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Evaluation de la pertinence du potentiel oxydant en tant que métrique sanitaire pour l'exposition à la pollution atmosphérique

Assessment of the relevance of the oxidative potential as a health metrics for exposure to air pollution

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Abstract

Air pollution is a major public health issue, estimated to be responsible for over 7 million premature deaths worldwide every year. Most of the chronic effects of exposure to air pollution are attributable to particulate matter (PM). Child development, including the fetal period and the first years of life, is a key exposure window, since early exposures can have long-term impacts on child health. Most epidemiological studies assess the health effects of exposure to particulate matter on the basis of PM mass concentration, which corresponds to the regulatory metric currently in force. However, one of the mechanisms primarily responsible for the deleterious effects of exposure to particulate matter is its ability to induce or generate reactive oxygen species. These species disrupt the redox balance in the lungs, generating oxidative stress. More than a dozen different tests have been developed to measure the oxidative potential (OP) of particles, i.e. their capacity to oxidize a pulmonary fluid, and which integrates the effects on the fluid of the size, surface properties and chemical composition of PM. Although this is a promising metric, there is currently a lack of epidemiological studies evaluating exposure to the OP of particulate matter, which limits the evaluation of this indicator as a proxy for the health effects of PM exposure. Through a multidisciplinary approach combining atmospheric sciences and epidemiology, this thesis aimed to improve our knowledge on the relationship between OP of PM and health. To this end, the research strategy was based on data from the SEPAGES cohort (Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé; Assessment of air pollution exposure during pregnancy and effect on health, in English), a research platform designed to characterize the effects of early exposure (including in utero) to a wide range of environmental factors on child health. Firstly, associations between prenatal exposure to PM OP and different respiratory health parameters in young children were investigated. The underlying mechanisms were then examined, identifying the short-term effects of personal exposure to PM OP on biological markers of oxidative stress and immune function. Finally, to improve the current knowledge on PM OP, a characterization of the chemical species contributing to PM OP in the indoor air of 41 homes was performed. Spatial and seasonal variations of PM OP in the Grenoble conurbation were then determined

from samples taken outside the homes. The results showed deleterious effects of prenatal exposure to PM OP on children's lung volumes, as well as on a DNA oxidative stress biomarker and on pro-inflammatory cytokines in pregnant women. These associations observed with the OP of PM were stronger than those with $PM_{2.5}$ mass concentration. Finally, activities within the home, such as the use of household appliances like vacuum cleaners, emitted and resuspended chemical species that contribute significantly to the OP of PM, but less to $PM_{2.5}$ mass concentration. Thus, this work points to sources contributing to PM OP that are specific to indoor air, and reinforces the interest of PM oxidative potential as a new metric of particulate exposure for assessing the health effects of air pollution.

Résumé

La pollution de l'air est un enjeu de santé publique majeur, puisqu'elle est estimée responsable de plus de 7 millions de décès prématurés par an dans le monde. La majeure partie des effets chroniques de l'exposition à la pollution de l'air est attribuable aux particules (PM). Le développement de l'enfant, qui inclut la période fœtale et les premières années de vie, est une fenêtre d'exposition clé, car les expositions précoces peuvent avoir des impacts sur la santé à long terme. La plupart des études épidémiologiques évalue les effets sanitaires de l'exposition aux particules à partir de la concentration massique des PM, qui correspond à la métrique réglementaire actuellement en vigueur. Cependant, un des mécanismes à l'origine des effets délétères de l'exposition aux particules est leur capacité à induire ou générer des espèces réactives de l'oxygène. Ces espèces perturbent l'équilibre redox des poumons et génèrent du stress oxydant. Plus d'une dizaine de tests différents ont été développés pour mesurer le potentiel oxydant (PO) des particules, c'est-à-dire leur capacité à oxyder un milieu pulmonaire, et qui intègre les effets sur ce milieu de la taille, des propriétés de surface des PM, ainsi que de leur composition chimique. Bien que cette métrique soit prometteuse, les études épidémiologiques évaluant l'exposition au PO des particules sont peu nombreuses, ce qui limite l'évaluation de cet indicateur en tant que métrique indicatrice des effets sanitaires de l'exposition aux particules. Par une approche multidisciplinaire combinant les sciences atmosphériques et l'épidémiologie, ces travaux de thèse ont visé à améliorer les connaissances des relations entre le PO des particules et la santé. Pour cela, la stratégie de recherche de ces travaux a reposé sur les données de la cohorte SEPAGES, (Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé), qui est une plateforme de recherche visant à caractériser les effets de l'exposition précoce (y compris in utero) à un large panel de facteurs environnementaux sur la santé de l'enfant. Tout d'abord, les associations entre l'exposition prénatale au PO des PM et différents paramètres de la santé respiratoire de jeunes enfants ont été étudiées. Les mécanismes sous-jacents ont ensuite été examinés, en identifiant les effets à court-terme de l'exposition personnelle au PO des PM sur des marqueurs biologiques du stress oxydant et de la fonction immunitaire. Enfin, afin d'améliorer les connaissances relatives au PO des PM, une caractérisation des

espèces chimiques contribuant au PO des PM dans l'air intérieur de 41 domiciles a été réalisée. Ensuite, les variations spatiales et saisonnières du PO des PM dans l'agglomération grenobloise ont pu être déterminées à partir des échantillons pris à l'extérieur des domiciles. Les résultats obtenus ont montré un effet délétère de l'exposition prénatale au PO des PM sur les volumes pulmonaires des enfants, ainsi que sur un marqueur biologique du stress oxydant de l'ADN et sur des cytokines pro-inflammatoires de la femme enceinte. Ces associations observées avec le PO de PM étaient plus fortes que celles avec les concentrations de $PM_{2.5}$. Enfin, les travaux ont mis en évidence que des activités au sein des domiciles, telles que l'utilisation d'appareils électroménagers comme l'aspirateur, émettaient et remettaient en suspension des espèces chimiques qui contribuent grandement au PO des $PM_{2.5}$, mais moins à la concentration massique des $PM_{2.5}$. Ainsi, ces travaux indiquent des sources contribuant au PO des PM bien spécifiques à l'air intérieur et renforcent l'intérêt du potentiel oxydant des PM comme nouvelle métrique de l'exposition aux particules pour évaluer les effets sanitaires de la pollution de l'air.

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Chapter I

Introduction

I. General introduction

"Fog everywhere. Fog up the river, where it flows among green aits and meadows; fog down the river, where it rolls defiled among the tiers of shipping and the waterside pollutions of a great (and dirty) city. […] Fog in the eyes and throats of ancient Greenwich pensioners, wheezing by the firesides of their wards." – Charles Dickens, Bleak House, 1852.

In cities, air pollution has been known to threaten human health for several decades. From the 19th to the mid-20th century, air pollution levels increased, following the growing industrial production. The first famous air pollution events occurred in Belgium (1930), in the USA (1948) and in London (1952), all of them taking place in winter, with a combination of intense coal burning emissions from industrial and home heating purposes, and exhaust emissions from road traffic, that led to important concentrations of SO_2, NO_x and particles. The unprecedented number of deaths, with 3000 excess deaths during the first three weeks of December 1952 (Bell and Davis, 2001), mainly caused by cardiovascular affections and respiratory infections made it very hard to ignore the role of the high pollution events in triggering these adverse health endpoints.

Today, the population is still facing threatening levels of air pollution, with a combined effect of ambient and household air pollution associated with 7 million premature deaths per year in the world (WHO, 2016), with great geographical inequalities since more than half of this burden is located in developing countries. In addition to short-term effects studies, several studies also demonstrated the effects of long-term exposure to particles on cardio-respiratory, neurological, metabolic and reproductive systems (Slama et al., 2008; Tang et al., 2022; Thurston et al., 2017; Wang et al., 2021). In particular, early-life exposure has been identified as a period of great vulnerability for the child's future health. Indeed, exposure during the prenatal and early childhood periods could influence health at birth, cognitive and

pulmonary capacities, and the future development of respiratory diseases such as asthma and chronic obstructive pulmonary disease (Bush, 2021; Hsu et al., 2015; Jedrychowski et al., 2010; Korten et al., 2017; Lavigne et al., 2018; Thurston et al., 2020).

While strategies have been implemented to reduce air pollution exposure, particularly in western countries (Hammer et al., 2020), more targeted approaches relying on the biological mechanisms of air pollution are also being investigated, in order to decrease exposure to specific components of particles, that would be more harmful than others. Since the 2000s, a large body of research identified the role of oxidative stress as common to the adverse action of air pollutants, occurring as soon as being inhaled, with a key role of lung antioxidants (Ayres et al., 2008; Cho et al., 2005; Hellack et al., 2014; Valavanidis et al., 2013). The measurement of oxidative potential (OP) tests of particles has been developed with the aim to use a more appropriate metric, that would specifically target the oxidizing capacity of particles, in order to better predict the adverse health endpoints than the sole mass concentration.

While there is mounting evidence of PM's health effects from epidemiological studies using different exposure assessment approaches, different populations and different periods of time, indicators of the OP of particles remain poorly utilized. More investigations are needed to have sufficient evidence regarding its relevance as an exposure parameter in epidemiological studies and as a health risk indicator to be implemented in air pollution monitoring. To achieve these objectives, it is particularly necessary to enhance our understanding of the relationship between early-life exposure to the OP of particles and its impact on children's health, while characterizing the potential biological mechanisms involved and investigating strategies to minimize exposure to this parameter. This can only be achieved through a multidisciplinary approach, relying on atmospheric biogeochemistry and epidemiology, each of them bringing unique perspectives and methodologies, and together providing a more holistic view to ultimately build robust scientific evidence.

The aim of this thesis was to provide a better understanding of OP, by addressing some research gaps in three complementary research domains:

1) *Etiological research*, by assessing the association between prenatal exposure to the OP of particles and newborn respiratory health

2) *Mechanistic research*, by assessing the association between personal exposure to the OP of particles and biomarkers of oxidative stress in pregnant women
3) *Aerosol research*, by characterizing OP of particles in indoor air and evaluate the eventual disparities with ambient OP exposure.

The chapter below will introduce different concepts and current state of knowledge, by giving elements of context regarding air pollution in ambient and indoor environments. A focus will then be made on the different exposure assessment techniques that are used in epidemiological studies. The short and long-term health effects of PM exposure will be developed in a third section, also providing elements on the complexity of evaluating these effects at the earliest stage of life. Finally, the oxidative stress pathway, a major biological mechanism involved in the air pollution health effects, will be presented, along with the current evidence supporting the use of OP of particles in epidemiological studies.

II. Air Pollution

Air pollution is a complex, ubiquitous concern. In ambient environments, its effects, particularly on cardiovascular and respiratory health, have been well-established. Moreover, an increasing body of evidence highlights its impact on various other health outcomes. Growing concerns also revolve around indoor exposure, which is less well understood. The sections below will define major air pollutants and their sources, the issues related to indoor air quality will be presented next, followed by an overview of the different regulations in place in Europe to mitigate exposure to air pollutants.

II.1. Ambient air pollutants

Ambient air pollution refers to solid, liquid or gaseous compounds that can originate from both natural and anthropogenic sources and that have negative impacts on the health, environment and ecosystems. Primary pollutants are emitted directly, while secondary pollutants originate from compounds transformed through chemical and photo-chemical reactions during their atmospheric transport. Pollutants that are targeted for their health effects by national and international health organizations include gases such as carbon monoxide (CO), ozone (O_3), nitrogen oxides (NO_x), sulfur dioxide (SO_2), and particulate matter (PM).

II.1.1. Gases

NO_x are measured as a proxy for exposure to traffic emissions. NO_x are composed of nitrogen oxide (NO) and nitrogen dioxide (NO_2) that are highly reactive gases formed by combustion processes such as heating, power generation, and engines in vehicles. Incomplete combustion of fuels also leads to the formation of CO, a gas present as traces, that is very toxic. SO_2 has been a major pollutant since the industrial revolution, being responsible of the famous pollution events occurring upon coal burning. It is a poisonous gas with an irritating odor and is key for PM formation. A natural source for SO_2 is volcanic activity. Another complex compound, targeted for its health effects is ozone. Ozone is a harmful secondary pollutant, formed by the photochemical reaction between NO_x and volatile organic compounds (VOCs). In the troposphere (located in the lower atmosphere, up to 10km above the earth surface), its high concentrations are of great concern for health. Stratospheric O_3 (located in the high atmosphere), not considered to be a pollutant, forms the ozone layer, that filters the sun's ultraviolet radiation and therefore attenuates the harmful effects of solar radiations. In Europe, from 1990 to 2019, about 10% of the death attributable to air pollution were associated with ambient gaseous air pollution (Juginović et al., 2021).

II.1.2. Particles

In addition to gases, the atmosphere is composed of solid and liquid airborne particles, that are called particles or particulate matter (PM). PM are very complex material, that can cover a wide size range from few nanometers to few micrometers and that can be composed of various chemical species (Seinfeld and Pandis, 2016). Both their size and composition are parameters that depend on the sources of PM, and that will determine PM behavior in the atmosphere, as well as their health effects.

Figure 1 summarizes the different mechanisms for formation, growth and removal from aerosols. Primary PM are emitted directly to the atmosphere under the action of wind that will resuspend dust, salts or plant debris, which are coarse particles (1-10 µm). Direct emissions from combustion processes such as wood burning or diesel and gasoline in engines will emit fine primary particles ($\leq$ 2.5 µm). Secondary PM are formed from precursor gases through gas-to-particle conversion. Precursor gas molecules create clusters that form ultrafine aerosols (nucleation phase). Atmospheric SO_2 is a key

precursor, because it is rapidly oxidized in sulfuric acid (H_2SO_4), that condenses to aqueous sulfate particles (sulfuric acid) because of its low vapor pressure (condensation). Other precursor gases such as ammonia (NH_3) or low-volatile organic compounds can also modify particles chemical composition. Particles will grow through collisions (coagulation), up to 1 µm, beyond which they are too large and have a slow collision rate. The 0.01-1 µm is therefore called the accumulation mode, because particles formed from gas precursors will accumulate in this size range. Coarse particles are large enough to sediment, and can also be removed from the atmosphere by rainout. Accumulation particles are too small to sediment, they can be removed by rainout too and they can be captured by water droplet or ice crystal (scavenging) or evaporate.

