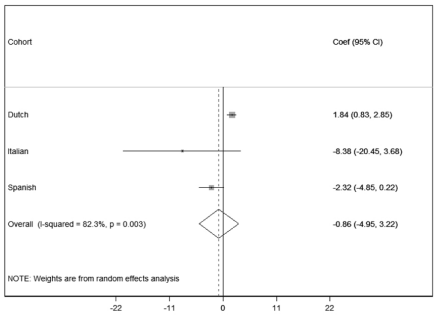
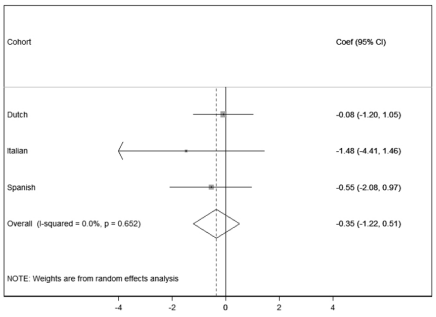


Figure 2. Fully adjusted associations of exposure to $PM_{2.5}$ elemental composition at birth and motorized traffic pollution with fine motor function at average age of 1y in Dutch cohort, 4y in Italian cohort and 4y in Spanish cohort. Region-specific and summary risk estimates (coefficient and 95% CI) for fine motor function expressed for an increase of (A) 5ng/m^3 in $PM_{2.5}$Cu levels, (B) 100ng/m^3 in $PM_{2.5}$Fe levels, (C) 50ng/m^3 in $PM_{2.5}$K levels, (D) 1ng/m^3 in $PM_{2.5}$Ni levels, (E) 200ng/m^3 in $PM_{2.5}$S levels, (F) 100ng/m^3 in $PM_{2.5}$Si levels, (G) 2ng/m^3 in $PM_{2.5}$V levels, (H) 10ng/m^3 in $PM_{2.5}$Zn levels, and (I) motorized traffic pollution levels during pregnancy, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.

G. $PM_{2.5}$ V (per Δ2 ng/m³)



H. $PM_{2.5}$ Zn (per Δ10 ng/m³)



I. Motorized Traffic Pollution

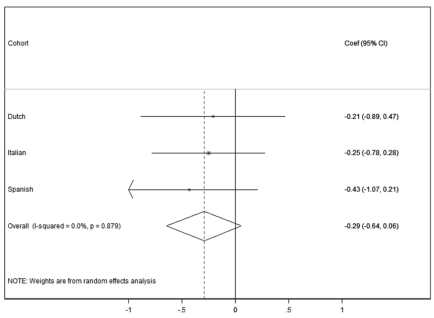


Figure 2. (Continued)

Material 7). Therefore, this component was not analyzed further. The proportion of participants with a higher socioeconomic status was larger in areas with higher levels of motorized traffic pollution expressed in tertiles (Table, Supplementary Material 8).

DISCUSSION

To our knowledge, no previous study focused on the association between exposure to elemental composition of outdoor $PM_{2.5}$ at birth and cognitive and psychomotor function in childhood. This study is based on 4 European birth cohorts with data on 7,246 children. Despite the lack of significant association between airborne $PM_{2.5}$ during pregnancy and cognitive and psychomotor development in childhood, we found an association with one of its elemental components. Higher estimated exposure at birth to airborne iron, a main element in motorized traffic pollution, was associated with lower fine motor function in children assessed between 1 and 4 years of age. Exposure to elemental composition of outdoor $PM_{2.5}$ at birth was not associated with gross motor function or cognitive functions, although the effect estimates of the latter were predominantly negative.

This study has considerable strengths: i) large sample size with western European geographical extent including two countries from the northern part of Europe and two

from the southern part, with varying levels and sources of air pollution; ii) standardized air pollution assessment which was based on validated measurements; exposure assessment of a large number of elemental components measured in airborne $PM_{2.5}$ and modeled to the individual level of each participant; iii) prospective neuropsychological function assessment during childhood using validated neuropsychological tests and questionnaires; iv) use of advanced statistical methods including multiple imputation combined with inverse probability weighting to reduce possible attrition bias in the study; v) adjustment for various socioeconomic and lifestyle variables that are known to be potentially associated with air pollution exposure during pregnancy and with neuropsychological performance of the offspring. However, we cannot completely discard residual confounding by sociodemographic and geographic factors since adjustment for parental education levels and marital status might not fully account for factors that may influence cognitive and psychomotor development.

There are also several other limitations in our study. The neuropsychological tests and the type of evaluators assessing cognitive and psychomotor functions, and the ages at which children were assessed, are heterogeneous across the 4 cohorts. Nevertheless, we carefully selected those tests that represent similar neuropsychological domains, adding to their comparability. Another limitation of our study is related to the exposure assessment. There is no historical element data available from routine monitoring stations in the study areas and therefore back extrapolation of the concentration levels to each individual pregnancy period was not possible. Since the temporal component was missing, we assumed that the relative composition of $PM_{2.5}$, including the relative concentration of the elements, has remained constant between births of the participants and the measurements, as it has been done in a previous study on birth outcomes (Pedersen et al., 2016), here covering a period of 3.5 to 9 years on average. Spatial stability over time has been demonstrated for other traffic related air pollutants for periods stretching from 8 to 18 years (Gulliver et al., 2013; Cesaroni et al., 2012; Eeftens et al., 2011). Nevertheless, this assumption could result in non-differential exposure misclassification which could lead to an underestimation of the associations. Furthermore, we also cannot discard the possibility that some of our findings occur due to chance because of the multiple comparisons performed. Similar studies are necessary to confirm or refute our findings.

We observed a negative association between the exposure to airborne iron, the main component of motorized traffic pollution, and fine motor function. Our previous published study found a significant negative association between prenatal exposure to NO_2, a well-known marker for traffic related air pollution, and psychomotor function assessed in children between 1 and 6 years of age (Guxens et al., 2014). The association between prenatal exposure to $PM_{2.5}$ and psychomotor function was also negative, although these results were at the margin of significance. Repetition of these analyses in our current study resulted in small changes attributable to the changes in the study populations. Other epidemiological studies also found negative associations between traffic related air pollution or some of its components such as PAHs, NO_2 and hydrocarbons and lower psychomotor function in early childhood (Xu et al., 2016). This is the first study to assess a relationship between airborne iron and psychomotor function. It is plausible that airborne iron is a marker for

traffic related air pollution and that the association that we found is in fact an association between traffic related air pollution and fine motor function. However, considering that iron is a documented, highly active oxidizer, and its excessive accumulation in the brain tissue can trigger neuroinflammation and oxidative stress which are linked to neurodegenerative diseases, neurodevelopmental disorders and decreased cognitive function (Block et al., 2012; Daugherty and Raz, 2015), we also cannot discard the possibility that the association found in the current study can be attributed to the environmental exposure to airborne iron. Moreover, a recent study found the presence of magnetite ultra-fine particles of external origin in human brain samples. Magnetite ultra-fine particles are highly pervasive and abundant in air pollution and they arise from combustion as iron-rich particlulates which, upon release in the air, condense and/or oxidize (Maher et al., 2016). Nevertheless, more research is needed to confirm that airborne iron is one of the primary neurotoxic components of motorized traffic pollution instead of a marker for a different neurotoxicant or a group of neurotoxicants present in traffic related air pollution.

The associations between the elemental components, and the motorized traffic pollution component, and cognitive function, were predominantly negative, but significance has not been reached in any case. Also in our previous published study we did not find an association between prenatal exposure to NO_2 or $PM_{2.5}$ and cognitive function (Guxens et al., 2014). Postnatal exposure at the average age of 8.5 years to source apportioned elemental components of outdoor PM at schools and child's working memory and attentional function at corresponding time point have been assessed in a recent study which found a negative relationship between exposure to source apportioned traffic pollution and the cognitive functions (Basagaña et al., 2016). That study assessed specific cognitive functions such as working memory and attentional function, instead of more global cognitive measurements like in the current study, which might be responsible for the differing results. Also, they assessed postnatal exposures in schools, as opposed to residential exposures at birth in our study. Pregnancy period is of a special interest due to the relatively immature detoxification mechanisms of fetuses and only partial protection of placenta and blood-brain barrier against entry of environmental toxicants, and therefore higher vulnerability of the developing brain. Still, brain maturation continues in childhood and adolescence and therefore a relationship with postnatal exposures is plausible as well. To our knowledge, that is the only other study to date that has assessed exposure to PM elements and/or source apportioned PM elements, and cognitive development. Previous epidemiologic studies assessing exposure to traffic related air pollutants during pregnancy and cognitive development in early childhood showed conflicting results (Guxens and Sunyer, 2012; Suades-González et al., 2015).

In summary, we found a negative association between estimated exposure to airborne iron, an element highly prevalent in motorized traffic air pollution, and fine motor function in childhood with a score decrease of 1.27 points for every 100 ng/m^3 increase in predicted iron levels at birth. Although this seemingly small decrease of 1.27% from the population average might not be noticeable at an individual level, taking the population level into account, this decrease will shift the distribution of fine motor performance to the left and increase the number of people performing below average. Gross motor function and the

cognitive functions were not significantly associated with any of the PM element exposures at birth, although the effect estimates of the latter were predominantly negative. Since this study is the first to focus on exposure to elemental composition of outdoor PM at birth and neuropsychological function in early childhood, the results require confirmation. Nevertheless, they are of potential concern due to the ubiquity of traffic related air pollution, which fortunately can be reduced through implementation of adequate policies worldwide.

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PAPER I – SUPPLEMENTARY MATERIAL

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

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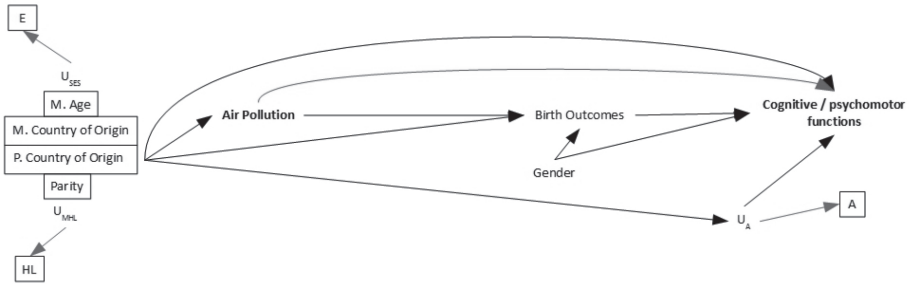


Supplementary Material 1. Figure illustrating simplified Direct Acyclic Graph (DAG), based on available data

Purple arrow indicated the association to be tested. Blue arrows indicate surrogates of unmeasured potential confounders: A, proxy for delay in attendance to the test: age of the child at the neuropsychological assessment; E, proxy for socio-economic status: parental education and marital status; HL, proxy for maternal health and lifestyle: smoking during pregnancy, alcohol consumption during pregnancy, maternal pre-pregnancy BMI and maternal height; M, maternal; MHL, maternal health and lifestyle; P, paternal; SES, socio-economic status; U, unmeasured covariate.

Supplementary Material 2. Table illustrating the details of the imputation modeling

Software used and key settings: STATA 14.0 software (Stata Corporation, College Station, Texas) – Ice command (with 10 cycles)
Number of imputed datasets created: 25
Variables included in the imputation procedure for the Dutch cohort: results from global psychomotor test, results from the verbal cognition test, results from the non-verbal cognition test, age at the different tests, elemental composition levels at birth address, maternal country of origin, paternal country of origin, age of mother at birth, number of siblings, maternal education, paternal education, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal body mass index before pregnancy, maternal height, the season of birth, child's sex, birth weight, breastfeeding, area SES, and parental income **Variables included in the imputation procedure for the German cohort:** results from global psychomotor test, results from the general cognition test, results from the verbal cognition test, results from the non-verbal cognition test, age at the different tests, elemental composition levels at birth address, maternal country of origin, paternal country of origin, age of mother at birth, number of siblings, maternal education, paternal education, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal body mass index before pregnancy, maternal height, maternal IQ, child's date of birth, the season of birth, child's sex, birth weight, breastfeeding, and area SES **Variables included in the imputation procedure for the Italian cohort:** results from global psychomotor test, results from gross motor test, results from fine motor test, results from the general cognition test, results from the verbal cognition test, results from the non-verbal cognition test, age at the different tests, elemental composition levels at birth address, maternal country of origin, paternal country of origin, age of mother at birth, number of siblings, maternal education, paternal education, marital status, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal body mass index before pregnancy, maternal height, the season of birth, child's sex, birth weight, gestational age based on last normal menstrual period, breastfeeding, and parental postnatal smoking **Variables included in the imputation procedure for the Spanish cohort:** results from global psychomotor test, results from gross motor test, results from fine motor test, results from the general cognition test, results from the verbal cognition test, results from the non-verbal cognition test, age at the different tests, elemental composition levels at birth address, maternal country of origin, paternal country of origin, age of mother at birth, number of siblings, maternal education, paternal education, marital status, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal body mass index before pregnancy, maternal height, maternal IQ, the season of birth, child's sex, birth weight, gestational age based on last normal menstrual period, breastfeeding, parental postnatal smoking, change of address from birth until 14-months visit, and change of address from birth until 4-years visit
Treatment of binary/categorical variables: logistic and multinomial models
Statistical interactions included in imputation models: none

Supplementary Material 3. Table illustrating the overview of the participation at different follow-ups in each cohort study

	enrolled during pregnancy or at birth of the child	*died neonatal / induced abortion / iuvd / withdrawal from the study*	**singleton live births**	*child's death / retired / loss to follow up*	**children in postnatal phase (no twins)**	age (years)	**participants with exposure and outcome**
Dutch cohort	8737	34	8633	1810	6823	1	4704
						1.5	4397
						6	4580
German cohort	232	36	196	0	196	1	186
						2	178
						8-10	95
Italian cohort	719	28	691	24	667	1.5	546
						4	551
						7	450
Spanish cohort	740	118	622	39	583	1.5	519
						4	439

Supplementary Material 4. Table illustrating the differences in the parental characteristics of population with available exposure and outcome data and population without exposure and outcome data

	Population of the Dutch cohort			Population of the German cohort		
	with e+o data	without e+o data	p-value	with e+o data	without e+o data	p-value
	n = 5911	n = 2826	difference	n = 190	n = 42	difference
maternal education level			<0.001			0.229
low	8.6	19.0		20.0	31.0	
medium	41.2	58.9		37.9	38.1	
high	50.2	22.1		40.1	31.0	
paternal education level			<0.001			0.642
low	6.2	16.0		32.3	24.5	
medium	38.0	52.7		19.4	23.9	
high	55.8	31.3		48.4	51.5	
maternal country of origin (foreign)	44.3	67.2	<0.001	13.2	19.0	0.323
paternal country of origin (foreign)	41.7	65.8	<0.001	18.5	25.0	0.349
maternal age at delivery (years)	30.5 (5.0)	27.7 (5.5)	<0.001	31.2 (4.7)	32.0 (5.3)	0.279
maternal pre-pregnancy BMI (kg/m^2)	22.5 (20.7 to 25.1)	22.9 (20.8 to 26.2)	<0.001	22.9 (20.8 to 25.7)	23.5 (21.3 to 26.6)	0.339
maternal height (cm)	167.9 (7.4)	165.4 (7.1)	<0.001	167.6 (6.2)	168.3 (6.5)	0.492
maternal smoking during pregnancy (yes)	25.0	32.4	<0.001	22.6	33.3	0.145
maternal alcohol consumption during pregnancy (yes)	42.5	23.1	<0.001	11.4	10.0	0.805
nulliparous	57.1	52.6	<0.001	56.8	38.1	0.007
marital status (living alone)	11.2	23.4	<0.001	2.7	7.5	0.140

	Population of the Italian cohort			Population of the Spanish cohort		
	with e+o data	without e+o data	p-value	with e+o data	without e+o data	p-value
	n = 614	n = 105	difference	n = 531	n = 209	difference
maternal education level			0.584			0.006
low	13.4	17.1		27.5	35.2	
medium	50.7	48.6		41.5	48.0	
high	36.0	34.3		31.1	16.8	
paternal education level			0.570			0.904
low	22.9	21.7		35.8	37.4	
medium	44.3	50.7		42.8	40.7	
high	32.8	27.5		21.3	22.0	
maternal country of origin (foreign)	1.0	1.9	0.397	10.1	17.6	0.017
paternal country of origin (foreign)	1.5	0.0	0.252	11.3	12.9	0.626
maternal age at delivery (years)	32.8 (4.4)	32.4 (5.0)	0.385	31.7 (4.2)	30.3 (5.3)	0.005
maternal pre-pregnancy BMI (kg/m^2)	21.3 (19.8 to 23.7)	20.8 (19.5 to 22.7)	0.036	22.7 (21.0 to 25.4)	22.7 (20.8 to 25.4)	0.657
maternal height (cm)	164.7 (5.8)	164.3 (6.8)	0.512	162.4 (6.0)	162.3 (6.3)	0.828
maternal smoking during pregnancy (yes)	11.3	20.4	0.010	29.4	36.0	0.213
maternal alcohol consumption during pregnancy (yes)	35.7	31.4	0.399	21.1	27.2	0.140
nulliparous	58.0	67.3	0.009	57.3	50.0	0.133
marital status (living alone)	1.5	0.0	<0.001	1.1	2.4	0.273

e+o, exposure and outcome

Values are percentages for the categorical variables, mean (standard deviation) for the continuous normally distributed variables, and median (interquartile range) for the continuous non-normally distributed variables

Supplementary Material 5. Table illustrating the variables used in forward selection logistic regression model to calculate inverse probability of attrition weights

Variables	Dutch cohort		German cohort		Italian cohort		Spanish cohort	
	Explored	Included	Explored	Included	Explored	Included	Explored	Included
age mother at birth of the child	X	X	X	X	X		X	X
age biological father at birth of the child	X	X					X	
participation of the partner in the study	X	X						
ethnicity mother	X	X	X		X		X	
ethnicity father			X		X		X	
ethnicity partner	X							
education mother	X	X	X		X		X	X
education father			X	X	X		X	
education partner	X	X						
marital status	X	X	X	X	X	X	X	X
household income	X	X						
enrollment period (prenatal or postnatal)	X	X						
weight of the mother just before the pregnancy	X							
weight of the mother at enrollment	X						X	
height of the mother at enrollment	X	X	X	X	X		X	
BMI of the mother just before the pregnancy	X							
BMI of the mother at enrollment	X		X		X		X	
BMI of the partner	X	X						
parity	X	X	X	X	X	X	X	X
gestational age at birth	X	X					X	X
weight child at birth	X						X	
child's birth season							X	X
maternal IQ							X	
maternal social class							X	X
paternal social class							X	X
maternal smoking during pregnancy	X	X	X		X		X	
maternal alcohol consumption during pregnancy	X	X	X		X		X	X

BMI=body mass index; IQ= intelligence quotient

Supplementary Material 6. Table illustrating the Spearman correlations between modeled elemental components

Dutch cohort	$PM_{2.5}$cu	$PM_{2.5}$fe	$PM_{2.5}$k	$PM_{2.5}$ni	$PM_{2.5}$s	$PM_{2.5}$si	$PM_{2.5}$v	$PM_{2.5}$zn
$PM_{2.5}$cu	1.00							
$PM_{2.5}$fe	0.91	1.00						
$PM_{2.5}$k	0.30	0.25	1.00					
$PM_{2.5}$ni	0.14	0.15	0.12	1.00				
$PM_{2.5}$s	0.13	0.15	0.16	0.42	1.00			
$PM_{2.5}$si	0.38	0.51	0.23	0.47	0.26	1.00		
$PM_{2.5}$v	0.15	0.16	0.12	1.00	0.42	0.47	1.00	
$PM_{2.5}$zn	0.33	0.29	0.99	0.08	0.14	0.21	0.08	1.00

German cohort	$PM_{2.5}$cu	$PM_{2.5}$fe	$PM_{2.5}$ni	$PM_{2.5}$s	$PM_{2.5}$si	$PM_{2.5}$v	$PM_{2.5}$zn	
$PM_{2.5}$cu	1.00							
$PM_{2.5}$fe	0.69	1.00						
$PM_{2.5}$ni	0.24	0.48	1.00					
$PM_{2.5}$s	0.29	0.58	0.62	1.00				
$PM_{2.5}$si	0.40	0.50	0.59	0.41	1.00			
$PM_{2.5}$v	0.55	0.50	0.19	0.16	0.31	1.00		
$PM_{2.5}$zn	0.49	0.58	0.53	0.58	0.57	0.50	1.00	

Italian cohort	$PM_{2.5}$cu	$PM_{2.5}$fe	$PM_{2.5}$k	$PM_{2.5}$ni	$PM_{2.5}$s	$PM_{2.5}$si	$PM_{2.5}$v	$PM_{2.5}$zn
$PM_{2.5}$cu	1.00							
$PM_{2.5}$fe	0.68	1.00						
$PM_{2.5}$k	0.44	0.41	1.00					
$PM_{2.5}$ni	0.23	0.39	0.07	1.00				
$PM_{2.5}$s	0.61	0.88	0.50	0.52	1.00			
$PM_{2.5}$si	0.43	0.64	0.21	0.53	0.67	1.00		
$PM_{2.5}$v	0.26	0.57	0.08	0.55	0.57	0.61	1,00	
$PM_{2.5}$zn	0.95	0.67	0.61	0.23	0.65	0.42	0.24	1.00

Spanish cohort	$PM_{2.5}$cu	$PM_{2.5}$fe	$PM_{2.5}$k	$PM_{2.5}$ni	$PM_{2.5}$s	$PM_{2.5}$si	$PM_{2.5}$v	$PM_{2.5}$zn
$PM_{2.5}$cu	1.00							
$PM_{2.5}$fe	0.81	1.00						
$PM_{2.5}$k	0.59	0.78	1.00					
$PM_{2.5}$ni	0.47	0.62	0.62	1.00				
$PM_{2.5}$s	0.46	0.56	0.51	0.81	1.00			
$PM_{2.5}$si	0.66	0.71	0.60	0.54	0.58	1.00		
$PM_{2.5}$v	0.47	0.60	0.54	0.76	0.68	0.51	1.00	
$PM_{2.5}$zn	0.62	0.75	0.59	0.75	0.75	0.62	0.62	1.00

Supplementary Material 7. Table illustrating the results of the principal component analysis

Elemental Component	PCA Component 1	PCA Component 2
Copper (Cu)	0.44	-0.16
Iron (Fe)	0.45	-0.10
Potassium (K)	0.38	-0.22
Nickel (Ni)	-0.08	0.58
Sulfur (S)	0.49	0.28
Silicon (Si)	0.31	-0.32
Vanadium (V)	0.03	0.62
Zinc (Zn)	0.34	0.13

Supplementary Material 8. Table illustrating the distribution of parental characteristics across different motorized traffic pollution concentrations expressed in tertiles

	motorized traffic pollution				
Dutch cohort	**1st tertile**	**2nd tertile**	**3rd tertile**	**p-value**	**p-trend**
m. education level (%)				<0.001	<0.001
low	11.3	10.7	5.9		
medium	46.7	41.6	37.5		
high	42.0	47.7	56.6		
p. education level (%)				<0.001	<0.001
low	12.6	10.3	6.8		
medium	44.3	43.1	38.0		
high	43.2	46.7	55.2		
m. country of origin (% foreign)	47.7	45.7	39.3	<0.001	<0.001
p. country of origin (% foreign)	45.6	43.3	37.6	<0.001	<0.001
m. age at delivery in years (mean (SD))	30.8 (5.1)	31.1 (5.0)	31.2 (4.7)	0.001	0.007
m. pre-pregnancy BMI in kg/m^2 (median (IQR))	22.8 (20.9-25.7)	22.6 (20.8-25.3)	22.3 (20.6-24.7)	<0.001	<0.001
m. height in cm (mean (SD))	167.3 (7.5)	167.6 (7.4)	168.9 (7.2)	0.225	<0.001
m. smoking pregnancy (% yes)	13.7	14.2	15.0	0.547	0.277
m. alcohol consumption pregnancy (% yes)	39.1	41.2	47.3	<0.001	<0.001
nulliparous (%)	53.3	56.6	61.3	<0.001	<0.001
marital status (% living alone)	13.0	11.9	8.7	<0.001	<0.001
Italian cohort	**1st tertile**	**2nd tertile**	**3rd tertile**	**p-value**	**p-trend**
m. education level (%)				<0.001	<0.001
low	21.1	11.2	8.8		
medium	52.9	52.2	46.3		
high	26.0	36.6	44.9		
p. education level (%)				<0.001	<0.001
low	2.9	2.0	1.0		

Supplementary Material 8. (Continued)

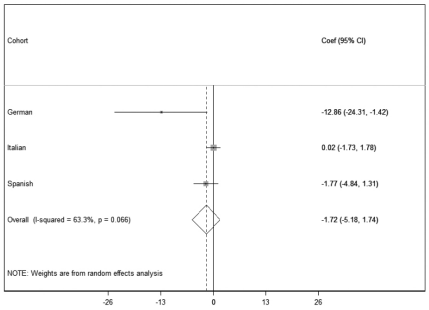
medium	78.9	71.2	48.8		
high	18.1	26.8	50.2		
m. country of origin (% foreign)	4.4	3.9	2.9	0.724	0.429
p. country of origin (% foreign)	2.0	2.0	3.4	0.677	0.341
m. age at delivery in years (mean (SD))	32.8 (4.4)	33.7 (4.6)	33.7 (4.1)	0.276	0.010
m. pre-pregnancy BMI in kg/m^2 (median (IQR))	21.8 (20.2-24.2)	21.3 (19.7-23.9)	21.2 (19.8-23.3)	0.048	0.016
m. height in cm (mean (SD))	163.9 (5.7)	164.57 (6.0)	165.8 (5.6)	0.552	0.001
m. smoking pregnancy (% yes)	11.3	12.2	10.2	0.822	0.741
m. alcohol consumption pregnancy (% yes)	34.8	35.1	38.1	0.752	0.494
nulliparous (%)	55.4	62.0	57.1	0.450	0.843
marital status (% living alone)	2.0	1.5	1.0	0.811	0.983

Spanish cohort	1st tertile	2nd tertile	3rd tertile	p-value	p-trend
m. education level (%)				0.146	0.035
low	29.4	28.8	24.3		
medium	44.1	42.9	37.3		
high	26.6	28.3	38.4		
p. education level (%)				0.005	<0.001
low	41.2	39.6	27.1		
medium	43.5	40.7	43.5		
high	15.3	19.8	29.4		
m. country of origin (% foreign)	11.3	5.7	13.6	0.040	0.482
p. country of origin (% foreign)	10.2	11.3	12.4	0.798	0.502
m. age at delivery in years (mean (SD))	31.5 (4.3)	31.8 (4.2)	31.8 (4.1)	0.888	0.551
m. pre-pregnancy BMI in kg/m^2 (median (IQR))	22.9 (21.3-25.6)	22.6 (20.7-25.1)	22.6 (21.0-25.2)	0.540	0.277
m. height in cm (mean (SD))	162.8 (6.1)	162.8 (6.1)	161.6 (5.8)	0.755	0.157
m. smoking pregnancy (% yes)	28.3	29.9	29.9	0.922	0.727
m. alcohol consumption pregnancy (% yes)	18.1	18.6	26.6	0.092	0.051
nulliparous (%)	57.6	50.9	63.3	0.201	0.242
marital status (% living alone)	0.6	1.7	1.1	0.875	0.615

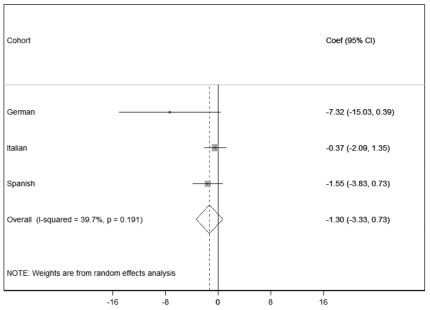
m., maternal; p., paternal

Values are percentages for the categorical variables, mean (standard deviation, SD) for the continuous normally distributed variables, and median (interquartile range, IQR) for the continuous non-normally distributed variables

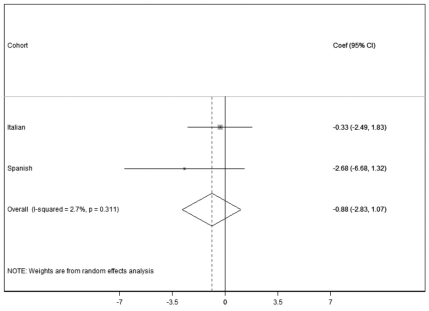
A. $PM_{2.5}$ Cu (per Δ5 ng/m³)



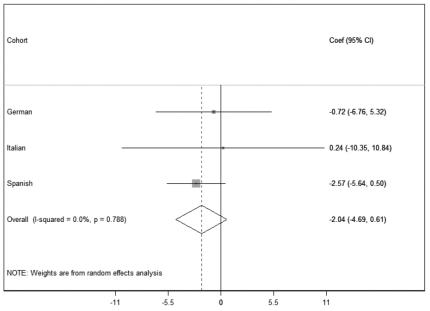
B. $PM_{2.5}$ Fe (per Δ100 ng/m³)



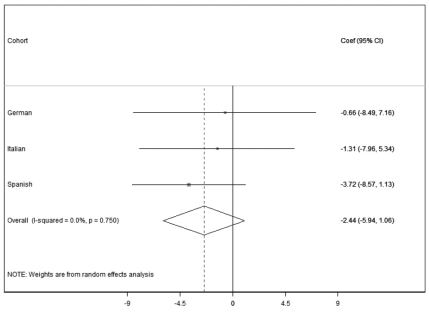
C. $PM_{2.5}$ K (per Δ50 ng/m³)



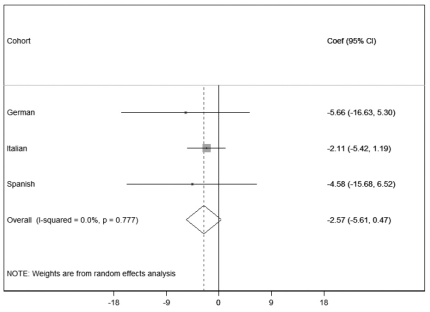
D. $PM_{2.5}$ Ni (per Δ1 ng/m³)



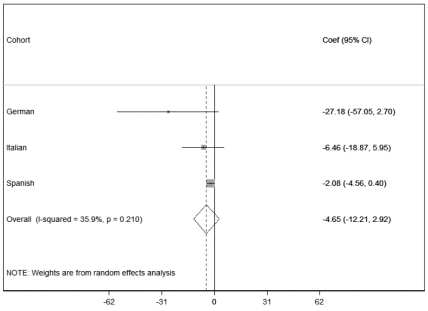
E. $PM_{2.5}$ S (per Δ200 ng/m³)



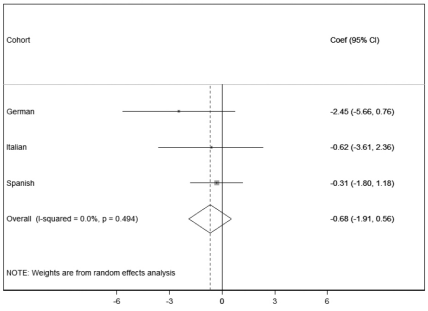
F. $PM_{2.5}$ Si (per Δ100 ng/m³)



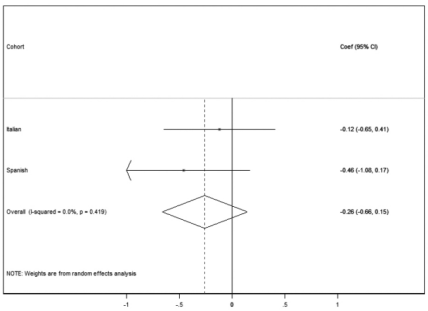
G. $PM_{2.5}$ V (per Δ2 ng/m³)



H. $PM_{2.5}$ Zn (per Δ10 ng/m³)

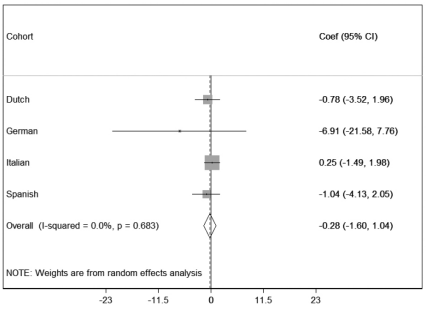


I. Motorized Traffic Pollution

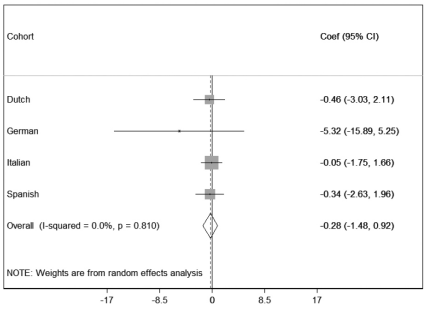


Supplementary Material 9. Figure illustrating the forest plots of associations between elemental components and general cognitive function. Fully adjusted associations of exposure at birth to $PM_{2.5}$ elemental composition and motorized traffic pollution with general cognitive function at average age of 2y in German cohort, 7y in Italian cohort and 4y in Spanish cohort. Region-specific and summary risk estimates (coefficient and 95% CI) for general cognitive function expressed for an increase of (A) 5ng/m³ in $PM_{2.5}$ Cu levels, (B) 100ng/m³ in $PM_{2.5}$ Fe levels, (C) 50ng/m³ in $PM_{2.5}$ K levels, (D) 5ng/m³ in $PM_{2.5}$ Ni levels, (E) 5ng/m³ in $PM_{2.5}$ S levels, (F) 5ng/m³ in $PM_{2.5}$ Si levels, (G) 5ng/m³ in $PM_{2.5}$ V levels, (H) 5ng/m³ in $PM_{2.5}$ Zn levels, and (I) motorized traffic pollution levels at birth, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.

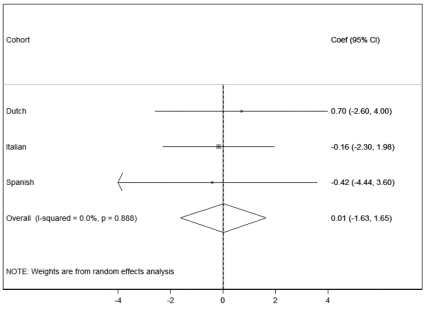
A. $PM_{2.5}$ Cu (per Δ5 ng/m^3)



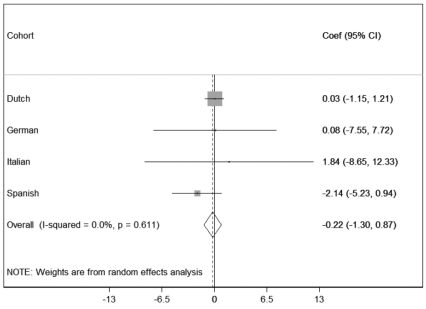
B. $PM_{2.5}$ Fe (per Δ100 ng/m^3)



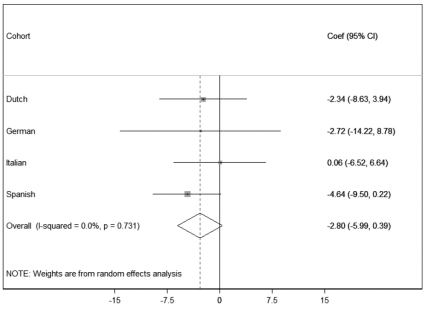
C. $PM_{2.5}$ K (per Δ50 ng/m^3)



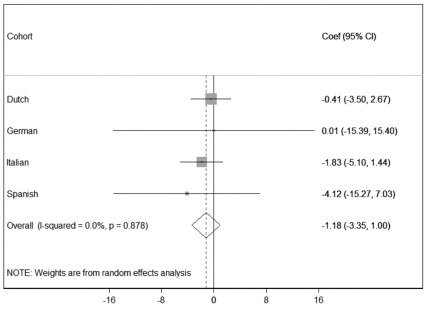
D. $PM_{2.5}$ Ni (per Δ1 ng/m^3)



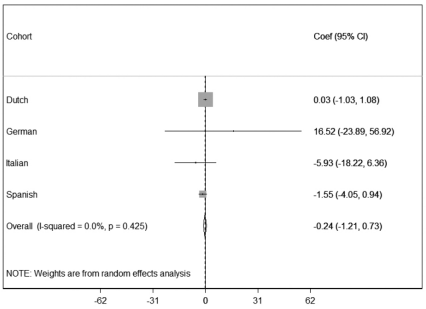
E. $PM_{2.5}$ S (per Δ200 ng/m^3)



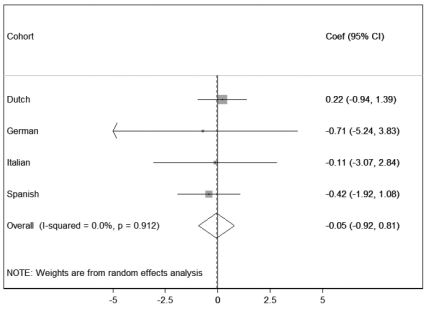
F. $PM_{2.5}$ Si (per Δ100 ng/m^3)



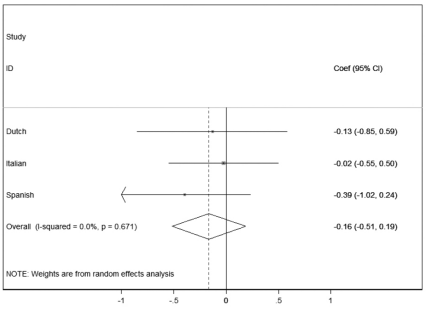
G. $PM_{2.5}$ V (per Δ2 ng/m^3)



H. $PM_{2.5}$ Zn (per Δ10 ng/m^3)

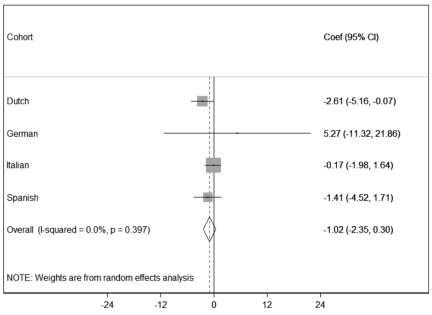


I. Motorized Traffic Pollution

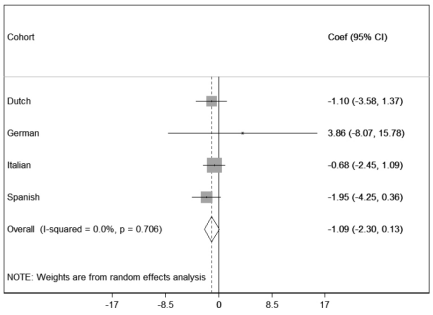


Supplementary Material 10. Figure illustrating the forest plots of associations between elemental components and verbal cognitive function. Fully adjusted associations of exposure at birth to $PM_{2.5}$ elemental composition and motorized traffic pollution with verbal cognitive function at average age of 1.5 years in Dutch cohort, 9y in German cohort, 7y in Italian cohort and 4y in Spanish cohort. Region-specific and summary risk estimates (coefficient and 95% CI) for verbal cognitive function expressed for an increase of (A) 5ng/m^3 in $PM_{2.5}$ Cu levels, (B) 100ng/m^3 in $PM_{2.5}$ Fe levels, (C) 50ng/m^3 in $PM_{2.5}$ K levels, (D) 5ng/m^3 in $PM_{2.5}$ Ni levels, (E) 5ng/m^3 in $PM_{2.5}$ S levels, (F) 5ng/m^3 in $PM_{2.5}$ Si levels, (G) 5ng/m^3 in $PM_{2.5}$ V levels, (H) 5ng/m^3 in $PM_{2.5}$ Zn levels, and (I) motorized traffic pollution levels at birth, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.