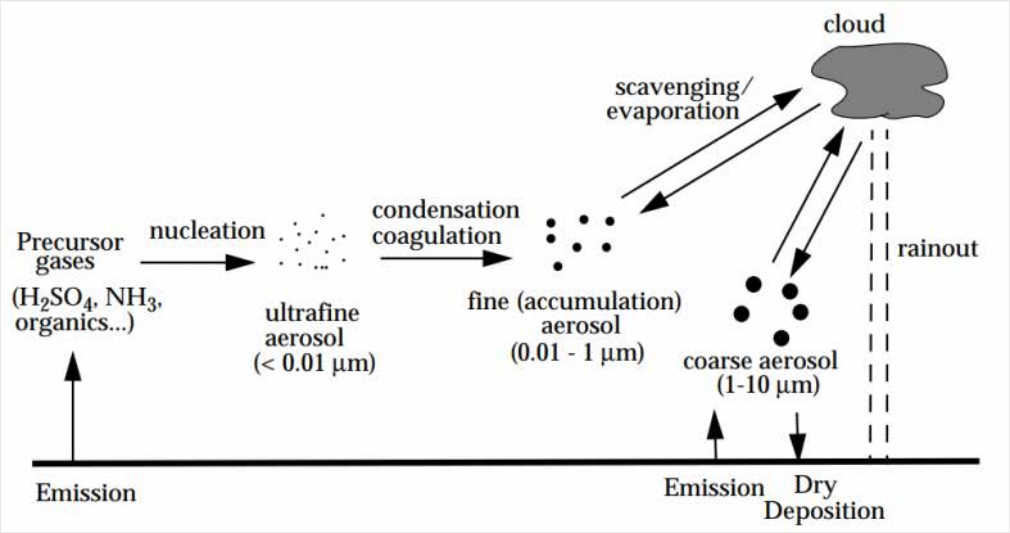


Figure 1. Formation, growth and removal of aerosols (Jacob, 1999).

Particle size is an important characteristic of PM since it is modulated by the sources and can lead to different reactivity (Gietl and Klemm, 2009; Manousakas et al., 2022), and health effects, considering that smaller particles are able to penetrate deeper in the lungs (Kelly and Fussell, 2012; Strak et al., 2012). The aerodynamic diameter is defined as the diameter of a sphere of density 1 g/cm^3 that has the same inertial properties as the atmospheric particle (Weiner, 2015), and is used to describe PM size given their complex shapes. PM_{10}, $PM_{2.5}$ and $PM_{0.1}$, therefore correspond to particles with an aerodynamic diameter smaller or equal to 10 µm, 2.5 µm and 0.1 µm, respectively. $PM_{0.1}$ are also called ultrafine particles (Figure 2).

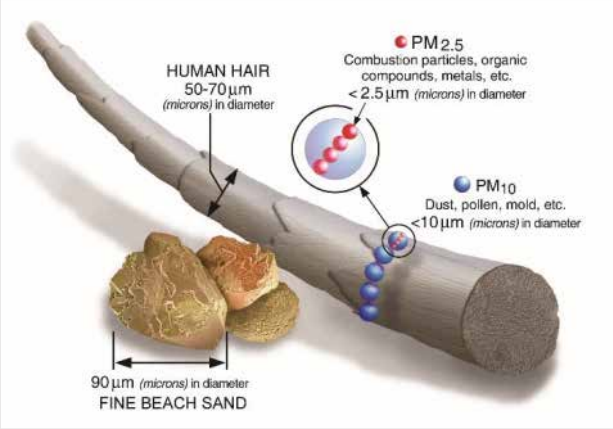


Figure 2. Size comparison between PM, hair and fine beach sand. (US EPA, 2016)

In order to determine the sources of particles, and to target chemical species that are potentially more harmful to human health, the chemical composition of PM is analyzed (Favez et al., 2020; Perrino et al., 2020; Terzi et al., 2010).

The most abundant chemical species in PM are ionic species formed in the atmosphere by condensation of precursor gases, namely sulfates (SO_4^{2-}), nitrates (NO_3^-) and ammonium (NH_4^+) that originate respectively from SO_2, NO_x and NH_3. Other ions such as sodium (Na^+), chloride (Cl^-) and magnesium (Mg^{2+}) are also found with smaller concentrations. An important fraction of PM is composed of organic carbon (OC), an umbrella term representing many chemical species formed of a carbon chain, oxygen and nitrogen. Some specific organic species can be determined in PM, such as levoglucosan, an anhydride sugar originating from the combustion of cellulose contained in wood. Carbon is also found in PM as elemental carbon (EC), a compound that is emitted during incomplete combustion processes and that influence greatly the optical properties of PM, since its dark color absorbs light radiation. Finally, some metals are also constitutive of PM chemical composition, such as aluminum (Al), copper (Cu), iron (Fe), lead (Pb), titanium (Ti), tin (Sn) or zinc (Zn). Although they only represent a small portion of the mass of PM, metals are of particular importance because of the potentially harmful effects they can have on health (Hernández-Pellón et al., 2018).

Many studies investigated the sources of PM by examining their chemical composition either directly at the emission, or from time series sampled in various typologies of environments (i.e. rural, urban, industrial, etc.) (Alleman et al., 2010a; Calzolai et al., 2015; Gietl and Klemm, 2009; Hadley, 2017; Hopke et al., 2020; Weber et al., 2019). Primary sources include biomass burning (originating from non-

efficient domestic heating, and emitting mostly OC, EC, levoglucosan, potassium and rubidium), road traffic (from exhaust or non-exhaust such as brake or tire abrasion, it will emit EC and several metals such as Cu, Fe, Sn and Pb), primary biogenic emissions (emissions due to the biological activities of soil and plants micro-organisms, their specific tracers are polyols: arabitol, mannitol, sorbitol), industries, agriculture (emission of NO_3^- and NH_4^+ contained in fertilizers), mineral dust (emitted by the resuspension of the crustal dust, this is mainly composed of metals or ions), sea-salts (emitted by sea sprays, when fresh, they are mainly composed of Na^+ and Cl^-). Although more sporadic, events such as volcanic eruptions or long-distance transportation of desert dust are also sources that can contribute to PM.

Aerosol aging or photochemical transformation, leading to secondary sources, is also an important factor to consider since it changes PM chemical composition, and therefore can potentially modify its health effect. Organic secondary aerosols correspond to natural or anthropogenic volatile organic compounds, that are transformed and transported during their atmospheric lifetime. Secondary inorganic aerosol is also an important source of PM. Agricultural NH_3 forms particulate ammonium nitrates (NO_3NH_4) when reacted with NO_x, and ammonium sulfates $(NH_4)_2SO_4$ with SO_2.

The better understanding of chemical composition and sources of PM are critical for mitigation strategies in ambient air. By determining the sources that contribute to PM, policy-makers can implement strategies targeting sources that contribute to both high episodes of PM and background levels.

II.2. Indoor air quality

Ambient air quality is damaged by anthropogenic activities. A growing concern has also emerged regarding indoor air, since people spend more than 80% of their time in indoor environments (Avery et al., 2010) such as homes, day care centers, public building, offices, schools or transports. Indoor environments are sometimes described as “ecosystems” (Goyal and Mukesh, 2010) because they are composed of inhabitants and their activities in a specific closed space that can be modulated by air ventilation, building material and the environmental settings, as illustrated on Figure 3.

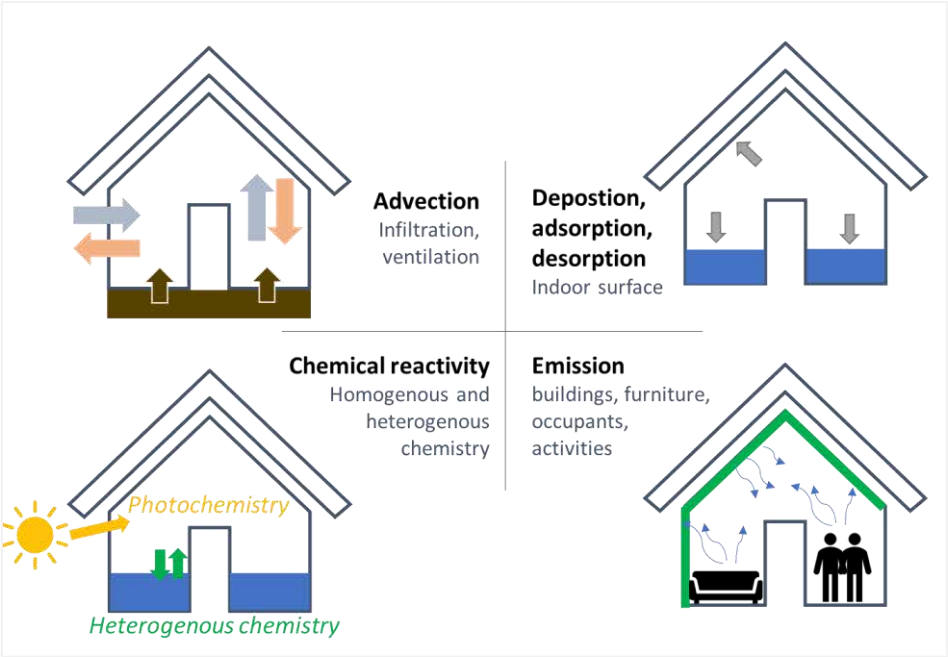


Figure 3. Processes affecting indoor air quality.

Outdoor air pollutants can penetrate indoor environments by infiltration through building cracks, or through mechanical or natural ventilation. Therefore, the environmental setting of the building is key for indoor concentration of pollutants. As an example, the distance to main road was found to be a predictor of indoor air quality in homes and schools (Janssen et al., 2001; Lawson et al., 2011). This is usually explained by poorly designed ventilation systems and airtightness. A negative pressure difference between the inside and the outside of the building then draws the pollutants through cracks, vents and openings (Goyal and Mukesh, 2010).

Indoors, a greater use of synthetic building materials and strengthened airtightness appeared with the greater insulation that improves the quality of life, by maintaining comfortable temperature indoors, further reducing thermic losses. The use of synthetic building materials contributes to indoor air pollutants, and the airtightness concentrates the pollutants indoors.

Typical indoor PM sources include building materials, cleaning products (Gerster et al., 2014), printers (Arı, 2020; He et al., 2007; Z.-M. Wang et al., 2011), activities such as cooking or smoking (Vardoulakis et al., 2020), and fire places (Tsakas et al., 2011). Indoor PM is usually enriched with organic compounds, that come from various sources, including the primary biological aerosols emitted by the occupants (hair, skin flakes) (Marcovecchio and Perrino, 2021) or its activities (cooking and cleaning, emitting volatile and semi-volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons

(PAHs) that sorb on PM) (Liu and Zhang, 2019; Tofful et al., 2021). Combustion processes (cooking, stoves) will additionally emit EC. Cigarette smoking were found to be potential sources of cadmium and lead (Matt et al., 2021) and copper was linked to the use of electric appliances (vacuum cleaner, hair dryer) (Tofful et al., 2021; Yli-Tuomi et al., 2008) and to the type of kitchen utensils (Molnár et al., 2007). Dust resuspension is also an important process taking place in indoor environments and contributes to the trace elements and inorganic fractions of PM. The wavelengths initiating outdoor photochemistry (~300 nm) are attenuated by windows, leading to a very different photochemistry indoors, which will impact both the outdoor aerosol aging after indoor penetration and the indoor secondary organic fraction (Abbatt and Wang, 2020; Young et al., 2019).

Although this work focuses on PM, it should be mentioned that volatile compounds can also threaten inhabitant's health. NO_x can penetrate the indoor environment but also be emitted by gas stoves or cigarette smoking. Inhabitants can be exposed to O_3 indoors, that is either from outdoor origin or that is formed indoors. CO and radon gas are two additional risks in indoor environments. Incomplete combustion generates an accumulation of CO indoors that easily reaches life-threatening concentrations. Because it is odorless and colorless, CO poisoning is very hard to detect, and caused 35 500 deaths worldwide as estimated by the 2017 Global Burden of Disease (Roth et al., 2018). Radon is an odorless and invisible radioactive gas, that can be released from the water, the soil and the rocks. Long-term exposure to radon was proven to increase the risk of lung cancer, in fact the United States Environmental Protection Agency estimates that radon causes 21 000 lung cancer (National Center for Environmental Health, 2023) deaths each year, making it the second leading cause of lung cancer.

II.3. Regulated metrics

Most studies investigating the health effects of particles used the mass concentration in the air to describe exposure. Policy-makers implemented regulations based on these studies, therefore using the same mass concentration metric for PM. In 2004, four metals and benzo(a)pyrene (as a tracer for the broader family of polycyclic aromatic hydrocarbons) measured in PM_{10} were further added in the list of regulated pollutants in the European Union, because of their carcinogenic and genotoxic effects on humans. Table

1 presents the current European air quality standards related to PM, that are the same as the French standards.

Air quality standards are quantitative limits for pollutants in the air, that are enshrined in European or national legislation and are legally binding, requiring the Member States to evaluate air quality based on common methods and to fix sanitary and environmental objectives. By contrast to the European or national standards, the WHO air quality guidelines do not represent a legal constraint, but reflect the current state of scientific knowledge on the health impact of air pollution. The WHO guidelines are long-term global targets to achieve, aiming to help the different regions putting in place air quality policies. WHO guidelines were updated in 2021, proposing interim-targets intended to guide the reduction efforts, with a final recommended yearly concentration of 5 µg/m^3 and 15 µg/m^3 for $PM_{2.5}$ and PM_{10}, respectively (Table 1). Efforts can be made to reach these concentrations, by reducing anthropogenic emissions, but natural emissions such as biogenic emissions or desert dusts cannot be controlled. In fact, during strong dust events, Saharan dusts have been estimated to account for up to 60% of total PM_{10} concentration in the Middle East and Mediterranean countries (Pey et al., 2013; Querol et al., 2019, 2009). This reinforces the need to further investigate PM using other properties that integrate their potential to harm human health.

Table 1. Air Pollutant Regulations: Comparing WHO guidelines, European standards.

Pollutant	Time interval	WHO 2021	European Air Quality Directives (2008)
$PM_{2.5}$ (µg/m^3)	Year	5	25
	24hrs	15	-
PM_{10} (µg/m^3)	Year	15	40
	24hrs	45	50[a]
BaP (ng/m^3)[b]	Annual	-	1
Pb (ng/m^3)[b]	Annual	0.5	0.5
As (ng/m^3)[b]	Annual	6.6	6
Cd (ng/m^3)[b]	Annual	5	5
Ni (ng/m^3)[b]	Annual	25	20

a: not to be exceeded on more than 35 d/yr; b: measured in PM_{10}

III. PM Exposure assessment

Exposure assessment is a key challenge for studies aiming at investigating effects of PM exposure, particularly for long-term exposure assessment. In the recent epidemiological studies, exposure levels are often estimated by exposure models (land-use regression models, dispersion models…) at the residential address, while in the past, data from the closest air quality monitoring station were used. Other smaller-scaled studies use personal samplers, to allow for the consideration of exposures in the various microenvironments visited by the participants, therefore providing a closer estimation of PM exposure. Depending on the exposure duration that is being studied (i.e. short- or long-term), the different approaches will present different advantages or limitations.