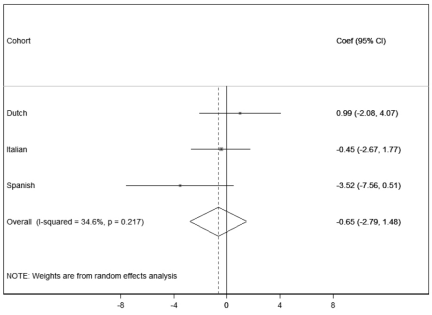
A. $PM_{2.5}$ Cu (per Δ5 ng/m^3)



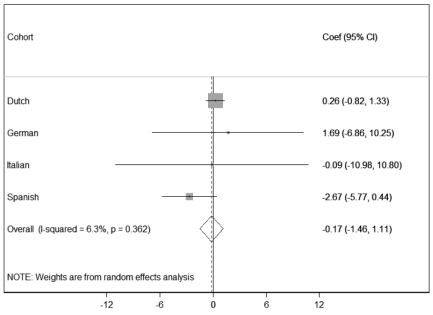
B. $PM_{2.5}$ Fe (per Δ100 ng/m^3)



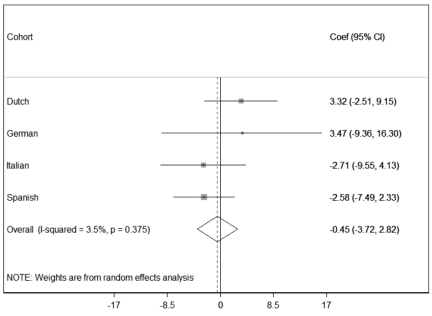
C. $PM_{2.5}$ K (per Δ50 ng/m^3)



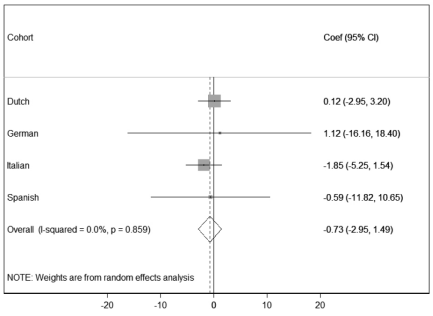
D. $PM_{2.5}$ Ni (per Δ1 ng/m^3)

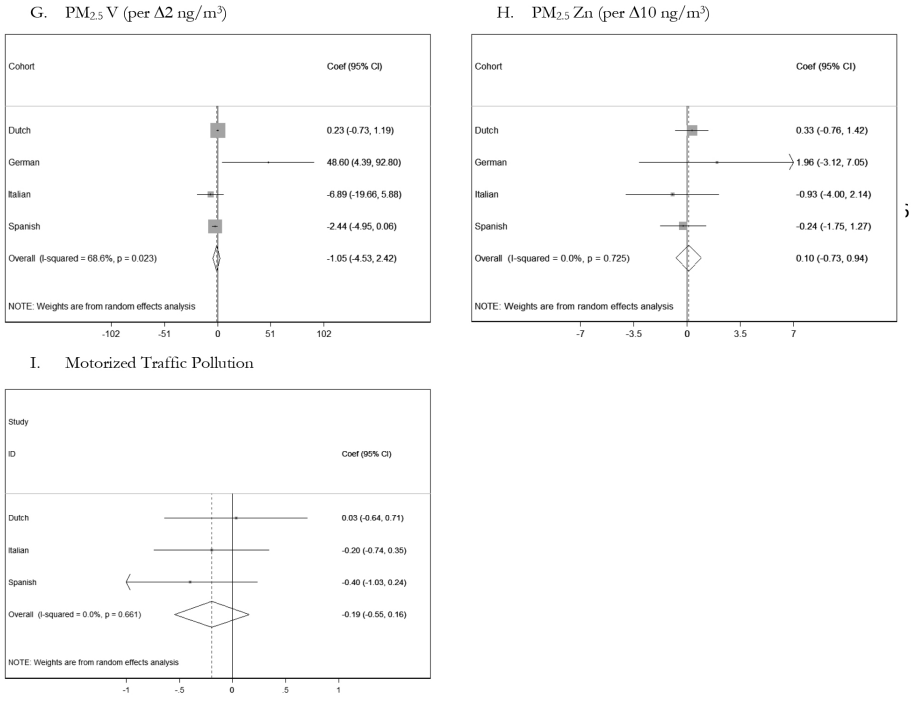


E. $PM_{2.5}$ S (per Δ200 ng/m^3)



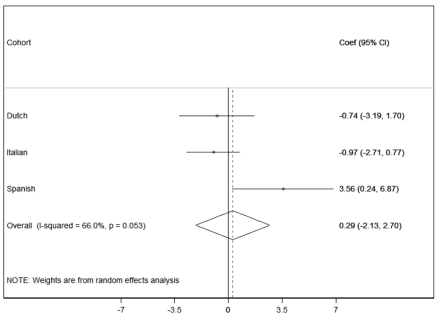
F. $PM_{2.5}$ Si (per Δ100 ng/m^3)



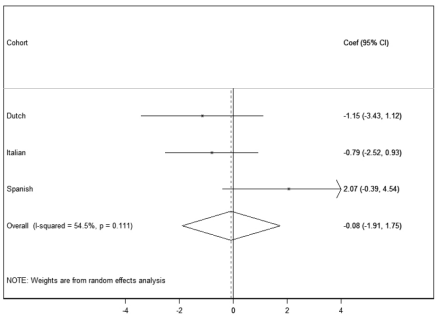


Supplementary Material 11. Figure illustrating the forest plots of associations between elemental components and non-verbal cognitive function. Fully adjusted associations of exposure at birth to $PM_{2.5}$ elemental composition and motorized traffic pollution with non-verbal cognitive function at average age of 6y in Dutch cohort, 9y in German cohort, 7y in Italian cohort and 4y in Spanish cohort. Region-specific and summary risk estimates (coefficient and 95% CI) for non-verbal cognitive function expressed for an increase of (A) 5ng/m^3 in $PM_{2.5}$ Cu levels, (B) 100ng/m^3 in $PM_{2.5}$ Fe levels, (C) 50ng/m^3 in $PM_{2.5}$ K levels, (D) 5ng/m^3 in $PM_{2.5}$ Ni levels, (E) 5ng/m^3 in $PM_{2.5}$ S levels, (F) 5ng/m^3 in $PM_{2.5}$Si levels, (G) 5ng/m^3 in $PM_{2.5}$ V levels, (H) 5ng/m^3 in $PM_{2.5}$ Zn levels, and (I) motorized traffic pollution levels at birth, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.

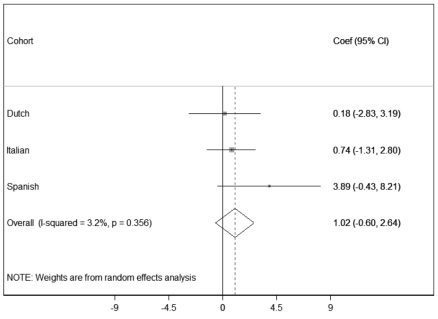
A. $PM_{2.5}$ Cu (per Δ5 ng/m^3)



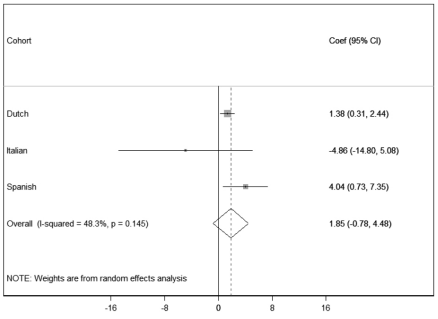
B. $PM_{2.5}$ Fe (per Δ100 ng/m^3)



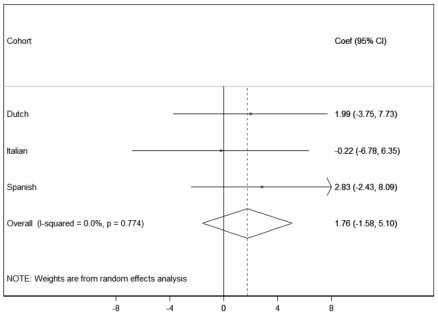
C. $PM_{2.5}$ K (per Δ50 ng/m^3)



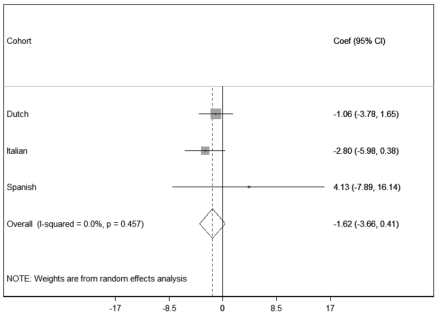
D. $PM_{2.5}$ Ni (per Δ1 ng/m^3)



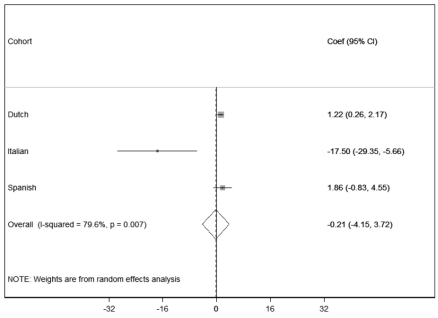
E. $PM_{2.5}$ S (per Δ200 ng/m^3)



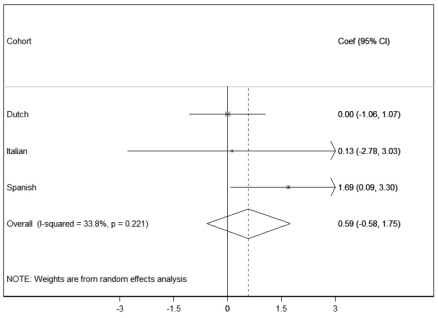
F. $PM_{2.5}$ Si (per Δ100 ng/m^3)



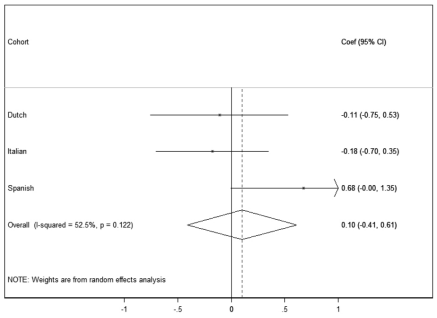
G. $PM_{2.5}$ V (per Δ2 ng/m^3)



H. $PM_{2.5}$ Zn (per Δ10 ng/m^3)



I. Motorized Traffic Pollution

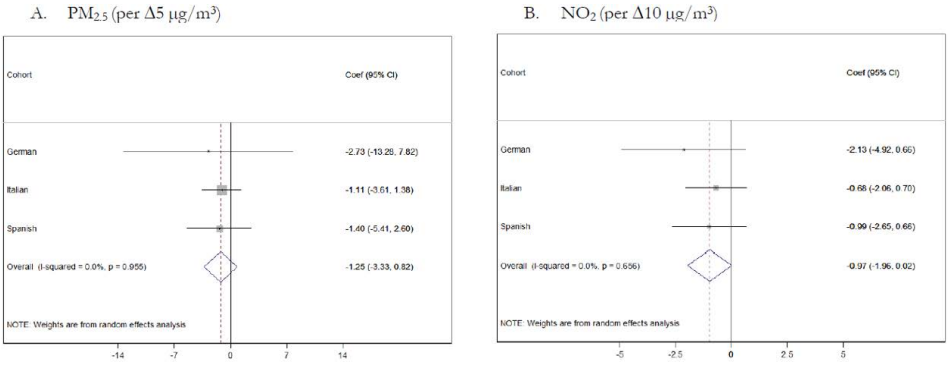


Supplementary Material 12. Figure illustrating the forest plots of associations between elemental components and gross motor function. Fully adjusted associations of exposure at birth to $PM_{2.5}$elemental composition and motorized traffic pollution with gross motor function at average age of 1y in Ducth cohort, 4y in Italian cohort and 4y in Spanish cohort. Region-specific and summary risk estimates (coefficient and 95% CI) for gross motor function expressed for an increase of (A) 5ng/m^3 in $PM_{2.5}$ Cu levels, (B) 100ng/m^3 in $PM_{2.5}$ Fe levels, (C) 50ng/m^3 in$PM_{2.5}$ K levels, (D) 5ng/m^3 in $PM_{2.5}$ Ni levels, (E) 5ng/m^3 in $PM_{2.5}$ S levels, (F) 5ng/m^3 in $PM_{2.5}$ Si levels, (G) 5ng/m^3 in $PM_{2.5}$V levels, (H) 5ng/m^3 in $PM_{2.5}$ Zn levels, and (I) motorized traffic pollution levels at birth, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.

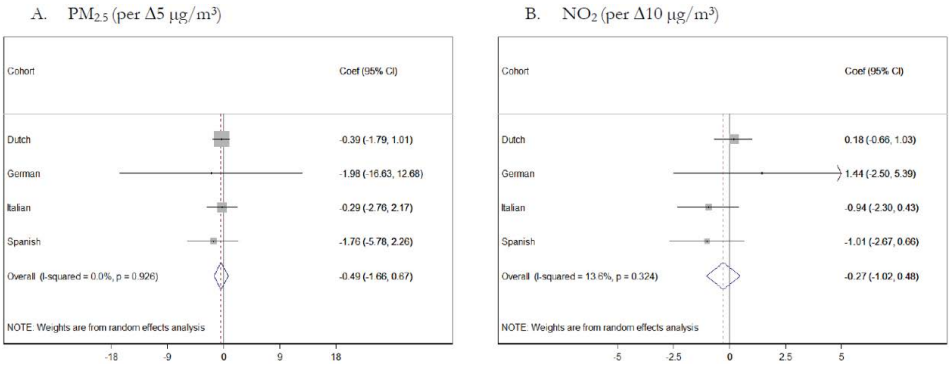
Supplementary Material 13. Table illustrating the associations between exposure to $PM_{2.5}$ and NO_2 during pregnancy and cognitive and psychomotor functions

	General cognitive function			Verbal cognitive function			Non-verbal cognitive function		
		Test for			Test for			Test for	
	Coef. (95% CI)	Heterogeneity, P	I^2 (%)	Coef. (95% CI)	Heterogeneity, P	I^2 (%)	Coef. (95% CI)	Heterogeneity, P	I^2 (%)
$PM_{2.5}$	-1.25 (-3.33 to 0.82)	0.955	0.0	-0.49 (-1.66 to 0.67)	0.926	0.0	-1.04 (-2.17 to 0.08)	0.965	0.0
NO_2	-0.97 (-1.96 to 0.02)	0.656	0.0	-0.27 (-1.02 to 0.48)	0.324	13.6	0.14 (-0.49 to 0.78)	0.529	0.0
	Fine motor function			Gross motor function					
		Test for			Test for				
	Coef. (95% CI)	Heterogeneity, P	I^2 (%)	Coef. (95% CI)	Heterogeneity, P	I^2 (%)			
$PM_{2.5}$	-1.18 (-3.80 to 1.45)	0.032	71.0	-1.02 (-3.47 to 1.42)	0.050	66.6			
NO_2	-0.59 (-1.23 to 0.05)	0.717	0.0	-0.36 (-1.50 to 0.77)	0.084	59.7			

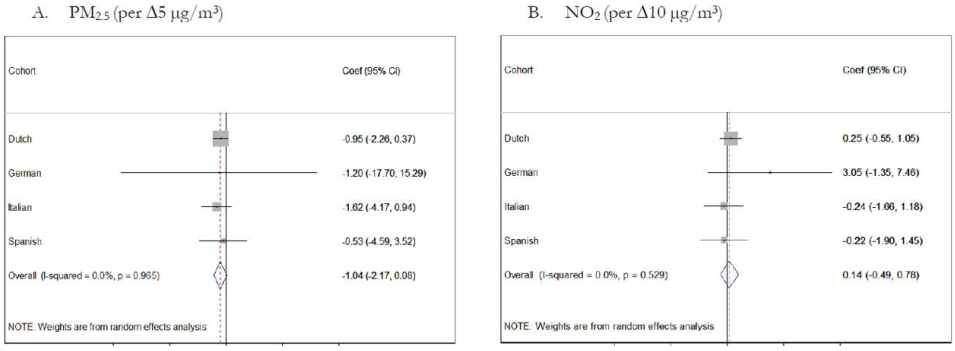
Coefficient and 95% CI were estimated by random-effects meta-analysis by cohort. Models were adjusted for parental education levels, parental countries of birth, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing, and expressed for an increase of 10µg/m^3 in NO_2 levels and 5µg/m^3 in $PM_{2.5}$ levels during pregnancy.



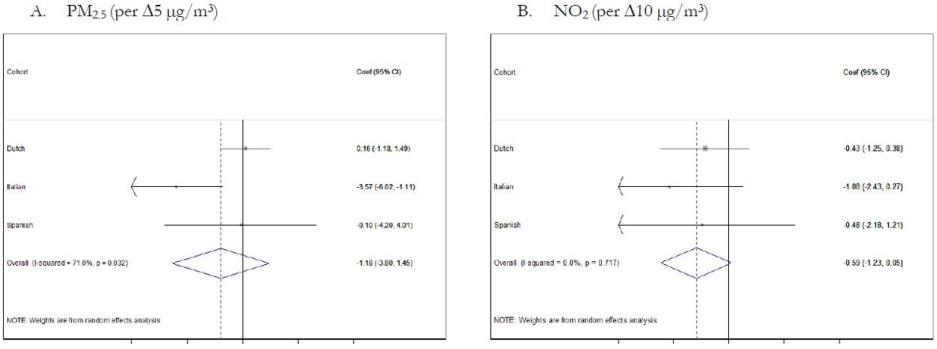
Supplementary Material 14. Figure illustrating the forest plots of associations between $PM_{2.5}$ and NO_2 and general cognitive function. Fully adjusted associations between air pollution exposure and general cognitive function. Region-specific and summary risk estimates (coefficient and 95% CI) for general cognitive function expressed for an increase of (A) 5μg/m^3 in $PM_{2.5}$ levels, and (B) 10μg/m^3 in NO_2 levels during pregnancy, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses



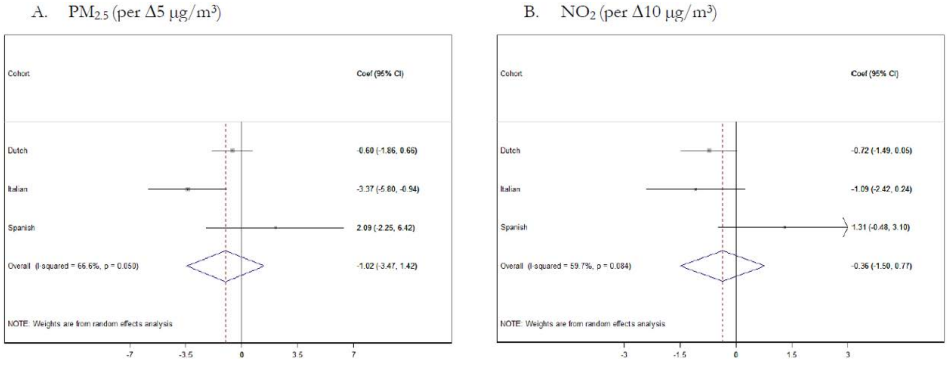
Supplementary Material 15. Figure illustrating the forest plots of associations between $PM_{2.5}$ and NO_2 and verbal cognitive function. Fully adjusted associations between air pollution exposure and verbal cognitive function. Region-specific and summary risk estimates (coefficient and 95% CI) for verbal cognitive function expressed for an increase of (A) 5μg/m^3 in $PM_{2.5}$ levels, and (B) 10μg/m^3 in NO_2 levels during pregnancy, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.



Supplementary Material 16. Figure illustrating the forest plots of associations between $PM_{2.5}$ and NO_2 and non-verbal cognitive function. Fully adjusted associations between air pollution exposure and non-verbal cognitive function. Region-specific and summary risk estimates (coefficient and 95% CI) for non-verbal cognitive function expressed for an increase of (A) 5μg/m^3 in $PM_{2.5}$ levels, and (B) 10μg/m^3 in NO_2 levels during pregnancy, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.



Supplementary Material 17. Figure illustrating the forest plots of associations between $PM_{2.5}$ and NO_2 and fine motor function. Fully adjusted associations between air pollution exposure and fine motor function. Region-specific and summary risk estimates (coefficient and 95% CI) for fine motor function expressed for an increase of (A) 5μg/m^3 in $PM_{2.5}$ levels, and (B) 10μg/m^3 in NO_2 levels during pregnancy, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.



Supplementary Material 18. Figure illustrating the forest plots of associations between $PM_{2.5}$ and NO_2 and gross motor function. Fully adjusted associations between air pollution exposure and gross motor function. Region-specific and summary risk estimates (coefficient and 95% CI) for gross motor function expressed for an increase of (A) 5μg/m^3 in $PM_{2.5}$ levels, and (B) 10μg/m^3 in NO_2 levels during pregnancy, adjusted for parental education levels, parental countries of origin, maternal age at delivery, maternal pre-pregnancy BMI, maternal height, maternal alcohol consumption during pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing. Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses.

Supplementary Material 19. Table illustrating the sensitivity analyses selecting younger ages, and the analysis wherein older ages in the German cohort were included

	General cognitive function - younger ages			Verbal cognitive function - younger ages			General cognitive function - older ages		
	Coef. (95% CI)	Test for Heterogeneity, P	I^2 (%)	Coef. (95% CI)	Test for Heterogeneity, P	I^2 (%)	Coef. (95% CI)	Test for Heterogeneity, P	I^2 (%)
Copper (Cu)	0.14 (-1.33 to 1.60)	0.725	0.0	-0.01 (-1.35 to 1.33)	0.515	0.0	-0.44 (-1.96 to 1.08)	0.587	0.0
Iron (Fe)	-0.57 (-1.86 to 0.73)	0.747	0.0	0.32 (-0.88 to 1.53)	0.430	0.0	-0.83 (-2.19 to 0.54)	0.681	0.0
Potassium (K)	-0.70 (-2.55 to 1.15)	0.517	0.0	0.50 (-1.07 to 2.07)	0.887	0.0	-0.88 (-2.83 to 1.07)	0.311	2.7
Nickel (Ni)	-0.19 (-3.02 to 2.63)	0.335	8.5	-0.23 (-1.32 to 0.85)	0.641	0.0	-2.03 (-4.79 to 0.74)	0.728	0.0
Sulfur (S)	1.42 (-1.83 to 4.66)	0.600	0.0	-2.19 (-5.37 to 0.99)	0.392	0.0	-2.55 (-6.27 to 1.17)	0.735	0.0
Silicon (Si)	-0.73 (-8.97 to 7.51)	0.040	68.8	0.66 (-1.46 to 2.77)	0.567	0.0	-2.21 (-5.32 to 0.89)	0.866	0.0
Vanadium (V)	0.42 (-1.70 to 2.54)	0.397	0.0	-0.20 (-1.17 to 0.77)	0.576	0.0	-2.22 (-5.62 to 1.19)	0.352	4.2
Zinc (Zn)	0.01 (-1.12 to 1.14)	0.554	0.0	0.02 (-0.84 to 0.88)	0.857	0.0	-0.29 (-1.58 to 0.99)	0.898	0.0
Motorized traffic	0.03 (-0.35 to 0.41)	0.431	0.0	-0.04 (-0.46 to 0.38)	0.250	27.8	n/a	n/a	n/a
	Fine motor function - younger ages			**Gross motor function - younger ages**					
	Coef. (95% CI)	Test for Heterogeneity, P	I^2 (%)	Coef. (95% CI)	Test for Heterogeneity, P	I^2 (%)			
Copper (Cu)	-0.51 (-1.84 to 0.82)	0.489	0.0	0.84 (-1.18 to 2.87)	0.122	52.5			
Iron (Fe)	-0.80 (-2.01 to 0.40)	0.423	0.0	0.38 (-1.24 to 2.01)	0.169	43.8			
Potassium (K)	-0.53 (-2.77 to 1.71)	0.181	41.5	0.70 (-1.18 to 2.59)	0.265	24.8			
Nickel (Ni)	-1.10 (-5.90 to 3.70)	0.003	83.0	1.92 (-0.08 to 3.92)	0.241	29.6			
Sulfur (S)	-0.68 (-4.00 to 2.63)	0.624	0.0	1.40 (-1.91 to 4.71)	0.586	0.0			
Silicon (Si)	-1.68 (-3.75 to 0.39)	0.984	0.0	0.00 (-1.99 to 1.99)	0.471	0.0			
Vanadium (V)	-0.51 (-4.35 to 3.32)	0.007	80.1	1.30 (0.41 to 2.20)	0.833	0.0			
Zinc (Zn)	-0.19 (-1.05 to 0.68)	0.813	0.0	0.62 (-0.51 to 1.76)	0.228	32.5			
Motorized traffic	-0.19 (-0.54 to 0.16)	0.606	0.0	0.19 (-0.24 to 0.62)	0.224	33.2			

Motorized traffic component was acquired using the principle component analysis (PCA). See Supplementary Table 6 for detailed configuration of the component. Coefficient and 95% CI were estimated by random-effects

pregnancy, maternal smoking during pregnancy, marital status, parity and age of the child at neuropsychological testing per increments of 5ng/m^3 for Cu $PM_{2.5}$; 100ng/m^3 for Fe $PM_{2.5}$; 50ng/m^3 for K $PM_{2.5}$; 1ng/m^3 for Ni $PM_{2.5}$; 200ng/m^3 for S $PM_{2.5}$; 100ng/m^3 for Si $PM_{2.5}$; 2ng/m^3 for V $PM_{2.5}$; and 10ng/m^3 for Zn $PM_{2.5}$.

Paper II

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

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Prenatal and Postnatal Exposure to Air Pollution, and Emotional and Aggressive Symptoms in children from 8 European birth cohorts

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ABSTRACT

Background: Little is known on the association between air pollution exposure and emotional and behavioural problems in children. We aimed to assess the relationship between prenatal and postnatal exposure to several air pollutants and child's depressive and anxiety symptoms, and aggressive symptoms in children of 7-11 years.

Methods: We analyzed data of 13,182 children from 8 European population-based cohorts. Concentrations of nitrogen dioxide (NO_2), nitrogen oxides (NO_X), particulate matter (PM) with diameters of ≤ 10μm (PM_{10}), ≤ 2.5μm ($PM_{2.5}$), and between 10 and 2.5μm (PM_{COARSE}), the absorbance of $PM_{2.5}$ filters ($PM_{2.5}$abs), and polycyclic aromatic hydrocarbons (PAHs) were estimated at residential addresses of each participant. Depressive and anxiety symptoms and aggressive symptoms were assessed at 7-11 years of age using parent-reported tests. Children were classified in borderline/clinical range or clinical range using validated cut offs. Region specific models were adjusted for various socio-economic and lifestyle characteristics of the participants and then combined using random effect meta-analysis. Multiple imputation and inverse probability weighting methods were applied to correct for potential attrition bias.

Results: A total of 1,896 (14.4%) children were classified as having depressive and anxiety symptoms in the borderline/clinical range, and 1,778 (13.4%) as having aggressive symptoms in the borderline/clinical range. Overall, 1,108 (8.4%) and 870 (6.6%) children were classified as having depressive and anxiety symptoms, and aggressive symptoms in the clinical range, respectively. Prenatal exposure to air pollution was not associated with depressive and anxiety symptoms in the borderline/clinical range (e.g. OR 1.02 [95%CI 0.95 to 1.10] per 10μg/m^3 increase in NO_2) nor with aggressive symptoms in the borderline/clinical range (e.g. OR 1.04 [95%CI 0.96 to 1.12] per 10μg/m^3 increase in NO_2). Similar results were observed for the symptoms in the clinical range, and for postnatal exposures to air pollution.

Conclusions: Overall, our results suggest that prenatal and postnatal exposure to air pollution is not associated with depressive and anxiety symptoms or aggressive symptoms in children of 7 to 11 years old.

Keywords: air pollution; depressive symptoms; anxiety symptoms; aggressive symptoms; children's mental health

ABBREVIATIONS

ABCD – Amsterdam Born Children and their Development study

BC – Black carbon

BMI – Body Mass Index

CBCL/6-18 - Child Behavior Checklist for ages 6-18

EC – Elemental carbon

EDEN - Étude des Déterminants pré et postnatals du développement et de la santé de l'Enfant

ESCAPE - European Study of Cohorts for Air Pollution Effects

GASPII – Genetica e Ambiente: Studio Prospettico dell'Infanzia in Italia

GINIplus – GINIplus Birth Cohort Study

LISA – LISA Birth Cohot Study

LUR – Land Use Regression

INMA – Infancia y Medio Ambiente project

NO_2 – Nitrogen dioxide

NO_X – Nitrogen oxides

OR – Odd Ratio

PM – Particulate matter

PM_{10} – Particulate matter with aerodynamic diameter of ≤ 10μm

$PM_{2.5}$ - Particulate matter with aerodynamic diameter of ≤ 2.5μm

PM_{COARSE} – Particulate matter with aerodynamic diameter between 10 and 2.5μm

$PM_{2.5}$abs – The absorbance of particulate matter with aerodynamic diameter of ≤ 2.5μm filters

PAHs - Polycyclic aromatic hydrocarbons

REPRO_PL – Polish Mother and Child Cohort Study

SDQ – Strength and Difficulties Questionnaire

INTRODUCTION

Exposure to air pollution is considered a potential hazard for healthy neurodevelopment (Grandjean and Landrigan, 2014). Neurodevelopment starts in early pregnancy with numerous processes continuing throughout the entire childhood (Hines, 2018). During this developmental period, the detoxification mechanisms are still maturing, making early life a critical window of vulnerability to environmental exposures such as air pollution (Block et al., 2012; Grandjean and Landrigan, 2014).

The majority of epidemiological studies in this field has been conducted on prenatal or postnatal exposure to air pollution and children's cognition, psychomotor skills, and some specific behavioural problems, such as autism spectrum disorders and attention deficit and hyperactivity disorders (Becerra et al., 2013; Forns et al., 2016; Freire et al., 2010; Guxens and Sunyer, 2012; Guxens et al., 2014, 2015; Jedrychowski et al., 2015; Lubczyńska et al., 2017; Min et al., 2017; Sentís et al., 2017; Suades-González et al., 2015; Volk et al., 2013). However, little is known whether prenatal or postnatal exposure to air pollution is associated with other common mental health problems in childhood, such as emotional and aggressive problems. Regarding prenatal exposure, the only existing studies have been conducted in New York City (Margolis et al., 2016) and in Krakow (Genkinger et al., 2015), showing that exposure to higher levels of airborne polycyclic aromatics hydrocarbons (PAHs) during pregnancy was associated with more depressive and anxiety symptoms in children of 4.8-11 years old, as well as with more aggressive symptoms in children of 6-11 years old. Conversely, three studies on the relationship between postnatal air pollution exposure including elemental carbon (EC), black carbon (BC), particulate matter (PM) with aerodynamic diameter of less than 2.5μm ($PM_{2.5}$), and nitrogen dioxide (NO_2), with depressive and anxiety symptoms, and aggressive symptoms in children of 7-12 years old, conducted in Barcelona (Forns et al., 2016), in Ohio (Newman et al., 2015), and in London (Roberts et al., 2019), showed no associations. However, the study from London, found that higher postnatal exposures to NO_2 and $PM_{2.5}$ was associated with an increased odds of major depressive disorders at age 18 (Roberts et al., 2019).

Awareness of, and concern about, mental health disorders in childhood, which are often chronic in nature, is increasing (Pitchforth et al., 2018). Worldwide prevalence of any anxiety disorder, depressive disorder or aggressive problems is currently around 6.5%, 2.6%, and 2.1% respectively (Polanczyk et al., 2015). Such disorders can often have serious negative consequences for children's development and wellbeing, academic achievement, and social development later in life (Polanczyk et al., 2015). Thus, the identification of potential risk factors for these mental health problems is crucial for their prevention. Therefore, the aim of the current study was to assess whether prenatal and postnatal exposure to air pollutants highly ubiquitous in urban settings, was associated with depressive and anxiety symptoms, and aggressive symptoms in childhood across Europe.

METHODS

Population and Study Design

We included 8 European population-based birth cohorts: Amsterdam Born Children and their Development study (ABCD) from the Netherlands (van Eijsden et al., 2011), the Generation R Study from the Netherlands (Kooijman et al., 2016), GINIplus Birth Cohort Study and LISA Birth Cohort Study from two regions in Germany (von Berg et al.,2010; Heinrich et al., 2002), Polish Mother and Child Cohort Study (REPRO_PL) from Poland (Polańska et al., 2016), Étude des Déterminants pré et postnatals du développement et de la santé de l'Enfant (EDEN) from two regions in France (Drouillet et al., 2009), Genetica e Ambiente: Studio Prospettico dell'Infanzia in Italia (GASPII) from Italy (Porta et al., 2007), and the INfancia y Medio Ambiente (INMA) project from five regions in Spain (Guxens et al., 2012) (Table 1). Mother-child pairs were recruited between 1995 to 2008, depending on the cohort (Table 1). A total of 13,182 children (from singleton births) with available data on exposures and outcomes were included in the current study. Informed consent was obtained from all participants, and local authorized Institutional Review Boards granted the ethical approval for the studies.

Air Pollution Exposure

Air pollution exposure data used in this study originated from the European Study of Cohorts for Air Pollution Effects (ESCAPE) project (http://www.escapeproject.eu), except for the REPRO_PL cohort and Gipuzkoa region of the INMA cohort where different air pollution exposure assessments were used, as described subsequently.

Within ESCAPE, land use regression (LUR) models were developed following a standardized procedure described elsewhere (Beelen et al., 2013; Eeftens et al., 2012a). Briefly, air pollution monitoring campaigns were performed in the study areas between October 2008 and January 2011, except in EDEN where they were done in 2002 (Nancy) and 2005 (Poitiers) (Sellier et al., 2014). In all regions, NO_2 and nitrogen oxides (NO_X) were measured in three 2 week periods within 1 year, with the exceptions of EDEN for which no NO_X measurements were done (Cyrys et al., 2012) (Table 1). PM with aerodynamic diameter of less than 10μm (PM_{10}) and $PM_{2.5}$ were measured 3 times during a 2 week period at 40 sites in the Netherlands/Belgium (applied for ABCD and the Generation R Study) and Sabadell region of INMA, and at 20 sites in Augsburg and the Ruhr area (GINIplus/ LISA) and in Rome (GASPII) (Eeftens et al., 2012b). PM measurements were not available in EDEN and Asturias, Valencia and Granada regions of INMA. Coarse particle concentration (PM_{COARSE}) was calculated as the difference between PM_{10} and $PM_{2.5}$. The absorbance of the $PM_{2.5}$ filters ($PM_{2.5}$abs) was measured to serve as a proxy for elemental carbon. Additionally, $PM_{2.5}$ filters were also analysed for PAHs in the Netherlands and the Sabadell region of INMA (Jedynska et al., 2014). Next, LUR models were developed for each pollutant, based on the measurements, and on a variety of potential land use predictors derived from geographic information systems (Beelen et al., 2013; Eeftens et al., 2012a, Jedynska et al., 2014, Sellier et al., 2014). These models were then used to assign annual average air pollution concentration to all the collected home addresses of each participant.

If more than one address was collected during the prenatal period, we calculated the weighted average concentration level of all the addresses according to the time spent at each address, resulting in one concentration level per pollutant for each participant. The same procedure was followed for the postnatal period. In this study, the postnatal period is defined as the period stretching from birth until the emotional and behavioral problems assessment. Due to data availability, no analyses relying on postnatal exposures could be performed in ABCD for NO_2, NO_X and PM, EDEN for NO_2 and Asturias, Gipuzkoa, Valencia and Granada regions of INMA cohort for NO_X.

In the REPRO_PL cohort, universal kriging methodology was used. Average concentrations of air pollutants from the entire country were used, covering the period between 2006 and 2016 for NO_2 and PM_{10}, and the period between 2010 and 2016 for $PM_{2.5}$ (http://www.gios.gov.pl/en/) and assigned to the residential addresses of the participants.

In the Gipuzkoa region of the INMA cohort, while NO_2 average concentrations were based on ESCAPE methodology, the average concentrations of $PM_{2.5}$ and PM_{10} were obtained through 24-h sampling campaigns, monthly rotating between Urola Medio Valley, Urola Alto Valley, and Oria Valley, covering the period between May 2006 and December 2007, and assigned to residential addresses for each participant (Lertxundi et al., 2010).

Emotional and Behavioural Problems Assessment

Emotional and behavioural problems were measured in each participating cohort/region using the Child Behaviour Checklist for ages 6-18 (CBCL/6-18) or the Strength and Difficulties Questionnaire (SDQ) (Table 1). All tests were reported by the parents.

CBCL/6-18 was administered when the children were between 7 and 10 years old, depending on the cohort/region. The CBCL/6-18 consists of 9 syndrome scales, from which we selected four scales. The anxious/depressed syndrome scale (13 items) and withdrawn/depressed syndrome scale (8 items) were selected as indicators of child's depressive and anxiety symptoms. The rule-breaking behaviour syndrome scale (17 items) and aggressive symptoms scale (18 items) were selected as measures of children's aggressive symptoms. Higher scores indicate more symptoms. We used the 93rd and 98th percentile of the region specific total population as cut off scores, which have been validated and standardized, to classify children with symptoms in the borderline and clinical range (from now on named borderline/clinical range) and in the clinical range, respectively (Achenbach and Rescorla, 2000). Validation studies reported high sensitivity (>0.80) for borderline/clinical cut off's and medium specificity (>0.60) for clinical cut offs (eMethods 1 and eTable 1).

The SDQ was administered when the children were between 7 and 11 years old, depending on the cohort/region. The SDQ comprises 5 scales from which we selected 2 scales. The emotional problem scale was selected as indicators of child's depressive and anxiety symptoms. The selected scale is composed of 5 items that can be scored with 0, 1 or 2, with higher scores indicating more symptoms. Validated and standardized cut offs were used to classify children (Goodman, 1997). A cut off of 4 points was considered as cut off

Table 1. Description of the participating cohort studies

							Depressive and anxiety symptoms		Aggressive symptoms	
	Pregnancy period	Pollutants	% urban area*	Test	Age	n[a]	% borderline/ clinical range	% clinical range	% borderline/ clinical range	% clinical range
ABCD, The Netherlands[1]	2003-2004	NO_2,NO_X, PM	100	SDQ	11y	2701	18.2	11.0	6.4	2.6
GENERATION R, The Netherlands[1]	2001-2005	NO_2, NO_X, PM, PAHs	100	CBCL	10y	3120	6.9	2.3	6.9	2.3
GINIplus/LISA, Germany-Wesel[1]	1995-1998	NO_2, NO_X, PM	0	SDQ	10y	1696	17.6	10.0	16.2	10.7
GINIplus/LISA, Germany-Munich[1]	1995-1998	NO_2, NO_X, PM	57	SDQ	10y	2514	17.5	11.7	26.4	13.0
REPRO_PL, Poland[b,2]	2007	NO_2,PM	85	SDQ	7y	327	22.9	13.7	28.7	9.8
EDEN, France-Nancy[3]	2003-2006	NO_2	66	SDQ	8y	323	29.4	20.8	26.6	14.2
EDEN France-Poitiers[3]	2003-2006	NO_2	52	SDQ	8y	247	27.1	17.4	23.0	13.0
GASPII, Italy[1]	2003-2004	NO_2, NO_X, PM	100	CBCL	7y	461	7.3	3.2	6.1	1.7
INMA, Spain-Asturias[1]	2004-2006	NO_2,NO_X	95	SDQ	7y	357	22.9	15.0	25.8	11.2
INMA, Spain-Gipuzkoa[4]	2006-2008	NO_2, NO_X, PM	89	CBCL	8y	397	7.1	2.4	4.5	2.6
INMA, Spain-Sabadell[1]	2004-2006	NO_2, NO_X, PM, PAHs	100	CBCL	9y	484	8.3	2.8	7.4	5.4
INMA, Spain-Valencia[1]	2003-2005	NO_2, NO_X	94	CBCL	9y	427	5.7	7.2	8.0	6.0
INMA, Spain-Granada[1]	2000-2002	NO_2, NO_X	85	CBCL	9y	153	5.2	0.0	5.9	0.0

CBCL/6-18, child behavior checklist school age 6-18; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; PM, particulate matter (PM); PAHs, polycyclic aromatic hydrocarbons; SDQ, Strengths and Difficulties Questionnaire.