III.1. Estimation methods in ambient air

Air quality monitoring stations are used by local or national authorities to evaluate the compliance with the current regulations and are equipped to measure various pollutants, including PM_{10} and $PM_{2.5}$ mass concentration. Because of the numerous advantages they offer, monitoring stations have been used to estimate long-term exposure to PM in earlier studies (Gauderman et al., 2004; He et al., 2019; Horak et al., 2002; Latzin et al., 2009). In time-series analyses, ambient monitoring stations are still being used. To address the uncertainties linked to their spatial resolution, study participants are increasingly enrolled within a certain buffer of the monitoring station (Weichenthal et al., 2016; Zhang et al., 2016) or an inverse weighting approach has been used to add more uncertainties on homes located further from the monitoring station (Mortimer et al., 2008). The main limitation of assessing exposure from the central monitoring station are the low spatial coverage of the stations (Table 2), therefore estimating exposure with low spatial resolution.

To improve the spatial resolution of exposures assessments, several numerical models and geostatistical models have been developed, to build fine-scale maps of particles concentrations. A large number of epidemiological studies calculated exposure at home address using chemistry-transport models (CTM) (Bergstra et al., 2018; Cai et al., 2020), that are based on physical and chemical representation of

atmospheric processes, such as the chemistry of gases and aerosols, the different types of emissions, the transformation of pollutants, and the mixing, dispersion and deposition of aerosols. The main advantages of CTM reside in the flexibility in terms of temporal scale, as well as fine spatial resolution, and the accuracy of the processes considered, making it possible to access specific PM components too.

Other studies used Land Use Regression (LUR) models to calculate exposure at home address (Hellack et al., 2017; MacIntyre et al., 2014; Pedersen et al., 2013; Stapleton et al., 2022), that are geostatistical models relying on geographical variables to predict pollutant concentrations and capture small-scale spatial variations attributable to the environment. LUR models use geographic information system-derived predictor variables such as distance to the closest road, traffic intensity, land-use, population distribution in multivariate regressions, to statistically model air pollutant concentrations measured in different sites. LUR models provide finely resolved concentration maps with less computational work than CTMs. Models, whether numerical or geostatistical, comprise certain levels of errors and uncertainties in exposure assessment that will impact the subsequent air pollutant-health regression models. Since this error is not linked to the air pollutant-health model itself, the parameters estimating the quality of the predicted associations will not be formally impacted, but regression coefficients could be attenuated or the confidence interval increased (Basagaña et al., 2013; Wang et al., 2015). Moreover, these exposure models estimate outdoor exposure levels, but do not account for indoor exposure, even though people spend more than 80% of their time indoors (Avery et al., 2010; Klepeis et al., 2001).

III.2. Personal measures

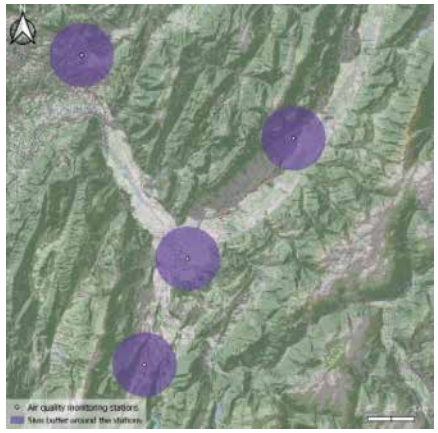
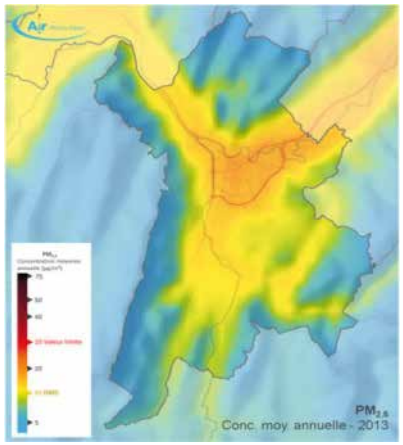
To further reduce the uncertainties linked to exposure assessment, several mixed approaches were used to estimate personal exposure to PM, which corresponds to concentrations experienced by an individual during normal daily activities and depends on the exposure experienced indoors, outdoors, and the "personal cloud" (i.e. the resuspension around the person, generated by its activity).

Some studies developed approaches to calculate personal exposure, that rely on: 1) the self-reporting of the time-activity patterns over the measurement periods (i.e.: time spent in their bedroom with open or close window, time spent in a car, outdoor…), 2) either indoor-outdoor ratios estimations for different

micro-environments (He et al., 2021) or an indoor PM sampler (Ouidir et al., 2015), 3) GPS-data to calculate outdoor exposure to PM using fine-scale CTM.

To avoid the potential errors due to the steps required to calculate personal PM concentrations, a few studies used portable PM samplers, that are considered the "gold standard", because they include all the different indoor and outdoor micro-environments (X.-C. Chen et al., 2020; Delfino et al., 2008; Dutta et al., 2021; Jedrychowski et al., 2010; Meng et al., 2005). Active PM samplers are equipped with a pump, and PM mass concentration is measured either using a nephelometer or by gravimetric analysis. PM personal monitors using the nephelometer technique rely on the optical (light-scattering) properties of PM, and gravimetric-based monitors are equipped with a filter that is weighted before and after the sampling period. The mass difference divided by the volume of air sampled during the measurement period provides the average mass concentration of PM over this period. Personal monitors are designed to be small and light, in order for the study participant to carry it for the whole measurement period. The main limitations of using active personal samplers are the cost, the acceptability for the participants, the complexity of using the samplers in larger-scale cohorts, the temporal coverage and the technical difficulties to handle filters. Compared to the other techniques, personal exposure reduces measurement errors in short-term exposure assessment, which augment the statistical power of the study and reduces the risk of failure in detecting effects (Armstrong, 1998). However, for studies investigating the effects of long-term exposure to PM, several measurement periods must be conducted to extrapolate an average exposure, which also incorporate some measurement error.

Table 2. Comparison of measurement and modelling strategies to estimate PM concentration.

Criteria	Monitoring stations	Chemistry-Transport Models	Land Use Regression models	Personal samplers
Ease and cost of use	+++	+++	+++	- - -
Temporal resolution	+++	+++	+	+++
Temporal coverage	+++ (long-term measurements)	+++ (possibility to hind- and forecast)	+ (1-2 yrs)	- (days-weeks)
Spatial resolution	- - - 	++[1]  (Gabet et al., 2018)		+++  ©Inserm
Access to the chemical complexity	+++	++	- - (geographical variables only partly represent the chemical complexity)	+++
Consideration of micro-environments	- - -	+ (atmospheric processes, buildings)	+ (local geographic features)	+++
Acceptability from participants	/	/	/	-

1: Please note that the same spatial resolution is used for CTMs and LUR in this representation, although this is only correct for fine-resolution CTMs applied to the city-scale.

IV. Health effects of exposure to PM

Air pollution is estimated to be responsible for 7 to 8.8 million deaths per year worldwide (Lelieveld et al., 2019; WHO, 2016) and 2.3 to 3.8 million deaths are attributable to indoor air pollution specifically.

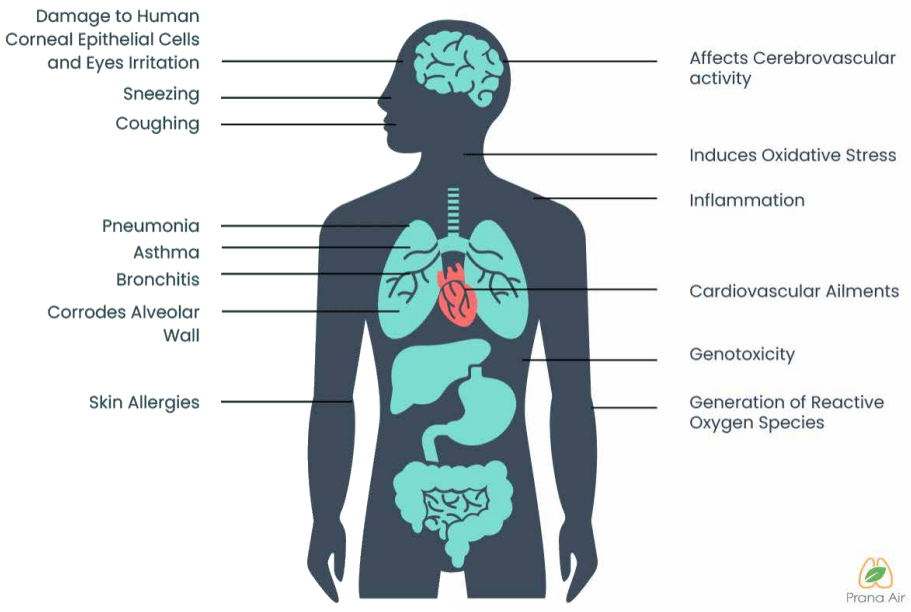


Figure 4. Health impacts of PM.
(Credit: Prana Air, adapted from: https://www.pranaair.com/blog/particulate-matter-pm-2-5-sources-impacts-measures)

PM has both short-term and long-term effects on health (Figure 4). Short-term effects are immediate or acute and may happen after a single intense exposure to high levels of PM, and long-term effects are cumulative or chronic and may cause the development of various diseases. Different categories of the population are suspected to be more susceptible to the health effects of air pollution, in particular children, and studying the respiratory effects on children is critical to understand their future respiratory health trajectories.

IV.1. Short term health effects of PM

Already by the early 1900s, several time-series studies highlighted effects of short-term changes in air pollution exposure during severe pollution events on the changes in daily mortality counts (Pope and Dockery, 2006). Short-term effects of exposure to PM mostly impact the cardiovascular and respiratory systems. Effects on the cardiovascular system include an increased rate of myocardial infarction (C.

Chen et al., 2020; Y. Liu et al., 2021) and ischemia, exacerbation of heart failure, increased incidence of stroke (C. Chen et al., 2020). For the respiratory system, there is evidence for increased wheezing (Bergstra et al., 2018), asthma exacerbation (Janssen et al., 2003; Rosenquist et al., 2020), increased symptoms of chronic obstructive pulmonary disease (C. Chen et al., 2020), bronchiolitis and other respiratory tract infections. In a review of acute effects of $PM_{2.5}$ constituents, Achilleos et al. (2017) found stronger association with mortality for combustion elements (elemental carbon, potassium), than with $PM_{2.5}$ mass concentration, supporting the use of additional PM properties in health studies.

Severe pollution events were found to be harmful for the vulnerable and at-risk people, including children and elderly people (Bell et al., 2013). However, chronic exposure is a risk for the general population, because the repetitive nature of exposure can lead to the dysregulation of critical biological processes, impairing the body's ability to maintain homeostasis, which in turn can lead to the development of various diseases.

IV.2. Long term health effects of PM

Effects of chronic exposure to PM on the cardiovascular and respiratory system are well established, with numerous studies showing increased mortality and morbidity related to cardiorespiratory effects (Abelsohn and Stieb, 2011; Abrams et al., 2017; Pope and Dockery, 2006). Recently, air pollution exposure has been estimated as a contributing factor for asthma and chronic obstructive pulmonary disease (COPD) onset (Thurston et al., 2020) and for other adverse outcomes, including diabetes (Liu et al., 2019), neurodegenerative diseases (Wang et al., 2021), in-utero growth and adverse birth outcomes (Jedrychowski et al., 2004; Lavigne et al., 2018).

IV.3. Increased vulnerability for children

Exposure to PM has unequal impact on population health and several subgroups have been identified as more susceptible, such as elders, or asthmatic individuals, for which short-term effect of exposure to PM can have very deleterious impacts. Continuous exposure can potentially have long-term implications, and children have been identified as a susceptible subgroup (Brumberg et al., 2021; Ha, 2021; Makri and Stilianakis, 2008; Maung et al., 2022).

Children are considered highly vulnerable to the effects of particulate air pollution exposure, mainly because their lungs are not fully developed until they are 6 to 8 years old. Moreover, the relatively small size of their lungs compared to their height leads to higher respiratory rates compared to adults, which in turns leads to higher amounts of pollutants inhaled. This is also worsened by their small height, making them closer to the ground, where particles are being resuspended. Alveoli are the smallest part of the airways, which enable gas exchanges from the lungs to the blood. They start to develop in-utero, at around 36 gestational weeks, their multiplication last until about 2-3 years, and their growth continues until adolescence or early adulthood (Bateson and Schwartz, 2007; Joshi and Kotecha, 2007; Korten et al., 2017). Since smaller particles accumulate in the alveoli, the alveolarization process can easily be disturbed during that relatively long time period.

Prenatal exposure has been identified as a critical exposure window for child health. Numerous studies showed that air pollution exposure as early as during the prenatal stage could affect future health trajectories of the fetuses, which supports the hypotheses of "developmental origins of health and diseases" (DOHaD), following which exposure to certain environmental factors during the prenatal and perinatal stages could have significant impacts on individual's short and long-term health. While the exact mechanisms by which *in-utero* exposure to PM affects the fetus remains unknown, the role of epigenetic mechanisms has been suggested (Bianco-Miotto et al., 2017; Michels et al., 2022). After inhaled by the pregnant mother, PM are thought to either cross the alveoli and placental barrier, acting directly on the fetus or to induce inflammatory or immune reactions of the mother, therefore impacting the nutrients or oxygen supply for the fetus (Kannan et al., 2006). The effects of maternal exposure to PM on children include preterm birth and low birth weight (Fleischer et al., 2014; Lavigne et al., 2018; Malley et al., 2017), child's neurodevelopment (Ha, 2021) and childhood and adult respiratory illnesses (Aithal et al., 2023; Bush, 2021).

IV.3.1. Measuring lung function in early childhood: a challenge for epidemiological studies

Investigating the effects of prenatal exposure to PM on health parameters measured in infants and children is a challenging task, because it requires mother-child cohorts enrolled during the pregnancy

stage, and a close follow-up in infancy. The health parameter of interest can also be challenging to get, especially at the youngest age because of parents' availability, newborns and infants' agitation. On a more technical aspect, while it is of great interest to measure objective health outcomes in very early children, it sometimes requires more advanced techniques. Lung function parameters are commonly assessed by spirometry, which requires to take in a deep breath, and then blow as hard and long as possible into the spirometer. This kind of cooperation cannot be expected from infants, and this is why most studies explored the effects of prenatal exposure to PM on lung function in children older than 4 years, when spirometry becomes feasible. Several noninvasive techniques relying on tidal breathing are more suited to assess lung function parameters in very young children, since they do not require any cooperation from the participant. Among them, tidal breathing flow-volume loops (TBFVL), multiple breath washout (MBW), thoracoabdominal compression and body plethysmography measure lung volumes and airway oscillometry (AOS) measures mechanical properties of the lung.