* Urbanicity at child's birth address.

[a] Number of children with air pollution, depressive and anxiety symptoms, and aggressive symptoms data available (n=13182).

[b] Monitoring campaigns used to estimate annual pollution concentrations were different than the rest of the cohorts that used land use regression models from the ESCAPE project.

Air pollution assessments were performed during the following years: [1]2008-2011; [2]2006-2016; [3]2002-2005; [4]2006-2007.

to classify children in the borderline/clinical range, and a cut off of 5 points was used to classify children in the clinical range. The conduct problems scale was selected as the scale measure of children's aggressive symptoms. The selected scale is composed of 5 items, with higher scores indicating higher number of symptoms. A cut off of 3 points was considered as the threshold to classify children in the borderline/clinical range, and a cut off of 4 points was used to classify children in the clinical range (Goodman, 1997). The cut offs used have a sensitivity of 0.64 for emotional disorders and 0.60 sensitivity for aggressive disorders, and a high specificity (0.95) for diagnostic cut offs (eMethods 1 and eTable 1).

Potential confounding variables

Potential confounding variables were defined a priori based on previous literature and selected as similarly as possible across the participating cohorts considering the availability of the data. The included potential confounding variables related to parental characteristics were: maternal and paternal age at child's birth (in years); maternal and paternal countries of birth (country of cohort/foreign country); household status during pregnancy (parents living together/single parent household), and maternal and paternal education levels child's at emotional/behavioural assessment (low/medium/high based on cohort specific classifications). We selected the following potential confounding maternal characteristics: tobacco use during pregnancy (no/yes); alcohol use during pregnancy (no/yes); and parity (nulliparous/one child/two or more children). All these variables were collected by tests during pregnancy or at the birth of the child. Maternal height and pre-pregnancy weight were measured or self-reported in the 1st trimester of the pregnancy or at birth. Pre-pregnancy body mass index (BMI) was then calculated (kg/m^2). Child's sex was obtained either from the hospital, from national registries, or from tests. Child's age at the emotional and behavioural symptoms assessment was also collected.

Statistical Analyses

Among children with available data on exposure and outcome variables, we performed multiple imputation of missing confounding variables using chained equations, where 25 completed data sets were generated and analysed using standard combination rules for multiple imputation (Spratt et al., 2010; Sterne et al., 2009). The percentage of missing covariates in all the cohorts was lower than 15% with exception of paternal country and education in Generation R, which had 19.6% and 26.8% of missing values respectively, and maternal alcohol use during pregnancy in GINIplus and LISA, which had 90.0% and 66.3% of missing values respectively. Distributions in the imputed datasets were very similar to those observed (data not shown).

Children included in this analysis (n=13,182) were more likely to have mothers who did not smoke during pregnancy, parents that were living together, and parents with higher educational levels, compared to children not included due to unavailability of data on exposure or outcome (n=8,494) (data not shown). We used inverse probability weighting to correct for the potential selection bias that can arise when only children with available exposure and outcome data are included as compared to a full initial cohort recruited at pregnancy (Weisskopf et al., 2015; Weuve et al., 2012). 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 populations of the cohorts.

Generalized additive models were used to assess the linearity of the relationships of each air pollutant with depressive, anxiety, and aggressive symptom scales, by visual examination and deviance comparison. In all cases linear function provided a good fit. We then applied logistic regression models to estimate the associations between each air pollutant and depressive and anxiety symptoms, and between each air pollutant and aggressive symptoms, with the borderline/clinical range and the clinical range being analysed as separate outcomes. For all analyses, children with a score below the borderline cut off were the reference group. Models were first minimally adjusted, only including child age and sex as potential confounding variables. We then performed fully adjusted regression analyses wherein all potential confounding variables described in the preceding paragraph were included. We applied a two-step approach: first, the associations were analysed separately for each cohort/region, and subsequently the cohorts/regions estimates were pooled using random-effects meta-analysis. We assessed the heterogeneity in the estimates using Cochran Q test and I^2 statistic.

To test the sensitivity of the results, we repeated the meta-analysis i) leaving out one cohort at the time to test the individual influence of that cohort; ii) using the 90th percentile of the depressive and anxiety symptoms scale, and of the aggressive symptoms scale, as cut off; iii) stratifying the results by test; iv) analyzing each symptom scales separately as quantitative scores using negative binomial regression models and performing meta-analyses grouping the cohorts by the test used; and v) analyzing the association between prenatal exposure to air pollution and depressive and anxiety symptoms, and aggressive symptoms only in the subset of cohort, for which the exposure measurements were carried out during pregnancy period or at most the first 2 years of life. After accepting a type I error of 5% in a two-sided test, we had an 80% power to detect ORs between 1.06 and 1.21 depending on the pollutant and the outcome variable. The statistical analyses were carried out using STATA (version 14.0; Stata Corporation, College Station, TX).

RESULTS

In our study population, 14.4% (n=1,896) of children were classified in the borderline/clinical range of depressive and anxiety symptoms, of whom 8.4% (n=1,108) were in the clinical range. Regarding aggressive symptoms, 13.4% (n=1,778) children were classified in the borderline/clinical range, of whom 6.6% (n=870) were in the clinical range (Table 1). Distribution of child, maternal and paternal characteristics varied across the cohorts (Table 2).

We observed a higher percentage of children in the borderline/clinical range of symptoms among mothers with lower education, as compared to mothers with higher education (with exception of the Nancy region of EDEN). Also, higher percentage of children was

observed in the borderline/clinical range of symptoms among mothers who smoked during pregnancy as compared to mothers non-smoking mothers (with exception of the Nancy region of EDEN, and the Granada and Valencia regions of INMA) (data not shown).

Regarding region-specific mean NO_2 levels, the prenatal levels ranged from 15.9μg/m^3 (the Poitiers region of EDEN) to 43.5μg/m^3 (GASPII), whereas postnatal levels ranged from 14.0μg/m^3 (the Gipuzkoa region of INMA) to 43.5μg/m^3 (GASPII) (eTable 2). The region specific prenatal mean $PM_{2.5}$ levels ranged from 13.9μg/m^3 (ABCD) to 23.0μg/m^3 (GASPII) while the postnatal levels ranged from 11.8μg/m^3 (the Gipuzkoa region of INMA) to 28.4μg/m^3 (REPRO_PL) (eTable2).

In our study population, higher educated mothers were more likely to live in areas with higher levels of NO_2 during pregnancy, except for Nancy region of EDEN, REPRO-PL and Gipuzkoa and Valencia regions of INMA (data not shown). The results with the postnatal exposures to NO_2 showed more variability across the cohorts. The population characteristics did not vary substantially across different $PM_{2.5}$ levels (data not shown).

Overall, we found that the correlations between prenatal levels of different pollutants in each cohort were stronger in the Generation R Study and in the Sabadell region of INMA as compared to other cohorts/regions. This was also observed with postnatal exposures (eTable 3 and eTable 4). We observed weaker correlations between prenatal and postnatal levels of pollutants in Generation R Study (0.47 between NO_2 prenatal and NO_2 postnatal) and in Gipuzkoa region of INMA (0.41 between NO_2 prenatal and NO_2 postnatal) in comparison to other cohorts/regions, such as in GASPII cohort (0.88 between NO_2 prenatal and NO_2 postnatal) or in Sabadell region of INMA (0.70 between NO_2 prenatal and NO_2 postnatal) (eTable 5).

Logistic regression analyses showed that prenatal exposures were not associated with depressive and anxiety symptoms in the borderline/clinical range (Table 3, Figure 1A-B), except for the Generation R Study, where we did observe an increase in odds ratio for depressive and anxiety symptoms (OR 1.15 [95%CI 1.01 to 1.30] per 10μg/m^3 increase in NO_2). The analysis on the relationship between prenatal exposures and aggressive symptoms in the borderline/clinical range also did not show any significant associations (Table 4, Figure 1C-D), but we did observe an increased odds ratio for aggressive symptoms in the Poitiers region of EDEN (OR 3.04 [95%CI 1.56 to 16.25] per 10μg/m^3 increase in NO_2). Similar results were observed when the analyses were restricted to clinical ranges of symptoms only (eTable 6 and eTable 7). Results based on postnatal exposures showed no associations with depressive and anxiety symptoms or aggressive symptoms in the borderline/clinical or in the clinical range. Overall, there was little to no heterogeneity in the analyses performed. When we tested the influence of confounding variables through minimally-adjusted models, the influence of each cohort on the overall estimates, and the influence of the validated and standardized cut off points in the symptom scales by changing it to the 90th percentile of the symptom scales, the results did not change meaningfully (eTable 8 – eTable 15). However, when we tested the influence of the stratification of the results by test, the analyses with postnatal exposure to air various pollutants showed lower

odds of depressive and anxiety symptoms in borderline/clinical range when the symptoms were assessed with CBCL test (OR 0.67 [95% 0.49;0.91] per 10μg/m^3 increase in PM_{10}, and OR 0.56 [95% 0.38;0.82] per 5μg/m^3 increase in $PM_{2.5}$) (eTable 16) as compared to SDQ test (OR 0.96 [95% 0.81;1.15] per 10μg/m^3 increase in PM_{10}, and OR 0.81 [95% 0.65;1.03] per 5μg/m^3 increase in $PM_{2.5}$) (eTable 17). Moreover, prenatal exposure, to air pollution was associated with increased odds of aggressive symptoms (OR 1.16 [95% 1.05;1.26] per 10μg/m^3 increase in NO_2, and OR 1.14 [95% 1.03;1.21] per 20μg/m^3 increase in NO_X) when only the cohorts using SDQ were included (eTable 18 and eTable 19). When we assessed the relationship of exposure to air pollution with depressive, anxiety, and aggressive symptoms using quantitative scores of the symptoms scales, the analysis did not show notable changes compared to the results using dichotomized outcomes (data not shown). When we tested the association of prenatal air pollution exposure and depressive and anxiety symptoms, and aggressive symptoms in those cohorts for which exposure measurements were carried out during pregnancy and within the first 2 years of life, the results did not change substantially (data not shown).

Table 2. Distribution of the child, maternal and paternal characteristics.

	N	Sex of the child	Maternal educational level			Paternal educational level			Maternal country of birth	Paternal country of birth	Maternal age at delivery (years)	Paternal age at delivery (years)	House-hold status during preg-nancy	Maternal pre-pregnancy body mass index (kg/m^2)	Maternal height (cm)	Maternal alcohol use during pregnancy	Maternal smoking during pregnancy	Parity
		% female	% low	% medium	% high	% low	% medium	% high	% foreign	% foreign	mean (SD)	mean (SD)	% parents	mean(SD)	mean (SD)	% yes	% yes	% nullip-arous
ABCD, The Netherlands	2701	5.8	9.9	34.2	55.8	7.7	17.8	74.5	20.6	21.9	33.1 (4.3)	40.5 (5.5)	8.3	22.7 (3.5)	169.8 (7.0)	0.0	6.9	57.9
GENERATION R, The Netherlands	3120	50.7	5.7	39.8	54.5	4.9	38.6	46.5	39.2	30.0	31.2 (4.7)	33.7 (4.2)	9.9	24.5 (4.2)	168.3 (7.3)	44.2	12.1	60.6
GINIplus/LISA, Germany-Wesel	1696	49.2	17.7	49.8	32.5	38.5	26.0	35.5	0.0	0.0	30.7 (3.7)	32.8 (4.5)	8.5	23.4(3.6)	169.4 (6.1)	45.6	13.3	45.7
GINIplus/LISA, Germany-Munich	2514	47.3	6.5	26.7	66.8	11.6	14.5	73.9	NA	NA	32.9 (3.8)	35.4 (5.1)	12.4	22.3(3.6)	168.5 (5.9)	63.2	12.7	52.9
REPRO_PL, Poland	327	52.0	2.8	33.6	63.6	2.6	54.4	43.0	NA	NA	29.1 (4.0)	31.3 (5.2)	23.2	22.1(3.7)	166.0 (5.7)	9.7	9.2	52.6
EDEN, France-Nancy	323	49.8	14.7	12.2	73.1	27.7	14.4	57.9	3.7	7.5	30.2 (4.4)	32.3 (5.2)	2.8	22.7(4.1)	164.8 (6.2)	58.2	15.0	50.2
EDEN, France-Poitiers	247	41.3	23.1	20.7	56.2	30.7	22.3	47.0	3.3	7.7	30.7 (4.6)	32.6 (5.3)	2.5	23.2(4.4)	163.0 (6.3)	66.8	19.8	50.0
GASPII, Italy	461	48.8	11.6	50.1	38.3	1.4	64.9	33.7	3.3	2.7	33.6 (4.2)	36.2 (4.9)	0.7	22.1(3.4)	164.7 (6.1)	36.4	11.1	59.0
INMA, Spain-Asturias	357	47.3	13.7	44.8	41.5	29.4	46.4	24.4	1.1	1.1	33.3 (4.1)	35.3 (5.2)	2.0	23.7(4.1)	162.4 (5.8)	7.2	27.1	61.3
INMA. Spain-Gipuzkoa	397	50.4	10.1	36.0	53.9	20.9	48.9	30.3	2.5	1.5	32.6 (3.2)	35.1 (4.5)	0.0	22.9(3.4)	163.8 (6.0)	6.3	21.8	56.9
INMA, Spain-Sabadell	484	47.9	23.7	41.8	34.5	32.2	42.9	24.9	7.7	8.9	31.9 (4.1)	33.7 (4.8)	1.4	23.8(4.6)	162.3 (6.1)	11.1	27.3	56.6
INMA, Spain-Valencia	427	49.4	27.2	43.8	29.0	43.1	39.5	17.4	5.6	9.8	31.5 (4.1)	33.5 (4.7)	1.2	23.6(4.3)	162.2 (6.2)	9.4	36.8	56.4
INMA, Spain-Granada	153	0.0	51.5	30.6	17.9	53.0	23.9	23.1	0.0	0.0	30.6 (4.6)	33.2 (4.9)	1.5	23.3 (3.5)	162.2 (6.2)	3.7	21.6	28.8

NA, not available

Table 3. Fully-adjusted combined associations[a] between exposure to each air pollutant and depressive and anxiety symptoms in the borderline/clinical range

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	13	1.02	0.95;1.10	0.421	2.5	9	0.92	0.82;1.03	0.891	0.0
NO_X	10	1.02	0.96;1.09	0.916	0.0	5	0.94	0.82;1.07	0.960	0.0
PM_{10}	7	0.93	0.76;1.15	0.378	6.4	6	0.77	0.57;1.03	0.438	0.0
$PM_{2.5}$	7	0.83	0.64;1.09	0.896	0.0	6	0.69	0.47;1.01	0.904	0.0
PM_{COARSE}	6	0.88	0.74;1.04	0.440	0.0	6	0.79	0.62;1.01	0.726	0.0
$PM_{2.5}$abs	6	0.92	0.76;1.10	0.569	0.0	5	0.79	0.58;1.06	0.711	0.0
PAH	2	0.93	0.66;1.31	0.664	0.0	2	0.93	0.67;1.22	0.452	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter ≤10μm; $PM_{2.5}$, particulate matter ≤ 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol use, parity), paternal characteristics (education level, country of birth, age at delivery), household status during pregnancy, and child's sex and age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with depressive and anxiety symptoms in the border/clinical were excluded.

Table 4. Fully-adjusted combined associations[a] between exposure to each air pollutant and aggressive symptoms in the borderline/clinical range.

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	13	1.07	0.97;1.19	0.354	9.2	9	0.93	0.82;1.06	0.709	0.0
NO_X	10	1.03	0.95;1.12	0.664	0.0	5	0.91	0.78;1.06	0.685	0.0
PM_{10}	7	0.98	0.72;1.34	0.231	25.9	6	0.81	0.59;1.12	0.473	0.0
$PM_{2.5}$	7	0.94	0.67;1.31	0.896	0.0	6	0.72	0.46;1.14	0.333	12.8
PM_{COARSE}	6	1.07	0.87;1.33	0.653	0.0	6	0.82	0.59;1.16	0.192	32.5
$PM_{2.5}$abs	6	0.98	0.78;1.25	0.659	0.0	5	0.92	0.67;1.25	0.466	0.0
PAH	2	0.78	0.54;1.13	0.625	0.0	2	0.83	0.53;1.32	0.160	46.5

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter ≤10μm; $PM_{2.5}$, particulate matter ≤ 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol use, parity), paternal characteristics (education level, country of birth, age at delivery), household status during pregnancy, and child's sex and age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with aggressive symptoms in the border/clinical were excluded.

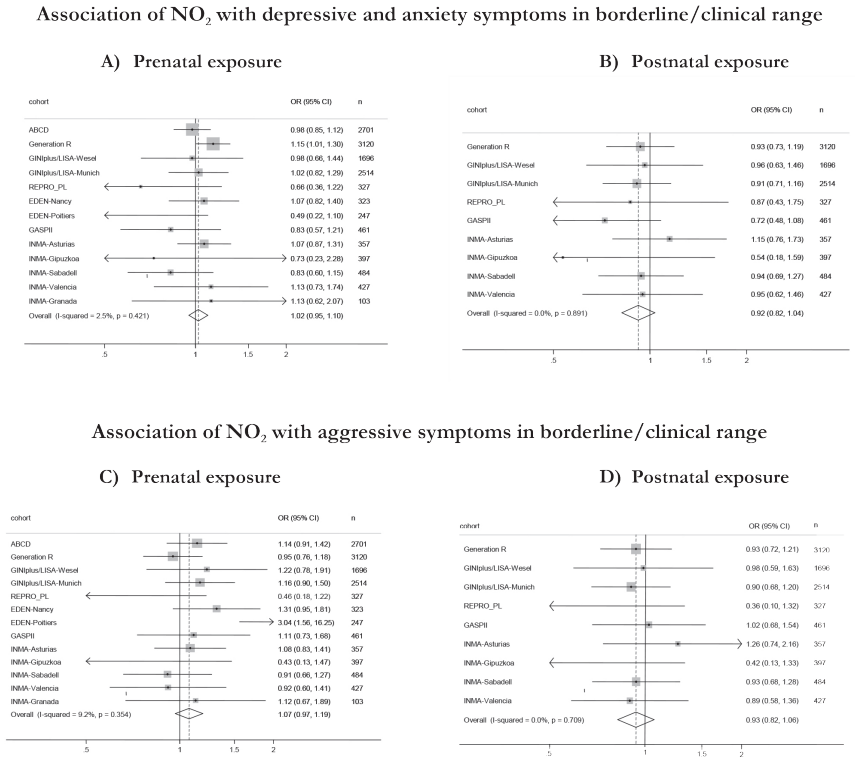

Figure 1. Fully-adjusted associations of prenatal and postnatal exposure to NO_2 and depressive and anxiety symptoms or aggressive symptoms in borderline/clinical range at average age of 11y in ABCD cohort, 10y in Generation R, GINIplus and LISA cohort, 9y in INMA Sabadell, Valencia and Granada cohorts, 8y in EDEN cohort and INMA Gipuzkoa cohort and 7y in REPRO_PL cohort and GASPII cohort. Cohort/region-specific and summary odd ratio estimates (coefficient and 95% confidence interval) expressed in 10 $\mu g/m^3$, adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol using, parity), paternal characteristics (education level, country of birth, age at delivery) child's sex and child's age at assessment.Grey squares around region-specific coefficients represent the relative weight that the estimate contributes to the summary coefficient. Weights are from random-effects analyses. Coef, coefficient; CI, confidence intervals; NO_2, nitrogen dioxide.

DISCUSSION

In this study of 13,182 children from population-based birth cohorts from across Europe, we did not observe an association between prenatal and postnatal exposure to several ubiquitous air pollutants with depressive and anxiety symptoms, and aggressive symptoms, in children between 7 and 11 years old.

This study has several strengths. One of the main strengths is the use of data from several prospective population-based birth cohorts with a wide European geographical extent, granting a large sample size and representativeness within Europe. Also, we used exposure data from pollutants during prenatal and postnatal exposure periods, taking into account residential moving. Seven key air pollutants were included, all highly ubiquitous in urban settings, where around 75% of the European population lives nowadays (Eurostat. 2019). Also, we used multiple imputation and inverse probability weighting to reduce a possible attrition bias in the cohort studies, thereby adding to the representativeness of the study population with respect to the full cohorts. Additionally, the models were adjusted for a large number of socioeconomic and lifestyle variables that are known to be associated with neuropsychological development in children. Regarding the assessment of the emotional and aggressive symptoms in childhood, two standardized and validated behavioural assessments were used, both equally suitable to distinguish between children with and without clinical symptoms (Goodman, 1997; Klasen et al., 2000). Although the use of clinical diagnostic data might be of greater importance for policy making and health interventions than the use of data with quantitatively assessed disorders, clinical data is often not available. Moreover, quantitatively assessed data allows examination of the symptoms on the whole spectrum, which, while often not qualifying for clinical diagnosis, might still have a great impact on individual's mental health and well-being (Kagee et al., 2013).

A limitation of our study is the slight inconsistency in exposure assessment as two cohorts (REPRO_PL and the Gipuzkoa region of INMA) used a different method to estimate air pollution levels at participant's residential addresses, as compared to the remaining cohorts. Both methodologies are commonly used to estimate air pollution exposure (Mercer et al.,2011; Xie et al., 2017) and our assessment of individual influences of each cohort did not show substantial differences in the results. Another limitation is that only NO_2 was available for all cohorts, whereas the other pollutants were available for only a selection of the included cohorts. A further limitation related to the exposure assessment is that the air pollution measurements were performed between 0 and 10 years after the pregnancies of the participating mothers, meaning that we had to assume that the spatial distribution of air pollutants remained stable over that period. This assumption is supported by previous research suggesting that the spatial distribution of air pollution concentrations and its predictors can indeed be considered stable over time for periods up to 10 or 20 years (Cesaroni et al., 2012; Eeftens et al., 2011, Gulliver et al., 2013). Moreover, the results did not change when we tested the associations between prenatal air pollution exposure and depressive and anxiety symptoms, and aggressive symptoms, using only a subset of cohorts which had the exposure measurements carried out either during pregnancy or the first 2

years of life, further supporting the assumption made. Another limitation related to the exposure assessment is that the postnatal period is defined as the period between birth and the emotional and behavioural assessment, which translates to a time window of 7 up to 11 years. Taking an average over such a long period of time, might prevent the identification of critical windows in postnatal exposure that would be identifiable if exposure data would be assessed on a finer time scale. However, such data were not available, and therefore we used one value for the entire postnatal period which might lead to more conservative results. The use of two different tests (CBCL and SDQ) to assess emotional and behavioural symptoms is another limitation of our study. Each of these tests includes a different number of items, gives a slightly different weight to various symptoms, and validated cut-offs lead to different proportion of children within the borderline and clinical range. Overall, the results did not change substantially when we stratified the cohorts by test, except for the associations between postnatal exposure to various pollutants and lower odds of depressive and anxiety symptoms assessed with the CBCL test. Another limitation was that socioeconomic area-level variables were not available to test the potential spatial autocorrelation.

In the current study, we did not observe associations of prenatal exposure to air pollution with depressive and anxiety symptoms or aggressive symptoms. The lack of associations is in line with the results of two previous meta-analyses on the relationships of prenatal exposure to air pollution and with autistic traits, and ADHD symptoms, including several European birth cohorts, in which also no associations were found (Guxens et al., 2016; Forns et al., 2018). However, the results of our current study are not consistent with two previous studies assessing air pollution and depression, anxiety, and aggressive symptoms, as they found an association between prenatal exposure to PAH and symptoms of depression and anxiety, and rule breaking and aggressive symptoms in children between 4.8 and 11 years of age (Margolis et al., 2016; Genkinger et al., 2015). A possible explanation for the discrepancy between these previous findings and ours might be the difference in exposure assessment. In our study we assessed air pollution levels at home addresses of the participants. In the previous study, PAHs exposure was measured using personal air monitors that pregnant mothers carried with them 48-hr in the third trimester of pregnancy (Margolis et al., 2016). These previously used methods are certainly more accurate to assess individual exposure, but are likely less representative as indicator of long-term exposure in comparison to the estimations at residential level assessed using land use regression or kriging methods (Park and Kwan, 2017).

Regarding the associations between postnatal exposure to air pollution and emotional and aggressive symptoms in children, three studies assessed the relationship between exposure to EC, BC, and NO_2 and depressive and anxiety symptoms and aggressive symptoms at ages 7-12 years (Newman et al 2014; Forns et al., 2016; Roberts et al., 2019). In the study done in Barcelona, NO_2 and EC levels were measured at the schools of the participating children by air pollution monitors, and BC levels were estimated at residential addresses using LUR models (Forns et al., 2016). The results showed that there was no association between EC, BC and NO_2 exposure and odds of depressive and anxiety symptoms, and aggressive symptoms. In the study in Ohio, residential levels of EC were estimated using LUR models and no association was found between EC and odds of aggressive symptoms.

In the study in London, residential levels of NO_2 and $PM_{2.5}$ were estimated using King's College London urban model (Roberts et al., 2019). The results showed that there was no association between NO_2 and $PM_{2.5}$, and odds of depressive and anxiety symptoms, and aggressive symptoms. In line with these previous findings, we did not find an association between postnatal exposure to NO_2, or any other pollutant, and depressive anxiety, or aggressive symptoms.

To date, studies on the association between exposure to air pollution and emotional symptoms, have been mainly carried out in adults. Overall, the results of these studies suggest that higher levels of NO_2 and $PM_{2.5}$ are positively associated with onset of depression, depressive symptoms, anxiety symptoms, and with antidepressant use (Kioumourtzoglou et al., 2017; Pun et al., 2017; Vert et al., 2017; Power et al., 2015). While the exact biological mechanisms underlying these associations are not yet fully understood, there is increasing evidence from animal studies suggesting that exposure to NO_2 or $PM_{2.5}$ is associated with increased inflammation in the brain, oxidative stress, cerebrovascular impairment and neurodegeneration (Block and Calderón, 2009, Mohankumar et al., 2008). These mechanisms have been shown to be associated with many neurological and neuropsychological disorders in humans, including depression and anxiety (Fonken et al., 2012). Therefore, in light of the results from the studies performed in adults, the lack of associations in our study might suggest that our study population is too young to already have developed emotional and behavioral problems related to air pollution exposure, and that such problems are likely to develop later in life. This hypothesis is supported by findings from a recent study from London, where exposure to NO_2 and $PM_{2.5}$ was not associated with mental health problems in school-age children, while it did predict higher odds of mental disorders in 18-year-old adolescents (Roberts et al., 2019). Therefore, we suggest focusing follow up studies on adolescents and young adults, which will give insight into the period between childhood and adulthood, and will potentially help to understand the discrepancies between the results of the studies carried out in these two life stages.

CONCLUSIONS

In conclusion, we did not find evidence for an association between prenatal and postnatal exposure to several air pollutants and emotional and aggressive symptoms in a large sample of children between 7 and 11 years from various regions across Europe.

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PAPER II – SUPPLEMENTARY MATERIAL

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

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eTable 1. Distribution of the emotional and aggressive symptoms scales.

Cohort Study	Domain	Test	Range	P10	P25	P50	P75	P90	Mean ± SD	Cut-off borderline or clinical range	Cut-off borderline or clinical range
ABCD, The Netherlands	Emotional	SDQ	0-10	0	0	2	3	5	1.9 ± 1.8	4	5
	Aggressive		0-9	0	0	1	2	3	1.3 ± 1.3	3	4
GENERATION R, The Netherlands	Emotional	CBCL	0-25	0	1	2	5	8	3.2 ± 3.7	9	14
	Aggressive		0-42	0	0	2	5	10	3.8 ± 4.7	12	17
GINIplus, Germany-Wesel	Emotional	SDQ	0-10	0	0	1	3	4	1.8 ± 1.8	4	5
	Aggressive		0-10	0	0	1	2	4	1.6 ± 1.5	3	4
LISA, Germany-Munich	Emotional	SDQ	0-10	0	0	1	3	5	1.9 ± 2.0	4	5
	Aggressive		0-10	0	1	1	3	4	1.7 ± 1.5	3	4
REPRO_PL, Poland	Emotional	SDQ	0-8	0	1	2	3	5	2.2 ± 1.8	4	5
	Aggressive		0-7	0	1	2	3	3	1.7 ± 1.4	3	4
EDEN, France-Nancy	Emotional	SDQ	0-10	0	1	2	4	6	2.5 ± 2.2	4	5
	Aggressive		0-7	0	0	1	3	4	1.7 ± 1.6	3	4
EDEN, France-Poitiers	Emotional	SDQ	0-10	0	1	2	4	5	2.4 ± 1.9	4	5
	Aggressive		0-10	0	0	1	3	4	1.7 ± 1.6	3	4
GASPII, Italy	Emotional	CBCL	0-25	1	2	5	8	11	5.5 ± 4.0	12	15
	Aggressive		0-38	1	3	6	10	14	7.3 ± 5.4	16	20
INMA, Spain-Asturias	Emotional	SDQ	0-9	0	1	2	3	5	2.2 ± 2.1	4	5
	Aggressive		0-7	0	0	1	3	4	1.6 ± 1.5	3	4
INMA, Spain-Gipuzkoa	Emotional	CBCL	0-27	0	1	3	7	10	4.4 ± 4.0	11	15
	Aggressive		0-29	0	2	4	8	14	5.9 ± 5.6	16	21
INMA, Spain-Sabadell	Emotional	CBCL	0-25	1	2	4	7	12	5.6 ± 4.9	14	19
	Aggressive		0-37	0	2	5	11	15	6.9 ± 6.6	19	26
INMA, Spain-Valencia	Emotional	CBCL	0-22	1	2	4	7	11	5.3 ± 4.3	13	17
	Aggressive		0-31	1	2	3	10.5	16	7.6 ± 6.0	19	23
INMA, Spain-Granada	Emotional	CBCL	0-23	2	3	6	9	12	6.2 ± 4.0	13	17
	Aggressive		0-30	1	3	6	10	16	7.5 ± 6.0	18	23

CBCL, child behaviour checklist 6/18; SD, standard deviation; SDQ, Strengths and Difficulties Questionnaire

eTable 2. Cohort specific NO_2 and $PM_{2.5}$ levels during prenatal and postnatal periods

	NO_2		$PM_{2.5}$	
	Prenatal	**Postnatal**	**Prenatal**	**Postnatal**
ABCD, The Netherlands	39.9 (7.3)	na	13.9 (1.3)	na
GENERATION R, The Netherlands	35.1 (7.7)	32.7 (6.0)	16.5 (0.5)	16.4 (0.4)
GINIplus, Germany-Wesel	23.0 (5.2)	22.1 (4.7)	15.3 (2.2)	15.7 (2.2)
LISA, Germany-Munich	22.0 (5.6)	20.5 (5.1)	14.1 (1.8)	14.1 (1.8)
REPRO_PL, Poland	25.7 (4.7)	24.0 (4.1)	na	28.4 (3.0)
EDEN, France-Nancy	30.3 (10.3)	na	na	na
EDEN, France-Poitiers	15.9 (5.2)	na	na	na
GASPII, Italy	43.3 (10.0)	43.5 (9.9)	23.0 (2.7)	19.4 (2.0)
INMA, Spain-Asturias	30.5 (13.4)	20.7 (6.7)	na	na
INMA, Spain-Gipuzkoa	18.9 (4.6)	14.0 (4.5)	16.9 (2.5)	11.8 (0.6)
INMA, Spain-Sabadell	43.1 (11.0)	36.7 (10.6)	15.1 (1.8)	14.8 (2.2)
INMA, Spain-Valencia	24.7 (10.6)	29.3 (10.7)	na	na
INMA, Spain-Granada	27.9 (13.9)	na	na	na

na, not available; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter less than 2.5μm
Values are mean (standard deviation).

eTable 3. Spearman correlations[a] between prenatal air pollution levels.

	NO_2 vs NO_X	**NO_2 vs $PM_{2.5}$**	**NO_2 vs $PM_{2.5}$ abs**	**NO_X vs $PM_{2.5}$**	**NO_X vs $PM_{2.5}$ abs**	**$PM_{2.5}$ vs PM**
ABCD, The Netherlands	0.87	0.19	0.78	0.17	0.73	0.62
GENERATION R, The Netherlands	0.85	0.82	0.85	0.74	0.87	0.72
GINIplus, Germany-Wesel	0.98	0.72	0.78	0.72	0.73	0.75
LISA, Germany-Munich	0.94	0.70	0.61	0.43	0.70	0.41
GASPII, Italy	0.70	na	0.7	na	0.74	na
INMA, Spain-Asturias	0.99	na	na	na	na	na
INMA. Spain-Gipuzkoa	0.96	na	na	na	na	na
INMA, Spain-Sabadell	0.92	0.73	0.82	0.78	0.95	0.82
INMA, Spain-Valencia	0.98	na	na	na	na	na
INMA, Spain - Granada	0.99	na	na	na	na	na

[a]all pvalues are ≤ 0.05

na, not available; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; $PM_{2.5}$, particulate matter less than 2.5μm;

$PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters.

eTable 4. Spearman correlations[a] between postnatal air pollution levels.

	NO_2 vs NO_X	NO_2 vs $PM_{2.5}$	NO_2 vs $PM_{2.5}$ abs	NO_X vs $PM_{2.5}$	NO_X vs $PM_{2.5}$ abs	$PM_{2.5}$ vs $PM_{2.5}$ abs
GENERATION R, The Netherlands	0.84	0.36	0.87	0.45	0.87	0.51
GINIplus, Germany-Wesel	0.97	0.71	0.78	0.72	0.72	0.75
LISA, Germany-Munich	0.93	0.20	0.55	0.35	0.66	0.41
REPRO_PL, Poland	na	0.60	na	na	na	na
GASPII, Italy	0.66	0.55	0.56	0.69	0.66	0.70
INMA, Spain-Gipuzkoa	na	0.30	na	na	na	na
INMA, Spain-Sabadell	0.99	0.68	0.93	0.71	0.94	0.78

[a]all pvalues are ≤ 0.05

na, not available; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; $PM_{2.5,}$ particulate matter less than 2.5μm;

$PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters.

eTable 5. Spearman correlations[a] between prenatal and postnatal air pollution levels.

	NO_2 pre vs NO_2 post	**NO_X pre vs NO_X post**	**PM_{10} pre vs PM_{10} post**	**$PM_{2.5}$ pre vs $PM_{2.5}$ post**	**PM_{COARSE} pre vs PM_{COARSE} post**	**$PM_{2.5}$abs pre vs $PM_{2.5}$abs post**	**PAH pre vs PAH post**
ABCD, The Netherlands	na	na	na	na	na	na	na
GENERATION R, The Netherlands	0.47	0.55	0.51	0.59	0.55	0.52	0.67
GINIplus, Germany-Wesel	0.69	0.66	0.86	0.93	0.79	0.86	na
LISA, Germany-Munich	0.66	0.62	0.73	0.75	0.70	0.71	na
REPRO_PL, Poland	0.70	na	0.13	na	na	na	na
EDEN, France-Nancy	na	na	na	na	na	na	na
EDEN, France-Poitiers	na	na	na	na	na	na	na
GASPII, Italy	0.88	0.80	0.80	0.78	0.72	0.67	na
INMA, Spain-Asturias	0.57	na	na	na	na	na	na
INMA. Spain-Gipuzkoa	0.41	na	na	0.33	na	na	na
INMA, Spain-Sabadell	0.73	0.66	0.64	0.59	0.59	0.58	0.82
INMA, Spain-Valencia	0.68	na	na	na	na	na	na
INMA, Spain-Granada	na	na	na	na	na	na	na

[a]all pvalues are ≤ 0.05

na, not available; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; ; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; PM_{COARSE}, particulate matter between 2.5 and 10μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters

eTable 6. Fully adjusted combined associations[a] between exposure to air pollution and depressive and anxiety symptoms in clinical range.

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	12	1.04	0.94;1.14	0.369	7.8	9	0.90	0.76;1.07	0.572	0.0
NO_X	9	1.03	0.94;1.13	0.797	0.0	5	0.97	0.80;1.18	0.933	0.0
PM_{10}	7	1.04	0.80;1.34	0.454	0.0	6	0.79	0.52;1.18	0.796	0.0
$PM_{2.5}$	7	0.83	0.58;1.20	0.949	0.0	6	0.66	0.39;1.11	0.702	0.0
PM_{COARSE}	6	0.88	0.70;1.10	0.908	0.0	6	0.75	0.53;1.06	0.890	0.0
$PM_{2.5}$abs	6	0.90	0.70;1.17	0.872	0.0	5	0.79	0.50;1.23	0.757	0.0
PAH	5	0.79	0.50;1.23	0.757	0.0	2	0.86	0.50;1.50	0.496	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol use, parity), paternal characteristics (education level, country of birth, age at delivery), household status during pregnancy, and child's sex and age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with depressive and anxiety symptoms in the clinical were excluded.

eTable 7. Fully adjusted combined associations[a] between exposure to air pollution and aggressive symptoms in clinical range.

	Prenatal exposure					Postnatal exposure				
	N[b]	OR	(95% CI)	p-heter	I^2	N[b]	OR	(95% CI)	p-heter	I^2
NO_2	12	1.08	0.93;1.24	0.211	23.7	9	0.99	0.82;1.20	0.314	14.5
NO_X	9	1.06	0.96;1.18	0.383	6.2	5	0.97	0.79;1.19	0.361	8.0
PM_{10}	7	1.05	0.61;1.81	0.007	66.0	6	1.13	0.69;1.87	0.218	28.9
$PM_{2.5}$	6	1.15	0.73;1.87	0.257	22.5	6	1.06	0.52;2.18	0.166	36.1
PM_{COARSE}	6	1.18	0.82;1.70	0.104	45.2	6	1.08	0.67;1.74	0.111	44.2
$PM_{2.5}$abs	6	1.04	0.72;1.49	0.179	34.3	5	1.21	0.78;1.88	0.258	24.5
PAH	2	0.74	0.36;1.53	0.190	41.9	2	0.95	0.52;1.74	0.188	42.3

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol use, parity), paternal characteristics (education level, country of birth, age at delivery), household status during pregnancy, and child's sex and age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with aggressive symptoms in the clinical were excluded.

eTable 8. Minimally-adjusted combined associations[a] between exposure to each air pollutant and depressive and anxiety symptoms in the borderline/clinical range

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	13	0.99	0.91;1.07	0.220	22.1	9	0.94	0.84;1.05	0.582	0.0
NO_X	10	1.01	0.95;1.08	0.682	0.0	5	0.98	0.86;1.10	0.719	0.0
PM_{10}	7	0.90	0.74;1.10	0.341	11.6	6	0.79	0.59;1.04	0.431	0.0
$PM_{2.5}$	7	0.85	0.65;1.09	0.868	0.0	6	0.73	0.51;1.04	0.837	0.0
PM_{COARSE}	6	0.88	0.73;1.06	0.313	16.1	6	0.82	0.65;1.02	0.960	0.0
$PM_{2.5}$abs	6	0.90	0.75;1.08	0.399	2.7	5	0.82	0.62;1.08	0.691	0.0
PAH	2	0.97	0.69;1.40	0.571	0.0	2	0.94	0.70;1.26	0.697	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2 =Percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol use, parity), paternal characteristics (education level, country of birth, age at delivery), household status during pregnancy, and child's sex and age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with depressive and anxiety symptoms in the border/clinical were excluded.

eTable 9. Minimally-adjusted combined associations[a] between exposure to each air pollutant and aggressive symptoms in the borderline/clinical range

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	13	1.05	0.99;1.12	0.434	1.1	9	0.97	0.88;1.07	0.530	0.0
NO_X	10	1.03	0.97;1.10	0.785	0.0	5	0.96	0.86;1.09	0.929	0.0
PM_{10}	7	0.96	0.79;1.18	0.794	0.0	6	0.85	0.66;1.09	0.740	0.0
$PM_{2.5}$	7	1.03	0.79;1.35	0.996	0.0	6	1.00	0.75;1.32	0.456	0.0
PM_{COARSE}	6	0.99	0.84;1.19	0.782	0.0	6	0.87	0.71;1.07	0.931	0.0
$PM_{2.5}$abs	6	0.95	0.79;1.16	0.767	0.0	5	0.95	0.74;1.22	0.569	0.0
PAH	2	0.94	0.68;1.30	0.608	0.0	2	0.94	0.71;1.24	0.966	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; $PM_{COARSE,}$ particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, prenatal alcohol use, parity), paternal characteristics (education level, country of birth, age at delivery), household status during pregnancy, and child's sex and age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with aggressive symptoms in the border/clinical were excluded.

eTable 10. Fully-adjusted combined associations[a] between prenatal air pollution exposure and depressive and anxiety symptoms in the borderline/clinical range: Assessing the influence of each cohort separately in the meta-analysis estimates

	NO_2		NO_X		PM_{10}		$PM_{2.5}$		PM_{COARSE}		$PM_{2.5}$abs		PAH	
	OR	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**		**OR**	**(95% CI)**
Combined estimate	1.02	0.95;1.10	1.02	0.96;1.09	0.93	0.76;1.15	0.83	0.64;1.09	0.88	0.74;1.04	0.92	0.76;1.10	0.90	0.67;1.22
Area omitted:														
ABCD, The Netherlands	1.03	0.94;1.13	1.03	0.95;1.11	1.06	0.83;1.35	0.90	0.64;1.25	0.97	0.76;1.25	0.97	0.77;1.24		
GENERATION R, The Netherlands	0.98	0.90;1.06	1.01	0.94;1.09	0.92	0.73;1.15	0.82	0.62;1.08	0.85	0.72;1.01	0.88	0.72;1.08	0.83	0.45;1.53
GINIplus, Germany-Wesel	1.02	0.84;1.10	1.02	0.96;1.10	0.94	0.74;1.18	0.83	0.63;1.09	0.89	0.73;1.08	0.92	0.76;1.11		
LISA, Germany-Munich	1.02	0.93;1.10	1.02	0.95;1.09	0.87	0.70;1.07	0.81	0.60;1.10	0.85	0.70;1.02	0.87	0.71;1.06		
REPRO_PL, Poland	1.03	0.96;1.10			0.91	0.71;1.17								
EDEN, France-Nancy	1.01	0.93;1.10												
EDEN, France-Poitiers	1.03	0.96;1.11												
GASPII, Italy	1.04	0.95;1.14	1.03	0.96;1.10	0.97	0.79;1.19	0.95	0.74;1.22	0.96	0.76;1.21	0.94	0.77;1.14		
INMA, Spain-Asturias	1.01	0.96;1.10	1.02	0.94;1.10										
INMA, Spain-Gipuzkoa	1.02	0.94;1.10	1.03	0.96;1.10			0.80	0.61;1.05						
INMA, Spain-Sabadell	1.04	0.97;1.11	1.04	0.97;1.12	0.94	0.74;1.20	0.84	0.64;1.11	0.86	0.72;1.04	0.95	0.78;1.15	0.98	0.65;1.47
INMA, Spain-Valencia	1.01	0.94;1.10	1.02	0.96;1.10										
INMA, Spain-Granada[b]	1.02	0.94;1.10	1.02	0.96;1.09										

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; PM_{10}. particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; PM_{COARSE}. particulate matter between 2.5 and 10μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; PAH, polycyclic aromatic hydrocarbons; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Region with less than 10 children with depressive and anxiety symptoms in the border/clinical were excluded.

eTable 12. Fully-adjusted combined associations[a] between prenatal air pollution exposure and aggressive symptoms in the borderline/clinical range: Assessing the influence of each cohort separately in the meta-analysis estimates.

	NO_2		NO_X		PM_{10}		$PM_{2.5}$		PM_{COARSE}		$PM_{2.5}$abs		PAH	
	OR	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Combined estimate	0.92	0.82;1.03	0.94	0.82;1.07	0.77	0.57;1.03	0.69	0.47;1.01	0.79	0.62;1.01	0.79	0.58;1.06	0.90	0.67;1.22
Area omitted:														
ABCD, The Netherlands														
GENERATION R, The Netherlands	0.92	0.80;1.05	0.92	0.77;1.10	0.80	0.58;1.10	0.70	0.47;1.03	0.82	0.63;1.06	0.82	0.58;1.16	0.98	0.68;1.43
GINIplus, Germany-Wesel	0.92	0.81;1.04	0.92	0.80;1.07	0.74	0.54;1.00	0.66	0.44;1.00	0.80	0.62;1.03	0.74	0.54;1.03		
LISA, Germany-Munich	0.93	0.81;1.06	0.95	0.81;1.10	0.68	0.47;0.99	0.63	0.39;1.02	0.77	0.58;1.02	0.75	0.53;1.06		
REPRO_PL, Poland	0.92	0.82;1.04			0.82	0.60;1.12								
EDEN, France-Nancy														
EDEN, France-Poitiers														
GASPII, Italy	0.98	0.87;1.10	0.95	0.86;1.10	0.82	0.60;1.12	0.80	0.55;1.17	0.84	0.64;1.09	0.84	0.61;1.15		
INMA, Spain-Asturias	0.90	0.80;1.02												
INMA, Spain-Gipuzkoa	0.92	0.82;1.04					0.69	0.47;1.01	0.79					
INMA, Spain-Sabadell	0.92	0.81;1.04	0.94	0.82;1.08	0.72	0.49;1.06	0.74	0.47;1.16	0.76	0.57;1.00	0.77	0.55;1.00	0.77	0.46;1.30
INMA, Spain-Valencia	0.92	0.81;1.04	0.94	0.81;1.07	0.77	0.57;1.03	0.69	0.47;1.01	0.79	0.62;1.01	0.79	0.58;1.06		
INMA, Spain-Granada[b]														

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; PM_{COARSE}· particulate matter between 2.5 and 10μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; PAH, polycyclic aromatic hydrocarbons; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Region with less than 10 children with aggressive symptoms in the border/clinical were excluded.

eTable 13. Fully-adjusted combined associations[a] between postnatal air pollution exposure and aggressive symptoms in the borderline/clinical range: Assessing the influence of each cohort separately in the meta-analysis estimates.

	NO_2		NO_X		PM_{10}		$PM_{2.5}$		PM_{COARSE}		$PM_{2.5}$abs		PAH	
	OR	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Combined estimate	0.94	0.84;1.05	0.92	0.80;1.04	0.85	0.65;1.13	0.95	0.68;1.32	0.83	0.64;1.08	0.94	0.72;1.23	0.82	0.57;1.17
Area omitted:														
ABCD, The Netherlands														
GENERATION R, The Netherlands	0.96	0.85;1.09	0.97	0.83;1.15	0.91	0.69;1.20	0.98	0.69;1.37	0.91	0.72;1.15	1.05	0.78;1.41	0.95	0.66;1.37
GINIplus, Germany-Wesel	0.94	0.84;1.06	0.92	0.79;1.07	0.82	0.61;1.11	0.94	0.63;1.42	0.82	0.61;1.00	0.90	0.65;1.23		
LISA, Germany-Munich	0.95	0.83;1.07	0.92	0.79;1.08	0.90	0.61;1.33	1.02	0.67;1.55	0.84	0.59;1.18	0.94	0.66;1.34		
REPRO_PL, Poland	0.94	0.84;1.05			0.83	0.60;1.14								
EDEN, France-Nancy														
EDEN, France-Poitiers														
GASPII, Italy	0.93	0.83;1.05	0.89	0.77;1.02	0.81	0.60;1.11	0.88	0.64;1.23	0.78	0.60;1.01	0.87	0.66;1.17		
INMA, Spain-Asturias	0.94	0.84;1.05												
INMA, Spain-Gipuzkoa	0.95	0;85;1.06					0.95	0.68;1.32						
INMA, Spain-Sabadell	0.92	0.82;1.04	0.91	0.79;1.04	0.87	0.60;1.27	1.04	0.73;1.50	0.82	0.58;1.16	0.98	0.69;1.38	0.65	0.39;1.07
INMA, Spain-Valencia	0.94	0.83;1.05	0.92	0.80;1.04	0.85	0.65;1.13	0.95	0.68;1.32	0.83	0.64;1.08	0.94	0.72;1.23		
INMA, Spain-Granada[b]														

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; PM_{10}. particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; PM_{COARSE}. particulate matter between 2.5 and 10μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; PAH, polycyclic aromatic hydrocarbons; OR, Odds Ratio.

[a]Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Region with less than 10 children with aggressive symptoms in the border/clinical were excluded.

eTable 14. Fully adjusted combined associations[a] between exposure to air pollution and depressive and anxiety symptoms in the 90th percentile of each test.

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	13	1.04	0.96;1.15	0.649	0.0	9	0.93	0.84;1.03	0.979	0.0
NO_X	10	1.02	0.95;1.09	0.925	0.0	5	0.95	0.85;1.08	0.887	0.0
PM_{10}	7	1.06	0.87;1.31	0.582	0.0	6	0.85	0.65;1.13	0.823	0.0
$PM_{2.5}$	7	0.94	0.72;1.23	0.672	0.0	6	0.78	0.54;1.11	0.988	0.0
PM_{COARSE}	6	0.98	0.83;1.17	0.735	0.0	6	0.83	0.66;1.04	0.901	0.0
$PM_{2.5}$abs	6	0.95	0.79;1.14	0.581	0.0	5	0.90	0.69;1.15	0.987	0.0
PAH	2	0.76	0.56;1.03	0.793	0.0	2	0.85	0.64;1.13	0.770	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal caracteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with depressive and anxiety symptoms in the border/clinical were excluded.

eTable 15. Fully adjusted combined associations[a] between exposure to air pollution and aggressive symptoms in the 90th percentile of each test.

	Prenatal exposure					Postnatal exposure				
	N[b]	OR	(95% CI)	p-heter	I^2	N[b]	OR	(95% CI)	p-heter	I^2
NO_2	13	1.02	0.90;1.50	0.095	36.0	9	0.95	0.79;1.15	0.964	40.7
NO_X	10	1.01	0.92;1.10	0.290	16.6	5	0.93	0.81;1.07	0.966	0.0
PM_{10}	7	0.92	0.74;1.15	0.481	0.0	6	0.87	0.66;1.19	0.873	0.0
$PM_{2.5}$	7	0.94	0.70;1.26	0.974	0.0	6	0.78	0.52;1.18	0.724	0.0
PM_{COARSE}	6	0.97	0.82;1.15	0.751	0.0	6	0.89	0.69;1.14	0.732	0.0
$PM_{2.5}$abs	6	0.94	0.78;1.14	0.605	0.0	5	1.06	0.78:1.44	0.335	12.4
PAH	2	0.77	0.57;1.05	0.941	0.0	2	0.84	0.64;1.11	0.338	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with aggressive symptoms in the border/clinical were excluded.

eTable 16. Fully adjusted combined associations[a] between exposure to air pollution and depressive and anxiety symptoms in the borderline/clinical range: Assessing the influence of CBCL test.

	Prenatal exposure					Postnatal exposure				
	N[b]	OR	(95% CI)	p-heter	I^2	N[b]	OR	(95% CI)	p-heter	I^2
NO_2	6	1.06	0.97;1.15	0.448	0.0	5	0.89	0.80;1.00	0.820	0.0
NO_X	6	1.01	0.93;1.09	0.738	0.0	3	0.92	0.82;1.05	0.941	0.0
PM_{10}	3	0.85	0.36;1.16	0.566	0.0	3	0.67	0.49;0.91	0.672	0.0
$PM_{2.5}$	4	0.87	0.62;1.22	0.783	0.0	4	0.56	0.38;0.82	0.977	0.0
PM_{COARSE}	3	0.95	0.70;1.24	0.334	11.8	3	0.72	0.57;0.91	0.678	0.0
$PM_{2.5}$abs	3	0.89	0.72;1.10	0.600	0.0	3	0.69	0.52;0.90	0.797	0.0
PAH	2	0.93	0.73;1.19	0.909	0.0	2	0.90	0.73;1.12	0.756	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with depressive and anxiety symptoms in the borderline/clinical were excluded.

eTable 17. Fully adjusted combined associations[a] between exposure to air pollution and depressive and anxiety symptoms in the borderline/clinical range: Assessing the influence of SDQ test.

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	7	1.04	0.96;1.12	0.315	14.6	4	0.96	0.85;1.09	0.908	0.0
NO_X	4	1.03	0.97;1.09	0.993	0.0	3	0.95	0.82;1.10	0.902	0.0
PM_{10}	4	0.96	0.81;1.15	0.379	4.9	3	0.85	0.63;1.17	0.451	0.0
$PM_{2.5}$	3	0.81	0.65;1.03	0.931	0.0	2	0.84	0.58;1.21	0.987	0.0
PM_{COARSE}	3	0.86	0.75;0.99	0.727	0.0	3	0.88	0.69;1.13	0.885	0.0
$PM_{2.5}$abs	3	0.94	0.79;1.11	0.592	0.0	2	0.94	0.67;1.31	0.953	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with depressive and anxiety symptoms in the borderline/clinical were excluded.

eTable 18. Fully adjusted combined associations[a] between exposure to air pollution and aggressive symptoms in the borderline/clinical range: Assessing the influence of CBCL test.

	Prenatal exposure					Postnatal exposure				
	N[b]	**OR**	**(95% CI)**	**p-heter**	**I^2**	**N[b]**	**OR**	**(95% CI)**	**p-heter**	**I^2**
NO_2	6	0.96	0.86;1.07	0.859	0.0	5	0.93	0.83;1.04	0.839	0.0
NO_X	6	0.93	0.86;1.03	0.939	0.0	3	0.93	0.81;1.07	0.542	0.0
PM_{10}	3	0.91	0.66;1.28	0.480	0.0	3	0.71	0.48;1.05	0.310	16.3
$PM_{2.5}$	4	0.95	0.66;1.36	0.814	0.0	4	0.82	0.46;1.47	0.262	23.9
PM_{COARSE}	3	0.98	0.77;1.24	0.533	0.0	3	0.78	0.55;1.09	0.227	30.9
$PM_{2.5}$abs	3	0.80	0.63;1.02	0.963	0.0	3	0.86	0.64;1.17	0.379	2.7
PAH	2	0.78	0.61;1.02	0.888	0.0	2	0.87	0.68;1.11	0.365	0.7

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; $PM_{10,}$ particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; PAH, polycyclic aromatic hydrocarbon; OR, Odds Ratio

[a]Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with aggressive symptoms in the borderline/clinical were excluded.

eTable 19. Fully adjusted combined associations[a] between exposure to air pollution and aggressive symptoms in the borderline/clinical range: Assessing the influence of SDQ test.

	Prenatal exposure					Postnatal exposure				
	N[b]	OR	(95% CI)	p-heter	I^2	N[b]	OR	(95% CI)	p-heter	I^2
NO_2	7	1.16	1.05;1.26	0.429	0.0	4	0.95	0.80;1.12	0.501	0.0
NO_X	4	1.14	1.03;1.21	0.904	0.0	3	0.88	0.74;1.05	0.982	0.0
PM_{10}	4	1.04	0.76;1.41	0.248	26.1	3	0.93	0.67;1.30	0.950	0.0
$PM_{2.5}$	3	0.93	0.68;1.27	0.879	0.0	2	0.63	0.41;0.97	0.897	0.0
PM_{COARSE}	3	1.14	0.93;1.39	0.892	0.0	3	0.89	0.62;1.30	0.387	0.0
$PM_{2.5}$abs	3	1.18	0.94;1.49	0.950	0.0	2	1.03	0.71;1.51	0.903	0.0

CI, Confidence Interval; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; p-heter, P value of heterogeneity using the Cochran's Q test; PM_{COARSE}, particulate matter between 2.5 and 10μm; PM_{10}, particulate matter less than 10μm; $PM_{2.5}$, particulate matter less than 2.5μm; $PM_{2.5}$abs, reflectance of $PM_{2.5}$ filters; I^2, percentage of the total variability due to between-areas heterogeneity; OR, Odds Ratio.

[a] Odds Ratio and 95% confidence interval estimated by random-effects meta-analysis by cohort/region, calculated per increments of: 10μg/m^3 for NO_2; 20μg/m^3 for NO_X; 10μg/m^3 for PM_{10}; 5μg/m^3 for $PM_{2.5}$; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m^3 for PAH. Models were adjusted for maternal characteristics (education level, country of birth, age at delivery, pre-pregnancy body mass index, height, prenatal smoking, alcohol use during pregnancy, parity), paternal characteristics (education level, country of birth, age at delivery) household status, child's sex and child's age at assessment.

[b] Number of cohorts/regions included in the meta-analysis. Cohorts/regions with less than 10 children with aggressive symptoms in the borderline/clinical were excluded.

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Paper III

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

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

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Short title: Air pollution and brain morphology in children

ABSTRACT

Objective. Air pollution exposure during fetal life has been related to impaired child neurodevelopment but it is unclear if brain structural alterations underlie this association. The authors assessed whether air pollution exposure during fetal life alters brain morphology and whether these alterations mediate the association between air pollution exposure during fetal life and cognitive function in school-age children.

Method. We used data from a population-based birth cohort set up in Rotterdam, The Netherlands (2002-2006). Residential levels of air pollution during the entire fetal period were calculated using land-use regression models. Structural neuroimaging and cognitive function were performed at age 6-10 years (n=783). Models were adjusted for several socioeconomic and life-style characteristics.

Results. Mean fine particle levels were 20.2μg/m^3 (range 16.8-28.1). Children exposed to higher particulate matter levels during fetal life had thinner cortex in several brain regions of both hemispheres (e.g. cerebral cortex of the precuneus region in the right hemisphere was 0.045mm thinner (95% Confidence Interval 0.028-0.062) for each 5μg/m^3 increase in fine particles). The reduced cerebral cortex in precuneus and rostral middle frontal regions partially mediated the association between exposure to fine particles and impaired inhibitory control. Air pollution exposure was not associated with global brain volumes.

Conclusions. Exposure to fine particles during fetal life was related to child brain structural alterations of the cerebral cortex and these alterations partially mediated the association between exposure to fine particles during fetal life and impaired child inhibitory control. Such cognitive impairment at early ages could have significant long-term consequences.

INTRODUCTION

Air pollution is a global risk factor for various adverse health effects in humans (1–7). There is increasing evidence indicating that air pollution exposure is also related to an impairment of the central nervous system through chronic neuroinflammation and microglia activation which can lead to neuronal damage (8). Since pregnancy and the first years of life are critical windows of developmental vulnerability for the brain, exposure to air pollution during this period could cause permanent changes in the brain even at low levels of exposure (9, 10).

Several epidemiological studies have assessed the association between air pollution exposure during early life and child neurodevelopment (11–16). These studies have found that air pollution exposure during pregnancy or during the first years of life was associated with lower cognitive or psychomotor function and higher behavior problems including autism spectrum disorders. However, they mainly used neuropsychological or clinical instruments to evaluate child neurodevelopment, limiting our understanding of which brain structural and functional alterations underlie these associations. Only few small studies have started using magnetic resonance imaging (MRI) techniques to assess relationships with air pollution (17–20). Three studies found an association between higher exposure to air pollution at home during fetal life or early childhood and white matter abnormalities in children at seven to thirteen years old (17–19). A fourth study in children aged eight to twelve years showed a relationship between air pollution exposure at school and lower functional integration and segregation in key brain networks (20). Despite the fact that prior studies have not found an association between air pollution exposure and cortical thickness, the study of brain morphology is key in providing insights in the underlying neurobiological pathways.

Therefore, the aims of the present study were i) to assess the association between air pollution exposure during fetal life and brain morphology in school-age children and ii) to assess the mediation role of brain morphology on the association between air pollution exposure during fetal life and cognitive function in school-age children. Cognitive function is the result of integration of functions of many different brain regions, and thus there was no a priori hypothesis on which specific brain regions would be affected by air pollution exposure during fetal life as no other similar studies have been performed so far. Thus, we used an exploratory approach to examine the association of exposure to air pollutants and brain surface measures.

METHODS AND MATERIALS

Population and study design

This study was embedded in the Generation R Study, a population-based birth cohort study from fetal life onwards in Rotterdam, the Netherlands (21). A total of 8,879 pregnant women were enrolled and children were born between April 2002 and January 2006. A subgroup of children aged between six and ten years participated in an MRI sub-study (22). Briefly, a total of 1,932 were invited to participate in this sub-study. Children were oversampled

based on certain maternal exposures during pregnancy (i.e. cannabis, nicotine, selective serotonin reuptake inhibitors, depressive symptoms, and plasma folate levels) and child behavior problems (i.e. attention deficit hyperactivity disorder, pervasive developmental problems, dysregulation problems, and aggressive problems). Exclusion criteria comprised contradictions for the MRI procedure, severe motor or sensory disorders, neurological disorders, head injuries with loss of consciousness, and claustrophobia. Among those invited, 155 did not answer the invitation call, 447 refused to participate, and 5 could not participate due to contraindications for the MRI procedure. Among the 1,325 that attended the MRI visit, after excluding those with poor MRI data quality and major abnormalities, MRI measurements were available for 1,070 children. Finally, after excluding those without air pollution estimations during fetal life, 783 children were included in the present study. This study was approved by the Medical Ethics Committee of the Erasmus Medical Centre in Rotterdam, The Netherlands. Written informed consent was obtained from parents.

Air pollution exposure

Air pollution levels at mothers' home addresses for the entire fetal period were estimated following a standardized procedure described elsewhere (23–25). Briefly, air pollution monitoring campaigns of three two-week periods of nitrogen dioxide (NO_2) in 80 sites and particulate matter (PM) with aerodynamic diameters <10μm (PM_{10}) and <2.5μm ($PM_{2.5}$ or fine particles), and absorbance of fine particles (a proxy for elemental carbon) in 40 sites were performed in 2009-2010 across The Netherlands and Belgium (26, 27). Coarse particle concentration was calculated as the difference between PM_{10} and $PM_{2.5}$. The three measurements were averaged, adjusting for temporal variation using data from a centrally located background monitoring site with year-round monitoring. Land-use regression models were developed using predictor variables on nearby traffic intensity, population/household density, and land use derived from Geographic Information Systems to explain spatial variation of annual average concentrations (23–25). These models were then used to assign air pollution levels at mothers' home addresses during the entire fetal period using the exact geographical x and y coordinates that corresponded to the addresses reported by each participant. Seven available routine background monitoring network sites were simultaneously used to back-extrapolate to the exact fetal period (6, 25) accounting for the changes of home address during pregnancy (Supplemental Methods S1). This resulted in a single, time-adjusted mean air pollution concentration for each participant for the entire fetal period. Previous research supports stability of measured and modeled spatial contrast in air pollutants for periods up to 18 years (28).

Magnetic Resonance Imaging

Structural MRI scans were obtained on a 3-Tesla scanner (Discovery MR750, GE Healthcare, Milwaukee, USA). Using an 8-channel head coil, a whole-brain high-resolution T1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained. The scan parameters were the following: repetition time=10.3ms, echo time=4.2ms, inversion time=350ms, flip angle=16°, 186 contiguous slices with a thickness of 0.9mm, and in-plane resolution = 0.9×0.9mm.

To minimize movement children participated in a mock scanning session prior to the actual MRI scanning to introduce them to the scanning environment (22). In the scanner, care was taken that children were comfortable and soft cushions were used to assist with head immobilization. However, it was still possible that children moved in the scanner. Image quality assurance was performed in 2 steps. First, a visual inspection of the image quality of the T1 sequence was done at the scanner. If the image quality was poor or unusable, the scan was repeated with extra instructions for children to lie still. Second, a visual inspection of the surface reconstruction quality was done after the images were processed through the FreeSurfer pipeline. Both steps of quality control had to be passed successfully for data to be included in the analyses.

Cortical reconstruction and volumetric segmentation of global brain measures was performed with the Freesurfer image analysis suite version 5.1.0, (http://surfer.nmr.mgh.harvard.edu/). Briefly, cortical thickness at each vertex was measured by calculating the shortest distance from the white matter to the pial surface. Procedures for the measurement of cortical thickness have been validated against histological analysis and manual measurements (29). Volumetric measures included total brain volume, cortical gray matter volume, cortical white matter volume, subcortical gray matter volumes (i.e., caudate, putamen, pallidum, accumbens, hippocampus, amygdala, and thalamus), and ventricular volume. Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (30). All Freesurfer output was visually inspected and rated for quality.

Cognitive function

Children's cognitive function was assessed on the day of the scanning or shortly after using an array of subtasks from the Dutch version of the Developmental Neuropsychological Assessment test (NEPSY-II) (31). Detailed description of the test has been published previously (22). Briefly, the subtasks were chosen to tap into specific domains, including: attention and executive functioning, language, memory and learning, sensorimotor function, and visuospatial processing. Children were individually tested in a quiet room by trained investigators.

Potential confounding variables

Potential confounding variables were defined a priori based on direct acyclic graph (DAG) (Supplemental Figure S1) and on previous literature (11, 12, 25). Parental characteristics during pregnancy were collected by questionnaires: parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, maternal parity, family status, and maternal psychological distress (using the Brief Symptom Inventory). Parental weights and heights were measured or self-reported at the first trimester of pregnancy in the research center. Pre-pregnancy body mass index (kg/m^2) was calculated. Child's sex and date of birth were obtained from hospital or national registries. Child genetics ancestry was estimated based on the genome-wide SNP data from whole blood at birth and 4 principal components of ancestry

were included to better correct for population stratification (32, 33). Maternal intelligence quotient was assessed at child's age of six years with the Ravens Advanced Progressive Matrices Test, set I. Child's age at scanning was also collected.

Statistical analyses

We performed whole-brain, vertex-wise statistics using the Freesurfer QDEC module (query, design, estimate contrast) for each air pollutant adjusting for child's sex and age. As there are many vertices per hemisphere (~160,000), analyses were corrected for multiple testing using the built-in Monte Carlo null-Z simulations with 10,000 iterations ($p<.01$). Due to limitations in modeling strategy with QDEC (types of variables, number of confounding variables, and inability to impute missingness in confounding variables), subject-level data from the identified regions associated with each air pollutant were imported into STATA (version 14; StataCorporation, College Station, TX, USA) for the following analysis.

Among children with available data on air pollution, neuroimaging, and cognitive function we performed multiple imputation of missing values of potential confounding variables using chained equations to generate 25 complete datasets (34). The percentage of missing values was relatively low and distributions in imputed datasets were similar to those observed (Supplemental Table S1). Children included in the analysis (n=783) were more likely to have mothers from a higher socioeconomic position compared to those that were not included, among children selected for the MRI sub-study (n=1,149) (Supplemental Table S2). This was also the case when we compared our study population to the not included children from the full cohort recruited in pregnancy (n=8,097) (Supplemental Table S3). We used inverse probability weighting to correct for lost to follow-up, i.e. to account for potential selection bias when including only participants with available data as compared to the full cohort recruited at pregnancy (35).

We used linear regression analyses to assess the associations between i) exposure to each air pollutant and global brain measures and ii) exposure to each air pollutant and the cortical thickness of each identified region in the QDEC analysis. Models were adjusted for all potential confounding variables described in the previous section.

Next we selected the tasks that assessed the cognitive function involved with each identified region based on the literature. We assessed whether both air pollution exposure and the cortical thinness of these regions were associated with the selected cognitive functions using adjusted negative binomial or linear regression models depending on the distribution of the outcome. We then applied causal mediation analysis providing estimation of the natural direct effect (NDE), the natural indirect effect (NIE), and the total effect (Supplemental Methods S2) (36). Briefly, we assessed the direct and indirect effects of air pollution exposure during fetal life on cognitive function. We tested whether part of the indirect effect was mediated by cortical thinness (Supplemental Figure S1). We used negative binomial regression for the outcome regression model and linear regression for the mediator regression model. Standard errors were calculated using bootstrapping. All models were adjusted for all potential confounding variables described in the previous

section. The total effect results as the product of the natural direct effect (NDE) and natural indirect effect (NIE). We also calculated the proportion mediated as incidence rate ratio $(IRR)^{NDE}(IRR^{NIE} - 1)/(IRR^{NDE}IRR^{NIE} - 1)$.

We performed sensitivity analysis of the association between air pollutants and the cortical thickness of each identified region in the whole-brain analysis: i) we restricted the analysis to those children without attention deficit hyperactivity disorder, pervasive developmental problems, dysregulation problems, and aggressive problems and ii) we restricted the analysis to those children from non-smoking mothers during pregnancy.

RESULTS

Participant characteristics of the study population are shown in Table 1 and Supplemental Table S4. Mean residential air pollution exposure during fetal life was 39.3μg/m^3 for NO_2 (range 25.3-73.3) and 20.2μg/m^3 for fine particles (range 16.8-28.1). Correlation between air pollutants was between 0.43 and 0.79 (Supplemental Table S5). Mothers exposed to higher air pollution levels during fetal life were more likely to have a higher level of education, to have a higher household income, and to be Dutch compared to those exposed to lower levels (Supplemental Table S6-9).

We did not find significant associations between air pollution exposure during fetal life and global brain volume measures (Table 2). Children exposed to higher particulate matter levels during fetal life had thinner cortices in several brain regions in both hemispheres (Figure 1). Sizes of associated brain regions varied between 532 and 2,995mm^2 (Supplemental Table S10). Mean thickness of these brain regions was between 2.31 and 3.17mm^2 (with a minimum thickness of 1.61 to 2.23mm^2 and a maximum thickness of 3.23 to 3.97mm^2). After adjusting for potential confounding variables, exposure to particulate matter levels remained strongly associated with thinner cortices of all identified regions (e.g. cerebral cortex of the precuneus region was 0.045mm thinner (95% Confidence Interval (CI) 0.028 to 0.062) for each 5μg/m^3 increase in fine particles) (Table 3). We observed similar results in the different sensitivity analysis (Supplemental Tables S11-12).

Based on the cognitive functions involved with each identified region, we selected the attention and executive functioning tasks for all regions except for the fusiform region where we selected the memory for faces tasks (Supplemental Methods S3). Fine particles exposure during fetal life was associated with a higher number of inhibition errors of the response set task (IRR 1.07; 95% CI 1.01 to 1.14 per each 5μg/m^3 increase in fine particles) (Table 4). No significant associations were observed for the other relationships. A thinner cortex in the precuneus region and the rostral middle frontal region was also associated with a higher number of inhibition errors of that tasks (IRR 1.32; 95% CI 1.00 to 1.77 per each 1mm decrease of the cortex in the precuneus region and IRR 1.69; 95% CI 1.09 to 2.61 per each 1mm decrease of the cortex in the rostral middle frontal region) (Table 5). We

Table 1. Participant characteristics and air pollution levels during fetal life

	Distribution		
Participant characteristics	**Percentage**	**Mean**	**(SD)**
Maternal education level			
Primary education	7.0		
Secondary education	44.8		
University education	48.2		
Paternal education level			
Primary education	5.7		
Secondary education	40.9		
University education	53.4		
Monthly household income			
<1,200€	14.1		
1,200€ - 2,000€	17.7		
>2,000€	68.1		
Maternal country of birth			
The Netherlands	65.2		
Cape Verde	4.7		
Morocco	4.7		
Surinam	6.5		
Turkey	4.5		
Other country of birth	14.5		
Paternal country of birth			
The Netherlands	72.7		
Cape Verde	2.6		
Morocco	1.9		
Surinam	5.0		
Turkey	3.4		
Other country of birth	14.4		
Maternal age (years)		30.7	(4.9)
Paternal age (years)		32.9	(5.3)
Family status (mono vs. biparental)	13.5		
Maternal parity (multi vs. nulliparous)	39.5		
Maternal smoking use during pregnancy			
Never	75.8		
Smoking use until pregnancy known	6.5		
Continued smoking use during pregnancy	18.2		
Maternal alcohol use during pregnancy			
Never	37.6		
Alcohol use until pregnancy know	14.3		
Continued alcohol use during pregnancy	48.1		
Maternal pre-pregnancy body mass index (kg/m^2)		24.6	(4.3)
Paternal pre-pregnancy body mass index (kg/m^2)		25.3	(3.3)

Table 1. (Continuation)

	Distribution		
Participant characteristics	**Percentage**	**Mean**	**(SD)**
Maternal height (cm)		168.6	(7.4)
Paternal height (cm)		182.9	(7.3)
Maternal overall psychological distress		0.3	(0.4)
Maternal intelligence quotient score		98.4	(13.9)
Air pollution levels during fetal life	**Median**	**(Min-Max)**	
NO_2 ($\mu g/m^3$)	39.3	(25.3-73.3)	
Fine particles ($\mu g/m^3$)	20.2	(16.8-28.1)	
Coarse particles ($\mu g/m^3$)	11.8	(9.2-17.8)	
Absorbance of fine particles ($10^{-5}\ m^{-1}$)	1.9	(1.2-3.6)	

Abbreviations: Max, maximum; Min, minimum; NO_2, nitrogen dioxide, SD, standard deviation.

finally found that the reduced cortical thickness in the precuneus and rostral middle frontal regions partially mediated the observed association between fine particles exposure during fetal life and the increase number of inhibition errors (natural indirect effect: IRR 1.01; 95% CI 1.00 to 1.02 per each 1mm decrease of the cortex in the precuneus region and in the rostral middle frontal region) (Figure 2). The proportion mediated through the reduced cortical thickness in each of the regions was estimated to be 15%.

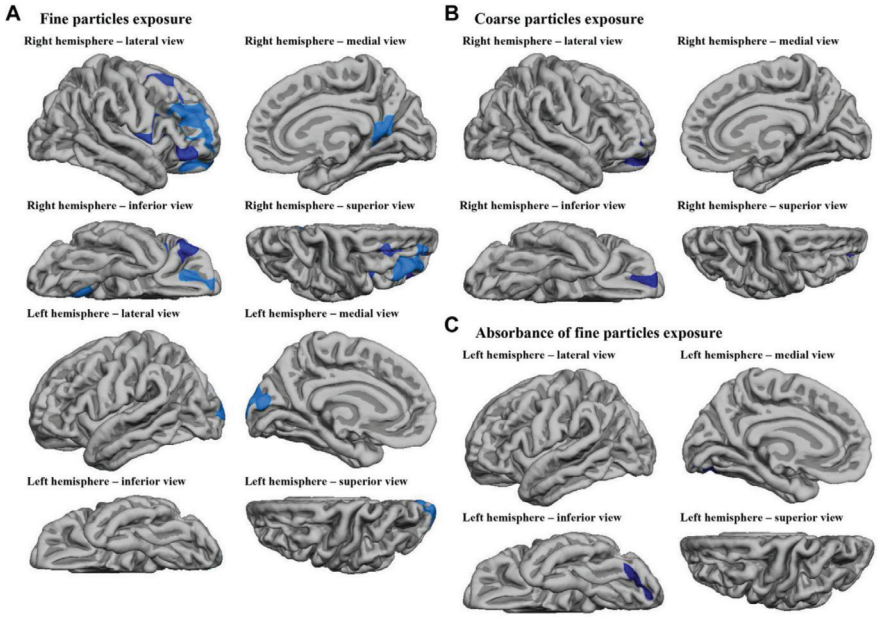
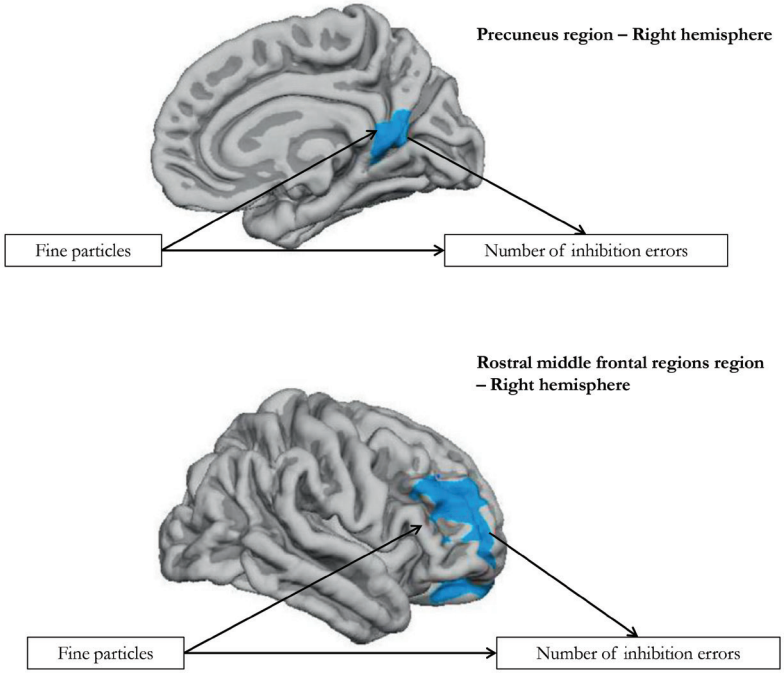


Figure 1. Differences in cortical thickness at 6-10 years of age associated with air pollution exposure during fetal life

The colored regions on the surface map represent brain regions that are thinner in relation to higher exposure to air pollution during fetal life in the right and left hemisphere (darker color indicates stronger association). Analyses were adjusted for child's sex and age. All brain regions survived the correction (Monte Carlo null-Z simulation with 10,000 iterations) for multiple comparisons ($p<.01$).



	IRR	(95% CI)
Natural direct effect	1.06	(1.00 to 1.12)
Natural indirect effect	1.01	(1.00 to 1.02)
Total effect	1.07	(1.00 to 1.13)

Figure 2. Causal mediation analyses between air pollution exposure during fetal life, cortical thickness (in mm) in precuneus and rostral middle frontal regions, and the number of inhibition errors of the response set task at 6-10 years of age

Abbreviations: CI, confidence interval; IRR, incidence risk ratio.

Incidence risk ratio (95% Confidence Interval) from negative binomial regression models adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol consumption, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry. As results for both causal mediation analyses were identical rounded to 2 decimal places, only one table is presented.