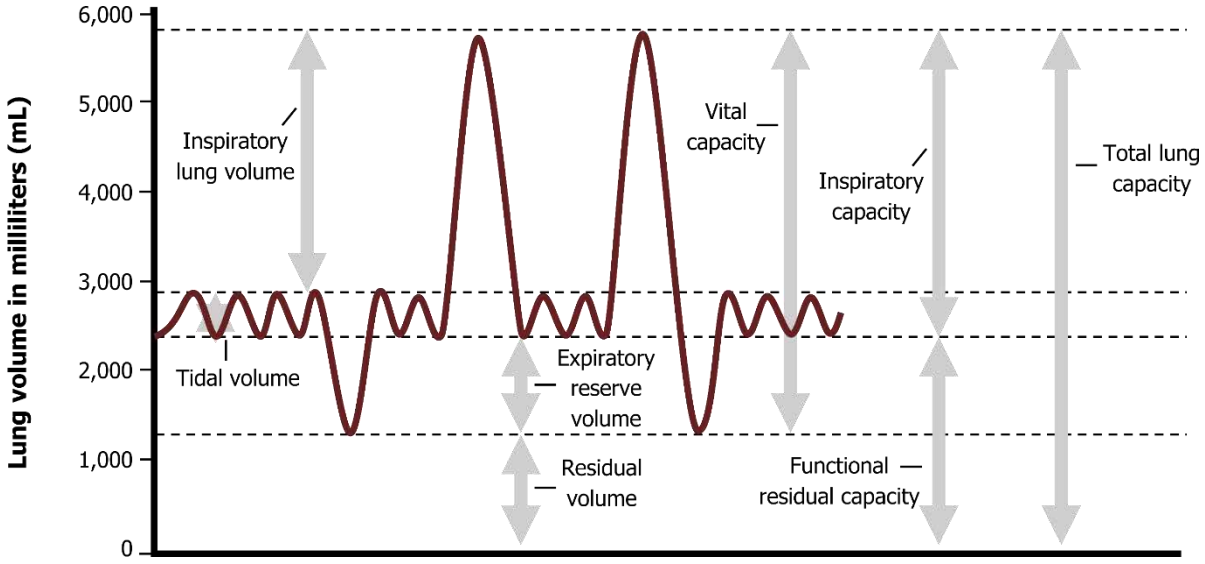


Figure 5. Definition of the different lung volumes (Binks, 2022).

For body plethysmography, patients are breathing through a spirometer and placed in a closed chamber with continuous measurement of pressure variation. Information collected through the spirometer and the pressure variation of the chamber enable the calculation of lung volumes. The thoracoabdominal compression technique requires the children to wear an inflatable vest inside an inextensible jacket around the chest, and to be equipped with a facial mask connected to the analyzer. Rapid compressions are applied by inflating the first jacket, thereby mechanically forcing the expiration, and the flow and volumes can be computed (Jat and Agarwal, 2023).

Tidal breathing refers to inhalation and exhalation during natural breathing, at rest. TBFVL analysis consists of analyzing the breathing pattern during tidal breathing, resulting in acquiring information regarding processes related to respiratory control and pulmonary mechanical function. The most straightforward measured parameters are the respiratory rate and the tidal volume, i.e. the volume of air inspired during each breath (Figure 5). TBFVL analysis also enables the calculation of the time to peak expiratory flow to expiratory time (t_{PTEF} /t_E, see Figure 6), a parameter able to reflect airway obstruction (van der Ent et al., 1996). For healthy patients, the peak flow of expiration should occur near the midpoint of exhaled tidal volume, but with airway obstruction, the peak expiratory flow occurs faster, near the beginning of expiration.

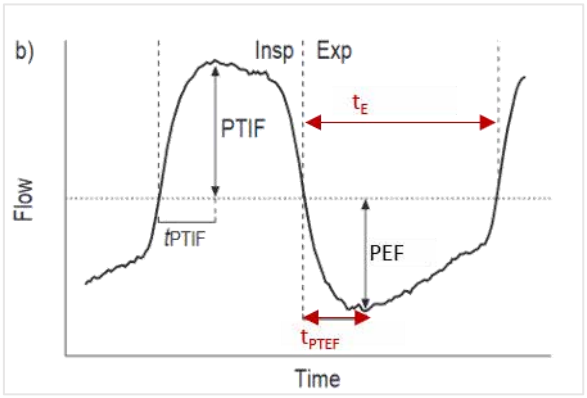


Figure 6. Flow to time component of the tidal breathing flow-volume loops analysis. (Bates et al., 2000)

Not all lung volumes are measured by TBFVL analysis, such as volumes linked to forced expiration or inhalation, or residual volume.

MBW is a technique performed during tidal breathing, and is a method used to assess the efficiency of gas exchange in the lungs, therefore providing information about the distribution of the ventilation. During the test, infants breathe a gas mixture containing a tracer gas (Beydon et al., 2007). The individual continues to breath the gas mixture for several breaths while the concentration of the tracer gas exhaled is measured. The MBW technique provides several important measurements, including functional residual capacity (FRC) and lung clearance index (LCI), which reflects heterogeneous obstruction of the distal airways. FRC corresponds to the sum of the residual volume, that prevents collapse caused by the elastic recoil of the lungs, and the expiratory reserve volume, that can be expired through forced expiration. LCI is calculated from the cumulative expired volume to clear the inert gas divided by the FRC, in other words it represents the number of "lung turnovers" (i.e., multiples of FRC)

required to complete the washout. The choice of inert gas can influence the resulting FRC and LCI, since some gases are soluble in blood and tissues. Sulfur hexafluoride is usually recommended to avoid any changes in the breathing pattern (Gustafsson et al., 2017), however the use of this gas is strictly regulated in the European Union, given its environmental persistence and its global warming potential accounting for 23 times the one of carbon dioxide (regulation (EU) 517/2014). To comply with the EU regulation, pure oxygen can be used and the concentration of nitrogen (N_2) during exhalation is monitored.

Airwave oscillometry (AOS), also known as forced oscillation technique, relies on the application of a pseudo-random oscillatory signal (pressure waves) on a tidal breathing to calculate the impedance of the lungs, which is related to its mechanical properties (Beydon et al., 2007). Impedance is decomposed in resistance and reactance, and is calculated for different frequencies. The resistance is representative of friction forces mainly in the airways and the reactance depends on the inertive and elastic behaviors of the respiratory system (Gosselink and Stam, 2005).

The effect of prenatal exposure to PM on infants' lung function was investigated using these non-invasive objective techniques in a few studies, compared to the body of literature relying on spirometry at a slightly older age. This is probably due to the commercial availability of the equipment, the fact that spirometry is the most common in clinical practices and to the complexity inherent to studying very young children.

To the best of our knowledge, 8 studies investigated the effects of prenatal exposure to PM on children's lung function. Most studies relied on spirometry parameters, assessed from 4 to 17.5 years, 2 assessed lung mechanics using AOS and 1 study relied on TBFVL and MBW. Among the studies assessing AOS, one in Australia aimed at investigating the effects of prenatal exposure to an intense pollution event caused by a coal mine fire on children's lung function at 7 yrs, assessed in 79 children (Hemstock et al., 2023). The other study, located in Nigeria measured 72-hrs personal sampling during the 2nd and 3rd pregnancy trimester and AOS in up to 223 children aged 2-3 yrs (Dutta et al., 2021). None of these two studies found an effect of prenatal exposure to PM on AOS parameters. Among studies that used spirometry, prenatal exposure to PM was associated with reduced lung volumes in most of them, except in a Chinese study investigating the effects of prenatal exposure to PM_{10} at a monitoring station on lung

function assessed at 17.5 yrs in China (He et al., 2019), and in a study on asthmatic children, where prenatal exposure was associated with ventilation rates, but not volumes (Mortimer et al., 2008). Finally, in a Swiss birth cohort study comprising 241 children, TBFVL parameters linked to ventilation rates, were associated with prenatal exposure to PM_{10} assessed at a monitoring station.

Overall, the existing literature on prenatal exposure to PM and children lung function presents contrasting results, that could be due to the different lung function and exposure assessment techniques, the differences in the population, age groups and number of participants. The use of PM mass concentration itself could also explain these contrasting results, since this indicator does not encompass its chemical constituents and sources, and ignores the underlying biological mechanisms.

Table 3. Articles investigating prenatal exposure to PM and lung function in children.

Author, publication year	Inclusion period	Country	Number of participants	PM size or source	PM estimation method	Lung function parameter	Conclusion
Cai et al. 2020	1990-1992	England	5272 at 8 yrs, 3446 at 15 yrs	PM_{10}, PM_{10} from road emissions, PM_{10} from local sources, estimated at each pregnancy trimester, and from 0-6 months, 7-12 months and average per year up to spirometry	Chemistry-transport model	Spirometry at 8 yrs and 15 yrs old	PM exposures in each time period in pregnancy and early life were associated with reduced lung function, in terms of forced expired volume and vital capacity, at age 8 years, but not at 15.
Dutta et al. 2021	2013-2015	Nigeria	223	Personal $PM_{2.5}$ during 2nd and 3rd pregnancy trimesters and indoor postnatal exposure	Personal monitors (MicroPEM, RTI International)	Airwave oscillometry measured at 2-3 yrs	Only postnatal $PM_{2.5}$ exposures were associated with increased airway reactance at 5 Hz.
He et al. 2019	1997	China	2942	PM_{10} monthly average exposure at different developmental averages, including *in-utero*	Monitoring station	Spirometry at 17.5 yrs	No clear association of *in-utero* exposure to PM_{10} with lung function.
Hemstock et al. 2023	2012-2015	Australia	79	*In-utero* $PM_{2.5}$ daily average and maximum during a coalmine fire period, calcuted at each reported location every 12 hrs	Chemistry-transport model	Airwave oscillometry at 7 yrs	No detectable effect at 7 yrs of *in-utero* exposure to $PM_{2.5}$ from the local coalmine fire.
Jedrychowki et al. 2010	2000-2004	Poland	176	48hrs personal $PM_{2.5}$ during 2nd trimester of pregnancy	Personal monitor (PEMS)	Spirometry at 5 yrs.	Prenatal exposure to $PM_{2.5}$ was associated with lung volume reduction (forced expired volume and vital capacity) at 5 yrs.

Author, publication year	Inclusion period	Country	Number of participants	PM size or source	PM estimation method	Lung function parameter	Conclusion
Latzin et al. 2009	1999	Switzerland	241	PM_{10} average exposure during pregnancy and between birth and lung function test.	Monitoring station	Tidal breathing, multiple breath washout at 5 weeks	Association of increased prenatal PM_{10} exposure with higher minute ventilation, tidal inspiratory flow and respiratory rate.
Mortimer et al. 2008	2000	USA	232 asthmatic children	PM_{10} mean of 24hrs averages during pregnancy, each trimester, 0-3 yrs, 0-6 yrs.	Monitoring station and spatial interpolation at home address (inverse distance weight on the residence)	Spirometry at 6-11 yrs	1st and 2nd trimester exposure to PM_{10} have detrimental effects on asthmatic children lung function, in terms of flows (peak expiratory flow rate and forced expiratory flow).
Stapleton et al. 2022	2004-2006	Spain	487	$PM_{2.5}$, PM_{10}, $PM_{2.5-10}$ average over pregnancy and for each trimester, average for preschool period (1-4yrs), for school period (5-11yrs)	Land Use Regression models at home address	Spirometry at 4, 7, 9, 11 yrs old.	Prenatal exposure to PM_{10} and $PM_{2.5-10}$ shows trends for reduced lung function growth, in terms of volume (forced vital capacity decrease).

V. Oxidative stress: a major mechanism underlying the health effects of exposure to PM

Aerosols' composition, size, solubility or number are physicochemical properties that all influence PM's health effects. Considering that these parameters are all intertwined and that the current regulated PM metric (mass concentration) does not integrated this diversity, there is a need to go beyond the measure of each parameter separately, and consider a property that both integrate the physicochemical properties of PM and its potential health effects. Another angle to better understand the accumulating deleterious PM effects consists in investigating the underlying biological mechanisms. In a recent study, Peters et al. (2021) proposed eight main mechanisms underlying environmental insults, namely genomic alterations, epigenetic alterations, mitochondrial dysfunction, endocrine disruption, altered intercellular communication, altered microbiome communities, impaired nervous system function and oxidative stress and inflammation. PM, as the largest environmental risk, can affect individuals by all these mechanisms (Figure 7).

The investigation of PM composition has enabled the identification of components that are directly or indirectly redox-active, such as metals or organic species. Moreover, considering that, according to the latest Global Burden of Diseases, the main causes of deaths to which PM exposure contributes the most are linked to cardiovascular and respiratory diseases (Murray et al., 2020), and given the role of oxidative stress in the pathogenesis of these diseases, PM's oxidative capacities have gained greater interest in the scientific community. In fact, several studies highlighted that one of the main pathways for PM induced toxicity was linked to its capacity of generating oxidative stress in the lungs and on the systemic scale, which in turn, triggers an inflammation cascade (Baeza and Marano, 2007; Baulig et al., 2003; Delfino et al., 2011; Kelly and Fussell, 2015; Li et al., 2003; Mudway et al., 2020; Valavanidis et al., 2013).

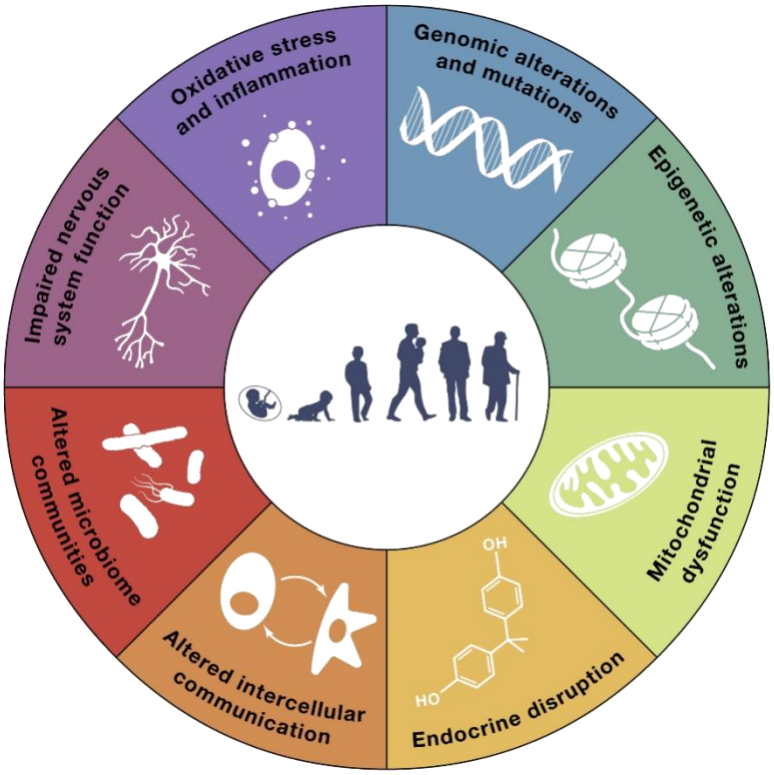


Figure 7. Biological mechanisms for environmental health effects, proposed by Peters et al. (2021).