Table 2. Fully-adjusted association between air pollution exposure during fetal life and global brain volume measures at 6-10 years of age

	Coef.	(95% CI)[a]	p value
NO_2			
Total brain volume	124	(-1118 to 1375)	0.84
Cortical gray matter volume	-60	(-853 to 733)	0.88
Cortical white matter volume	199	(-287 to 685)	0.42
Subcortical gray matter volume	36	(-17 to 89)	0.18
Ventricular volume	4	(-57 to 64)	0.90
Fine particles			
Total brain volume	-3079	(-7790 to 1632)	0.20
Cortical gray matter volume	-2598	(-5583 to 387)	0.09
Cortical white matter volume	-268	(-2096 to 1559)	0.77
Subcortical gray matter volume	-60	(-258 to 138)	0.55
Ventricular volume	-96	(-323 to 131)	0.40
Coarse particles			
Total brain volume	-4868	(-10337 to 822)	0.09
Cortical gray matter volume	-3542	(-7059 to 8)	0.05
Cortical white matter volume	-1129	(-3215 to 1127)	0.34
Subcortical gray matter volume	-92	(-325 to 148)	0.46
Ventricular volume	-100	(-372 to 168)	0.45
Absorbance of fine particles			
Total brain volume	-2861	(-18745 to 24467)	0.79
Cortical gray matter volume	-2683	(-16377 to 11012)	0.70
Cortical white matter volume	5807	(-2566 to 14180)	0.17
Subcortical gray matter volume	418	(-497 to 1334)	0.36
Ventricular volume	-64	(-1108 to 979)	0.90

Abbreviations: CI, confidence interval; Coef, beta coefficient; NO_2, nitrogen dioxide.

[a]Beta coefficient (95% Confidence Interval) from linear regression model adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, marital status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry. Coefficients represent the differences in volumes (cm^3) per each increase of $10\mu g/m^3$ of NO_2, $5\mu g/m^3$ of fine particles, $5\mu g/m^3$ of coarse particles, and $10^{-5}m^{-1}$ of absorbance of fine particles.

Table 3. Fully-adjusted association between air pollution exposure during fetal life and cortical thickness (in mm) at 6-10 years of age

	Hemisphere	Size brain region (mm^2)	Coef.	(95% CI)[a]	p value
Fine particles exposure					
Precuneus region	Right	936	-0.045	(-0.062 to -0.028)	<.001
Pars opercularis region	Right	753	-0.024	(-0.033 to -0.014)	<.001
Pars orbitalis region	Right	651	-0.028	(-0.043 to -0.012)	.001
Rostral middle frontal region	Right	2,995	-0.029	(-0.041 to -0.018)	<.001
Superior frontal region	Right	722	-0.029	(-0.043 to -0.016)	<.001
Cuneus region	Left	843	-0.022	(-0.035 to -0.009)	.002
Coarse particles exposure					
Lateral orbitofrontal region	Right	565	-0.037	(-0.059 to -0.016)	.001
Absorbance of fine particles exposure					
Fusiform region	Left	532	-0.105	(-0.160 to -0.049)	<.001

Abbreviations: CI, confidence interval; Coef, beta coefficient.

[a]Beta coefficient (95% Confidence Interval) from linear regression model adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry. Coefficients represent the differences in thickness (mm) per each increase of $5\mu g/m^3$ of fine particles, $5\mu g/m^3$ of coarse particles, and $10^{-5}m^{-1}$ of absorbance of fine particles.

Table 4. Adjusted association between air pollution levels during fetal life and cognitive function at 6-10 years of age

	IRR	(95% CI)[a]	p value
Fine particles exposure			
Auditory attention task			
Correct responses	1.00	(0.99 to 1.01)	0.61
Commission errors	1.00	(0.89 to 1.16)	0.95
Omission errors	0.98	(0.92 to 1.03)	0.38
Inhibition errors	1.10	(0.63 to 1.93)	0.73
Response set task			
Correct responses	1.01	(1.00 to 1.02)	0.17
Commission errors	1.00	(0.96 to 1.04)	0.79
Omission errors	0.97	(0.94 to 1.00)	0.07
Inhibition errors	1.07	(1.01 to 1.14)	0.02
Coarse particles exposure			
Auditory attention task			
Correct responses	1.00	(0.99 to 1.01)	0.71
Commission errors	0.99	(0.87 to 1.13)	0.88
Omission errors	0.98	(0.92 to 1.05)	0.63
Inhibition errors	0.98	(0.55 to 1.76)	0.95
Response set task			
Correct responses	1.01	(0.99 to 1.02)	0.39
Commission errors	0.97	(0.92 to 1.02)	0.19
Omission errors	0.98	(0.94 to 1.02)	0.28
Inhibition errors	1.04	(0.97 to 1.12)	0.24
	Coef.	**(95% CI)[b]**	**p value**
Absorbance of fine particles exposure			
Memory for faces task	0.22	(-0.24 to 0.69)	0.34
Memory for faces delayed task	0.29	(-0.23 to 0.81)	0.27

Abbreviations: CI, confidence interval; Coef, beta coefficient; IRR, incidence rate ratio.

[a] Incidence rate ratio values (95% Confidence Interval) from negative binomial regression model or [b] beta coefficients (95% Confidence Interval) from linear regression model adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry.

Table 5. Adjusted association between thinner cortical thickness (in mm) and the total number of inhibitory numbers of the response set task at 6-10 years of age

	IRR	(95% CI)[a]	p value
Precuneus region	1.32	(1.00 to 1.77)	0.05
Pars opercularis region	0.83	(0.49 to 1.42)	0.49
Pars orbitalis region	1.16	(0.83 to 1.61)	0.38
Rostral middle frontal region	1.69	(1.09 to 2.61)	0.02
Superior frontal region	1.28	(0.89 to 1.86)	0.18

Abbreviations: CI, confidence interval; IRR, incidence rate ratio.

[a] Incidence rate ratio values (95% Confidence Interval) from negative binomial regression model adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry. CI denotes confidence interval, IRR denotes incidence risk ratio.

DISCUSSION

The present study suggests that particulate matter exposure during fetal life was associated with a thinner cortex in several brain regions and to an impaired inhibitory control in school-age children. The structural alterations in the precuneus and the rostral middle frontal regions partially mediated the association between fine particles exposure and impaired inhibitory control. No association was found between air pollution exposure and global brain volume measures.

Several epidemiological studies have found that air pollution exposure during fetal life was associated with lower cognitive function (11–14). However, very few studies have investigated which brain structural and functional alterations underlie these associations. Child cognitive function is the result of integration of functions of many different brain regions, and thus we did not have a priori hypothesis on which specific brain regions could be affected by air pollution exposure during fetal life. In our study we identified that some specific brain regions had thinner cortex in relation to air pollution exposure during fetal life. We do not have a hypothesis why air pollution exposure during fetal life is affecting the grey matter of specific brain regions instead of having a more wide-spread effect. One explanation would be that this is due to the different development of each brain region across adolescence. For example, cortical volume of the frontal lobe showed a relatively stable trajectory in late childhood and an accelerated thinning in adolescence, while decelerating trajectories with increasing age were seen for thickness in the parietal and occipital lobes (37). Further longitudinal studies are warranted to better understand the potential associations at different ages.

To date, only one small study assessed the relationship between air pollution exposure during fetal life and structural brain morphology in 40 children at seven to nine years old from New York City, taking also an exploratory approach as we did in our study (17). Peterson et al. did not find an association between personal polycyclic aromatic hydrocarbons exposure during the third trimester of pregnancy and any measure of cortical thickness. However, they found an association between higher personal polycyclic aromatic hydrocarbons exposure during the third trimester of pregnancy and a lower white matter surface, almost exclusively to the left hemisphere of the brain (17). In contrast with this previous study, we did not find a relationship between exposure to air pollutants during fetal life and white matter volume using a much larger sample of children at a similar age. As there is indication that white matter could be one of the brain structural affected by air pollution exposure during fetal life, future research should focus on white matter microstructure which could uncover deficits that are not apparent with simple white matter volumetric measures.

During pregnancy, the detoxification mechanisms of the developing fetus are still immature and the placenta grants only a partial protection against the entry of environmental toxicants (10, 9). Hence, when the mother is exposed to air pollution, air pollutants might alter the prenatal brain development as a result of oxidative stress and systemic inflammation leading to chronic neuroinflammation, microglia activation, and neuronal migration damage (8). Early disturbances in neuronal path finding, abnormalities in cell proliferation,

and differentiation eventually result in a thinner cortex during childhood. Although the prenatal period is considered particularly vulnerable period for brain development, the brain continues to develop until adolescence and postnatal air pollution exposure could also play a role on brain development (8, 11, 12). In the New York City study, they also explored the relationship between postnatal urinary polycyclic aromatic hydrocarbon metabolites and structural brain morphology not finding an association with cortical thickness but showing a lower white matter surface in dorsal prefrontal regions bilaterally (17). Two small studies including around 30 children at six to fourteen years old found that children living in Mexico City had lower white matter volumes and higher rates of subcortical prefrontal white matter hyperintensities compared to those living in a low polluted city of Mexico (18, 19). Again, white matter seems to be influenced by air pollution exposure. Furthermore, in 263 children aged eight to twelve from Barcelona, Spain, higher elemental carbon and NO_2 exposure at school was not associated with brain structure but associated with lower functional integration and segregation in key brain networks relevant to both inner mental processes and stimulus-driven mental operations (20). That study was the first to shown that air pollution exposure might also alter brain functionality which leads to a slower brain maturation. Overall, air pollution exposure to both prenatal and postnatal periods has shown to impair brain development. Further studies are needed to disentangle the specific brain alterations due to prenatal and postnatal air pollution exposure.

Interestingly, our study is the first study showing that fine particles exposure during fetal life was associated with an impaired inhibitory control in school-age children and that thinner cortex in the precuneus and the rostral middle frontal regions partially mediated this association. Inhibitory control, a key component of executive functions, regulates the self-control of resisting temptations and acting impulsively and the selective attention (38). Impaired inhibitory control has been related to several mental health problems such as addictive behaviors (39) or attention deficit hyperactivity disorder (40). The previous study carried out in New York City found that the white matter disruption partially mediated the association between prenatal polycyclic aromatic hydrocarbons exposure and a slower information processing speed in children (17). Therefore, we hypothesize that air pollution exposure during fetal life could lead to brain structural changes and these to specific cognitive delays.

In our study, mean residential NO_2 levels during fetal life were just at the EU limit of 40$\mu g/m^3$, with 45% of our population having higher levels. Regarding fine particles, mean residential levels were clearly below the EU limit of 25$\mu g/m^3$, with only 0.5% of our population above that limit (41). However, as we observed in our study brain development effects in relationship to fine particles levels below the current EU limit, as well as other studies have found relationships with several health endpoints including natural-cause mortality, cardiovascular and respiratory diseases, cognitive decline, and fetal growth development (1–7), we cannot warrant that this limit is safe. The World Health Organization set a lower limit of 10$\mu g/m^3$ for fine particles (42), and in our study we have all our population above this limit. Further health effect research needs to bring more insight into the safety of the current levels of air pollution in our cities.

The strengths of our study are the large number of study participants with imaging data, the prospective and longitudinal nature of the study, the detailed information of air pollution estimations at the individual level during the entire fetal period, and the availability of adjusting the imaging analysis for a large number of socioeconomic and lifestyle factors known to be associated with both air pollution exposure and brain development. Nevertheless, we cannot discard that our results might still be affected by residual confounding due to the unavailability of other relevant potential confounding variables. Another limitation of our study was that children with exposure and outcome data were more likely to have mothers from higher socioeconomic position than those without these data but recruited at the beginning of the cohort in early pregnancy, which could lead to selection bias in our results. To reduce this possible selection bias, we used advanced statistical methods including multiple imputation combined with inverse probability weighting. However, we could have missed variables related to this potential selection bias that that would have a stronger effect in the results. In addition, there is the possibility of chance findings in the observed associations in the current study. The imaging analysis was corrected for multiple testing of the whole-brain, vertex-wise statistics as we have many vertices per hemisphere. However, the causal mediation analysis was hypothesis-driven and we decided not to correct for multiple testing as this could increase type 2 error (43, 44). Instead, our conclusions were based on the general patterns of associations observed in the study. This has been the first study showing that brain structural alterations seem to partially mediate the association between air pollution exposure during fetal life and an impaired cognitive function. Further studies are warranted to replicate these findings and better understand this association.

CONCLUSIONS

We showed that fine particles exposure during fetal life was both related to child brain structural alterations of the cerebral cortex and to an impairment of an essential executive function such as inhibitory control. Moreover, the identified structural alterations in two specific regions partially mediated the association between fine particles exposure during fetal life and the impaired inhibitory control. Such cognitive impairment at early ages could have significant long-term consequences including increased risk of mental disorders, low academic achievement, and diminished economic productivity (38), in particular due to the ubiquity of the exposure.

Conflict of interest Disclosures: None reported

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PAPER III – SUPPLEMENTARY MATERIAL

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

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Methods S1. Description of the back-extrapolation methodology of the air pollution levels

We used a back-extrapolation procedure to estimate the levels back in time during each fetal period of each woman in order to assess if fetal period is a relevant exposure period (1, 2). The estimated yearly concentrations (Cyearly,i) at each home address i were combined with time-specific measurements from seven available routine background monitoring network sites by averaging the daily concentrations during 1) the year corresponding to the LUR yearly concentration (Cyearly) and 2) each fetal period pi considered (Cp_i). The ratio Cp_i/Cyearly constituted the temporal component of the model. For each pollutant, the concentration (Cp_i, i) estimated at the home address i during the fetal period for woman i was estimated as the product of the temporal (Cp_i/Cyearly) and spatial (Cyearly, i) components. In cases when air quality monitoring data from background station was unavailable for a given pollutant, we used measurements for another pollutant during the same time period as a replacement; the choice of that pollutant used to back-extrapolate another pollutant was based on an extensive study of temporal correlations between pollutants simultaneously available (i.e. PM_{10} was used as a proxy for $PM_{2.5}$ and back smoke as a proxy for $PM_{2.5}$ absorbance). We accounted for change of home address during the whole fetal period since the date of moving and new address was available.

Methods S2. Description of the causal mediation analysis

The causal mediation analysis provides a better understanding of the causal chain by which an independent variable (X) influences a dependent variable (Y) through a mediator (M). Consistent with its conceptual definition (3), this involves sequential testing of the following: i) the effect of the exposure (X) on the outcome (Y); ii) the effect of the exposure e (X) on the mediator (M); iii) the effect of the mediator (M) on the outcome (Y) controlling for the exposure (X), and iv) the effect of the exposure (X) on the outcome (Y) controlling for the mediator (M). The causal mediation analysis provides estimation of the natural direct effect (NDE), the natural indirect effect (NIE), and the total effect (3). The natural direct effect (NDE) expresses how much the outcome (Y) would change if the exposure (X) is set at a level *a=1* to level *a=0* but for each individual the mediator (M) is kept at the level it would have taken in the absence of the exposure. The natural indirect effect (NIE) expresses how much the outcome (Y) would change on average if the exposure (X) is controlled at level a=1, but the mediator (M) is changed from the level it would take if a=0 to the level it would take if a=1. The total effect can be defined as how much the outcome (Y) would change overall for a change in the exposure (X) from level *a=0* to level *a=1*.

In our study, we applied causal mediation analysis to assess the direct and indirect effects of air pollution exposure during fetal life (X) on cognitive function (Y) where we tested whether part of the indirect effect was mediated by cortical thinness (M) (Figure S1). We used negative binomial regression for the outcome regression model and linear regression for the mediator regression model. Standard errors were calculated using bootstrapping. All models were adjusted for all potential confounding variables described in the section "Potential confounding variables" of the manuscript. The total effect results as the product

of the natural direct effect (NDE) and natural indirect effect (NIE). We also calculated the proportion mediated as incidence rate ratio $(IRR)^{NDE}(IRR^{NIE} - 1)/(IRR^{NDE}IRR^{NIE} - 1)$.

Methods S3. Cognitive function tests selected based on the identified regions

In the first analysis, we found that higher particulate matter levels during fetal life were associated with thinner cortices in specific regions of the frontal, parietal and occipital brain regions (Table 3). Post-hoc, we went back to the literature to find out in which cognitive processes these regions were involved. The frontal brain regions and the (pre)cuneus are known to be involved in attention and executive functions (4, 5) while the fusiform gyrus is known to be involved in the face perception, object recognition, and memory (6). Therefore, we selected two specific tasks of the NEPSY-II test for the mediation analysis: the attention and executive functioning task and the memory for faces task.

Attention and executive functioning task

Children were assessed with two different tasks from the attention and executive functioning domain of the NEPSY-II: auditory attention task and response set task (7–9). The auditory attention task was administered first. It is designed to assess selective auditory attention and the ability to sustain it (vigilance). Selective attention refers to the ability to focus on a specific task while suppressing irrelevant stimuli. Sustained attention refers to the ability to attend to a task for a long(er) period of time. In the auditory attention task, the children were presented with recording of a long list of color words and other words and were asked to only respond to the word "red" by touching the red circle on the sheet in front of them. The sheet also contained a blue, black, and yellow circle, but these circles had to be ignored. Touching the red circle within two seconds indicated a correct response.

The response set task was then administered. This task taps into response inhibition and working memory. Inhibition is the ability to suppress (automatic) behavior. Working memory is required to keep information actively in mind for as long as needed to complete a task. In this task, children must respond to the word "red" by touching the yellow circle, respond to "yellow" by touching the red circle, and lastly, respond to the word "blue" by touching the blue circle. All of the other colors or words should be ignored. Touching the correct circle within two seconds indicates a correct response. Touching another color is incorrect, as is having delayed response (not within a 2 seconds interval).

For each task, four scores were calculated: total number of correct responses, total number of commission errors (i.e. the number of times that the child responded erroneously to a non-target), total number of omission errors (i.e. the number of target to which the children failed to respond), and inhibition errors (i.e. the number of times that the child responded to a color word inappropriately; in other words, fails to inhibit an inappropriate response).

Memory for faces task

Children were assessed with two different tasks from the memory and learning domain of the NEPSY-II: memory for faces task and memory for faces delayed task (7–9). The memory for faces test is designed to assess encoding of facial features, as well as face discrimination and recognition. The child was first presented with multiple series of three faces and was asked to look closely at each face (for five seconds). The child was then provided with another set of three faces and was asked which face he or she had seen before. Immediate recall is the skill to retrieve information from memory immediately after learning.

The memory for faces delayed task is designed to assess long-term memory for faces. This task was assessed after a delay period of 15 to 25 minutes and measured the ability to retrieve information after a longer period of time.

For both tasks, all presented faces showed a neutral expression. A total correct score was calculated for both tasks.

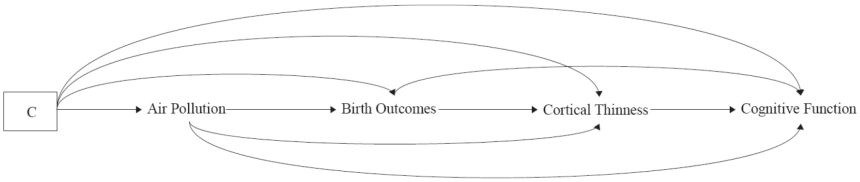

Figure S1. Direct Acyclic Graph

C denotes all the potential confounding variables in the relationship between air pollution exposure in fetal life and cognitive function in childhood, such as SES, parental lifestyle and ethnicity. This theoretical selection of confounders was reflected as completely as the data availability allowed. In our study we included: parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child genetic ancestry. Additionally, the models were adjusted for child's sex and child's age at the MRI session. The box indicates the conditioning on the potential confounders. Solid arrows represent existing pathways indicating thereby the direction of the associations.

Table S1. Distribution of participant characteristics in observed and imputed datasets

	Observed dataset[a]		Imputed datasets[a]		% data imputed
Maternal education level					2.8
Primary education	7.0		7.4		
Secondary education	44.8		45.1		
University education	48.2		47.4		
Paternal education level					26.6
Primary education	5.7		9.2		
Secondary education	40.9		43.9		
University education	53.4		46.9		
Monthly household income					10.6
<1,200€	14.1		15.8		
1,200€ - 2,000€	17.7		18.4		
>2,000€	68.1		65.8		
Maternal country of birth					1.4
The Netherlands	65.2		64.7		
Cape Verde	4.7		4.8		
Morocco	4.7		4.8		
Surinam	6.5		6.6		
Turkey	4.5		4.7		
Other country of birth	14.5		14.5		
Paternal country of birth					20.8
The Netherlands	72.7		66.3		
Cape Verde	2.6		4.2		
Morocco	1.9		3.1		
Surinam	5.0		6.7		
Turkey	3.4		4.9		
Other country of birth	14.4		14.8		
Maternal age (years)	---		---		0.0
Paternal age (years)	32.9	(5.3)	32.8	(5.5)	18.3
Family status (mono vs. biparental)	13.5		13.9		2.8
Maternal parity (multi vs. nulliparous)	39.5		39.4		0.4
Maternal smoking use during pregnancy					7.2
Never	75.4		74.8		
Smoking use until pregnancy known	6.5		6.6		
Continued smoking use during pregnancy	18.1		18.6		
Maternal alcohol use during pregnancy					6.9
Never	37.6		38.0		
Alcohol use until pregnancy known	14.3		14.3		
Continued alcohol use during pregnancy	48.1		47.7		
Maternal pre-pregnancy body mass index (kg/m^2)	---		---		0.0
Paternal pre-pregnancy body mass index (kg/m^2)	25.3	(3.3)	25.3	(3.4)	18.3
Maternal height (cm)	---		---		0.0

Table S1. (Continued)

	Observed dataset[a]		Imputed datasets[a]		% data imputed
Paternal height (cm)	182.9	(7.3)	182.3	(7.5)	18.3
Maternal overall psychological distress	0.3	(0.4)	0.3	(0.4)	12.1
Maternal intelligence quotient score	98.4	(13.9)	98.1	(13.9)	5.4

[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables

Table S2. Comparison of participant characteristics between included and not included subjects in the study among the 1,932 subjects selected for the MRI sub-study

	Included[a] (n=783)	Not included[a] (n=1,149)	p value[b]
Maternal education level			<.001
Primary education	7.0	11.0	
Secondary education	44.8	50.5	
University education	48.2	38.5	
Paternal education level			0.006
Primary education	5.7	8.5	
Secondary education	40.9	46.4	
University education	53.4	45.1	
Monthly Household income			0.001
<1,200€	14.1	19.5	
1,200€ -2,000€	17.7	21.3	
>2,000€	68.1	59.2	
Maternal country of birth			<.001
The Netherlands	65.2	53.9	
Cape Verde	4.7	4.8	
Morocco	4.7	4.8	
Surinam	6.5	7.8	
Turkey	4.5	9.0	
Other country of birth	14.5	19.7	
Paternal country of birth			0.02
The Netherlands	72.7	66.2	
Cape Verde	2.6	2.7	
Morocco	1.9	3.5	
Surinam	5.0	6.3	
Turkey	3.4	6.7	
Other country of birth	14.4	14.5	

Table S2. (Continued)

	Included[a] (n=783)		Not included[a] (n=1,149)		p value[b]
Maternal age (years)	30.7	(4.9)	29.6	(5.2)	<.001
Paternal age (years)	32.9	(5.3)	32.7	(5.8)	0.42
Family status (mono vs. biparental)	13.5		15.8		0.18
Maternal parity (multi vs. nulliparous)	39.5		40.3		0.87
Maternal smoking use during pregnancy					0.02
Never	75.4		69.1		
Smoking use until pregnancy known	6.5		7.9		
Continued smoking use during pregnancy	18.2		22.9		
Maternal alcohol use during pregnancy					<.001
Never	37.6		46.6		
Alcohol use until pregnancy known	14.3		15.0		
Continued alcohol use during pregnancy	48.1		38.3		
Maternal pre-pregnancy body mass index (kg/m^2)	24.6	(4.3)	24.9	(4.6)	0.23
Paternal pre-pregnancy body mass index (kg/m^2)	25.3	(3.3)	25.1	(3.5)	0.25
Maternal height (cm)	168.6	(7.4)	167.7	(7.5)	0.01
Paternal height (cm)	182.9	(7.3)	181.9	(8.1)	0.02
Maternal overall psychological distress	0.3	(0.4)	0.4	(0.5)	<.001
Maternal intelligence quotient score	98.4	(13.9)	94.2	(14.7)	<.001

[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.
[b]$\chi 2$ test for categorical variables and t-student test for continuous variables

Table S3. Comparison of participant characteristics between included and not included subjects in the study among the 8,879 subjects recruited in the full cohort in pregnancy

	Included[a] (n=783)		Not included[a] (n=8,097)		p value[b]
Maternal education level					<.001
Primary education	7.0		12.1		
Secondary education	44.8		46.6		
University education	48.2		41.3		
Paternal education level					0.05
Primary education	5.7		8.6		
Secondary education	40.9		41.1		
University education	53.4		50.3		
Monthly Household income					<.001
<1,200€	14.1		21.6		
1,200€ - 2,000€	17.7		18.7		
>2,000€	68.1		59.7		
Maternal country of birth					<.001
The Netherlands	65.2		47.8		
Cape Verde	4.7		4.1		
Morocco	4.7		6.9		
Surinam	6.5		9.3		
Turkey	4.5		9.6		
Other country of birth	14.5		22.3		
Paternal country of birth					<.001
The Netherlands	72.7		60.2		
Cape Verde	2.6		2.5		
Morocco	1.9		4.5		
Surinam	5.0		7.0		
Turkey	3.4		7.2		
Other country of birth	14.4		18.5		
Maternal age (years)	30.7	(4.9)	29.5	(5.3)	<.001
Paternal age (years)	32.9	(5.3)	32.7	(5.8)	0.30
Family status (mono vs. biparental)	13.5		14.9		0.30
Maternal parity (multi vs. nulliparous)	39.5		44.9		0.005
Maternal smoking use during pregnancy					0.14
Never	75.4		74.4		
Smoking use until pregnancy known	6.5		8.5		
Continued smoking use during pregnancy	18.1		17.1		
Maternal alcohol use during pregnancy					<.001
Never	37.6		51.2		

Table S3. (Continued)

	Included[a] (n=783)		Not included[a] (n=8,097)		p value[b]
Alcohol use until pregnancy known	14.3		13.6		
Continued alcohol use during pregnancy	48.1		35.2		
Maternal pre-pregnancy body mass index (kg/m^2)	24.6	(4.3)	24.9	(4.6)	0.08
Paternal pre-pregnancy body mass index (kg/m^2)	25.3	(3.3)	25.3	(3.5)	0.78
Maternal height (cm)	168.6	(7.4)	167.0	(7.4)	<.001
Paternal height (cm)	182.9	(7.3)	181.4	(8.0)	<.001
Maternal overall psychological distress	0.3	(0.4)	0.3	(0.4)	0.24
Maternal intelligence quotient score	98.4	(13.9)	95.3	(15.5)	<.001

[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.
[b]$\chi 2$ test for categorical variables and t-student test for continuous variables

Table S4. Global brain volume measures (in mm) in children at 6-10 years of age

	Mean	(SD)	Minimum	Percentile 25	Median	Percentile 75	Maximum
Total brain volume	1146063	-121154	709551	1062833	1141372	1227930	1549471
Cortical gray matter volume	551350	-65306	300283	508679	551737	597360	746402
Cortical white matter volume	381212	-47090	227365	347471	379598	412231	565693
Subcortical gray matter volume	61961	-4940	45762	58555	61801	65086	77729
Ventricular volume	11119	-5045	3752	7700	9871	13240	39891

Table S5. Spearman correlations between air pollution levels during fetal life

	NO_2	Fine particles	Coarse particles	Absorbance of fine particles
NO_2	1.00			
Fine particles	0.43	1.00		
Coarse particles	0.66	0.68	1.00	
Absorbance of fine particles	0.79	0.69	0.75	1.00

Abbreviation: NO_2, nitrogen dioxide

Table S6. Participant characteristics according to NO_2 levels during fetal life

	NO_2 levels (μg/m³)						
	Low (<37.1)[a]		Medium (37.1-41.7)[a]		High (>41.7)[a]		p value[b]
Maternal education level							0.24
Primary education	7.8		8.7		4.4		
Secondary education	47.3		43.3		43.8		
University education	44.9		48.0		51.8		
Paternal education level							0.02
Primary education	4.1		10.2		3.0		
Secondary education	44.3		36.0		42.2		
University education	51.6		53.8		54.8		
Monthly household income							0.3
<1,200€	16.5		16.5		9.4		
1,200€ - 2,000€	16.5		19.0		17.6		
>2,000€	67.0		64.6		73.0		
Maternal country of birth							0.24
The Netherlands	69.0		58.5		68.0		
Cape Verde	5.4		6.6		2.0		
Morocco	4.3		6.2		3.5		
Surinam	6,.2		6.6		6.6		
Turkey	3.5		4.7		5.5		
Other country of birth	11.6		17.4		14.5		
Paternal country of birth							0.55
The Netherlands	76.0		63.4		78.7		
Cape Verde	2.9		3.4		1.4		
Morocco	1.5		3.4		0.9		
Surinam	6.4		6.4		2.4		
Turkey	2.9		3.9		3.3		
Other country of birth	10.3		19.5		13.3		
Maternal age (years)	30.3	(5.0)	30.6	(5.2)	31.3	(4.6)	0.04
Paternal age (years)	32.9	(5.3)	32.8	(5.4)	33.1	(5.2)	0.89
Family status (mono vs. biparental)	13.0		14.4		13.1		0.88
Maternal parity (multi vs. nulliparous)	39.8		41.2		37.5		0.90
Maternal smoking use during pregnancy							0.83
Never	77.3		75.3		73.4		
Smoking use until pregnancy known	6.1		5.8		7.6		
Continued smoking use during pregnancy	16.6		18.9		19.0		
Maternal alcohol use during pregnancy							0.48
Never	39.3		36.9		36.6		
Alcohol use until pregnancy known	11.3		14.3		17.2		
Continued alcohol use during pregnancy	49.4		48.8		46.2		
Maternal pre-pregnancy BMI (kg/m²)	24.7	(4.4)	24.7	(4.4)	24.5	(4.1)	0.84
Paternal pre-pregnancy BMI (kg/m²)	25.7	(3.4)	25.3	(3.5)	25.0	(3.2)	0.11
Maternal height (cm)	169.4	(6.9)	167.6	(7.4)	168.7	(7.7)	0.03

Table S6. (Continued)

	NO_2 levels (μg/m³)						
	Low (<37.1)[a]		Medium (37.1-41.7)[a]		High (>41.7)[a]		p value[b]
Paternal height (cm)	183.5	(7.2)	182.1	(7.9)	182.9	(6.7)	0.12
Maternal overall psychological distress	0.3	(0.3)	0.3	(0.5)	0.3	(0.4)	0.16
Maternal intelligence quotient score	98.8	(14.2)	97.6	(13.5)	98.7	(13.8)	0.55

Abbreviation: BMI, body mass index; NO_2, nitrogen dioxide.
[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.
[b]$\chi 2$ test for categorical variables and one-way ANOVA test for continuous variables

Table S7. Participant characteristics according to fine particles levels during fetal life

	Fine particles levels (μg/m³)			
	Low (<19.7)[a]	Medium (19.7-21.0)[a]	High (>21.0)[a]	p value[b]
Maternal education level				0.05
Primary education	10.8	5.1	5.1	
Secondary education	41.4	44.4	48.6	
University education	47.8	50.6	46.2	
Paternal education level				0.41
Primary education	8.0	3.6	5.7	
Secondary education	38.0	42.0	42.5	
University education	54.0	54.4	51.8	
Monthly household income				0.003
<1,200€	20.7	13.0	8.7	
1,200€ - 2,000€	13.8	18.0	21.4	
>2,000€	65.5	69.0	69.9	
Maternal country of birth				0.8
The Netherlands	64.5	64.5	66.5	
Cape Verde	7.8	3.5	2.7	
Morocco	5.1	5.0	3.9	
Surinam	7.0	7.3	5.1	
Turkey	3.9	5.4	4.3	
Other country of birth	11.7	14.3	17.5	
Paternal country of birth				0.25
The Netherlands	69.9	73.5	74.7	
Cape Verde	6.0	0.5	1.4	
Morocco	1.0	3.0	1.8	
Surinam	6.5	4.4	4.1	
Turkey	4.0	3.4	2.8	

Table S7. (Continued)

	Fine particles levels (μg/m³)						
	Low (<19.7)[a]		**Medium (19.7-21.0)[a]**		**High (>21.0)[a]**		**p value[b]**
Other country of birth	12.6		15.2		15.2		
Maternal age (years)	30.9	(5.1)	30.8	(4.9)	30.5	(4.8)	0.57
Paternal age (years)	33.1	(5.2)	33.0	(5.2)	32.7	(5.5)	0.79
Family status (mono vs. biparental)	15.6		12.8		12.2		0.50
Maternal parity (multi vs. nulliparous)	42.4		35.0		40.9		0.03
Maternal smoking use during pregnancy							0.011
Never	74.6		77.9		73.7		
Smoking use until pregnancy known	10.2		5.8		3.6		
Continued smoking use during pregnancy	15.2		16.3		22.7		
Maternal alcohol use during pregnancy							0.5
Never	36.3		38.0		38.4		
Alcohol use until pregnancy known	10.1		15.7		16.8		
Continued alcohol use during pregnancy	53.6		46.3		44.8		
Maternal pre-pregnancy body mass index (kg/m²)	24.5	(4.4)	24.6	(4.3)	24.8	(4.2)	0.66
Paternal pre-pregnancy body mass index (kg/m²)	25.8	(3.5)	25.0	(3.2)	25.2	(3.3)	0.07
Maternal height (cm)	168.1	(7.2)	168.6	(7.4)	168.9	(7.6)	0.45
Paternal height (cm)	182.7	(6.6)	182.9	(7.6)	183.0	(7.6)	0.87
Maternal overall psychological distress	0.3	(0.4)	0.3	(0.4)	0.3	(0.4)	0.61
Maternal intelligence quotient score	98.4	(15.0)	98.8	(13.5)	97.9	(13.1)	0.77

[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.

[b]$\chi 2$ test for categorical variables and one-way ANOVA test for continuous variables

Table S8. Participant characteristics according to coarse particles levels during fetal life

	Coarse particles levels (μg/m³)						
	Low (<11.3)[a]		**Medium (11.3-12.4)[a]**		**High (>12.4)[a]**		**p value[b]**
Maternal education level							0.04
Primary education	10.2		6.3		4.3		
Secondary education	43.9		48.6		41.9		
University education	45.9		45.1		53.8		
Paternal education level							0.27
Primary education	8.5		4.9		3.9		
Secondary education	40.2		43.7		38.9		
University education	51.3		51.4		57.2		
Monthly household income							<.001
<1,200€	21.1		16.0		5.5		
1,200€ - 2,000€	17.2		18.2		17.7		
>2,000€	61.6		65.8		76.8		
Maternal country of birth							0.007
The Netherlands	65.0		60.3		70.2		
Cape Verde	7.0		5.1		1.9		
Morocco	4.3		7.4		2.3		
Surinam	6.6		8.2		4.7		
Turkey	4.7		5.4		3.5		
Other country of birth	12.5		13.6		17.4		
Paternal country of birth							0.008
The Netherlands	69.7		72.6		75.7		
Cape Verde	5.0		2.0		0.9		
Morocco	2.0		3.0		0.9		
Surinam	6.5		7.6		1.4		
Turkey	3.5		4.6		2.3		
Other country of birth	13.3		10.2		18.8		
Maternal age (years)	30.3	(5.1)	30.8	(5.2)	31.0	(4.4)	0.28
Paternal age (years)	32.7	(5.6)	33,.2	(5.4)	33.0	(5.0)	0.67
Family status (mono vs. biparental)	16.1		13.9		10.6		0.19
Maternal parity (multi vs. nulliparous)	39.1		42.5		36.9		0.61
Maternal smoking use during pregnancy							0.007
Never	72.4		79.2		74.6		
Smoking use until pregnancy known	10.7		5.1		3.6		
Continued smoking use during pregnancy	16.9		15.7		21.8		
Maternal alcohol use during pregnancy							0.08
Never	39.3		41.1		32.5		
Alcohol use until pregnancy known	11.9		11.9		18.9		
Continued alcohol use during pregnancy	48.8		47.0		48.6		
Maternal pre-pregnancy body mass index (kg/m²)	24.3	(4.0)	25.1	(4.7)	24.6	(4.1)	0.09

Table S8. (Continued)

	Coarse particles levels (µg/m³)						
	Low (<11.3)[a]		Medium (11.3-12.4)[a]		High (>12.4)[a]		p value[b]
Paternal pre-pregnancy body mass index (kg/m²)	25.4	(3.4)	25.2	(3.5)	25.4	(3.1)	0.87
Maternal height (cm)	168.7	(7.5)	168.4	(7.0)	168.5	(7.6)	0.89
Paternal height (cm)	182.8	(6.9)	183.0	(7.6)	182.9	(7.4)	0.96
Maternal overall psychological distress	0.3	(0.4)	0.3	(0.4)	0.3	(0.4)	0.39
Maternal intelligence quotient score	98.6	(14.5)	98.0	(14.0)	98.5	(13.2)	0.88

[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.
[b]$\chi 2$ test for categorical variables and one-way ANOVA test for continuous variables

Table S9. Participant characteristics according to absorbance of fine particles levels during fetal life

	Absorbance of fine particles levels (µg/m³)			
	Low (<1.8)[a]	Medium (1.8-2.0)[a]	High (>2.0)[a]	p value[b]
Maternal education level				0.16
Primary education	9.1	7.4	4.4	
Secondary education	47.2	41.8	45.4	
University education	43.7	50.8	50.2	
Paternal education level				0.40
Primary education	7.9	3.8	5.5	
Secondary education	38.9	44.6	39.2	
University education	53.2	51.6	55.3	
Monthly household income				0.08
<1,200€	18.6	14.1	9.8	
1,200€ - 2,000€	18.2	17.9	17.0	
>2,000€	63.2	67.9	73.2	
Maternal country of birth				0.08
The Netherlands	63.4	65.3	66.8	
Cape Verde	7.0	3.9	3.1	
Morocco	6.6	4.6	2.7	
Surinam	8.6	5.4	5.5	
Turkey	2.7	5.8	5.1	
Other country of birth	11.7	15.1	16.8	
Paternal country of birth				0.31
The Netherlands	70.8	73.1	74.2	

Table S9. (Continued)

	Absorbance of fine particles levels (μg/m³)						
	Low (<1.8)[a]		**Medium (1.8-2.0)[a]**		**High (>2.0)[a]**		**p value[b]**
Cape Verde	4.5		1.5		1.8		
Morocco	2.5		2.0		1.4		
Surinam	6.9		5.0		3.2		
Turkey	3.5		2.5		4.2		
Other country of birth	11.8		15.9		15.2		
Maternal age (years)	30.1	(5.1)	31.2	(4.8)	30.8	(4.8)	0.04
Paternal age (years)	32.7	(5.1)	33.0	(5.7)	33.1	(5.2)	0.75
Family status (mono vs. biparental)	14.2		14.1		12.3		0.78
Maternal parity (multi vs. nullipa-rous)	40.4		41.9		36.2		0.30
Maternal smoking use during pregnancy							0.25
Never	77.8		76.1		72.2		
Smoking use until pregnancy known	7.8		5.8		5.8		
Continued smoking use during pregnancy	14.4		18.1		22.0		
Maternal alcohol use during pregnancy							0.33
Never	39.5		34.8		38.4		
Alcohol use until pregnancy known	10.7		16.0		16.1		
Continued alcohol use during pregnancy	49.8		49.2		45.5		
Maternal pre-pregnancy body mass index (kg/m²)	24.8	(4.4)	24.7	(4.6)	24.4	(3.9)	0.58
Paternal pre-pregnancy body mass index (kg/m²)	25.5	(3.4)	25.0	(3.2)	25.5	(3.4)	0.28
Maternal height (cm)	168.5	(7.2)	168.1	(7.0)	169.1	(7.9)	0.32
Paternal height (cm)	182.7	(7.1)	183.1	(7.4)	182.8	(7.5)	0.84
Maternal overall psychological distress	0.3	(0.5)	0.3	(0.4)	0.3	(0.4)	0.12
Maternal intelligence quotient score	98.5	(14.8)	98.3	(13.2)	98.3	(13.6)	0.99

[a]Values are percentages for the categorical variables and mean (standard deviation) for the continuous variables.
[b]$\chi 2$ test for categorical variables and one-way ANOVA test for continuous variables

Table S10. Thickness (in mm) of the identified thinner brain regions in relation to higher exposure to air pollution during fetal life

	Hemisphere	Size (mm^2)	Mean	(SD)	Minimum	Percentile 25	Median	Percentile 75	Maximum
Precuneus region	Right	936	3.14	(0.32)	1.61	3.01	3.22	3.36	3.97
Pars opercularis region	Right	753	3.00	(0.19)	2.23	2.88	3.02	3.14	3.46
Pars orbitalis region	Right	651	2.92	(0.30)	2.02	2.73	2.93	3.12	3.71
Rostral middle frontal region	Right	2995	2.73	(0.22)	2.00	2.60	2.76	2.89	3.23
Superior frontal region	Right	722	2.63	(0.27)	1.85	2.43	2.65	2.83	3.31
Cuneus region	Left	843	2.31	(0.25)	1.67	2.14	2.29	2.46	3.28
Lateral orbitofrontal region	Right	565	2.82	(0.33)	1.86	2.59	2.83	3.06	3.88
Fusiform region	Left	532	2.37	(0.24)	1.62	2.21	2.37	2.51	3.42

Abbreviations: SD, standard deviation

Table S11. Adjusted association between air pollution exposure during fetal life and cortical thickness (in mm) at 6-10 years of age restricting to those children without attention deficit hyperactivity, pervasive developmental, dysregulation, and aggressive problems

	Hemisphere	Size brain region (mm^2)	Coef.	(95% CI)[a]	p value
Fine particles exposure					
Precuneus region	Right	936	-0.045	(-0.062 to -0.028)	<.001
Pars opercularis region	Right	753	-0.024	(-0.033 to -0.014)	<.001
Pars orbitalis region	Right	651	-0.028	(-0.043 to -0.012)	.001
Rostral middle frontal region	Right	2,995	-0.029	(-0.041 to -0.018)	<.001
Superior frontal region	Right	722	-0.029	(-0.043 to -0.016)	<.001
Cuneus region	Left	843	-0.022	(-0.035 to -0.009)	.002
Coarse particles exposure					
Lateral orbitofrontal region	Right	565	-0.037	(-0.059 to -0.016)	.001
Absorbance of fine particles exposure					
Fusiform region	Left	532	-0.105	(-0.160 to -0.049)	<.001

Abbreviations: CI, confidence interval; Coef, beta coefficient.

[a] Beta coefficient (95% Confidence Interval) from linear regression model adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry. Coefficients represent the differences in thickness (mm) per each increase of $5\mu g/m^3$ of fine particles, $5\mu g/m^3$ of coarse particles, and $10^{-5}m^{-1}$ of absorbance of fine particles.

Table S12. Adjusted association between air pollution exposure during fetal life and cortical thickness (in mm) at 6-10 years of age restricting to those children from non-smoking mothers during pregnancy

	Hemisphere	Size brain region (mm^2)	Coef.	(95% CI)[a]	p value
Fine particles exposure					
Precuneus region	Right	936	-0.048	(-0.065 to -0.032)	<.001
Pars opercularis region	Right	753	-0.026	(-0.035 to -0.016)	<.001
Pars orbitalis region	Right	651	-0.026	(-0.041 to -0.011)	<.001
Rostral middle frontal region	Right	2,995	-0.028	(-0.040 to -0.017)	<.001
Superior frontal region	Right	722	-0.027	(-0.041 to -0.013)	<.001
Cuneus region	Left	843	-0.016	(-0.029 to -0.003)	.016
Coarse particles exposure					
Lateral orbitofrontal region	Right	565	-0.042	(-0.063 to -0.022)	<.001
Absorbance of fine particles exposure					
Fusiform region	Left	532	-0.082	(-0.136 to -0.029)	.003

Abbreviations: CI, confidence interval; Coef, beta coefficient.

[a] Beta coefficient (95% Confidence Interval) from linear regression model adjusted for parental educational levels, monthly household income, parental countries of birth, parental ages, maternal prenatal smoking, maternal prenatal alcohol use, parental body mass indexes and heights, maternal parity, family status, maternal psychological distress, maternal intelligence quotient, and child sex, age, and genetic ancestry. Coefficients represent the differences in thickness (mm) per each increase of $5\mu g/m^3$ of fine particles, $5\mu g/m^3$ of coarse particles, and $10^{-5}m^{-1}$ of absorbance of fine particles.

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Paper IV

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

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ABSTRACT

Background. Studies investigating the relationship between exposure to air pollution and brain development using magnetic resonance images are emerging. However, most studies have focused only on prenatal exposures, and have included a limited selection of pollutants. Here, we aim to expand the current knowledge by studying fetal and childhood exposure to a wider selection of pollutants, and brain morphology in preadolescents.

Methods. We used data from 3,133 preadolescents from a birth cohort from Rotterdam, the Netherlands (enrollment: 2002-2006). Concentrations of nitrogen oxides, coarse, fine, and ultrafine particles, and composition of fine particles were estimated for participant's home addresses from fetal life and childhood, using land use regression models. Structural brain images were obtained at age 9-12 years. We assessed the relationships between air pollution exposure and (sub)cortical brain volumes, ventricular volume, and surface-based morphometric data, adjusting for socioeconomic and life-style characteristics.

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

Conclusion. Higher fetal and childhood exposure to air pollution was associated with brain morphology in preadolescents of 9-12 years old. The associations with brain volumes were predominantly observed in girls.

Keywords: neuroimaging, air pollutants, environmental pollution, cohort studies, brain development

INTRODUCTION

The evidence for effects of air pollution on health is accumulating (1,2). 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 (3,4). Moreover, the early years of life are characterized by numerous vital and fragile developmental processes crucial for a proper development. 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 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. 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 fetal exposure to air pollution 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 children of 6 to 10 years old. This association partially mediated the relationship between the exposure and cognitive impairment manifested by weakened inhibitory control. In the current study, we build on this previous work including a fourfold population of slightly older children. Moreover, we include air pollution exposure during fetal life as well as childhood to a wider selection of air pollutants, including components of fine particles and their oxidative potential, as well as ultrafine particles, 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. Moreover, we examined whether the associations differed between girls and boys. Our hypothesis was that 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 Rotterdam, the Netherlands (12). In total, 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 9 and 12 years old, they were invited to undergo an MRI (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 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, with fetal life stretching from conception to birth, and childhood from birth to MRI session, using a standardized procedure described elsewhere (14–17). Briefly, within 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 various 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 ($PM_{2.5}$abs), 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 after correction for temporal variability, by 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, using land use regression models, air pollution levels were estimated at each address that the participants have lived (14–17,19,20). Considering the time spent at each address and weighting the pollution levels accordingly, we then obtained mean air pollution concentration of each pollutant for each participant for the fetal period (i.e. conception to birth) and for the childhood period (i.e. birth to MRI). For those participants that were recruited 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 extrapolation of the concentrations to match the exact periods of interest, we assumed that the spatial contrast remained constant over time as demonstrated in previous studies (21).

Structural Magnetic Resonance Imaging

To familiarize the participants 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 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 volume metrics included total brain, cerebellum, cortical and sub-cortical gray matter including thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens, cerebral and cerebellar white matter, 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 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 scientific literature on the association between air pollution exposure and brain development (12). Parental educational level, monthly household income, parental country of birth, parental age at intake, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, parity, marital status, and parental psychological distress were collected by questionnaires during pregnancy. Parental 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. 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 to better correct for population stratification (24,25). Child's sex was obtained from 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 exposure and outcome data. The percentage of missing values was below 30% except for paternal education level, paternal psychological distress, and child genetic ancestry which had 36%, 39%, and 35% of missing values respectively.

Non-response analysis

Participants (n=3,133) were more likely to have parents from a higher socioeconomic position compared to non-included children (n=6,477) (eTable 1). We used inverse probability weighting to correct for selection bias that potentially arises when only population with available exposure and outcome data is included as compared to the full cohort (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

First, we 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. 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, total grey matter, cortical gray matter, subcortical grey matter, and cerebral white matter volumes 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), correcting the analyses for the number of tests with respect to the multiplicity of included pollutants using effective number of independent tests correction (28). The 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, 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.

Secondly, 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 studying each pollutant was separately. Next, we ran multi-pollutant analyses using the ExWAS approach (27), correcting all the analyses for the number of tests with respect to the exposure using the effective number of independent tests correction (28). The correction 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 $\mu g/m^3$ for NO_2 and 16.8 $\mu g/m^3$ for $PM_{2.5}$, and 32.4 $\mu g/m^3$ for NO_2 and 16.7 $\mu g/m^3$ 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 (eFigures 1 and 2). Mothers with higher education, 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 different pollutants (data not shown).

In the global approach we studied the associations between fetal and childhood exposure to each pollutant separately, 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 mm^3 [95%CI -76.3 to -14.3] per 1unit/m^3 increase of OP_{ESR}). Higher childhood exposure to PM_{10} and PM_{COARSE} also remained associated with larger volume of the putamen (470.3 mm^3 [95%CI 172.5 to 768.2] per 10$\mu g/m^3$ increase of PM_{10}, and (357.8 mm^3 [95%CI 142.4 to 573.1] per 5$\mu g/m^3$ 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 mm^3 [95%CI -38.9 to -7.9] per 1ng/m^3 increase of OC). When we simultaneously assessed fetal exposure to OP_{ESR} and childhood exposure to OC with the volume of the anterior part of 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 were driven predominantly by associations in girls (eTable 2 and 3). We also observed associations between fetal exposure to several pollutants with larger volumes in girls that were not observed when both sexes were analyzed together (eTable 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

Table 1. Participant characteristics

	Distribution		
Participant characteristics	**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)

Table 1. (Continued)

		Distribution	
Participant characteristics	**Percentage**	**Mean**	**(SD)**
Maternal pre-pregnancy body mass index (kg/m^2)		23.4	(4.1)
Paternal body mass index at intake (kg/m^2)		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 the continuous variables.

region of the left hemisphere (240.0 mm^2 and 289.0 mm^2 respectively, per 10ng/m^3 increase in elemental Zn).

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 to 12 years old. Overall, higher fetal and childhood exposure to air pollution 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 brain volumes were predominantly observed in girls. After the multi-pollutant analysis, only few associations remained.

Higher fetal and childhood exposure to various air pollutants 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 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 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

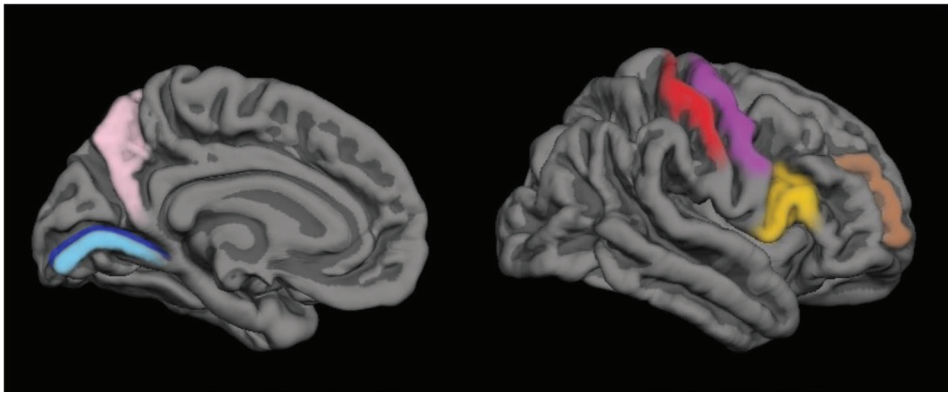

Figure 1. Medial view of the left hemisphere (on the left) and lateral view of the right hemisphere (on the right) of the brain

Exposure to air pollution showed 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 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
$PM_{2.5}$abs	1.5	1.6	1.8	1.4	1.5	1.7	0.53
PAH	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	1039.2	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 (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.

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 (n=3,133)

		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
	$PM_{2.5}$abs	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
	$PM_{2.5}$abs	---	---		---	---	175.9	17.9	;	333.8	0.029

Table 3. (Continued)

		fetal life					childhood				
Brain region	**Pollutant**	**Coef.**	**95% CI**			**p-value**	**Coef.**	**95% CI**			**p-value**
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

Table 3. (Continued)

		fetal life					childhood				
Brain region	**Pollutant**	**Coef.**	**95% CI**			**p-value**	**Coef.**	**95% CI**			**p-value**
	$PM_{2.5}$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 (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; $1ng/m^3$ for PAHs; $0.1ng/m^3$ for B[a]P; $1\mu g/m^3$ for OC; $5ng/m^3$ for Cu in $PM_{2.5}$; $100ng/m^3$ for Fe in $PM_{2.5}$; $50ng/m^3$ for K in $PM_{2.5}$; $100ng/m^3$ for Si in $PM_{2.5}$; $10ng/m^3$ for Zn in $PM_{2.5}$; 1nmol 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 4. 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 (n=3,133)

		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 (PM_{COARSE}); 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: 10μg/m^3 for NO_2; 5μg/m^3 for PM_{COARSE}; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1μg/m^3 for OC; 5ng/m^3 for Cu in $PM_{2.5}$; 50ng/m^3 for K in $PM_{2.5}$; 10ng/m^3 for Zn in $PM_{2.5}$; 1nmol 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.

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 fetal exposure to various air pollutants 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 influence estrogen receptor genes and estrogen receptor signaling (30,31), thereby evoking different responses between the two sexes. 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 slightly later. 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 putamen volume remained in the multi-pollutant analysis. After stratifying by sex, we again found that the associations were driven predominantly by results observed in girls.

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 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 be of transient nature (35). Repeated assessments of neuroimaging across childhood and adolescence will allow for further investigation.

We also observed a smaller volume of corpus callosum, 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. 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 brain aging, neurodegenerative diseases, and other 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 increase in 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 during fetal life and childhood, taking into account changes of residence; 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; v) two independent, complementing approaches to quantify brain structures, namely global measures and surface-based vertex-wise method, and vi) adjustment for socioeconomic and lifestyle variables that are known to be potentially associated with air pollution exposure and brain structure.

Several limitations should also be considered. First, sampling campaigns were carried out when participants were between 3.5 and 9 years old and historical pollution data from routine monitoring stations was not available for all the pollutants to extrapolate the levels to the periods of interest 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 such spatial stability is demonstrated for different air pollutants (21). Nevertheless, we cannot discard the possibility of misclassification, which is more likely to occur in fetal 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, the results might still be influenced by residual confounding by other relevant, yet unavailable or not inferred, variables. Third, we observed that children with data on exposure and outcome were more likely to have parents with higher socioeconomic status, 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 could have an important effect on the results. Finally, our study is based on a single measurement of the brain structural morphology in preadolescence. Repeated measurements 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 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 childhood exposure to air pollution 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. 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.

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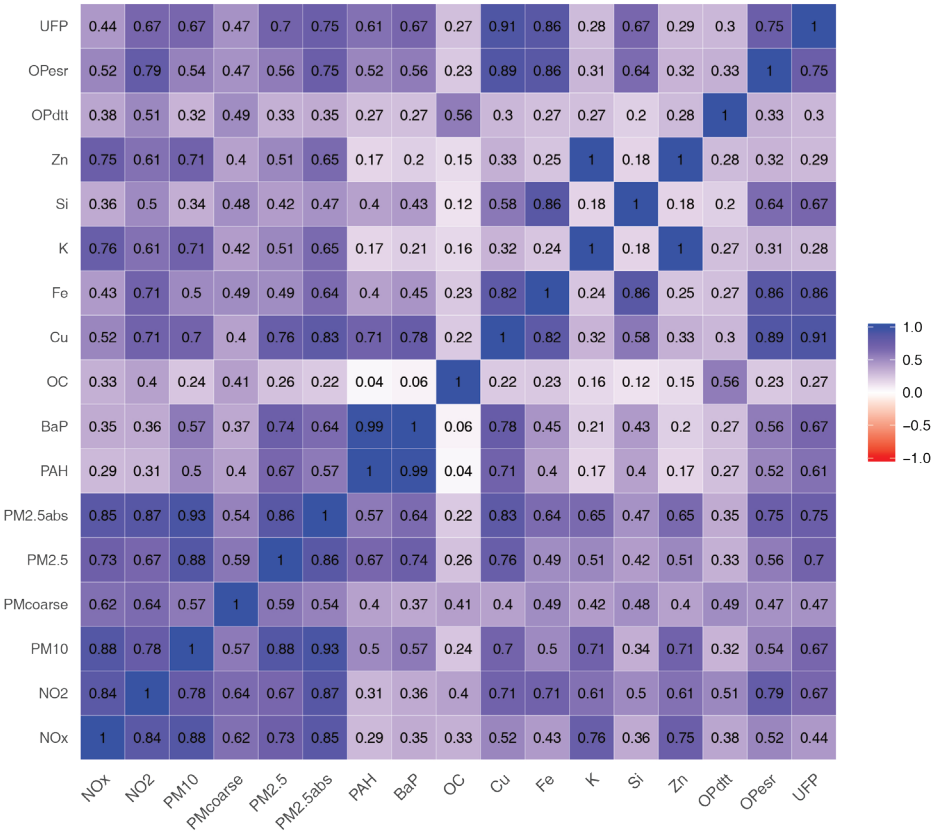
Supplementary Table 1. Comparison of participant characteristics between included and non-included subjects in the study among the 9,610 eligible subjects

Participant characteristics	Distribution		p-value
	Included (n = 3,133)	Not included (n = 6,477)	
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

Supplementary Table 1. (Continued)

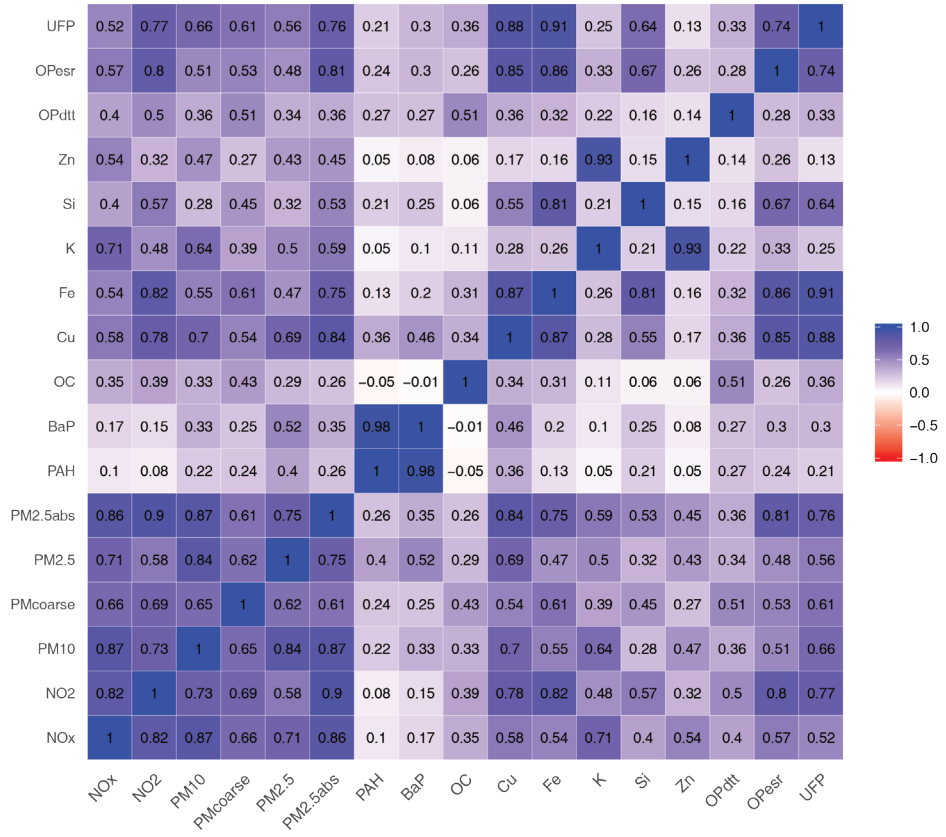
Participant characteristics	Distribution		p-value
	Included (n = 3,133)	Not included (n = 6,477)	
Maternal pre-pregnancy body mass index (kg/m^2)	23.4 (4.1)	23.8 (4.5)	0.003
Paternal body mass index (kg/m^2)	25.3 (3.6)	25.3 (3.4)	0.444
Maternal height (cm)	168.1 (7.3)	166.6 (7.4)	<.001
Paternal height (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.
$\chi 2$ 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	---	---	---	---

Supplementary Table 2. (Continued)

		girls				boys			
brain region	**pollutant**	**estimate**	**min95**	**max95**	**p**	**estimate**	**min95**	**max95**	**p**
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	---	---	---	---

Supplementary Table 2. (Continued)

		girls				boys			
brain region	**pollutant**	**estimate**	**min95**	**max95**	**p**	**estimate**	**min95**	**max95**	**p**
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: 20μg/m^3 for NO_X; 10μg/m^3 for NO_2; 10μg/m^3 for PM_{10}; 5μg/m^3 for PM_{COARSE}; 5μg/m^3 for $PM_{2.5}$; 10^{-5}/m^{-1} for $PM_{2.5}$abs; 1ng/m^3 for PAHs; 0.1ng/m^3 for B[a]P; 1μg/m^3 for OC; 5ng/m^3 for Cu in $PM_{2.5}$; 100ng/m^3 for Fe in $PM_{2.5}$; 50ng/m^3 for K in $PM_{2.5}$; 100ng/m^3 for Si in $PM_{2.5}$; 10ng/m^3 for Zn in $PM_{2.5}$; 1nmol 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 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: 20µg/m³ for NO_X; 10µg/m³ for NO_2; 10µg/m³for PM_{10}; 5µg/m³ for PM_{COARSE}; 5µg/m³ for $PM_{2.5}$; $10^{-5}m^{-1}$ for $PM_{2.5}$abs; 1ng/m³ for PAHs; 0.1ng/m³ for B[a]P; 1µg/m³ for OC; 5ng/m³ for Cu in $PM_{2.5}$; 100ng/m³ for Fe in $PM_{2.5}$; 50ng/m³ for K in $PM_{2.5}$; 100ng/m³ for Si in $PM_{2.5}$; 10ng/m³ for Zn in $PM_{2.5}$; 1nmol 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.

Paper V

Exposure to air pollution during pregnancy and childhood, and white matter microstructure in preadolescents

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ABSTRACT

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

Objectives. We aimed to assess whether pregnancy 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 pregnancy 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 (DSA) algorithm.

Results. In the single-pollutant analyses, higher concentrations of several air pollutants during pregnancy or childhood were associated with significantly lower FA or significantly higher MD ($p < 0.05$). In multi-pollutant models selected by the DSA algorithm, higher concentration of fine particles during pregnancy was associated with significantly lower FA (-0.71 [95%CI -1.26, -0.16] per 5$\mu g/m^3$ fine particles) and higher concentration of elemental silicon during pregnancy was associated with significantly higher MD (0.06 [95%CI 0.01, 0.11] per 100ng/m^3 silicon). Multi-pollutant models of childhood exposures indicated significant associations of nitrogen oxides with FA (-0.14 [95%CI -0.23, -0.04] per 20$\mu g/m^3$ increase in nitrogen oxides), and of elemental zinc and the oxidative potential of PM with MD (0.03 [95%CI 0.01, 0.04] per 10ng/m^3 increase in zinc, and 0.07 [95%CI 0.00, 0.44] per 1nmol DTT/min/m^3 increase in oxidative potential). Mutually-adjusted models of selected exposures during pregnancy and childhood indicated significant associations of silicon during pregnancy, and zinc during childhood, with MD.

Discussion. Exposure in pregnancy and childhood 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 pregnancy 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 pregnancy 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). To our knowledge, the use of diffusion tensor imaging to quantify white matter microstructure in relation to air pollution exposures has been limited to a single study, which showed that airborne elemental copper was associated with differences in white matter microstructure adjacent to the 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 pregnancy 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 pregnancy 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 pregnancy onwards, based in the urban area of Rotterdam, the Netherlands (Kooijman et al. 2016). A total of 8,879 women were enrolled during pregnancy, and additionally 899 women were recruited shortly after the delivery. The children were born between April 2002 and January 2006, and we only included singleton pregnancies in our study, resulting in 9,610 children. When the children were between 9 and 12 years old, those still involved in the study 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 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 Air Pollution

Air pollution concentrations were estimated for all reported home addresses of each participant during the pregnancy and childhood 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 (PM_{COARSE}), less than 2.5μm ($PM_{2.5}$), absorbance of $PM_{2.5}$ fraction ($PM_{2.5}$abs), 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 concentrations 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 concentrations accordingly, we then obtained a single, mean air pollution concentration of each pollutant for each participant for the pregnancy period (i.e. since conception until birth) and for the childhood period (i.e. since birth until the MRI session). From the 899 participants that were recruited shortly after birth, 310 were included in this analysis, and we considered their 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 has been previously demonstrated in the Netherlands for a period up to 8 years (1999 – 2007) (Eeftens et al. 2011), and in Great Britain for a period up to 18 years (1991 – 2009) (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 = 72ms, field of view = 240mm x 240mm, Acquisition Matrix = 120 x 120, slice thickness = 2mm, number of slices = 65, Asset Acceleration Factor = 2, b = 900 s/mm^2).

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, the images were modified to exclude non-brain tissue, and then 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, indicating a substantial presence of artifacts, these cases were excluded from analyses. This was denoted by a structured-pattern high signal intensity in the SSE map on one or more slices, not including for example the ventricles or non-brain tissue. Examples

include substantial ghosting artifact, entire slices with high signal intensity (indicative of substantial motion). Ratings of 1 or 2 (mild and moderate artifacts, respectively) was rated when data contained no more than 3 slices with mildly increased structured signal (i.e., not high/strong, not in ventricles/non-brain areas) in the SSE map. SSE maps were rated independently of the automated DTIPrep results (and vice versa), and thus data could be excluded due to failing any of the checks done (i.e., some datasets were excluded for only SSE issues, only DTIPrep issues, only registration issues, or some combination of issues). 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. Nonlinear registration was checked by building a 4-dimensional nifti file containing all subjects' co-registered FA maps, such that the 4th dimension was "subject". Images were visually inspected one at a time for major deviations from the template, either in rotations, translations, or over-warping in certain areas (more than ~2 voxels of shift from the template). Proper whole-brain coverage was also inspected during this step, and some subjects missing substantial portions of the brain (leading to over-warping of the nonlinear registration) were also flagged.

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.

Potential confounding variables

Potential confounding variables included in the models were selected based on scientific literature and on availability of data within the Generation R cohort (Guxens et al. 2018). Maternal and paternal educational level (categorical: primary education or lower / secondary education / higher education), monthly household income (categorical: <900€ / 900€-1600€ / 1600€-2200€ / >2200€), maternal and paternal country of birth (categorical: the Netherlands / other Western / non-Western), maternal and paternal age at enrollment

in the cohort (continuous in years), maternal smoking during pregnancy (categorical: never / smoking use until pregnancy known / continued smoking during pregnancy), maternal alcohol consumption during pregnancy (categorical: never / alcohol use until pregnancy known / continued alcohol use during pregnancy), parity (categorical: nulliparous / one child / two or more children), marital status (categorical: married / living together / no partner), and maternal and paternal psychological distress (continuous), using Brief Symptom Inventory (De Beurs 2004) were collected by questionnaires during pregnancy. Maternal and paternal weight and height (continuous in kilograms and centimeters, respectively) were measured or self-reported in the 1st trimester of pregnancy, and maternal and paternal body mass index was calculated based on the collected weight and height data. Maternal and paternal height were included in the models as potential confounding variables separately from body mass index as they could be associated with the outcome variables independently from body mass index. Maternal intelligence quotient (continuous) was assessed at child's age of 6 years with Ravens Advanced Progressive Matrices Test, set I (Prieler 2003). Using multidimensional scaling, 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 (continuous) were included here to better correct for population stratification (Neumann et al. 2017; Price e al. 2006). Child's sex (categorical: boy / girl) was obtained from hospital records at birth and child's age (continuous in years) was collected at the scanning session.

Statistical Analyses

We first 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 (Table S1). 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 first imputed missing covariates for all eligible subjects (n=9,610), and we then used all the available information to predict the probability of participation in the current study, and used the inverse of those probabilities as weights in the analyses, which were then applied to the imputed datasets obtained in the previous step, 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 S2.

After visual inspection of the distributions, we used linear regression models to analyze the relationships between concentrations of air pollutants first during pregnancy and then during childhood, with 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 (DSA) 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 DSA 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 fixed the potential confounders, allowing only the air pollution exposures to participate in the selection process. When two

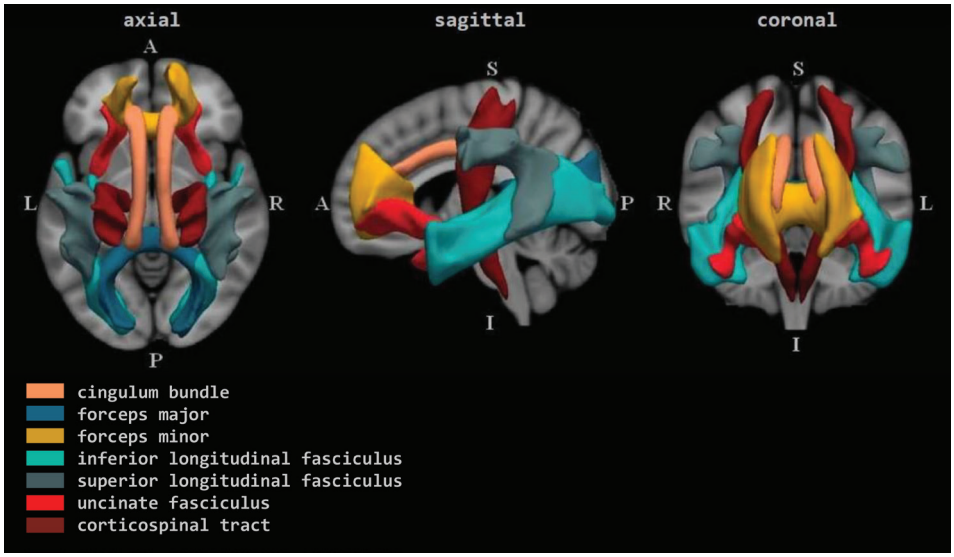

Figure1. Group average representations of the tracts in standard coordinate space. R, right; L, left; A, anterior; P, posterior; I, inferior; S, superior.

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 (Table S2). As the DSA 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 DSA algorithm: one including only air pollution exposures in pregnancy; and the second one including only the childhood air pollution exposures. In addition, for each global outcome, we performed a linear regression model that included all pregnancy and childhood exposures that were significant predictors of the outcome in a single pollutant model and significant predictors of the outcome in a DSA-selected multi-pollutant model of pregnancy exposures or childhood exposures. Additionally, the pollutants that were

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

Participant characteristics	Distribution		p-value
	Included (n=2,954)	Not included (n=6,656)	
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%)	
Missings	233	949	
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%)	
Missings	1,093	3,359	
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%)	
Missings	648	2,334	
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%)	
Missings	56	613	
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%)	
Missings	913	2,798	
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%)	
Missings	245	931	
Maternal parity (nulli vs. multiparous)	1,630 (57.2%)	3,473 (54.3%)	<.001
Missings	103	259	
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%)	
Missings	390	1,107	

Table 1. (Continued)

Participant characteristics	Distribution		p-value
	Included (n=2,954)	Not included (n=6,656)	
Maternal alcohol use during pregnancy			<.001
Never	973 (41.7%)	2,773 (53.4%)	
Alcohol use until pregnancy known	335 (14.4%)	691 (13.3%)	
Continued alcohol use during pregnancy	1,023 (43.9%)	1,728 (33.3%)	
Missings	623	1,464	
Maternal age at intake (years)	31.2 (4.8)	29.3 (5.5)	<.001
Missings	0	2	
Paternal age at intake (years)	33.5 (5.3)	32.3 (5.9)	<.001
Missings	877	2,477	
Maternal body mass index (kg/m^2)	23.4 (4.0)	23.8 (4.5)	0.003
Missings	773	1,815	
Paternal body mass index (kg/m^2)	25.2 (3.3)	25.4 (3.6)	0.141
Missings	884	2,485	
Maternal height (cm)	168.1 (7.4)	166.7 (7.4)	<.001
Missings	316	591	
Paternal height (cm)	182.6 (7.7)	181.1 (8.0)	<.001
Missings	880	2,475	
Maternal psychological distress during pregnancy	0.3 (0.3)	0.3 (0.4)	<.001
Missings	717	2,333	
Paternal psychological distress during pregnancy	0.1 (0.2)	0.2 (0.3)	<.001
Missings	1169	3,539	
Maternal intelligence quotient score	97.9 (14.7)	94.0 (15.7)	<.001
Missings	266	3,077	
Child's sex (boy vs. girl)	1,472 (49.8%)	3,339 (50.2)	0.298
Missings	0	107	
Child's genetic ancestry*			
Principal component 1	7.4 (40.5)	-4.0 (48.1)	<.001
Principal component 2	1.3 (20.9)	-0.7 (23.8)	0.002
Principal component 3	-2.6 (13.4)	1.4 (17.1)	<.001
Principal component 4	-0.4 (10.4)	0.2 (12.6)	0.045
Missings	1,073	2,851	
Child's age at scanning session (years)	10.1 (0.6)	10.1 (0.6)	<.001
Missings	0	5,722	

Values are counts (percentages) for the categorical variables and mean (standard deviation) for the continuous variables.
$\chi 2$ test for categorical variables and t-student test for continuous variables
* values multiplied by 1000

nominally significant in the multi-pollutant models of global FA or MD, as well as nominally significant in the single-pollutant models, were analyzed in separate single-pollutant models of FA and MD in twelve individual white matter tracts (Figure 1). Finally, if more than one pollutant remained significant for FA or MD in the same tract after application of false discovery rate (FDR) correction (Benjamini and Hochberg 1995), we performed multi-pollutant models for FA or MD in the tract.

As we considered the address at birth as representative for the pregnancy period for those participants that were recruited shortly after birth, and as their mothers were of slightly higher mean age (33.2 years (SD = 4.8) vs. 30.9 years (SD = 4.8)), and from a higher socioeconomic position as compared to mothers recruited during pregnancy (e.g. highest category education 57% vs. 53%; highest category household income: 76% vs. 64%), we repeated the pregnancy analyses excluding the children from mothers recruited shortly after birth, to test the sensitivity of the results. The combinations of pollutants analyzed were the same as those selected by the DSA algorithm in the analyses that included the full study population.

Finally, to quantify the measurement error in the air pollution assessment (LUR model predictions), and to transfer the resulting uncertainty to the exposure-outcome associations, we used a bootstrap method (Szpiro et al. 2011). Briefly, this method iteratively simulates a new health outcome variable and the exposure at the monitoring locations based on the fitted models and residual errors; builds a new LUR model that predicts the simulated exposure; uses the new LUR model to predict exposure for the whole cohort; and estimates the exposure-outcome association with the newly generated health outcome variable and predicted exposure. The variance in the estimates resulting from the different iterations is used as the measurement error corrected variance. This variance or, equivalently, the confidence intervals, was compared to the variance obtained when measurement error was not taken into account. As the measurement error is expected to be mostly of Berkson type, bias in exposure-outcome coefficient estimates was not expected and was therefore not corrected (Szpiro et al. 2011).

All models were carried out with all imputed datasets (except for the DSA selection process, and the measurement error calculations, which were carried out with the 25th 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. We present beta coefficients and their 95% confidence intervals per 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; 1ng/m^3 for PAHs; 0.1ng/m^3 for B[a]P; 1$\mu g/m^3$ for OC; 5ng/m^3 for Cu in $PM_{2.5}$; 100ng/m^3 for Fe in $PM_{2.5}$; 50ng/m^3 for K in $PM_{2.5}$; 100ng/m^3 for Si in $PM_{2.5}$; 10ng/m^3 for Zn in $PM_{2.5}$; 1nmol DTT/min/m^3 for OP_{DTT}; 1,000 arbitrary units/m^3 for OP_{ESR}; and 10,000 particles/cm^3 for UFP, based on the distribution of each exposure variable. Statistical tests of hypotheses were two-tailed with significance set at p-value<0.05. Statistical analyses were carried out using STATA (version 14.0; StataCorporation, College Station, TX) and R (version 3.4.2; R Core Team (2017)).

RESULTS

Participant characteristics are shown in Table 1. The percentage of missing values was below 30% except for paternal country of birth, paternal education level, paternal psychological distress, and child genetics ancestry which had 31%, 37%, 40% and 36% of missing values respectively. Based on observations with known values, mothers of the included participants (n=2,954) were more likely to have higher education, higher household income, be Dutch, and have a partner, as compared to mothers of participants that were not included (n=6,656). Mean air pollution exposure concentrations during pregnancy 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 who were nulliparous were exposed to higher average NO_2 concentrations during pregnancy. These associations were however not consistent between the different pollutants (Tables S3 - S11). 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 $PM_{2.5}$abs, PAHs, Zn, and Cu respectively, but had a poorer performing land use regression model (with exception of B[a]P which had a better performing land use regression model than PAHs (Table S2), but was excluded for the reason that PAHs comprises of various polycyclic aromatic hydrocarbons including B[a]P, and was therefore considered more comprehensive).

In the single-pollutant analysis, higher concentrations of NO_X, PM_{10}, $PM_{2.5}$, and $PM_{2.5}$abs during pregnancy were significantly associated with lower global FA (Table 3). Higher concentrations of NO_X, NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5}$abs, Cu, Fe, Si, OP_{ESR}, and UFP during pregnancy showed significant associations with higher global MD (Table 4). In the multi-pollutant analysis, $PM_{2.5}$ exposure during pregnancy remained significantly associated with global FA (0.71 lower global FA [95% CI: -1.26 to -0.16] per 5μg/m^3 increase of $PM_{2.5}$) (Table 5). $PM_{2.5}$ and PAHs exposures during pregnancy were both significant predictors of global FA when included in the same model, showing inverse and positive associations, respectively. Exposure in pregnancy to Si remained significantly associated with global MD in the multi-pollutant analysis (0.06 higher global MD [95% CI 0.01 to 0.11] per 100ng/m^3 increase of Si). Exclusion of children with mothers recruited shortly after the pregnancy (n=310), did not lead to notable changes in the effect estimates (Table S12).

Regarding air pollution exposure during childhood, higher concentrations of NO_X, NO_2, $PM_{2.5}$abs, OC, and K were significantly associated with lower global FA (Table 3). Higher concentrations of NO_X, NO_2, PM_{10}, PM_{COARSE}, $PM_{2.5}$, $PM_{2.5}$abs, K, Si, Zn, and OP_{DTT} showed significant associations with higher global MD (Table 4). In the multi-pollutant analysis, childhood exposure to NO_X remained significantly associated with global FA (0.14 lower global FA [95% CI: -0.23 to -0.04] per 20μg/m^3 increase of NO_X) while Zn and OP_{DTT} remained significantly associated with global MD (0.03 higher global MD [95%

CI: 0.01 to 0.04] per 10ng/m^3 increase in Zn, and 0.07 higher MD [95% CI 0.00, 0.44] per 1nmol DTT/min/m^3 increase in OP_{DTT}) (Table 5).