V.1. Oxidative stress

Oxidative stress occurs when there is an excess of reactive oxygen species (ROS) in comparison to antioxidants. In a normal situation, antioxidants naturally present in the body can neutralize ROS, but an excess of ROS, a deficiency of antioxidants, or a combination of both creates an imbalance between ROS and antioxidants, leading to oxidative stress.

The modulation of the immune system is a mechanism that closely interact with oxidative stress (Figure 8). When the body detects a threat such as pathogens, damaged cells, or irritants, the immune system triggers an inflammatory response. This involves immune cells like neutrophils and macrophages that release cytokines, which play a key role in the regulation of the immune response. Moreover, blood vessels dilate in the affected area, increasing blood flow, and becoming more permeable. This allows immune cells and plasma proteins to enter the tissue, aiding in the isolation and elimination of the threat. In cases where oxidative stress becomes prominent and the inflammation process is prolonged or excessive, immune cells release inflammation mediators, further escalating the response (Mudway et

al., 2020; Peters et al., 2021). These mediators include cytokines and chemokines, which attract more immune cells to the site and modify cellular properties to combat the threat effectively.

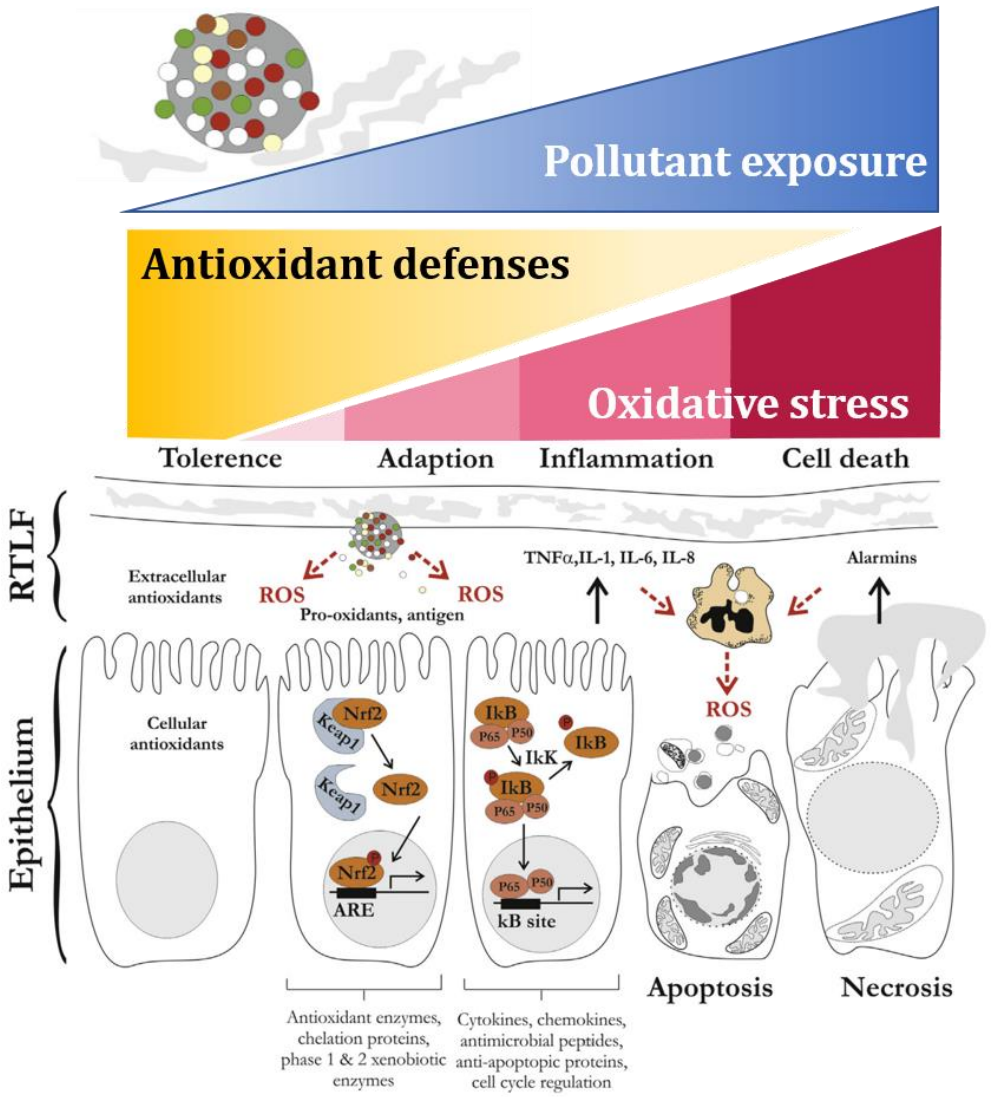


Figure 8. Biological response to oxidative stress at the air-lung interface. (adapted from (Mudway et al., 2020)

The interconnection between oxidative stress and inflammation amplifies the response to harmful stimuli. Excess ROS can enhance the inflammatory mechanism and the resulting inflammatory environment can, in turn, exacerbate oxidative stress, leading to a cyclic generation of oxidative stress and inflammation (Kelly and Fussell, 2015).

ROS are highly reactive species, which often comprise unpaired electrons. This term includes radical species such as superoxide ($O_2^{\cdot-}$) and the hydroxyl radical ($HO^{\cdot}$), the strongest oxidant in biological systems. Hydrogen peroxide (H_2O_2), while not a radical, possesses significant oxidant capacity and is involved in several redox reactions that lead to additional ROS formation. These reactive species are

byproducts of natural metabolic processes, but can also result from exogenous sources such as ultraviolet light, heat, cigarette smoke or exposure to PM (Castro and Freeman, 2001).

PM can carry or generate ROS in the body through various mechanisms. A direct acellular process is due to PM's physicochemical properties, driven by transition metals (Fe, Cu, Ni) or quinones that are redox active species. Additionally, ROS can adsorb onto PM surface, facilitating penetration into the air-lung interface. Furthermore, PM can activate cellular processes that lead to ROS generation. Indeed, the proinflammatory situation caused by the direct particle-lung surface interaction leads to the formation of ROS. Although they are not redox active, PAHs are metabolized, which in turns produces ROS (Kelly and Fussell, 2012; Lodovici and Bigagli, 2011) or are rapidly processed in the atmosphere to result in derivatives like oxo-PAH (quinones), nitro-PAHs, methyl-PAHs, that are redox active for most of them (Keyte et al., 2013; Li et al., 2003; Zeng et al., 2020).

The first step of oxidative stress, occurring when ROS levels are relatively low, activates the cellular antioxidants (Figure 8). These antioxidants initiate an adaptative process, striving to restore cellular redox balance (homeostasis) (Baeza and Marano, 2007). If the antioxidant response is insufficient, or if ROS levels increase, immune cells release cytokines, triggering inflammation. This inflammatory response recruits other immune cells, and modify cell properties to effectively combat the threat. However, prolonged inflammation and heightened oxidative stress can result in severe damage to cellular components such as DNA, lipids, and proteins. In cases of excessively high oxidative stress levels, this damage can lead to cell death processes through apoptosis or necrosis (Lodovici & Bigagli, 2011).

Oxidative damage can lead to various health problems and has been linked to the development of several chronic diseases. Accumulation of oxidative damage over time has been linked to aging and age-related diseases, such as neurodegenerative diseases that contribute to the progression of conditions like Alzheimer's and Parkinson's disease (Halliwell, 2006; Markesbery, 1999), as well as cardiovascular disease such as atherosclerosis, hypertension, and heart failure (Valko et al., 2007). Additionally, oxidative stress plays a role in diabetes and cancer (Castro and Freeman, 2001), primarily due to DNA modifications that can promote cell dysfunction and proliferation. Cytokine levels, which are signaling molecules released by immune cells during inflammation, often reflect the severity of oxidative stress

and its impact on the body's physiological processes. Biomarkers play a crucial role in assessing redox imbalance or oxidative damage. For instance, levels of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase can provide insights into the body's ability to counterbalance oxidative stress (Delfino et al., 2011). Stable oxidation products of damaged cellular components can also serve as biomarkers to measure the effects of oxidative stress.

Isoprostanes are stable chemical species formed *in vivo* by lipid peroxidation of arachidonic acid, which is present in the membrane phospholipids of cells (Cracowski et al., 2002; Milne et al., 2005). Isoprostanes are isomers of prostaglandins (Figure 9), meaning they have the same chemical formula but different structure and physicochemical properties. The two lateral chains in the isoprostanes are in *cis* configuration, while they are *trans* in prostaglandins. The peroxidation of arachidonic acid intermediate products can be fully reduced to form four regioisomers F_2-isoprostanes: the 5-series, 12-series, 8-series and 15-series F2-isoprostanes, names after the position of the hydroxyl function (Figure 9). Each of the regioisomer has eight diastereoisomers, leading to 64 different F2-isoprostanes isomers, the predominant one being the 8-isoprostane (also abbreviated as 15-F_2t-IsoP, 8-iso-$PGF_{2\alpha}$, 8-epi-PGF2α, or $iPF_{2\alpha}$-III), which is from the 15-series F_2-isoP. This molecule is released in several biological fluids, namely plasma, urine, exhaled breath condensate but is most stable and easily analyzed in urine (Dahl and van Breemen, 2010).

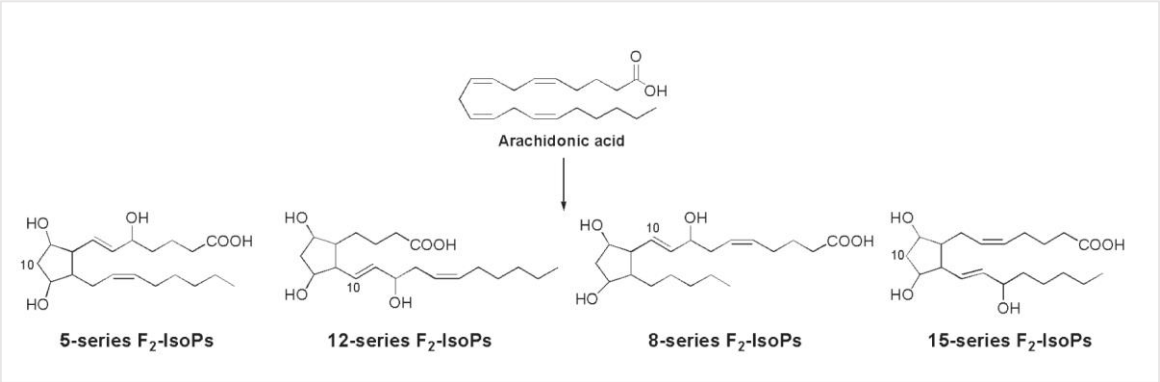


Figure 9. Structure of arachidonic acid and its peroxidation products (Milne et al., 2007).

Malondialdehyde (MDA) is also a biomarker for lipid peroxidation. In contrary of isoprostanes that only have one precursor (arachidonic acid), MDA can be formed by peroxidation of several polyunsaturated fatty acids. MDA is widely used when studying oxidative stress because it is relatively stable, and

because it is abundant in human biofluids even at low levels of oxidative stress (Cui et al., 2018; Delfino et al., 2011).

Several DNA products are produced by oxidative stress to DNA (covalent crosslinks, bases modifications), but nucleobase modifications and specifically the 8-hydroxy-2-deoxyguanosine (8-OHdG) lesion is the most abundant and has a mutagenic potential that makes it of key interest for carcinogenesis research (Kasai, 1997; Valavanidis et al., 2009). 8-OHdG is formed by the interaction of the $HO^{\cdot}$ radical with the nucleobases of DNA (Valavanidis et al., 2009), and can be analyzed in several human biofluids.

Other molecules can be used as biomarkers for oxidative stress, such as o,o'-dityrosine, fluorescent oxidation products, leukocyte telomer length or other chemokines, but 8-iso-$PGF_{2\alpha}$, MDA and 8-OHdG are well-known biomarkers, with the necessary insights for the feasibility of their analysis. Additionally, these biomarkers were associated with exposure to PM in several studies (Bin et al., 2016; Hashemzadeh et al., 2019; He et al., 2020a; Li et al., 2020; Liu et al., 2018), as well as with various lung function parameters (Andrianjafimasy et al., 2017; Graille et al., 2020; Hashemzadeh et al., 2019; He et al., 2020a; Montuschi et al., 1999). Moreover, 8-iso-$PGF_{2\alpha}$, MDA and 8-OHdG also comply with key parameters that must be considered when selecting the molecules of interest, namely low intra- and interindividual variation (Pelletier et al., 2017; Wu et al., 2010), high stability in the biofluids and over time (Barregard et al., 2013; Janicka et al., 2012; Martinez and Kannan, 2018), low circadian variations and available analytical techniques that lead to results with high reproducibility, repeatability and specificity (Graille et al., 2020; Martinez and Kannan, 2018; Martinez-Moral and Kannan, 2019; Sambiagio et al., 2021).

V.2. Oxidative potential of particles

Given the complexity of considering separately PM's chemical composition, size or specific surface area, several techniques have been developed to measure the oxidative properties of PM. There are two main categories for oxidative potential assays: cellular and acellular tests. Cellular assays have been developed to mimic the different steps of oxidative stress *in-vitro*, and further understand the biological

mechanism in place (Ayres et al., 2008). Compared to cellular assays, acellular assays are less time-consuming, cheaper and suitable for automation (Ayres et al., 2008; Gupta et al., 2020).

Acellular tests of oxidative potential were developed with the aim of reproducing physiological reactions with a shorter duration and lower cost of analysis in order to be able to use them widely and gain a better understanding of the oxidative properties of PM in long time series and various site typologies. In order to measure OP, a portion of sampled PM is extracted in a fluid, and the tests measures the ability of PM to either deplete an antioxidant or generate ROS in the extract. Extraction fluids include organic solvents (methanol), Milli-Q water, or a simulated lung fluid, which is a mix of several salts that mimic the epithelial fluid that cover the lungs (Calas et al., 2017). The most common acellular OP tests include ascorbic acid (AA), dithiothreitol (DTT), glutathione (GSH) and electron spin resonance (ESR) assays (Bates et al., 2019; Rao et al., 2020; Shahpoury et al., 2022). They were each developed to be representative of a different oxidation pathway in the lungs and therefore present different sensitivities to chemical components of PM. AA, DTT and GSH assays rely on the quantification of the reduced form of AA, DTT and GSH respectively (Cho et al., 2005; Rao et al., 2020; Yang et al., 2014). ESR aims at detecting directly materials containing unpaired electrons, namely $HO^{\cdot}$ in presence of H_2O_2 (D. Gao et al., 2020a). OP assays have been developed in order to closely mimic the biological mechanisms taking place in the lungs. Therefore, AA and GSH are used because they are present in high concentrations in the airway lining fluid, which is the first detoxifying environment of inhaled particles (Kelly and Mudway, 2003; Mudway et al., 2004). There is currently no consensus on a potentially better assay, and a large body of literature focuses on DTT and AA assays.