When pregnancy $PM_{2.5}$ and childhood NO_X exposures that were nominally significant in the multi-pollutant models, as well as nominally significant in the single-pollutant models, were analyzed simultaneously, they no longer showed statistically significant associations with global FA (Table S13), and the beta coefficients approached zero. However, the associations between pregnancy exposure to Si and childhood exposure to Zn, and global MD remained significant after mutual adjustment, and the beta coefficients did not change notably.

Analyses of FA in the twelve specific white matter tracts did not indicate FDR-significant associations with pregnancy $PM_{2.5}$ or childhood NO_X in any tract (Table S14). In analyses of MD in specific white matter tracts, FDR-significant associations were estimated for pregnancy Si and MD in the cingulate gyrus part of the cingulum of the left hemisphere, the superior longitudinal fasciculus of the left hemisphere, and the forceps minor. Associations between childhood Zn and MD were FDR-significant for six tracts: the uncinate fasciculus tract of the right hemisphere, the cingulate gyrus part of the cingulum of both hemispheres, the superior longitudinal fasciculus of both hemispheres, and the forceps minor (Table S15). None of the coefficients for childhood OP_{DTT} and MD in specific tracts were FDR-significant. When we simultaneously modeled pregnancy Si and childhood Zn in association with MD in the three tracts that were FDR-significant for both pollutants in single-pollutant models, associations were nominally significant for both exposures in all three tracts (Table S16).

Accounting for measurement error only slightly increased the standard errors and did not alter the main conclusions (Table S17).

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

	Pregnancy				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 (PM_{COARSE}) in μg/m^3; less than 2.5μm ($PM_{2.5}$) in μg/m^3; $PM_{2.5}$abs, 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 in ng/m^3; Fe in ng/m^3; K in ng/m^3; Si in ng/m^3; Zn 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 pregnancy and childhood to single air pollutants and global fractional anisotropy at 9-12y

		Global fractional anisotropy					
		Pregnancy			Childhood		
Pollutant	**Contrast**	**Coef.**	**95% CI**	**p-value**	**Coef.**	**95% CI**	**p-value**
NO_X	20 μg/m³	-0.11	-0.20 ; -0.02	0.018	-0.14	-0.23 ; -0.04	0.007
NO_2	10 μg/m³	-0.11	-0.25 ; 0.03	0.109	-0.13	-0.25 ; -0.01	0.029
PM_{10}	10 μg/m³	-0.49	-0.90 ; -0.08	0.018	-0.45	-0.91 ; 0.01	0.056
PM_{COARSE}	5 μg/m³	-0.05	-0.37 ; 0.27	0.757	-0.29	-0.63 ; 0.04	0.086
$PM_{2.5}$	5 μg/m³	-0.71	-1.26 ; -0.16	0.012	-0.46	-1.14 ; 0.21	0.179
$PM_{2.5}$abs	$10^{-5}m^{-1}$	-0.29	-0.51 ; -0.07	0.012	-0.27	-0.51 ; -0.02	0.032
PAHs	1 ng/m³	0.01	-0.19 ; 0.21	0.952	0.15	-0.09 ; 0.38	0.216
B[a]P	0.1 ng/m³	-0.06	-0.24 ; 0.13	0.563	0.11	-0.14 ; 0.35	0.382
OC	1 μg/m³	-0.12	-0.29 ; 0.05	0.175	-0.20	-0.38 ; -0.03	0.024
Cu	5 ng/m³	-0.32	-0.71 ; 0.06	0.097	-0.22	-0.65 ; 0.21	0.323
Fe	100 ng/m³	-0.20	-0.54 ; 0.14	0.247	-0.22	-0.53 ; 0.09	0.156
K	50 ng/m³	-0.38	-0.84 ; 0.08	0.103	-0.53	-1.03 ; -0.03	0.039
Si	100 ng/m³	-0.28	-0.70 ; 0.15	0.198	-0.24	-0.66 ; 0.19	0.277
Zn	10 ng/m³	-0.12	-0.28 ; 0.04	0.130	-0.13	-0.27 ; 0.02	0.098
OP_{DTT}	1 nmol DTT/min/m³	0.21	-0.34 ; 0.75	0.448	-0.14	-0.69 ; 0.42	0.622
OP_{ESR}	1,000 units/m³	-0.19	-0.55 ; 0.17	0.299	-0.21	-0.57 ; 0.16	0.259
UFP	10,000 particles/cm³	-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 (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; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficients and 95% CI from linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any

missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

Table 4. Adjusted associations between exposure during pregnancy and childhood to single air pollutants and global mean diffusivity at 9-12y

		Global mean diffusivity					
		Pregnancy			Childhood		
Pollutant	**Contrast**	**Coef.**	**95% CI**	**p-value**	**Coef.**	**95% CI**	**p-value**
NO_X	20 μg/m^3	0.01	0.00 ; 0.02	0.050	0.02	0.01 ; 0.03	0.005
NO_2	10 μg/m^3	0.02	0.00 ; 0.04	0.021	0.02	0.00 ; 0.03	0.011
PM_{10}	10 μg/m^3	0.05	0.00 ; 0.10	0.042	0.07	0.01 ; 0.12	0.027
PM_{COARSE}	5 μg/m^3	0.03	-0.01 ; 0.07	0.186	0.04	0.00 ; 0.09	0.038
$PM_{2.5}$	5 μg/m^3	0.09	0.02 ; 0.15	0.014	0.11	0.03 ; 0.20	0.010
$PM_{2.5}$abs	10^{-5}m^{-1}	0.04	0.01 ; 0.06	0.012	0.04	0.01 ; 0.07	0.009
PAHs	1 ng/m^3	0.01	-0.01 ; 0.04	0.259	0.01	-0.02 ; 0.04	0.477
B[a]P	0.1 ng/m^3	0.02	-0.01 ; 0.04	0.149	0.01	-0.02 ; 0.04	0.342
OC	1 μg/m^3	0.02	-0.01 ; 0.04	0.153	0.02	0.00 ; 0.04	0.088
Cu	5 ng/m^3	0.05	0.01 ; 0.10	0.030	0.03	-0.02 ; 0.09	0.221
Fe	100 ng/m^3	0.05	0.01 ; 0.09	0.018	0.03	-0.01 ; 0.07	0.102
K	50 ng/m^3	0.04	-0.02 ; 0.09	0.185	0.09	0.03 ; 0.15	0.006
Si	100 ng/m^3	0.07	0.02 ; 0.12	0.010	0.05	0.00 ; 0.11	0.047
Zn	10 ng/m^3	0.01	-0.01 ; 0.03	0.195	0.03	0.01 ; 0.05	0.003
OP_{DTT}	1 nmol DTT/min/m^3	0.06	-0.01 ; 0.13	0.069	0.09	0.02 ; 0.16	0.016
OP_{ESR}	1,000 units/m^3	0.04	0.00 ; 0.09	0.047	0.04	0.00 ; 0.09	0.080
UFP	10,000 particles/cm^3	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 (PM_{COARSE}); 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; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Coefficients and 95% CI from linear regression models adjusted for both maternal and

paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

Table 5. Results of multi-pollutant models selected by the Deletion/Substitution/Addition algorithm for pregnancy and childhood exposures in relation to global fractional anisotropy and global mean diffusivity, respectively.

	Contrast	Coef. (95% CI)	p-value
Global fractional anisotropy			
Pregnancy exposure models (% of runs)			
Model 1 (24.5%)			
$PM_{2.5}$	5 μg/m^3	-1.49 (-2.25 ; -0.73)	<0.001
PAHs	1 ng/m^3	0.33 (0.06 ; 0.59)	0.017
OP_{DTT}	1 nmol DTT/min/m^3	0.50 (-0.07 ; 1.07)	0.087
Model 2 (20%)			
$PM_{2.5}$	5 μg/m^3	-1.32 (-2.06 ; -0.58)	<0.001
PAHs	1 ng/m^3	0.33 (0.06 ; 0.60)	0.017
Model 3 (13%)			
$PM_{2.5}$	5 μg/m^3	-0.71 (-1.26 ; -0.16)	0.012
Childhood exposure models (% of runs)			
Model 1 (22.5%)			
NO_X	20 μg/m^3	-0.14 (-0.23 ; -0.04)	0.007
Model 2 (10.5%)			
NO_X	20 μg/m^3	-0.13 (-0.24 ; -0.03)	0.015
OP_{DTT}	1 nmol DTT/min/m^3	0.46 (-0.19 ; 1.11)	0.163
OC	1 μg/m^3	-0.19 (-0.40 ; 0.01)	0.059
Global mean diffusivity			
Pregnancy exposure models (% of runs)			
Model 1 (13.5%)			
Si	100 ng/m^3	0.06 (0.01 ; 0.11)	0.018
OP_{DTT}	1 nmol DTT/min/m^3	0.05 (-0.02 ; 0.11)	0.171
Childhood exposure models (% of runs)			
Model 1 (46.5%)			
Zn	10 ng/m^3	0.03 (0.01 ; 0.04)	0.005
OP_{DTT}	1 nmol DTT/min/m^3	0.07 (0.00 ; 0.14)	0.046
Model 2 (23%)			
Zn	10 ng/m^3	0.02 (0.01 ; 0.04)	0.008
OP_{DTT}	1 nmol DTT/min/m^3	0.06 (-0.01 ; 0.13)	0.078
Si	100 ng/m^3	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. Model selection is performed using Deletion/Substitution/Addition algorithm. PM_{10}, B[a]P, K, and UFP were excluded due to a correlation of 0.90 or more with $PM_{2.5}$abs, PAHs, Zn, and Cu respectively. For each combination of period of exposure and outcome, 200 runs were performed and the final model was selected based on frequency of occurrence (% of runs, at least 10% to be reported here). Coefficients and 95% CI from (multiple) linear regression models adjusted for both maternal and paternal

education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

DISCUSSION

We observed associations between exposure to air pollutants in two critical periods of brain development, pregnancy and childhood, and white matter microstructure in preadolescents aged 9-12 years. Our multi-pollutant analysis identified statistically significant associations between exposure to $PM_{2.5}$ and elemental Si during pregnancy, and exposure to nitrogen oxides, elemental Zn, and OP_{DTT} during childhood, and white matter microstructure, associations that were also statistically significant in the single-pollutant model analyses. When pregnancy $PM_{2.5}$ and childhood NO_X were included in the same model, the associations with global FA were no longer statistically significant. However, when pregnancy Si, and childhood Zn and OP_{DTT} were included in the same model, associations of pregnancy Si and childhood Zn with global MD remained statistically significant. Higher exposures to pollutants were predominantly 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 pregnancy exposures that were significantly associated with white matter microstructure in single-pollutant models and were selected for multi-pollutant models by the DSA algorithm, $PM_{2.5}$ remained significantly associated with lower global FA. Exposure to $PM_{2.5}$ is a human health concern, 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). Although single-pollutant models of global FA were not significant for pregnancy PAHs, the DSA algorithm selected models that estimated significant associations for both pregnancy $PM_{2.5}$ and pregnancy PAHs, with PAHs showing a significant positive association with global FA in the multi-pollutant model, while it showed no significant inverse association in the single-pollutant model. One possible explanation for these unexpected results with PAHs could be that the mutually-adjusted estimates may have been affected by collinearity. However, the two exposures were only moderately correlated ($r = 0.66$).

Pregnancy exposure to Si was a significant predictor of global MD in a multi-pollutant model that also included childhood Zn and OP_{DTT}. Pregnancy exposure to Si was also an FDR-significant predictor for MD in three white matter tracts based on single-pollutant models, and the associations remained statistically significant when we adjusted the models for childhood exposure to Zn. Si has not been documented as a potential neurotoxicant to date. However, Si may be a marker of exposure to resuspended road dust (Viana et al. 2008), and associations with Si may therefore reflect associations with exposure to high traffic, rather than exposure to Si specifically.

In analyses of exposures to air pollution during childhood, the association between higher concentrations of NO_X and lower global FA remained significant 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 higher 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. These results are location-wise moderately in accordance with findings of our previous study, wherein we found an association between higher concentrations 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). 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 in single-pollutant, as well as multi-pollutant models, 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 our knowledge, there has been only one previous epidemiological study of associations between air pollution and white matter microstructure (Pujol et al. 2016b). In that study, that exposure to higher concentrations of Cu at schools was associated with higher FA in regions adjacent to caudate nucleus in children aged 8-12 years. Similar to Zn, Cu reflects brake linings (Viana et al. 2008). In our study, we did not find a significant association between pregnancy or childhood exposure to Cu and FA, and the obtained non-significant associations were inverse, relating higher exposure to Cu to lower 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 pregnancy 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 pregnancy 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 concentrations 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 demonstrated in the Netherlands for a period up to 8 years (1999 – 2007) (Eeftens et al. 2011), and in Great Britain for a period up to 18 years (1991 – 2009) (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 performance with reference to a compromise between sensitivity and false discovery proportion compared to alternative methods in settings comparable to ours (Agier et al. 2016). Nevertheless, we still obtained an implausible result with pregnancy PAHs being selected by the DSA algorithm and showing a significant positive association with global FA when analyzed simultaneously with pregnancy $PM_{2.5}$ in a multi-pollutant model, while it showed no significant inverse association in the single pollutant analysis. 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). Additionally, several potential confounding variables, as well as variables used to predict participation in the study, had a high percentage of missing values. We applied multiple imputation, followed by inverse probability weighting to reduce possible selection and attrition bias in the study, but it is possible that this might not be sufficient to eliminate the bias due to missing covariates, as well as missing variables used to calculate the inverse probability weights, entirely. 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. The observed associations involved exposure to air pollution during both key developmental periods, namely pregnancy 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

Exposure to air pollution during pregnancy and childhood, and white matter microstructure in preadolescents

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Table S1: Table illustrating the details of the imputation modeling

Software used and key settings:
STATA 14.0 software (Stata Corporation, College Station, Texas) – Ice command (with 10 cycles)
Number of imputed datasets created:
25
Variables included in the imputation procedure:
global fractional anisotropy, global mean diffusivity, global axial diffusivity, and global radial diffusivity; concentration levels of the pollutants during pregnancy and childhood; maternal and paternal education, country of birth, age, height, weight, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, gestational age, parity, marital status, intelligence quotient, and household income; and child's ethnicity, genetic ancestry (10 principal components), gender, and age at the scanning session.
Treatment of binary/categorical variables:
logistic and multinomial models
Statistical interactions included in imputation models:
none

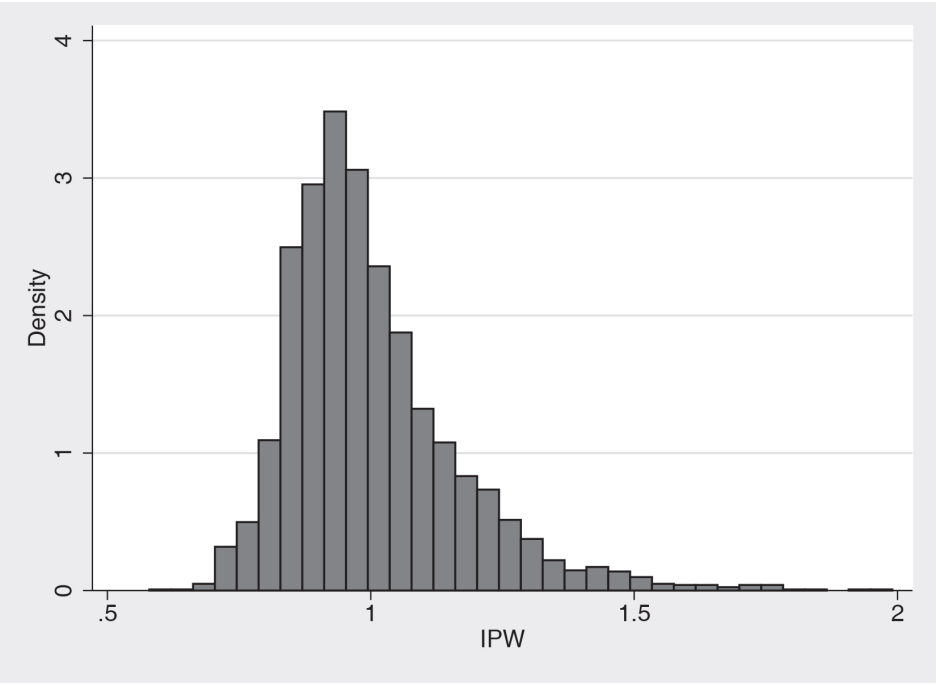

Figure S1: Description of the obtained inverse probability weights (IPW)

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 S2: LUR R^2 and R^2 cross validation

LUR model	R^2	R^2 CV
NO_X	87	82
NO_2	86	81
PM_{10}	68	60
PM_{COARSE}	51	38
$PM_{2.5}$	67	61
$PM_{2.5}$abs	92	89
PAHs	58	31
B[a]P	64	39
OC	80	71
Cu	83	81
Fe	78	73
K	31	25
Si	46	39
Zn	66	58
OP_{DTT}	60	47
OP_{ESR}	67	60
UFP	42	20

Abbreviations: LUR, land use regression; CV, cross validation; 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; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles. Source: NO_X and NO_2: Beelen et al. 2013; PM_{10}, $PM_{2.5}$, $PM_{2.5}$abs: Eeftens et al. 2012; $PM_{2.5}$ composition: de Hoogh et al. 2013; OP: Yang et al. 2015; OC and PAHs: Jedynska et al. 2014; UFP: Montagne et al. 2015.

Table S3. Exposure levels to NO_X and NO_2 during pregnancy by participant characteristics

Participant characteristics	n	NO_X	p-value	NO_2	p-value
Maternal education level			<.001		0.001
Primary education or lower	176	47.6 (10.6)		33.9 (3.9)	
Secondary education	1,092	50.7 (15.5)		34.5 (5.2)	
Higher education	1,453	52.2 (15.0)		35.0 (4.9)	
Paternal education level			0.441		0.190
Primary education or lower	92	50.2 (16.5)		34.2 (5.5)	
Secondary education	700	52.4 (16.6)		34.8 (5.7)	
Higher education	1,069	52.0 (14.9)		35.1 (4.8)	
Monthly household income at intake			<.001		0.002
<900€	172	47.8 (12.7)		33.8 (4.5)	
900€ - 1,600€	319	48.9 (13.6)		34.3 (4.3)	
1,600€ - 2,200€	329	53.6 (15.6)		35.2 (4.7)	
>2,200€	1,486	52.2 (15.5)		35.0 (5.2)	
Maternal country of birth			<.001		0.623
The Netherlands	1,702	51.9 (15.6)		34.7 (5.1)	
Other Western	252	52.0 (15.6)		34.9 (4.8)	
Non-Western	944	49.6 (13.3)		34.6 (4.6)	
Paternal country of birth			0.003		0.017
The Netherlands	1,419	52.6 (15.9)		35.0 (5.2)	
Other Western	120	52.8 (15.9)		35.7 (6.2)	
Non-Western	502	49.9 (13.9)		34.4 (4.7)	
Family status at intake			<.001		<.001
Married	1,394	50.4 (14.6)		34.4 (4.7)	
Living together	1,023	52.9 (15.8)		35.2 (5.3)	
No partner	292	49.0 (13.1)		34.6 (5.0)	
Maternal parity			<.001		<.001
nulliparous	1,630	52.4 (15.4)		35.2 (5.2)	
1 child	883	49.8 (14.2)		34.2 (4.4)	
2 or more children	338	49.1 (13.2)		34.0 (4.2)	
Maternal smoking use during pregnancy			0.525		0.851
Never	2,004	51.3 (14.9)		34.7 (5.0)	
Smoking use until pregnancy known	222	50.2 (14.2)		34.9 (5.2)	
Continued smoking use during pregnancy	338	51.5 (16.1)		34.7 (5.0)	
Maternal alcohol use during pregnancy			0.041		0,004
Never	973	50.5 (14.7)		34.4 (4.8)	
Alcohol use until pregnancy known	335	52.7 (16.0)		35.4 (5.6)	
Continued alcohol use during pregnancy	1,023	51.7 (14.9)		34.9 (4.9)	
Maternal age at intake (years)	2,954	0.05	0.007	0.04	0.015
Paternal age at intake (years)	2,077	0.00	0.905	0.01	0.637
Maternal body mass index (kg/m^2)	2,181	-0.02	0.437	-0.02	0.362
Paternal body mass index (kg/m^2)	2,070	-0.04	0.042	-0.05	0.013
Maternal height (cm)	2,638	0.06	0.002	0.03	0.192
Paternal height (cm)	2,074	0.06	0.010	0.01	0.537

Table S3. (Continued)

Participant characteristics	n	NO_X	p-value	NO_2	p-value
Maternal psychological distress during pregnancy	2,237	0.01	0.656	0.04	0.060
Paternal psychological distress during pregnancy	1,785	-0.05	0.036	-0.01	0.704
Maternal intelligence quotient score	2,688	0.05	0.009	0.03	0.097

Abbreviations: NO_X, nitrogen oxide; NO_2, nitrogen dioxide.
Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

Table S4. Exposure levels to PM_{10} and PM_{COARSE} during pregnancy by participant characteristics

Participant characteristics	n	PM_{10}	p-value	PM_{COARSE}	p-value
Maternal education level			<.001		0.036
Primary education or lower	176	26.7 (1.1)		10.1 (0.9)	
Secondary education	1,092	27.0 (1.7)		9.9 (1.0)	
Higher education	1,453	27.3 (1.7)		9.9 (1.1)	
Paternal education level			0.040		0.863
Primary education or lower	92	26.9 (1.7)		9.9 (1.0)	
Secondary education	700	27.2 (1.9)		9.9 (1.1)	
Higher education	1,069	27.3 (1.7)		9.9 (1.1)	
Monthly household income at enrollment			<.001		0.001
<900€	172	26.8 (1.4)		10.0 (0.9)	
900€ - 1,600€	319	26.9 (1.5)		10.1 (0.9)	
1,600€ - 2,200€	329	27.4 (1.7)		10.1 (1.1)	
>2,200€	1,486	27.3 (1.7)		9.9 (1.1)	
Maternal country of birth			<.001		<.001
The Netherlands	1,702	27.3 (1.7)		9.8 (1.1)	
Other Western	252	27.2 (1.7)		9.9 (1.1)	
Non-Western	944	27.0 (1.4)		10.1 (0.9)	
Paternal country of birth			0.001		0.010
The Netherlands	1,419	27.3 (1.8)		9.9 (1.1)	
Other Western	120	27.3 (1.7)		10.1 (1.2)	
Non-Western	502	27.0 (1.5)		10.0 (0.9)	
Family status at enrollment			<.001		<.001
Married	1,394	27.1 (1.6)		9.8 (1.1)	
Living together	1,023	27.4 (1.8)		10.0 (1.1)	
No partner	292	26.9 (1.4)		10.0 (0.9)	
Maternal parity			<.001		<.001
nulliparous	1,630	27.3 (1.7)		10.0 (1.1)	
1 child	883	27.0 (1.6)		9.8 (1.0)	
2 or more children	338	27.0 (1.4)		9.9 (1.0)	
Maternal smoking use during pregnancy			0.814		0.059
Never	2,004	27.2 (1.6)		9.9 (1.1)	
Smoking use until pregnancy known	222	27.1 (1.6)		9.8 (1.1)	
Continued smoking use during pregnancy	338	27.2 (1.8)		10.0 (1.0)	
Maternal alcohol use during pregnancy			<.001		0.935
Never	973	27.0 (1.5)		9.9 (1.0)	
Alcohol use until pregnancy known	335	27.4 (1.8)		9.9 (1.1)	
Continued alcohol use during pregnancy	1,023	27.3 (1.7)		9.9 (1.1)	
Maternal age at enrollment (years)	2,954	0.06	<.001	-0.03	0.022
Paternal age at enrollment (years)	2,077	0.03	0.182	-0.04	0.098
Maternal body mass index (kg/m^2)	2,181	-0.03	0.165	0.01	0.507
Paternal body mass index (kg/m^2)	2,070	-0.03	0.175	-0.08	<.001
Maternal height (cm)	2,638	0.07	0.001	-0.06	0.003

Table S4. (Continued)

Participant characteristics	n	PM_{10}	p-value	PM_{COARSE}	p-value
Paternal height (cm)	2,074	0.08	<.001	-0.05	0.039
Maternal psychological distress during pregnancy	2,237	-0.02	0.429	0.07	0.001
Paternal psychological distress during pregnancy	1,785	-0.05	0.025	0.03	0.179
Maternal intelligence quotient score	2,688	0.09	<.001	-0.04	0.025

Abbreviations: PM, particulate matter with aerodynamic diameters of less than 10μm (PM_{10}); between 10μm and 2.5μm (PM_{COARSE}).
Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

Table S5. Exposure levels to $PM_{2.5}$ and $PM_{2.5}$abs during pregnancy by participant characteristics

Participant characteristics	n	$PM_{2.5}$	p-value	$PM_{2.5}$abs	p-value
Maternal education level			<.001		<.001
Primary education or lower	176	16.8 (0.4)		1.6 (0.2)	
Secondary education	1,092	17.0 (0.6)		1.6 (0.3)	
Higher education	1,453	17.0 (0.6)		1.7 (0.3)	
Paternal education level			0.187		0.265
Primary education or lower	92	16.9 (0.6)		1.6 (0.4)	
Secondary education	700	17.0 (0.7)		1.7 (0.3)	
Higher education	1,069	17.0 (0.6)		1.7 (0.3)	
Monthly household income at intake			<.001		<.001
<900€	172	16.9 (0.5)		1.6 (0.3)	
900€ - 1,600€	319	16.9 (0.6)		1.6 (0.3)	
1,600€ - 2,200€	329	17.1 (0.6)		1.7 (0.3)	
>2,200€	1,486	17.0 (0.7)		1.7 (0.3)	
Maternal country of birth			<.001		<.001
The Netherlands	1,702	17.0 (0.7)		1.7 (0.3)	
Other Western	252	17.0 (0.6)		1.7 (0.3)	
Non-Western	944	16.9 (0.5)		1.6 (0.3)	
Paternal country of birth			<.001		0.001
The Netherlands	1,419	17.1 (0.7)		1.7 (0.3)	
Other Western	120	17.0 (0.7)		1.7 (0.4)	
Non-Western	502	16.9 (0.5)		1.6 (0.3)	
Family status at intake			<.001		<.001
Married	1,394	17.0 (0.6)		1.6 (0.3)	
Living together	1,023	17.1 (0.7)		1.7 (0.3)	
No partner	292	16.9 (0.5)		1.6 (0.3)	
Maternal parity			<.001		<.001
nulliparous	1,630	17.0 (0.7)		1.7 (0.3)	
1 child	883	16.9 (0.6)		1.6 (0.3)	
2 or more children	338	16.9 (0.5)		1.6 (0.2)	
Maternal smoking use during pregnancy			0.434		0.984
Never	2,004	17.0 (0.6)		1.7 (0.3)	
Smoking use until pregnancy known	222	16.9 (0.6)		1.7 (0.3)	
Continued smoking use during pregnancy	338	17.0 (0.7)		1.7 (0.3)	
Maternal alcohol use during pregnancy			<.001		<.001
Never	973	16.9 (0.6)		1.6 (0.3)	
Alcohol use until pregnancy known	335	17.1 (0.7)		1.7 (0.3)	
Continued alcohol use during pregnancy	1,023	17.0 (0.7)		1.7 (0.3)	
Maternal age at intake (years)	2,954	0.03	0.162	0,05	0.007
Paternal age at intake (years)	2,077	0.00	0.926	0.02	0.342
Maternal body mass index (kg/m^2)	2,181	-0.02	0.263	-0.04	0.080
Paternal body mass index (kg/m^2)	2,070	-0.05	0.015	-0.03	0.130

Table S5. (Continued)

Participant characteristics	n	$PM_{2.5}$	p-value	$PM_{2.5}$abs	p-value
Maternal height (cm)	2,638	0.07	<.001	0.07	0.001
Paternal height (cm)	2,074	0.07	0.001	0.06	0.011
Maternal psychological distress during pregnancy	2,237	-0.01	0.500	0.01	0.782
Paternal psychological distress during pregnancy	1,785	-0.04	0.074	-0.04	0.102
Maternal intelligence quotient score	2,688	0.08	<.001	0.07	<.001

Abbreviations: PM, particulate matter with aerodynamic diameters of less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters. Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

Table S6. Exposure levels to PAHs and B[a]P during pregnancy by participant characteristics

Participant characteristics	n	PAHs	p-value	B[a]P	p-value
Maternal education level			0.256		0.740
Primary education or lower	176	1.0 (0.2)		0.1 (0.0)	
Secondary education	1,092	1.0 (0.3)		0.1 (0.0)	
Higher education	1,453	1.0 (0.3)		0.1 (0.0)	
Paternal education level			0.512		0.843
Primary education or lower	92	1.0 (0.3)		0.1 (0.0)	
Secondary education	700	1.0 (0.4)		0.1 (0.0)	
Higher education	1,069	1.0 (0.4)		0.1 (0.0)	
Monthly household income at enrollment			<.001		0.025
<900€	172	1.0 (0.3)		0.1 (0.0)	
900€ - 1,600€	319	1.0 (0.3)		0.1 (0.0)	
1,600€ - 2,200€	329	1.0 (0.3)		0.1 (0.0)	
>2,200€	1,486	0.9 (0.4)		0.1 (0.0)	
Maternal country of birth			0.001		0.112
The Netherlands	1,702	1.0 (0.3)		0.1 (0.0)	
Other Western	252	0.9 (0.3)		0.1 (0.0)	
Non-Western	944	1.0 (0.3)		0.1 (0.0)	
Paternal country of birth			0.048		0.433
The Netherlands	1,419	1.0 (0.4)		0.1 (0.0)	
Other Western	120	1.0 (0.4)		0.1 (0.0)	
Non-Western	502	1.0 (0.3)		0.1 (0.0)	
Family status at enrollment			<.001		0.001
Married	1,394	0.9 (0.3)		0.1 (0.0)	
Living together	1,023	1.0 (0.4)		0.1 (0.0)	
No partner	292	1.0 (0.3)		0.1 (0.0)	
Maternal parity			<.001		<.001
nulliparous	1,630	1.0 (0.4)		0.1 (0.0)	
1 child	883	0.9 (0.3)		0.1 (0.0)	
2 or more children	338	0.9 (0.3)		0.1 (0.0)	
Maternal smoking use during pregnancy			0.031		0.116
Never	2,004	1.0 (0.3)		0.1 (0.0)	
Smoking use until pregnancy known	222	0.9 (0.3)		0.1 (0.0)	
Continued smoking use during pregnancy	338	1.0 (0.4)		0.1 (0.0)	
Maternal alcohol use during pregnancy			0.713		0.518
Never	973	1.0 (0.3)		0.1 (0.0)	
Alcohol use until pregnancy known	335	1.0 (0.4)		0.1 (0.0)	
Continued alcohol use during pregnancy	1,023	1.0 (0.4)		0.1 (0.0)	
Maternal age at enrollment (years)	2,954	-0.11	<.001	-0.08	<.001
Paternal age at enrollment (years)	2,077	-0.07	0.003	-0.05	0.013
Maternal body mass index (kg/m^2)	2,181	0.01	0.734	-0.00	0.900

Table S6. (Continued)

Participant characteristics	n	PAHs	p-value	B[a]P	p-value
Paternal body mass index (kg/m²)	2,070	-0.02	0.322	-0.02	0.336
Maternal height (cm)	2,638	-0.02	0.246	-0.00	0.915
Paternal height (cm)	2,074	-0.03	0.192	-0.00	0.716
Maternal psychological distress during pregnancy	2,237	0.05	0.020	0.04	0.094
Paternal psychological distress during pregnancy	1,785	0.03	0.203	0.02	0.472
Maternal intelligence quotient score	2,688	-0.03	0.166	-0.00	0.849

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene.
Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

Table S7. Exposure levels to OC and Cu during pregnancy by participant characteristics

Participant characteristics	n	OC	p-value	Cu	p-value
Maternal education level			0.018		<.001
Primary education or lower	176	1.8 (0.3)		4.7 (0.5)	
Secondary education	1,092	1.7 (0.4)		4.8 (0.9)	
Higher education	1,453	1.7 (0.4)		4.9 (0.9)	
Paternal education level			0.568		0.033
Primary education or lower	92	1.8 (0.4)		4.9 (1.0)	
Secondary education	700	1.7 (0.4)		4.8 (0.9)	
Higher education	1,069	1.7 (0.4)		5.0 (0.9)	
Monthly household income at enrollment			<.001		0.491
<900€	172	1.8 (0.3)		4.8 (0.8)	
900€ - 1,600€	319	1.8 (0.3)		4.9 (0.8)	
1,600€ - 2,200€	329	1.8 (0.4)		4.9 (0.9)	
>2,200€	1,486	1.7 (0.4)		4.9 (0.9)	
Maternal country of birth			<.001		0.732
The Netherlands	1,702	1.7 (0.4)		4.9 (0.9)	
Other Western	252	1.8 (0.4)		4.9 (0.8)	
Non-Western	944	1.8 (0.3)		4.8 (0.7)	
Paternal country of birth			0.006		0.143
The Netherlands	1,419	1.7 (0.4)		4.9 (0.9)	
Other Western	120	1.8 (0.4)		5.0 (1.1)	
Non-Western	502	1.8 (0.4)		4.8 (0.8)	
Family status at enrollment			0.002		0.006
Married	1,394	1.7 (0.4)		4.8 (0.8)	
Living together	1,023	1.8 (0.4)		4.9 (1.0)	
No partner	292	1.8 (0.3)		4.9 (0.8)	
Maternal parity			<.001		0.001
nulliparous	1,630	1.8 (0.4)		4.9 (0.9)	
1 child	883	1.7 (0.4)		4.8 (0.8)	
2 or more children	338	1.7 (0.4)		4.8 (0.6)	
Maternal smoking use during pregnancy			0.073		0.923
Never	2,004	1.7 (0.4)		4.9 (0.9)	
Smoking use until pregnancy known	222	1.7 (0.4)		4.9 (0.8)	
Continued smoking use during pregnancy	338	1.8 (0.4)		4.9 (0.9)	
Maternal alcohol use during pregnancy			0.330		<.001
Never	973	1.7 (0.4)		4.8 (0.8)	
Alcohol use until pregnancy known	335	1.8 (0.4)		5.0 (1.0)	
Continued alcohol use during pregnancy	1,023	1.7 (0.4)		4.9 (0.9)	
Maternal age at enrollment (years)	2,954	-0.06	0.001	0.01	0.581
Paternal age at enrollment (years)	2,077	-0.07	0.001	0.01	0.707
Maternal body mass index (kg/m^2)	2,181	0.02	0.245	-0.03	0.129

Table S7. (Continued)

Participant characteristics	n	OC	p-value	Cu	p-value
Paternal body mass index (kg/m^2)	2,070	-0.08	<.001	-0.04	0.043
Maternal height (cm)	2,638	-0.07	<.001	0.04	0.046
Paternal height (cm)	2,074	-0.04	0.058	0.02	0.261
Maternal psychological distress during pregnancy	2,237	0.08	0.029	0.01	0.510
Paternal psychological distress during pregnancy	1,785	0.05	0.029	0.01	0.757
Maternal intelligence quotient score	2,688	-0.06	0.003	0.05	0.007

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

Table S8. Exposure levels to Fe and K during pregnancy by participant characteristics

Participant characteristics	n	Fe	p-value	K	p-value
Maternal education level			<.001		0.009
Primary education or lower	176	121.7 (13.1)		111.5 (4.9)	
Secondary education	1,092	121.6 (18.2)		113.1 (7.8)	
Higher education	1,453	125.2 (21.1)		113.3 (7.2)	
Paternal education level			0.005		0.140
Primary education or lower	92	122.5 (16.2)		111.8 (6.7)	
Secondary education	700	122.5 (22.8)		113.5 (8.3)	
Higher education	1,069	125.8 (21.0)		113.4 (7.1)	
Monthly household income at enrollment			0.303		<.001
<900€	172	123.0 (17.1)		111.6 (6.3)	
900€ - 1,600€	319	123.1 (16.5)		111.8 (6.4)	
1,600€ - 2,200€	329	123.1 (19.0)		113.8 (7.8)	
>2,200€	1,486	124.8 (21.2)		113.6 (7.7)	
Maternal country of birth			0.865		0.001
The Netherlands	1,702	123.4 (21.0)		113.4 (7.6)	
Other Western	252	124.0 (21.3)		113.3 (7.9)	
Non-Western	944	123.3 (15.0)		112.3 (6.6)	
Paternal country of birth			0.122		0.010
The Netherlands	1,419	124.3 (21.6)		113.6 (7.7)	
Other Western	120	127.4 (28.5)		112.7 (6.5)	
Non-Western	502	123.1 (16.6)		112.5 (7.2)	
Family status at enrollment			0.024		<.001
Married	1,394	122.5 (17.8)		112.8 (7.3)	
Living together	1,023	124.3 (21.7)		113.7 (7.6)	
No partner	292	125.1 (19.5)		111.8 (5.8)	
Maternal parity			0.046		<.001
nulliparous	1,630	124.4 (21.2)		113.5 (7.5)	
1 child	883	122.7 (17.8)		112.5 (7.0)	
2 or more children	338	122.2 (13.6)		112.1 (6.8)	
Maternal smoking use during pregnancy			0.229		0.838
Never	2,004	123.7 (20.3)		113.1 (7.3)	
Smoking use until pregnancy known	222	124.8 (17.9)		112.9 (7.0)	
Continued smoking use during pregnancy	338	122.1 (16.1)		113.2 (8.2)	
Maternal alcohol use during pregnancy			0.001		0.030
Never	973	122.2 (18.1)		112.8 (7.4)	
Alcohol use until pregnancy known	335	125.3 (22.7)		114.0 (8.1)	
Continued alcohol use during pregnancy	1,023	125.4 (20.2)		113.1 (7.0)	
Maternal age at enrollment (years)	2,954	0,02	0.216	0.05	0.005
Paternal age at enrollment (years)	2,077	0.03	0.250	0.00	0.911
Maternal body mass index (kg/m^2)	2,181	-0.02	0.269	-0.02	0.387

Table S8. (Continued)

Participant characteristics	n	Fe	p-value	K	p-value
Paternal body mass index (kg/m^2)	2,070	-0.05	0.039	-0.02	0.409
Maternal height (cm)	2,638	0.03	0.126	0.06	0.002
Paternal height (cm)	2,074	0.00	0.935	0.07	0.002
Maternal psychological distress during pregnancy	2,237	0.01	0.483	-0.02	0.432
Paternal psychological distress during pregnancy	1,785	0.02	0.507	-0.04	0.118
Maternal intelligence quotient score	2,688	0.04	0.040	0.05	0.016

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

Table S9. 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 known	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 body mass index (kg/m^2)	2,181	-0.02	0.476	-0.02	0.444

Table S9. (Continued)

Participant characteristics	n	Si	p-value	Zn	p-value
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

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

Table S10. Exposure levels to OP_{DTT} and OP_{ESR} during pregnancy by participant characteristics