AA is one of the most abundant antioxidants in the lungs, and this assay was developed to mimic the antioxidant depletion in the lungs following PM inhalation. Historically, this test was developed to measure OP of transition metals (Fang et al., 2016; Maikawa et al., 2016; Yang et al., 2014), but it proved to be sensitive to oxidation by some organic species too (Daellenbach et al., 2020; Visentin et al., 2016).

DTT assay was developed to quantify OP induced by organic species (quinones, PAHs), and mimic $O2^{\cdot-}$ production in vivo (Cho et al., 2005) but it is also sensitive to some transition metals (Charrier and

Anastasio, 2012). DTT is used as a surrogate for cellular reductant agents, producing ROS with catalysis by PM redox active species. It is currently the most utilized OP assay in the literature.

OP can be normalized either by the mass of PM, or by the volume of air sampled. Intrinsic mass-normalized OP (OP_m) quantifies the OP of 1 µg of PM and is therefore representative of the reactivity of PM, whereas the volume-normalized OP_v (OP_m multiplied by $PM_{2.5}$ mass concentration), is a proxy for exposure levels to humans (Weichenthal et al., 2016).

V.3. Evidence for OP effects on health and biological parameters

Since OP tests are intended to predict PM's capacity to generate oxidative stress upon inhalation, several studies have been conducted with the aim of better understanding the OP of PM:

- by characterizing the chemical species and the associated sources most sensitive to the OP assays ;
- by evaluating its predictive character towards the health and biological effects attributed to PM exposure ;
- by comparing the findings with what is observed for PM mass concentration.

Regarding OP of PM sources, findings indicate that OP^{AA} is mostly sensitive to vehicular emissions, because of the contribution of copper (Cu), iron (Fe) and zinc (Zn) to this source. OP^{DTT} is also sensitive to vehicular emissions, but it is mostly associated to Cu and manganese (Mn) rather than Fe, and this sensitivity is also explained by the organic species composing the traffic emissions, such as soot-bound PAHs that oxidize to quinones once metabolized. Additionally, OP^{DTT} is also sensitive to biomass burning and trash burning emissions, due to the large fraction of reactive organic species emitted by these sources, including humic-like substances or levoglucosan. Moreover, studies showed that both OP^{AA} and OP^{DTT} responses could be modified due to the interaction between organic species and transition metals. Higher Fe and Cu indeed led to a decrease in OP^{AA} (antagonistic effect) while the presence of quinone and Cu increased the OP^{DTT} response (synergetic effect) (Borlaza et al., 2021a; Pietrogrande et al., 2022; Yu et al., 2018). Given the different photochemistry indoors, the different penetration rate depending on the chemical constituent of PM, and the different indoor activities, the OP of PM indoors has also been investigated, in European offices for OP^{GSH} and OP^{AA} (Szigeti et al., 2016), Chinese student dormitories for OP^{DTT} (Yang et al., 2021), Chinese homes for a cellular assay (Brehmer

et al., 2020; Secrest et al., 2016; Zhan et al., 2018) and OP^{DTT} (Secrest et al., 2016), and Indian slums for OP^{DTT} (Anand et al., 2022). Results show high variability in OP during outdoor-indoor transport, due the change in water-soluble iron and sulfate concentrations, that modifies particles' pH and therefore metals solubilities (Yang et al., 2021). While lower indoor OP^{AA} and OP^{GSH} were found in European offices, this was not the case for OP^{DTT} in Chinese homes. The role of transition metals, mainly copper and iron, sulfates, and PAHs were pointed in most studies, as well as the high variability between indoor environments of the studies. A recent study performed in an environmental chamber was able to show that nine indoor sources of PM could significantly contribute to OP^{DTT}, among which the combustion related contributed greater (all related to incense, candles, or different cigarette types) (Hu et al., 2023). In most epidemiological studies, exposure to OP relied on LUR models developed in Europe (Gulliver et al., 2018; Hellack et al., 2017; Strak et al., 2017; Tonne et al., 2012; Yang et al., 2016), and North America (Weichenthal et al., 2019). However, most of the studies that developed these LUR highlighted that there were no specific geographical tracers for OP. For this reason, monitoring stations were sometimes preferred in time-series analysis studies (Korsiak et al., 2022; Weichenthal et al., 2016), using several stations in order to have a small residence-station distance (5km). Personal PM samplers providing gravimetric measurements, have the additional advantage of enabling OP analysis on the filter. It is therefore possible to estimate the integrated personal OP over the measurement period, but to the best of our knowledge, only one study relied on personal samplers to address OP impacts on health (Maikawa et al., 2016).

In terms of health effects, OP was found associated with several cardiorespiratory outcomes in population of various ages (Table 4). OP^{DTT} was found associated with cardiovascular health (Abrams et al., 2017; Bates et al., 2015; Fang et al., 2016; Weichenthal et al., 2021, 2016), and OP^{GSH} was associated with adverse birth outcomes (Lavigne et al., 2018). More specifically, exposure to OP was used in relationship with lung function, airway inflammation and oxidative stress biomarkers in several cohort studies, interventional studies and time series studies. The assays used in these studies comprise the DTT, GSH, ESR, AA and a cellular test, and OP was standardized by air volume (OP_v) in most cases. Most of these studies assessed the effects of short-term exposure, except in the PIAMA birth cohort study that estimated annual averages of OP using a LUR model. This study conducted on 3701

children found consistent associations of OP_v^{DTT} with decreased lung volumes assessed by spirometry at 12 years, and this association was not observed with $PM_{2.5}$ exposure. Airway inflammation, assessed by the fraction of exhaled nitric oxide (FeNO), was frequently investigated, with no association for long-term exposure to OP_v^{DTT} and OP_v^{ESR} in the PIAMA cohort, whereas some other studies showed increased FeNO for increased short-term OP_v^{DTT} (Delfino et al., 2013; Janssen et al., 2015), OP^{GSH} (Maikawa et al., 2016), OP^{ESR} and OP^{AA} (Janssen et al., 2015) and OP^{cell} (Delfino et al., 2013, 2010). In a study, where healthy adults were exposed for 5 hours to air pollution at different sites in the Netherlands, OP exposure (OP^{AA} and OP^{GSH} based on measurement in different size fractions) was not associated with either FeNO, nor lung function assessed by spirometry.(Strak et al., 2012). However, a study relying on the same exposure design directly measured OP in $PM_{2.5}$ and PM_{10}, and found decreased lung volumes upon OP^{AA} and OP^{ESR} exposure, although associations varied with site of exposure and PM size fraction (Janssen et al., 2015). All other studies found decreased lung volumes measured by spirometry for increased OP exposure, whereas this effect was not observed, or was on the opposite side for increased PM exposure (He et al., 2021; Hogervorst et al., 2006; Yang et al., 2016). Only one study investigated short-term OP effects in airway mechanical parameters, and found increased resistance of small, large and total airways for higher OP^{cell} and increased total airways resistance for higher $PM_{2.5}$ (He et al., 2021). Mechanistic studies investigated the effects of short-term exposure to OP on oxidative stress and immune function biomarkers. Exposure duration ranged from a few hours post-exposure in interventional studies (Janssen et al., 2015; Liu et al., 2018; Steenhof et al., 2013), to 5 days in a cohort study (Delfino et al., 2010), and the population studied did not exceed 101 participants. Findings tended to converge towards an increase in pro-inflammatory biomarker interleukin 6 (IL-6), but associations varied depending on the biofluid used and therefore the type of inflammation considered, i.e. systemic or in the airways, for blood and nasal fluid respectively. Among the three studies investigating OP effects on oxidative stress biomarkers (Liu et al., 2018; Zhang et al., 2016, 2021), only one interventional study found an effect, with increased urinary MDA and 8-OHdG for higher personal exposure to OP_m^{AA} and OP_m^{GSH} in concentrated ambient particles, respectively (Liu et al., 2018).

Currently, there is no standardized protocol regulating OP analysis, which leads to results or ambient levels of OP that are hard to compare, in addition to the existing differences between the assays.

Table 4. Articles investigating exposure to OP and endpoints linked to lung function, airway inflammation or biomarkers of oxidative stress.

Author and publication year	Type of study	Country	Number of participants	OP assay	Follow-up duration[a], exposure duration[b], or time intervals[c]	Health endpoint	Conclusion
Yang et al. (2016)	Cohort study	Netherlands and Belgium	3701 children	OP_v^{DTT} and OP_v^{ESR} of $PM_{2.5}$, estimated by LUR	14 years	Lung function (spirometry), airway inflammation (FeNO)	Increased 1-year OP^{DTT} was associated with decreased lung volumes at 12 years, whereas $PM_{2.5}$ was not. There was no association with FeNO.
Delfino et al. (2010)	Panel study	USA	60 elderly nonsmoker participants with coronary artery disease	$OP^{cellular}$ of outdoor $PM_{0.25}$	12 weeks	serum IL-6, FeNO	Increased OP and PM concentrations over the last 5 days were associated with increased IL-6 and FeNO. Associations for PM varied by size fraction.
Delfino et al. (2013)	Panel study	Canada, USA	45 asthmatic children	OP_v^{DTT} and $OP^{cellular}$ of $PM_{2.5}$ at central monitoring site	10 days	Airway inflammation (FeNO)	Increased lag 1-day and 2-day OP was associated with increased FeNO, for both assays, but not $PM_{2.5}$.
He et al. (2021)	Panel study (relying on interventional study)	China	43 asthmatic children	calculated 24-h averages of personal $OP_v^{cellular}$ in $PM_{2.5}$	8 weeks	Airway mechanics (impulse oscillometry), lung function (spirometry), airway inflammation (FeNO) measured at each visit	Higher lag 0-day and lag 3-day OP was associated with decreased lung function in terms of airway mechanics, lung flow and volumes, whereas $PM_{2.5}$ was only associated with an increased lung resistance.
Hogervorst et al. (2006)	Panel study	Netherlands	342 children	OP_m^{ESR} and OP_v^{ESR} in Total Suspended Particles (TSP), PM_{10} and $PM_{2.5}$ in 6 primary schools	1 year	Lung function (spirometry)	Increased 4-day average TSP and PM_{10} were associated with increased lung volumes, whereas increased 4-day average OP^{ESR} of $PM_{2.5}$ was associated with decreased lung volumes.
Maikawa et al. (2016)	Panel study	Canada	62 asthmatic children	personal OP_v^{GSH}, OP_v^{AA} of $PM_{2.5}$	10 days	Airway inflammation (FeNO)	Increased lag 0 and lag 2-day OP^{GSH} was associated with increase in FeNO, whereas OP^{AA} and $PM_{2.5}$ were not.
Zhang et al. (2016)	Panel study	USA	97 elderly non-smoking adults	OP_v^{DTT} and $OP_v^{cellular}$ measured in $PM_{0.18}$, $PM_{0.18\text{-}2.5}$ at central monitoring site	12 weeks	MDA in exhaled breath condensate, airway inflammation (FeNO)	Increased 5-day average $PM_{0.18}$ was associated with increased MDA and FeNO, whereas increased $PM_{2.5}$ was associated with decreased FeNO.

Author and publication year	Type of study	Country	Number of participants	OP assay	Follow-up duration[a], exposure duration[b], or time intervals[c]	Health endpoint	Conclusion
							Associations with OP exposures were not significant.
Zhang et al. (2021)	Transversal study	China	101 families	OP_m^{DTT} in indoor dust	Dust collection duration not available	urinary 8-OHdG	None significant trend observed between OP in dust and urinary 8-OHdG.
Janssen et al. (2015)	Interventional study	Netherlands	31 healthy non-smoking adults	personal exposure OP_v^{DTT}, OP_v^{ESR}, OP_v^{AA} in $PM_{2.5}$ and PM_{10}	5-hour exposures; Participants exposed multiple times, separated for at least 14 days between two exposures	Lung function (spirometry), airway inflammation (FeNO), nasal lavage IL-6, serum IL-6 and C-reactive protein	Positive changes in FeNO and IL-6 in nasal lavage were associated with all OP exposures. Negative changes in lung volumes were associated with OP^{AA} and OP^{ESR} exposure, but associations varied with sites and PM size fraction.
Liu et al. (2018)	Interventional study	Canada	53 healthy non-smoker volunteers	OP_m^{GSH} and OP_m^{AA} measured in personal concentrated ambient aerosol	130-min exposures; Participants exposed multiple times, separated for at least 14 days between two exposures	blood and urinary MDA, urinary 8-OHdG, plasma IL-6	The percent change of urinary MDA and 8-OHdG, were significantly increased post-exposure to OP_m^{AA} and OP_m^{GSH}, respectively. Percent change in blood MDA and urinary 8-OHdG were significantly increased post-exposure to $PM_{2.5}$.
Steenhof et al. (2013)	Interventional study	Netherlands	31 healthy adults	calculated OP_v^{GSH}, OP_v^{AA} of PM_{10} from measurements in different PM size fractions	5-hour exposures; Participants exposed multiple times, separated for at least 14 days between two exposures	serum IL-6, nasal lavage IL-6 and IL-8	Changes in IL-6 and IL-8 were not associated with exposure to OP. Changes upon PM exposure varied by co-adjustment on PM components.
Strak et al. (2012)	Interventional study	Netherlands	31 healthy adults	similar to Steenhof et al. (2013)	5-hour exposures; Participants exposed multiple times, separated for at least 14 days between two exposures	Lung function (spirometry), airway inflammation (FeNO)	PM mass concentration and OP exposures were not associated with the studied endpoints.
Abrams et al. (2017)	Time series	USA	730,000 ED visits (general population)	OP_v^{DTT} of $PM_{2.5}$ at central monitoring site	10 months	Respiratory diseases related ED visit	Increased lag 0 and lag 2-day OP^{DTT} was associated with increased risks of ED visits for respiratory diseases, with stronger effect than $PM_{2.5}$.
Bates et al. (2015)	Time series	USA	263,665 ED visits (general population)	modelled OP_v^{DTT} of $PM_{2.5}$ at central monitoring site	10.5 years	Respiratory diseases related ED visit	Increased lag 0 and lag 2-day OP^{DTT} and $PM_{2.5}$ were associated with

Author and publication year	Type of study	Country	Number of participants	OP assay	Follow-up duration[a], exposure duration[b], or time intervals[c]	Health endpoint	Conclusion
							increased risk of ED visits for respiratory diseases, with higher risks for OP than PM.
Fang et al. (2016)	Time series	USA	458,526 ED visits (general population)	modelled OP_v^{DTT}, OP_v^{AA} of $PM_{2.5}$ at central monitoring site	10.5 years	Respiratory diseases related ED visit	Increased lag 0 and lag 2-day OP^{DTT} was associated with increased risks of ED visits for respiratory diseases, but not OP^{AA}.
Weichenthal et al. (2016)	Time series	Canada	426,587 ED visits (general population)	OP_v^{AA} and OP_v^{GSH} of $PM_{2.5}$ at central monitoring site	7,75 years	Respiratory diseases related ED visit	Increase in both OP assays for lag 0 and lag 2-day and in lag 0 and lag 2-day $PM_{2.5}$ were associated with increased risks of ED visits for respiratory diseases.

a: follow-up duration for cohort and panel studies, b: exposure duration for interventional studies, c: time intervals for time-series studies. Abbreviation: ED, emergency department.