Participant characteristics	n	OP_{DTT}	p-value	OP_{ESR}	p-value
Maternal education level			<.001		0.031
Primary education or lower	176	1.3 (0.1)		1064.5 (99.5)	
Secondary education	1,092	1.3 (0.1)		1072.3 (203.5)	
Higher education	1,453	1.3 (0.1)		1089.7 (181.2)	
Paternal education level			0.007		0.251
Primary education or lower	92	1.3 (0.1)		1093.3 (248.8)	
Secondary education	700	1.3 (0.1)		1076.3 (212.4)	
Higher education	1,069	1.3 (0.1)		1091.5 (174.7)	
Monthly household income at enrollment			<.001		0.963
<900€	172	1.3 (0.1)		1085.1 (155.2)	
900€ - 1,600€	319	1.3 (0.1)		1086.9 (170.7)	
1,600€ - 2,200€	329	1.3 (0.1)		1080.5 (165.9)	
>2,200€	1,486	1.3 (0.1)		1086.2 (194.9)	
Maternal country of birth			<.001		0.273
The Netherlands	1,702	1.3 (0.1)		1075.8 (193.9)	
Other Western	252	1.3 (0.1)		1074.2 (157.6)	
Non-Western	944	1.3 (0.1)		1087.3 (169.2)	
Paternal country of birth			0.001		0.158
The Netherlands	1,419	1.3 (0.1)		1082.7 (192.9)	
Other Western	120	1.3 (0.1)		1117.4 (242.5)	
Non-Western	502	1.3 (0.1)		1083.0 (172.3)	
Family status at enrollment			<.001		0.002
Married	1,394	1.3 (0.1)		1068.7 (168.1)	
Living together	1,023	1.3 (0.1)		1090.8 (206.3)	
No partner	292	1.3 (0.1)		1100.6 (189.4)	
Maternal parity			<.001		0.002
nulliparous	1,630	1.3 (0.1)		1091.2 (198.3)	
1 child	883	1.3 (0.1)		1069.6 (159.0)	
2 or more children	338	1.3 (0.1)		1061.1 (126.8)	
Maternal smoking use during pregnancy			0.038		0.905
Never	2,004	1.3 (0.1)		1080.7 (186.3)	
Smoking use until pregnancy known	222	1.3 (0.1)		1086.2 (160.1)	
Continued smoking use during pregnancy	338	1.3 (0.1)		1083.2 (211.7)	
Maternal alcohol use during pregnancy			<.001		0.014
Never	973	1.3 (0.1)		1071.6 (174.8)	
Alcohol use until pregnancy known	335	1.3 (0.1)		1098.3 (215.9)	
Continued alcohol use during pregnancy	1,023	1.3 (0.1)		1092.6 (182.9)	
Maternal age at enrollment (years)	2,954	-0.14	<.001	-0.00	0.920
Paternal age at enrollment (years)	2,077	-0.11	<.001	-0.00	0.939
Maternal body mass index (kg/m^2)	2,181	0.05	0.029	-0.03	0.136

Table S10. (Continued)

Participant characteristics	n	OP_{DTT}	p-value	OP_{ESR}	p-value
Paternal body mass index (kg/m²)	2,070	-0.05	0.031	-0.06	0.006
Maternal height (cm)	2,638	-0.09	<.001	0.02	0.327
Paternal height (cm)	2,074	-0.07	0.001	-0.01	0.528
Maternal psychological distress during pregnancy	2,237	0.12	<.001	0.04	0.036
Paternal psychological distress during pregnancy	1,785	0.06	0.015	0.03	0.248
Maternal intelligence quotient score	2,688	-0.11	<.001	0.02	0.398

Abbreviations: OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance).
Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

Table S11. Exposure levels to UFP during pregnancy by participant characteristics

Participant characteristics	n	UFP	p-value
Maternal education level			<.001
Primary education or lower	176	10141.3 (1153.8)	
Secondary education	1,092	10148.3 (1743.0)	
Higher education	1,453	10513.9 (1933.5)	
Paternal education level			<.001
Primary education or lower	92	10152.7 (1398.8)	
Secondary education	700	10223.9 (2067.4)	
Higher education	1,069	10583.7 (1928.8)	
Monthly household income at enrollment			0.506
<900€	172	10247.4 (1524.5)	
900€ - 1,600€	319	10358.0 (1507.4)	
1,600€ - 2,200€	329	10381.3 (1821.2)	
>2,200€	1,486	10450.1 (1965.9)	
Maternal country of birth			0.831
The Netherlands	1,702	10326.9 (1979.4)	
Other Western	252	10393.3 (1819.6)	
Non-Western	944	10316.4 (1417.5)	
Paternal country of birth			0.144
The Netherlands	1,419	10430.6 (2013.3)	
Other Western	120	10659.4 (2357.3)	
Non-Western	502	10297.4 (1534.2)	
Family status at enrollment			0.003
Married	1,394	10221.4 (1695.7)	
Living together	1,023	10458.8 (2013.0)	
No partner	292	10449.9 (1598.9)	
Maternal parity			0.002
nulliparous	1,630	10459.5 (1954.4)	
1 child	883	10215.7 (1666.5)	
2 or more children	338	10227.8 (1296.9)	
Maternal smoking use during pregnancy			0.596
Never	2,004	10353.4 (1864.3)	
Smoking use until pregnancy known	222	10427.3 (1750.2)	
Continued smoking use during pregnancy	338	10271.2 (1616.8)	
Maternal alcohol use during pregnancy			<.001
Never	973	10203.5 (1700.2)	
Alcohol use until pregnancy known	335	10547.1 (2016.4)	
Continued alcohol use during pregnancy	1,023	10518.5 (1874.5)	

Table S11. (Continued)

Participant characteristics	n	UFP	p-value
Maternal age at enrollment (years)	2,954	0.02	0.400
Paternal age at enrollment (years)	2,077	0.01	0.542
Maternal body mass index (kg/m^2)	2,181	-0.02	0.339
Paternal body mass index (kg/m^2)	2,070	-0.04	0.103
Maternal height (cm)	2,638	0.02	0.276
Paternal height (cm)	2,074	0.02	0.386
Maternal psychological distress during pregnancy	2,237	0.01	0.775
Paternal psychological distress during pregnancy	1,785	0.00	0.897
Maternal intelligence quotient score	2,688	0.06	0.002

Abbreviations: UFP, ultra fine particles.
Values are mean (standard deviation) per category using one-way ANOVA test for categorical variables, and are pairwise correlation coefficients for continuous variables

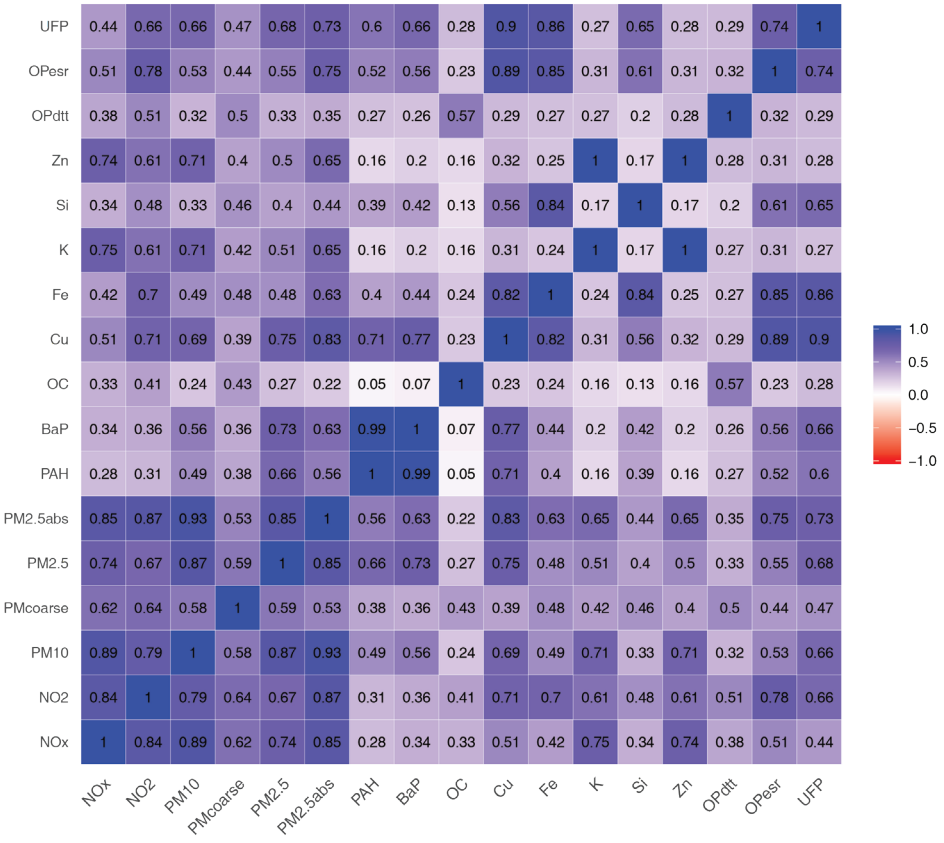

Figure S2. Correlations between levels of the pollutants during pregnancy

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 (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; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles.

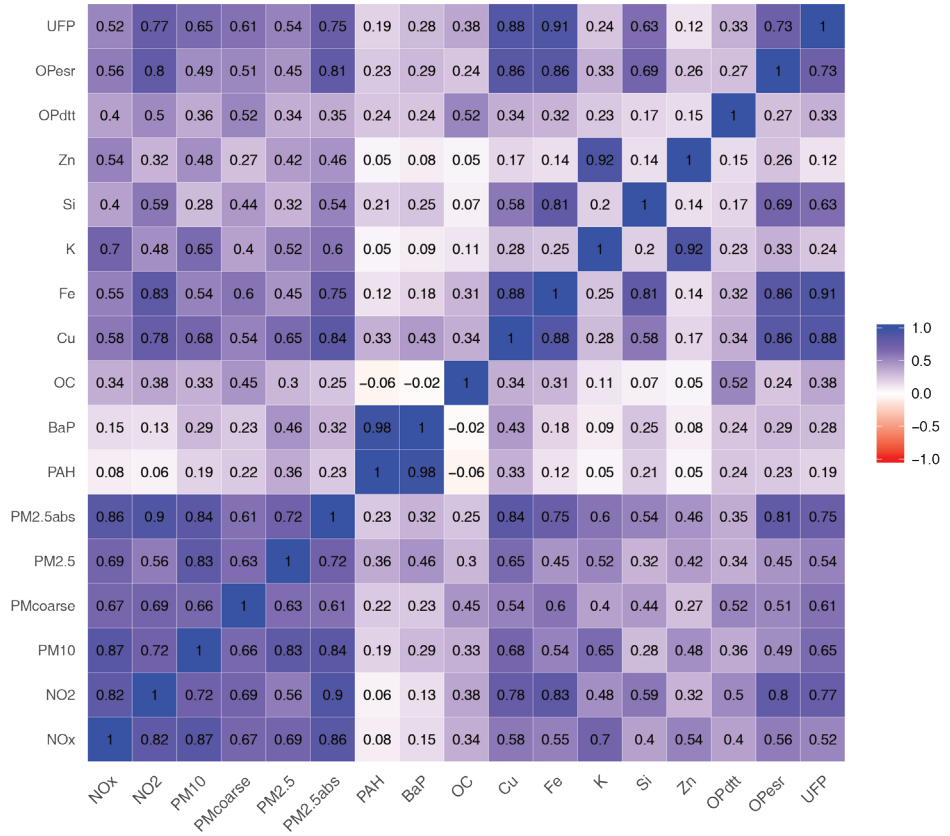

Figure S3. Correlations between levels of the pollutants during childhood

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 (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; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT} – dithiothreitol and OP_{ESR} – electron spin resonance); UFP, ultra-fine particles.

Table S12. Results of multi-pollutant models selected by the Deletion/Substitution/Addition algorithm for pregnancy exposures in relation to global fractional anisotropy and global mean diffusivity, respectively, excluding participants of mothers recruited after birth (n=310)

		Contrast	Coef. (95% CI)	p-value
Global fractional anisotropy				
Pregnancy exposure models (% of runs)				
Model 1 (24.5%)				
	$PM_{2.5}$	5 μg/m^3	-1.53 (-2.34 ; -0.73)	<0.001
	PAHs	1 ng/m^3	0.33 (0.05 ; 0.61)	0.022
	OP_{DTT}	1 nmol DTT/min/m^3	0.52 (-0.08 ; 1.12)	0.091
Model 2 (20%)				
	$PM_{2.5}$	5 μg/m^3	-1.36 (-2.14 ; -0.58)	0.001
	PAHs	1 ng/m^3	0.33 (0.05 ; 0.62)	0.020
Model 3 (13%)				
	$PM_{2.5}$	5 μg/m^3	-0.73 (-1.29 ; -0.16)	0.012
Global mean diffusivity				
Pregnancy exposure models (% of runs)				
Model 1 (13.5%)				
	Si	100 ng/m^3	0.05 (0.00 ; 0.11)	0.049
	OP_{DTT}	1 nmol DTT/min/m^3	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. Model selection is performed using Deletion/Substitution/Addition algorithm. PM_{10}, B[a]P, K, and UFP were excluded due to a correlation of 0.90 or more with $PM_{2.5}$abs, PAHs, Zn, and Cu respectively. For each combination of period of exposure and outcome, 200 runs were performed and the final model was selected based on frequency of occurrence (% of runs, at least 10% to be reported here). Coefficients and 95% CI from (multiple) linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

Table S13. Results of analyses in which pregnancy and childhood exposures selected by Deletion/ Substitution/Addition algorithm were introduced simultaneously in the model in relation to global fractional anisotropy and global mean diffusivity, respectively

	Contrast	Coef. (95% CI)	p-value
Global fractional anisotropy			
pregnancy $PM_{2.5}$	5 $\mu g/m^3$	-0.48 (-1.07 ; 0.10)	0.105
childhood NO_X	20 $\mu g/m^3$	-0.10 (-0.21 ; 0.00)	0.053
Global mean diffusivity			
pregnancy Si	100 ng/m^3	0.06 (0.01 ; 0.11)	0.024
childhood Zn	10 ng/m^3	0.02 (0.01 ; 0.04)	0.009
childhood OP_{DTT}	1 nmol DTT/min/m^3	0.06 (-0.01 ; 0.13)	0.069

Abbreviations: Coef., coefficient; CI, confidence intervals; $PM_{2.5}$, particulate matter with diameter of <2.5μm; NO_X, nitrogen oxides; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol). Coefficients and 95% CI from multiple linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

Table S14. Adjusted linear regression analyses of fractional anisotropy in twelve individual white matter tracts in relation to pregnancy $PM_{2.5}$, and childhood NO_X exposures

Fractional anisotropy	Fetal life exposure to $PM_{2.5}$			
	Coef.	**95% CI**	**p-value**	**q-value**
uncinate fasciculus left hemisphere	-0.009	-0.018 ; 0.000	0.056	0.134
uncinate fasciculus right hemisphere	-0.005	-0.013 ; 0.003	0.258	0.387
cingulate gyrus part of cingulum left hemisphere	-0.010	-0.023 ; 0.003	0.140	0.240
cingulate gyrus part of cingulum right hemisphere	-0.006	-0.018 ; 0.006	0.301	0.401
superior longitudinal fasciculus left hemisphere	-0.006	-0.013 ; 0.001	0.089	0.178
superior longitudinal fasciculus right hemisphere	-0.009	-0.016 ; -0.002	0.018	0.060
forceps minor	-0.013	-0.023 ; -0.003	0.012	0.060
forceps major	-0.003	-0.014 ; 0.008	0.578	0.578
inferior longitudinal fasciculus left hemisphere	-0.003	-0.009 ; 0.004	0.390	0.468
inferior longitudinal fasciculus right hemisphere	-0.002	-0.009 ; 0.005	0.564	0.578
corticospinal tract left hemisphere	-0.008	-0.014 ; -0.001	0.020	0.060
corticospinal tract right hemisphere	-0.008	-0.014 ; -0.002	0.015	0.060

Fractional anisotropy	Childhood exposure to NO_x			
	Coef.	**95% CI**	**p-value**	**q-value**
uncinate fasciculus left hemisphere	-0.002	-0.003 ; -0.000	0.027	0.065
uncinate fasciculus right hemisphere	-0.002	-0.003 ; -0.000	0.026	0.065
cingulate gyrus part of cingulum left hemisphere	-0.002	-0.004 ; 0.001	0.137	0.206
cingulate gyrus part of cingulum right hemisphere	0.000	-0.002 ; 0.002	0.886	0.886
superior longitudinal fasciculus left hemisphere	-0.001	-0.002 ; 0.001	0.302	0.362
superior longitudinal fasciculus right hemisphere	-0.002	-0.003 ; -0.000	0.012	0.065
forceps minor	-0.002	-0.003 ; 0.000	0.052	0.104
forceps major	-0.000	-0.002 ; 0.002	0.794	0.866
inferior longitudinal fasciculus left hemisphere	-0.001	-0.002 ; 0.000	0.086	0.147
inferior longitudinal fasciculus right hemisphere	-0.001	-0.003 ; -0.000	0.019	0.065
corticospinal tract left hemisphere	-0.001	-0.002 ; -0.000	0.024	0.065
corticospinal tract right hemisphere	-0.001	-0.002 ; 0.000	0.223	0.297

Abbreviations: Coef, coefficient; CI, confidence intervals; NO_X, nitrogen oxides; $PM_{2.5}$, particulate matter with diameter of <2.5µm. Coefficients and 95% CI from linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

Pregnancy $PM_{2.5}$ (5µg/m^3 increment) and childhood NO_X (20µg/m^3 increment) were selected for this analysis as significant predictors of global FA (nominal $p<0.05$) in DSA-selected multipollutant models of pregnancy or childhood exposures (respectively), and as significant predictors of global FA in the single pollutant models. To obtain the q-value, false discovery rate correction for multiple testing was applied using Benjamini and Hochberg method (Benjamini and Hochberg 1995).

The FDR significant exposures were then included in multipollutant models of individual white matter tracts.

Table S15. Adjusted linear regression analyses of mean diffusivity in twelve individual white matter tracts in relation to pregnancy Si, and childhood Zn and OP_{DTT} exposures

Mean diffusivity**	Fetal life exposure to Si			
	Coef.	**95% CI**	**p-value**	**q-value**
uncinate fasciculus left hemisphere	0.003	-0.003 ; 0.008	0.323	0.431
uncinate fasciculus right hemisphere	0.004	-0.002 ; 0.009	0.167	0.251
cingulate gyrus part of cingulum left hemisphere	0.009	0.002 ; 0.016	0.008	0.032
cingulate gyrus part of cingulum right hemisphere	0.008	0.001 ; 0.014	0.027	0.081
superior longitudinal fasciculus left hemisphere	0.009	0.004 ; 0.014	0.002	0.012
superior longitudinal fasciculus right hemisphere	0.006	-0.000 ; 0.012	0.053	0.106
forceps minor	0.017	0.009 ; 0.024	<.001	<.001
forceps major	-0.001	-0.016 ; 0.015	0.910	0.910
inferior longitudinal fasciculus left hemisphere	0.007	0.000 ; 0.014	0.040	0.096
inferior longitudinal fasciculus right hemisphere	0.007	-0.001 ; 0.014	0.076	0.130
corticospinal tract left hemisphere	0.002	-0.009 ; 0.013	0.697	0.836
corticospinal tract right hemisphere	-0.001	-0.011 ; 0.009	0.840	0.910

Mean diffusivity**	Childhood exposure to Zn			
	Coef.	**95% CI**	**p-value**	**q-value**
uncinate fasciculus left hemisphere	0.002	0.000 ; 0.004	0.046	0.061
uncinate fasciculus right hemisphere	0.002	0.001 ; 0.004	0.009	0.018
cingulate gyrus part of cingulum left hemisphere	0.004	0.002 ; 0.007	<.001	<.001
cingulate gyrus part of cingulum right hemisphere	0.004	0.001 ; 0.006	0.003	0.012
superior longitudinal fasciculus left hemisphere	0.003	0.001 ; 0.004	0.009	0.018
superior longitudinal fasciculus right hemisphere	0.003	0.001 ; 0.005	0.006	0.018
forceps minor	0.005	0.002 ; 0.007	0.001	0.006
forceps major	0.000	-0.005 ; 0.006	0.915	0.915
inferior longitudinal fasciculus left hemisphere	0.002	0.000 ; 0.005	0.037	0.056
inferior longitudinal fasciculus right hemisphere	0.003	0.000 ; 0.005	0.034	0.056
corticospinal tract left hemisphere	0.001	-0.003 ; 0.005	0.586	0.639
corticospinal tract right hemisphere	0.002	-0.001 ; 0.006	0.165	0.198

Mean diffusivity**	Childhood exposure to OP_{DTT}			
	Coef.	**95% CI**	**p-value**	**q-value**
uncinate fasciculus left hemisphere	0.004	-0.003 ; 0.011	0.265	0.454
uncinate fasciculus right hemisphere	0.002	-0.004 ; 0.009	0.472	0.515
cingulate gyrus part of cingulum left hemisphere	0.010	0.001 ; 0.019	0.028	0.321
cingulate gyrus part of cingulum right hemisphere	0.007	-0.001 ; 0.016	0.100	0.321
superior longitudinal fasciculus left hemisphere	0.003	-0.004 ; 0.010	0.414	0.515
superior longitudinal fasciculus right hemisphere	0.005	-0.003 ; 0.013	0.199	0.454
forceps minor	0.008	-0.002 ; 0.018	0.107	0.321
forceps major	0.008	-0.012 ; 0.028	0.437	0.515

Table S15. (Continued)

inferior longitudinal fasciculus left hemisphere	0.007	-0.001 ; 0.016	0.093	0.321
inferior longitudinal fasciculus right hemisphere	0.006	-0.004 ; 0.015	0.264	0.454
corticospinal tract left hemisphere	0.006	-0.008 ; 0.020	0.391	0.515
corticospinal tract right hemisphere	0.002	-0.011 ; 0.015	0.753	0.753

Abbreviations: Coef, coefficient; CI, confidence intervals; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol). Coefficients and 95% CI from linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

Pregnancy Si (100 ng/m^3 increment) and childhood Zn (10ng/m^3 increment) and OP_{DTT} (1nmol DTT/min/m^3 increment) were selected for this analysis as significant predictors of global MD (nominal $p<0.05$) in DSA-selected multipollutant models of pregnancy or childhood exposures (respectively), and as significant predictors of global MD in the single pollutant models.

To obtain the q-value, false discovery rate correction for multiple testing was applied using Benjamini and Hochberg method (Benjamini and Hochberg 1995).

The FDR significant exposures were then included in multipollutant models of individual white matter tracts.

*Values of MD were multiplied by 10^9 (concerns only individual tracts analyses)

Table S16. Results of analyses in which pregnancy and childhood exposures selected by Deletion/Substitution/Addition algorithm were introduced simultaneously in the model in relation to global mean diffusivity in three white matter tracts

	Contrast	Coef. (95% CI)	p-value
Mean diffusivity in:			
Cingulate gyrus part of cingulum of the left hemisphere			
pregnancy Si	100 ng/m^3	0.0082 (0.0015 ; 0.0151)	0.017
childhood Zn	10 ng/m^3	0.0041 (0.0017 ; 0.0065)	0.001
Superior longitudinal fasciculus of the left hemisphere			
pregnancy Si	100 ng/m^3	0.0083 (0.0030 ; 0.0137)	0.002
childhood Zn	10 ng/m^3	0.0023 (0.0004 ; 0.0042)	0.016
Forceps minor			
pregnancy Si	100 ng/m^3	0.0158 (0.0085 ; 0.0232)	<.001
childhood Zn	10 ng/m^3	0.0041 (0.0015 ; 0.0067)	0.002

Abbreviations: Coef., coefficient; CI, confidence intervals; $PM_{2.5}$, particulate matter with diameter of <2.5µm; NO_X, nitrogen oxides; OP_{DTT}, oxidative potential of $PM_{2.5}$ (DTT: evaluated using dithiothreitol). Coefficients and 95% CI from multiple linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

The selection of the pollutants is based on FDR-significance in single-pollutant models. If more than one pollutant was FDR-significant for FA or MD in the same tract, multi-pollutant models were performed for FA or MD in the tract.

Table S17. Results of the adjusted associations between exposure during pregnancy 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

	Pregnancy				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
$PM_{2.5}$	-1.26 ; -0.16	0.277	-0.99 ; -0.11	0.224	-1.14 ; 0.21	0.340	-1.06 ; 0.14	0.306
$PM_{2.5}$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	-0.71 ; 0.06	0.193	-0.69 ; 0.05	0.189	-0.65 ; 0.21	0.217	-0.66 ; 0.18	0.215
Fe	-0.54 ; 0.14	0.172	-0.55 ; 0.14	0.175	-0.53 ; 0.09	0.156	-0.55 ; 0.06	0.155
K	-0.84 ; 0.08	0.230	-0.97 ; 0.25	0.310	-1.03 ; -0.03	0.253	-0.70 ; 0.17	0.221
Si	-0.70 ; 0.15	0.214	-0.73 ; 0.15	0.225	-0.66 ; 0.19	0.216	-0.34 ; 0.06	0.102
Zn	-0.28 ; 0.04	0.081	-0.48 ; 0.20	0.174	-0.27 ; 0.02	0.075	-0.18 ; 0.00	0.047

Mean diffusivity

	Pregnancy				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
$PM_{2.5}$	0.02 ; 0.15	0.034	0.01 ; 0.12	0.028	0.03 ; 0.20	0.041	0.02 ; 0.18	0.040
$PM_{2.5}$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

Table S17. (Continued)

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 (PM_{COARSE}); less than 2.5μm ($PM_{2.5}$); $PM_{2.5}$abs, absorbance of $PM_{2.5}$ filters. Coefficients and 95% CI from linear regression models adjusted for both maternal and paternal education, country of birth, age, height, BMI, and psychological distress during pregnancy; maternal smoking and alcohol consumption during pregnancy, parity, marital status, intelligence quotient, and household income; and child's genetic ancestry, gender, and age at the scanning session. Any missing covariates were imputed through multiple imputation, and inverse probability weighting technique was used to account for potential selection bias.

GENERAL DISCUSSION

GENERAL DISCUSSION

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.

Main findings

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.

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).

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.

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.

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.

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).

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 true effects are being discarded as not significant 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.

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.

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. 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.

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.

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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CONCLUSIONS

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 pregnancy 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.

−Pregnancy 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 pregnancy exposure to various air pollutants and larger cortical and subcortical volumes in preadolescents from 9 to 12 years old, was mainly observed in girls.

−Pregnancy 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.

SUMMARY/SAMENVATTING

SUMMARY

Exposure to outdoor air pollution is an increasingly recognized risk factor for neuropsychological disorders. Understanding the associations between early life exposure to outdoor air pollution and neuropsychological development, as well as understanding the mechanisms underlying such associations, is crucial, yet insufficient to date. The objective of the work presented in this thesis, was to expand the current body of evidence and fill several identified gaps in knowledge. For this aim, five studies were carried out which are presented in the results section of this thesis.

In the first study, presented in **Paper I**, we investigated the association between elemental components of fine particulate matter with cognitive and psychomotor function in children of 1 to 9 years old from 4 European birth cohorts. We studied the elemental components one by one, as well as combined into latent variables depending on their source of origin. 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, meaning that children exposed to higher concentrations at birth were performing less well on tasks requiring fine motor skills than less exposed children. No associations were found between any elemental component with gross motor function or cognitive function, although the effect estimates of the latter were predominantly negative.

In **Paper II**, we looked at the emotional and behavioral domain of neuropsychological development. We assessed whether prenatal and postnatal exposure to various air pollutants was related to depressive and anxiety symptoms, and aggressive symptoms in children of 7 to 11 years old from 8 European birth cohorts. We did not observe such relationship. As studies in adults generally report positive associations between exposure to air pollution and the odds of emotional problems, including depression and anxiety, we hypothesize 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 possibly too young to already have developed such problems.

Next, we examined the relationship between exposure to air pollution during fetal life and brain morphological alterations in children of 6 to 10 years old in a subset of Generation R Study, a population-based birth cohort from Rotterdam, the Netherlands, and reported the findings in **Paper III**. The study population was oversampled for certain maternal and child characteristics. We found that exposure during pregnancy to fine particulate matter was associated with a thinner cortex in various regions of the brain of the children. Moreover, thinner cortex in the precuneus and the rostral middle frontal regions partially mediated the association between exposure to fine particles and impaired inhibitory control. From the study population, only 0.5% of the participants was exposed to concentrations of fine particulate matter higher than the current EU annual limits.

Paper IV was a follow up on the previous study. By increasing the population size fourfold, assessing exposures during fetal life as well as during childhood, and including a larger number of pollutants, we made the study more comprehensive. Also, the study population was not oversampled, and was approximately 4 years older than the study population from Paper III. We found that a higher fetal and childhood exposure to pollutants representative of traffic related sources, was associated with thinner cortex, larger cortical and subcortical volumes, and lower volume of corpus callosum in preadolescents of 9 to 12 years old. Moreover, higher exposure to air pollution during childhood was 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.

We then looked into the association between pregnancy and childhood exposures to air pollution and white matter microstructure in the same large population of preadolescents of 9 to 12 years old, 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, was associated with alterations in white matter microstructure, manifested by lower fractional anisotropy and higher mean diffusivity. Generally, normal white matter microstructure development is characterized by gradually increasing fractional anisotropy and decreasing mean diffusivity, thus the alterations related to air pollution exposure observed in our study could indicate a compromised white matter integrity and possibly thereto related developmental delay.

All the results of the five papers are summed up in the general discussion, where we also discuss various methodological considerations, as well as implications for public health and policy making. Finally, several recommendations for future directions in research are proposed.

SAMENVATTING

Blootstelling aan luchtverontreiniging is een erkende risicofactor voor neuropsychologische stoornissen. Het begrijpen van de relatie tussen vroegtijdige blootstelling en neuropsychologische ontwikkeling, evenals het krijgen van inzicht in de onderliggende mechanismen van dergelijke associaties, is cruciaal, maar tot op heden gering. Het doel van het werk gepresenteerd in dit proefschrift was om het huidige bewijsmateriaal uit te breiden, en verschillende geïdentificeerde leemten in kennis te vullen. Omwille hiervan hebben wij een vijftal studies uitgevoerd, welke worden gepresenteerd in de resultatensectie van dit proefschrift.

In de eerste studie, gepresenteerd in **Paper I**, hebben we de associatie tussen elementaire componenten van fijnstof met cognitieve en psychomotorische functie bij kinderen van 1 tot 9 jaar oud uit 4 Europese geboortecohorten onderzocht. We hebben de elementaire componenten eerst één voor één bestudeerd, en vervolgens, afhankelijk van de bron, gecombineerd in latente variabelen. We vonden een negatief verband tussen blootstelling bij de geboorte aan elementair ijzer, en fijne motoriek. Elementair ijzer is een element dat veel voorkomt in de lucht die verontreinigd is door gemotoriseerd wegverkeer. Deze bevinding betekent dat kinderen die bij de geboorte werden blootgesteld aan hogere concentraties, minder goed presteerden op taken waarvoor fijne motoriek was vereist in vergelijking met kinderen met lage blootstelling. Er werd geen verband gevonden tussen enig elementair component met grove motorische functie of cognitieve functie. De geschatte effecten van de laatstgenoemde waren echter wel overwegend negatief.

In **Paper II** hebben we gekeken naar het emotionele- en gedragsdomein van neuropsychologische ontwikkeling. We hebben onderzocht of prenatale en postnatale blootstelling aan verschillende luchtverontreinigende stoffen verband hield met symptomen van depressie en angst, evenals met symtomen van agressie, bij kinderen van 7 tot 11 jaar oud uit 8 Europese geboortecohorten. Geen van deze relaties werd geobserveerd. Aangezien studies bij volwassenen over het algemeen blootstelling aan luchtverontreiniging associeren met een verhoogd risico op emotionele problemen, waaronder depressie en angst, veronderstellen we dat emotionele en gedragsproblemen in verband met blootstelling aan luchtvervuiling zich pas later in het leven manifesteren, en onze studiepopulatie ergo mogelijk te jong is om dergelijke problemen al ontwikkeld te hebben.

Vervolgens onderzochten we de relatie tussen blootstelling aan luchtvervuiling tijdens het foetale leven en morfologische veranderingen in de hersenen bij kinderen van 6 tot 10 jaar oud in een subgroep van Generation R Study, een bevolkingscohort uit Rotterdam, en rapporteerden de bevindingen in **Paper III**. De studiepopulatie was overgeselecteerd op bepaalde kenmerken van de moeder en het kind. We vonden dat blootstelling aan fijnstof tijdens de zwangerschap verband had met een dunnere hersenschors in verschillende delen van de hersenen van de kinderen. Bovendien verklaarde de dunnere hersenschors in de precuneus en de rostrale middenfrontale gebieden gedeeltelijk de associatie tussen

blootstelling aan fijnstof en inhibitieproblemen. Van de studiepopulatie werd slechts 0.5% van de deelnemers blootgesteld aan concentraties fijnstof hoger dan de huidige jaarlimieten van de EU.

Paper IV was een vervolg op het voorgaande onderzoek. We hebben dat onderzoek uitgebreid door een vier keer grotere populatie te bestuderen, blootstellingen tijdens het foetale leven evenals tijdens de kindertijd te beoordelen, en een groter aantal verontreinigende stoffen op te nemen. Ook was de onderzoekspopulatie niet overgeselecteerd op bepaalde kenmerken van de moeder en het kind, en was deze ongeveer 4 jaar ouder dan de onderzoekspopulatie uit Paper III. We hebben geconstateerd dat zowel een hogere foetale- als kindertijd blootstelling aan verontreinigende stoffen die representatief zijn voor wegverkeer, geassocieerd was met een dunnere hersenschors, grotere corticale en subcorticale volumes, en een lager volume van corpus callosum, in preadolescenten van 9 tot 12 jaar oud. Bovendien werd een hogere blootstelling aan luchtverontreiniging tijdens de kindertijd in verband gebracht met een groter oppervlak van de corticale pia. De associaties met foetale blootstelling aan luchtvervuiling werden voornamelijk waargenomen bij meisjes. De gebieden van de herschenschors waar de veranderingen werden waargenomen, kwamen overeen met de gebieden uit Paper III.

Vervolgens hebben we gekeken naar de samenhang van foetale en kindertijd blootstelling aan luchtvervuiling met microstructuur van het witte stof in dezelfde grote populatie van preadolescenten van 9 tot 12 jaar oud, en rapporteerden de resultaten in **Paper V**. We namen waar dat een hogere blootstelling aan verontreinigende stoffen representatief voor remvoeringen, bandslijtage, en uitlaatstoffen afkomstig voornamelijk van diesel verbranding, was geassocieerd met veranderingen in de microstructuur van de witte stof, wat zich manifesteerde in een lagere fractionele anisotropie en een hogere gemiddelde diffusiviteit. Over het algemeen wordt de normale ontwikkeling van de microstructuur van witte stof gekenmerkt door geleidelijk toenemende fractionele anisotropie en afnemende gemiddelde diffusiviteit. De in onze studie geobserveerde veranderingen, kunnen dus wijzen op een aangetaste integriteit van de witte stof en mogelijk op de daarmee samenhangende ontwikkelingsachterstand.

Alle resultaten van de vijf papers worden samengevat in de algemene discussie, waar we ook verschillende methodologische overwegingen bespreken, evenals implicaties voor de volksgezondheid en beleidsvorming. Ten slotte worden enkele aanbevelingen voor toekomstige richtingen in onderzoek gedaan.

APPENDICES

WORDS OF THANKS

WORDS OF THANKS

While it is my name that is on the cover of this book, it was not solely my effort that led to its completion. Many people helped in many different ways, and I would like to express my gratitude for that.

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ABOUT THE AUTHOR

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Malgorzata (Gosia) Lubczyńska was born on 11th of March 1984 in Wroclaw, Poland. In 1993 she moved to the Netherlands, where she continued her education. In 2002, she graduated from Het Stedelijk Lyceum Zuid in Enschede, and enrolled to Civil Engineering study at University of Twente. After the completion of the first year, she switched studies to obtain a Bachelor's Degree in Soil, Water, and Atmosphere at Wageningen University and Research Center, and thereafter a Master's Degree in Meteorology and Air Quality, which included an overseas program of one trimester at the University of Wisconsin-Madison, USA. Following her graduation in 2009, Gosia was selected to participate in a competitive research trainee program at Joint Research Center of the European Commission in Ispra, Italy. After completion of the traineeship, she was selected for a position of Research Assistant at the Cyprus Institute in Nicosia, Cyprus. Following a presentation of her work at the Cyprus International Institute for Environmental and Public Health in association with the Harvard School of Public Health in Limassol, Cyprus, she was invited to participate in selected courses of a Masters program in Biostatistics and Epidemiology which she gladly accepted. This led to her growing interest and knowledge in the field of environmental epidemiology. After participating in a conference hosted at CREAL, she was sure that undertaking a PhD in environmental epidemiology at CREAL was her next ambition and the right step in her career. She got accepted for a joint-PhD position between CREAL (now ISGlobal) and Erasmus Medical Center, and you are holding the "fruit" of this step in your hands right now.

PORTFOLIO

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Name PhD student:	Malgorzata Joanna Lubczyńska
UPF Department:	Experimental and Health Sciences
Erasmus MC Department:	Child & Adolescent Psychiatry
PhD period:	October 2015 – September 2019
Promotors:	Henning Tiemeier
	Mònica Guxens

Joint-PhD training UPF-EMC	Year	ECTS
MSc - Biostatistics and Epidemiology courses		
Introduction to Biostatistics	2014	8
Introduction to Epidemiology	2014	4
Advanced Epidemiological Methods I	2014	4
Regression Analysis	2014	8
Environmental Epidemiology	2014	4
PhD - Biomedicine program courses		
Digital competence	2015	0.3
Scientific Integrity	2016	0.3
Practice and politics of publication	2016	0.3
Animal Research	2016	0.3
Human Research	2016	0.3
International courses		
FSL course: functional and structural brain image analysis	2016	1.4
Causal inference course	2017	1.4
Skills courses		
Spanish Language B1	2017	3
Spanish Language B2	2018	3
Introduction to STATA	2015	
PRBB Interval courses		
Negotiating with confidence, inside or outside science		
Say it so it stays: oral presentation skills for scientists		
Mindfulness for improved self-mastery		
Interview and job application skills		

Joint-PhD training UPF-EMC	Year	ECTS
International conferences		
ISEE: 29th Annual International Conference, Utrecht, the Netherlands (oral and poster presentation)	2019	1.2
74ª Jornada de Primavera de la SCN, Barcelona, Spain (oral presentation)	2019	0.3
ISEE-Europe: Young Researchers Conference, Freising, Germany (oral presentation)	2018	0.6
DOHaD: 10th International Congres, Rotterdam, the Netherlands (oral presentation)	2017	1.2
13ª Jornadas Científicas, Sabadell, Spain (poster presentation)	2016	0.6
INCHES: 8th International Conference, Barcelona, Spain (oral presentation)	2016	0.9
ISEE: 28th Annual International Conference, Rome, Italy (oral presentation)	2016	1.2
Symposia, Meetings, and Workshops		
5th ISGlobal PhDSymposium, Barcelona, Spain (poster presentation)	2018	0.3
2nd ISGlobal-CREAL PhDSymposium, Barcelona, Spain (poster presentation)	2015	0.3
Child and Environment Meetings, Barcelona, Spain (oral presentations)	2017	1

Air Pollution Meetings, Barcelona, Spain (oral presentations)	2017	1
Psychiatry Research Meetings, Rotterdam, the Netherlands (oral presentation	2016	0.3

Teaching and educational activities		
Master thesis supervision of Ainhoa Jorcano *Air Pollution and Emotional and Aggressive Symptoms*	2018	4

Other activities	
Generation R data collection	2016 – 2018
MRI quality assessment	2017
INMA data collection	2015 – 2016
INMA data management	2016 – 2017