VI. Objectives

The general objective of this work is to make significant progress in the validation of oxidative potential as a relevant indicator of the health impacts of exposure to particulate matter. With the aim of achieving this objective, this thesis proposes a multidisciplinary approach based on a strategy that involves a combination of atmospheric sciences and epidemiology to:

- Characterize the associations between prenatal personal measurements of OP and respiratory health endpoints in early childhood,
- Characterize the associations between personal measurements of OP and biological markers of systemic oxidative stress and immune function,
- Characterize the chemical species determining OP in indoor air, and OP spatial and seasonal variability in homes in the Grenoble area.

The general methodology of this work will be the object of chapter II and the three main research axes will be developed in chapters III, IV and V, and VI. A general discussion of the work and its perspective will be conducted in chapters VII.

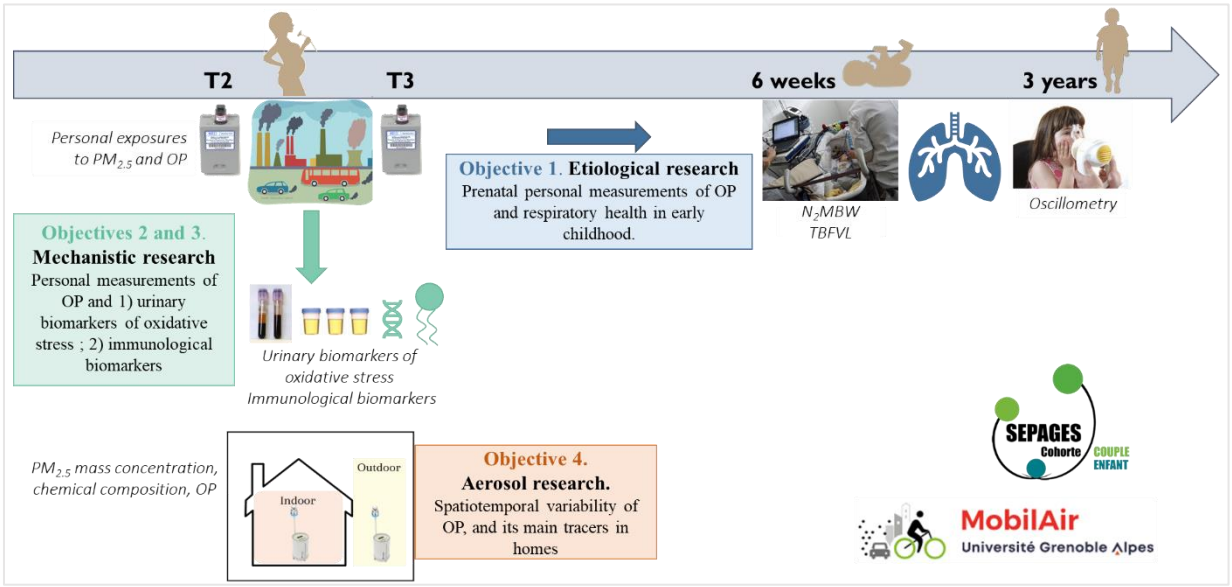


Figure 10. Schematic of the thesis work.

Chapter II

Methodology

This work was based on the couple-child SEPAGES cohort based in the area of Grenoble, France. This chapter will provide an overview of the measurements utilized, and more details specific to each research question will be provided in the corresponding chapters.

I. Study site

Grenoble is a city located in South-East of France. Together with 49 surrounding municipalities, it forms the Grenoble-Alpes Métropole territory, that occupies a total area of 545,5 km^2 and comprised a total of 448 457 inhabitants in 2020 (INSEE, 2023). The territory lies in the French Alps, and is surrounded by three mountain ranges: Chartreuse in the North, Vercors in the West and Belledonne in the East, however the city of Grenoble itself is flat, and is located at an altitude of about 200 m above sea level. The particular topography, with a Y shape formed by the valleys, and the proximity of mountains (peaking at up to 2000 to 3000m) strongly influences the climate, with irregular temperatures and rain patterns (Figure 11, Figure 12). The Grenoble basin is influenced by the oceanic and semi-continental climate and by the Mediterranean climate towards the South, favoring cold winters and hot summers. Because of the three valleys with main road axes and the surrounding vegetation, the presence of NO_x and VOCs favors the formation of O_3, leading to important O_3 events in summer. In winter, the temperature, orography and anticyclonic conditions lead to important thermal inversions (Largeron & Staquet, 2016), increasing ground concentration of pollutants, and the number of days exceeding the PM_{10} daily threshold.

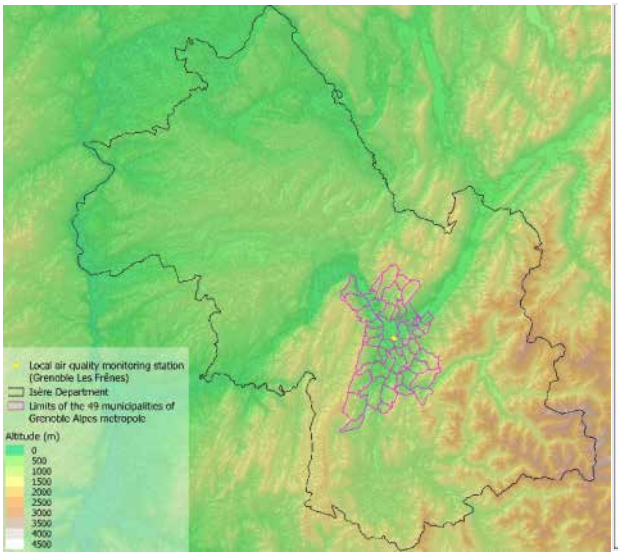


Figure 11. Topography of the Isère department, and location of the Grenoble basin. Credits: RGE Alti, IGN.

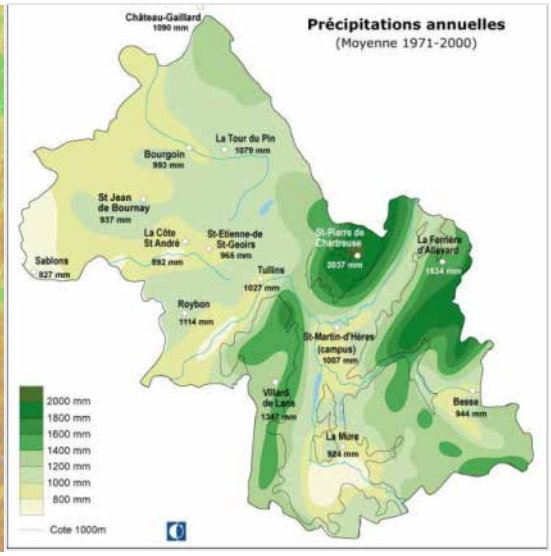


Figure 12. Yearly average precipitations over the Isère department. Credits: Meteo France.

The population of Grenoble is rather young with 30.2% of the population being between 15 and 29 years old, and is highly educated, with 25.2% of the population in 2020 having a higher education diploma, i.e. 5 years of study after high school, while this proportion is of around 11% for the whole metropolitan France (INSEE, RP2020).

II. SEPAGES cohort

This work is based on the data collected in the SEPAGES cohort (https://cohorte-sepages.fr/), whose design is detailed by Lyon-Caen et al. (2019). SEPAGES (Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé; Assessment of air pollution exposure during pregnancy and effect on health, in English) is a research platform aiming at characterizing the effect of early-life (including *in utero*) exposure to a large panel of environmental factors on child health, and specifically child growth, respiratory health and neurodevelopment. This cohort relates to the Developmental Origins of Health and Disease (DOHaD) research, which explores how the interplay between maternal and environmental factors modifies fetal and child growth and influence developmental trajectories and susceptibility to disease throughout the life course (Carraro et al., 2014). The finely characterized environmental exposure mainly include air pollutants (NO_2, BC, VOCs, $PM_{2.5}$), temperature, noise, UV, and chemical exposures such as phenols, phthalates and perfluorinated

compounds. The SEPAGES cohort is also aiming at investigating various biological mechanisms involved in the health effects of early-life environmental factors, including DNA methylation, gut microbiota, thyroid hormones, immunological parameters, and oxidative stress.

Briefly, 484 women were recruited at the beginning of their pregnancy between July 2014 and July 2017 in eight obstetrical ultrasonography practices located in Grenoble area. To be included, women had to be of legal age (18 or more), to be pregnant by less than 19 gestational weeks, to be living in the Grenoble area, and to be planning to give birth in one of the four partner maternity hospitals. A recruitment questionnaire allowed to compare SEPAGES women to the population of pregnant women approached but not included, and to pregnant women of Grenoble and France (Table 5). Compared to the non-included women, the 484 included women were older (74% were above 30, against 60% for the approached but not included women), had a lower parity, had a higher education level (94% undergraduate or graduate, against 70%), and were more frequently employed. Compared to the pregnant women in whole France, participants had a lower BMI (82% with a BMI <25 kg/m^2 against 68% in France) and smoked less before (89% did not smoke against 70% in France) and during pregnancy.

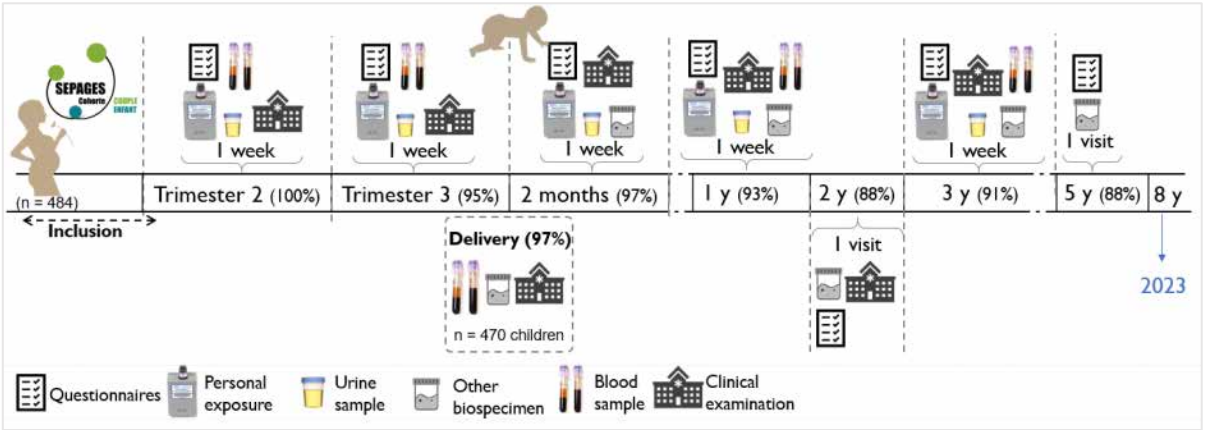


Figure 13. Design of the SEPAGES cohort.

Table 5. Characteristics of the women in SEPAGES cohort, and comparison with pregnant women from Grenoble and France. Table extracted from Lyon-Caen et al. (2019)

Characteristic	Population of pregnant women							
	SEPAGES Women		Approached but Not Included [1]		Whole Grenoble Area [2]		Whole France [3]	
	n = 484		*n* = 1841		*n* = 17,899		*n* = 12,950	
Age (years), mean ± SD	32.7 ± 3.9		31.0 ± 4.7 [4]		31.1 ± 5.0 5		30.3 ± 5.2	
Age (categories)			(<0.001) [4]		(<0.001) [5]		(<0.001) [6]	
<20	0	0	13	(0.7)	113	(0.6)	204	(2.5)
20–24	13	(2.7)	128	(7.0)	1483	(8.3)	1553	(12.0)
25–29	113	(23.3)	589	(32.0)	5116	(28.6)	4052	(31.3)
30–34	230	(47.5)	683	(37.1)	6656	(37.2)	4377	(33.8)
35–39	117	(24.2)	353	(19.2)	3626	(20.3)	2236	(17.3)
≥40	11	(2.3)	74	(4.0)	900	(5.0)	519	(4.0)
Maternal Parity [7]			(0.002) [4]		(<0.001) [5]		(<0.001) [6]	
0	222	(45.9)	816	(44.6)	6036	(39.7)	5464	(42.2)
1 child	214	(44.2)	721	(39.4)	6098	(40.1)	4609	(35.6)
≥2 children	48	(9.9)	294	(16.1)	3071	(20.2)	2872	(22.2)
Marital status			(0.005) [4]		NA		(<0.001) [6]	
In a relationship (cohabitation or married)	483	(99.8)	1808	(98.2)			9593	(81.9)
No relationship	1	(0.2)	33	(1.8)			2123	(18.1)
Education level			(<0.001) [4]		(<0.001) 5		(<0.001) [6]	
Primary school	0	(0.0)	4	(0.2)	106	(1.4)	187	(1.6)
Secondary Education	6	(1.2)	226	(12.3)	677	(9.2)	2489	(21.3)
High School education	23	(4.8)	316	(17.3)	1404	(19.2)	2521	(21.6)
Undergraduate or graduate	452	(94)	1285	(70.2)	5141	(70.2)	6464	(55.4)
Nationality							(<0.001)6	
French	394	(94.7)	NA		NA		10,083	(85.9)
Other European country [8]	18	(4.3)					416	(3.5)
African country	0	(0.0)					993	(8.5)
Other nationality	4	(1.0)					243	(2.1)
Working status during pregnancy			(<0.001) [4]		(<0.001) [5]		(<0.001) [6]	
Employed	434	(92.9)	1532	(85.0)	6806	(75.0)	7830	(68.1)
Unemployed	13	(2.8)	79	(4.4)	508	(5.6)	1928	(16.8)
Housewife/parental leave/in training	20	(4.3)	191	(10.6)	1757	(19.4)	1630	(14.2)
Not working, other	0	(0.0)	0	(0.0)	NA		108	(0.9)
BMI before pregnancy							(<0.001) [6]	
<18.5 kg/m^2	29	(6.0)	NA		NA		863	(7.4)
18.5–24.9 kg/m^2	364	(75.8)					7045	(60.8)
25–29.9 kg/m^2	67	(14.0)					2312	(20.0)
≥ 30 kg/m^2	20	(4.2)					1368	(11.8)
Smoking before pregnancy							(<0.001) [6]	
0	385	(89.1)	NA		NA		8217	(69.5)
1–9 cig./day	37	(8.6)					1350	(10.9)
≥ 10 cig./day	10	(2.3)					2132	(19.6)
Smoking during pregnancy [9]							(<0.001) [6]	
0	402	(93.3)	NA		NA		9798	(83.4)
1–10 cig./day	29	(6.7)					1447	(12.3)
>10 cig./day	0	(0.0)					499	(4.2)

Values reported are numbers (%), unless stated otherwise. BMI: Body Mass Index. [1]Pregnant woman interviewed by a SEPAGES fieldworker who met the SEPAGES inclusion criteria and did not want to participate to the study. [2]Database of birth certificates provided 8 days after birth, covering Isère département, where Grenoble is located. The population was restricted to women (1) who gave birth in one of the 4 maternity wards of Grenoble area, (2) who were older than 18 years old when they gave birth and (3) whose date of last menstrual period was between March 2014 and February 2017 (no data were available after this date). [3]Source: 2016 French Perinatal Survey [62]. [4]P-value; X^2-test (or Fisher exact test when needed) comparing the characteristics of pregnant women included in SEPAGES and the pregnant women not included in SEPAGES and interviewed by a SEPAGES fieldworker. [5]P-value; X^2-test (or Fisher exact test when needed) comparing the characteristics of pregnant women included in SEPAGES and pregnant women living in Grenoble area. [6]P-value; X^2-test (or Fisher exact test when needed) comparing the characteristics of pregnant women included in SEPAGES and pregnant women living in France. [7]Before the index pregnancy. [8] Including Turkish. [9] For the pregnant women included in SEPAGES, smoking during pregnancy was defined as smoking any time during pregnancy. For the pregnant women living in France, smoking during pregnancy was defined as smoking during third trimester of pregnancy.

The follow-up of women included several online or interview-based questionnaires, to provide a detailed description of the living environment, the habits of each participant, and the health conditions identified in the infant (wheezing, waking up short of breath, bronchitis, asthma), clinical examinations, and environmental exposures measurements (Figure 13). Environmental exposures were assessed at different timepoints: during the second and third trimesters of pregnancy, and two months, 1 year and 3 years after child birth. During the measurement weeks, and at birth, several biological samples were collected (urine, blood, placenta, nails, hair, milk, stools), and a clinical examination was conducted at the end of each measurement week (Table 5).

A subset of the cohort (41 families) also volunteered to participate in an intensive campaign to measure the chemical composition of PM samples collected from the indoor and outdoor air of their homes, using 4 low-volume samplers, and taking place when the child was about 3 years old. Figure 14 provides an overview of the measurements particularly used in the frame of this work. The maternal personal $PM_{2.5}$ measurements took place at during the second and third pregnancy trimesters, starting from 2015-06-22 to 2017-12-20. The indoor-outdoor measurement campaign took place at two seasons in the years 2018-2019, with a cold season ranging from 2018-11-26 to 2019-04-18 and the warm season ranging from 2019-05-09 to 2019-10-31, and lung function measurements in children took place at the end of the 2-months and 3-years measurement weeks.

Figure 14. Summary of the measurements used in this work, and their chronological sequence.

II.1. Pregnancy period

II.1.1. Exposure assessment

$PM_{2.5}$ was sampled using active air samplers equipped with internal 25mm PTFE filters (MicroPEM™; RTI International), installed in a backpack kept at close proximity during 7 to 8 consecutive days. Measurement periods took place at two different stages of the pregnancy: once during early pregnancy and once towards the end of the pregnancy, with a median (quartile 1, quartile 3) of time between the first and second measurement of 16 (14, 18) weeks, with a minimum of 4 weeks and a maximum of 23 weeks, mainly due to the availability of the samplers or the volunteers. The filters were subjected to gravimetric analysis (Mettler Toledo UMX2 ultramicrobalance) in controlled hygrometric conditions (21°C, 25% relative humidity), prior and after the 7-days $PM_{2.5}$ sampling, to measure the collected net mass (µg), and were then stored at -20°C.

II.1.2. Urine pools

On the 7th day of the first $PM_{2.5}$ measurement week (during early pregnancy), the participant collected three urine samples in the morning, midday and evening and stored them in her freezer (-20°C). When the study field worker visited the participants at the end of the PM collection week, the samples were picked up and transported to Grenoble University Hospital's (CHU-GA) certified biobank (bb-0033-00069), where they were stored at -20°C until pooled. One urine daily-pool per participant was created, by mixing equal-volumes of the three spot samples (Philippat and Calafat, 2021), that were thawed overnight at 4°C prior to pooling procedure. Aliquots of individual urine pools were then stored at -80°C until biomarker analysis in 2022 (storage of 5.9 ± 0.5 yrs, with one other thawing-freezing cycle).

II.1.3. Blood samples

Blood samples were collected by trained filed workers, within a maximum of 48 hours after the end of the first $PM_{2.5}$ measurement week. Blood was collected in BD Medical 368886 vacutainer tube (lithium heparin) for immunological analyses (cell culture and plasma separation), and in BD Medical 368861 vacutainer tube (EDTA) for cell counting. They were transported to the Etablissement Français du Sang (EFS) on ice, placed on a rotating device for at least 5 min to ensure homogeneous cell content, and were then processed within 24 hours after collection.

II.2. Lung function measurements

Objective measurements of children's lung function were performed by trained field workers at 6-12 weeks using the tidal breathing flow-volume loops (TBFVL) and nitrogen multiple-breath washout techniques (N_2MBW), and at 3 years using airwave oscillometry (AOS, see Figure 15). All measurements were performed in compliance with the current guidelines from the European Respiratory Society (ERS) and American Thoracic Society (ATS) (Bates et al., 2000; Beydon et al., 2007).

Figure 15. Pictures of the lung function measurements at 6 weeks (left), and three years (right). Credits for the pictures are: Inserm (left), Thorasis (right).

The three techniques used are noninvasive and do not require active participation of the children, making them particularly suitable for very young children. The parameters used are summarized and described in Table 6.

Table 6. Lung function parameters measured at 6 weeks and 3 years.

Age	Technique	Parameter	Description
6 weeks	TBFVL	Tidal volume (V_T)	Volume of a tidal breath.
		t_{PTEF}/t_E	Ratio of time to peak tidal expiratory flow (t_{PTEF}) to expiratory time (t_E)
	N_2MBW	Functional residual capacity (FRC)	Volume of air remaining in the lungs at the end of a tidal breath.
		Lung clearance index (LCI)	Number of "lung turnovers" (i.e., multiples of FRC) required to complete the washout
3 years	AOS	Resistance at 7 Hz (Rrs_7)	Associated with frictional losses in the large airways.
		Reactance at 7 Hz (Xrs_7)	Depends on the inertive and elastic behaviors of the respiratory system. Measures lung stiffness and heterogeneous ventilation.
		Area under the reactance curve (AX)	Represents the peripheral airways and measures the loss of elastic recoil of increased stiffness of the lung.
		Frequency dependence of the resistance (Rrs_{7-19})	Defined by the resistance difference between 7 and 19 Hz, this parameter evaluates the resistance of the small airways and the heterogenous obstruction of the distal bronchi.

II.3. Indoor-outdoor campaign

41 families volunteered for the concurrent evaluation of the indoor and outdoor $PM_{2.5}$ chemical characterization. In each home, indoor and outdoor sampling were carried out simultaneously, at two different seasons (cold and warm). Two indoor samplers were placed in the main living area while two outdoor samplers were placed on the adjacent balcony, terrace or garden when available. If there was none available, sampling was performed indoors only (N=12). The adjacent samplers were respectively equipped with a Teflon and a Quartz filter, to have sufficient material for chemical speciation. Figure 16 summarizes the available data.

Teflon filters were double-weighted in ATMO SUD's gravimetric laboratory, under strict environmental conditions before and after sampling, following standard NF EN 12341, respecting 12h (before sampling) and 24-72h (after sampling) between the two analyses.

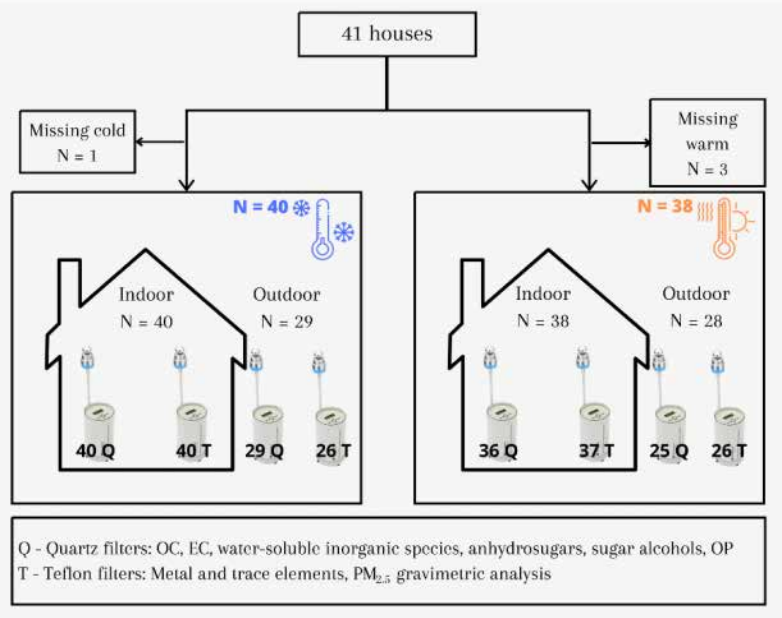


Figure 16. Schematic of the indoor-outdoor measurement campaign.

In 2018-2019, PM_{10} were collected daily on 150 mm-diameter quartz fiber filters at the central monitoring site "Les Frênes" of the regional monitoring Air Quality agency (Atmo Aura) using a Digitel DA-80 (30 m^3/h).

III. Chemical analyses

III.1. OP analysis

Personal $PM_{2.5}$ filters, weekly $PM_{2.5}$ indoor and outdoor quartz filters, and ambient PM_{10} filters were analyzed for OP following the same protocol established by Calas et al (Calas et al., 2018, 2017). PM were extracted from the filters in a simulated lung fluid (SLF), composed of a mixture of Gamble and 1,2-dipalmitoylphosphatidylcholine (DPPC), to reach a final concentration of 25 µg/mL for ambient PM_{10} extracts, and of 10 µg/mL for the other filter extracts. After vortex mixing at 37°C during 75min, OP was measured using the dithiothreitol (DTT) and the ascorbic acid (AA) assays, by mixing reagent solutions in a 96-well plate (CELLSTAR, Greiner-Bio) and measuring absorbance using Infinite M200 Pro TECAN spectrophotometer, with a total reaction time of 30 min. The DTT assay relies on the consumption of DTT by $PM_{2.5}$, and the titration of remaining DTT by dithionitrobenzoic acid (DTNB), forming 2-nitro-5-thiobenzoic acid (TNB) that is titrated every 10min. Since AA has an absorbance

spectrum in the UV-Visible, its absorbance at 265 nm, measured every 4 min, directly measures its depletion upon consumption by $PM_{2.5}$. For both assays, analyses are performed in triplicates, a positive control of 1,4-naphtoquinone is performed for every experiment, with the coefficient of variation of the positive control tests <3% . OP^{DTT} and OP^{AA} was normalized by the sampled air volume (nmol/min/m^3) and by the extracted PM mass (nmol/min/µg).

III.2. Indoor and outdoor $PM_{2.5}$ filters

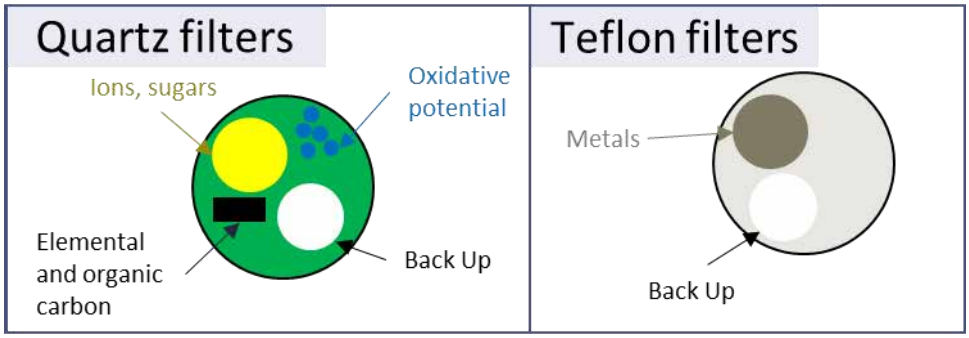


Figure 17. Summary of the chemical analyses conducted on the quartz and Teflon filters.

Filter samples were subjected to chemical analysis to measure the carbonaceous fraction, water-soluble ions, anhydrosugars and polyols, on the quartz filter; trace elements analysis was performed on the Teflon filter (Figure 17).

Organic and elemental carbon were analyzed on a 1.5 cm^2 punch of the quartz filter, by thermo-optical analysis following the EUSAAR2 protocol, using the Sunset Lab EC/OC analyzer (Birch and Cary, 1996; Cavalli et al., 2010). Briefly, the sample is placed in a quartz furnace and subjected to a prescribed temperature protocols, under a more or less oxidizing atmosphere. A gas stream carries the volatilized carbon through several steps, converting them into methane, analyzed by flame ionization detection.

Water-soluble ions, anhydrosugars, and polyols were analyzed on the same water extract of the sample. Briefly, 10 mL ultrapure water is used for the solid/liquid extraction of the filter during 20 minutes under vortex agitation, prior to filtration using a 0.25 µm Acrodisc filter (Milipore Millex-EIMF).

Ionic fraction is measured by ion chromatography (IC, Thermo Fisher ICS 3000), following a standard protocol previously described (CEN, 2017; Jaffrezo et al., 2005), with a CS16 column for cations analysis (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) and an AS11HC column for anions (SO_4^{2-}, NO_3^-, Cl^-).

The analysis of anhydrosugars (levoglucosan, mannosan, galactosan), polyols (arabitol, sorbitol, mannitol) is performed on the water extract, using high-performance liquid chromatography (HPLC), with pulsed amperometric detection (PAD) (model Thermo Fisher 5000+) with Metrosep columns (Carb 1 – Guard+A Supp 15 – 150+Carb 1 – 150), following the procedure described by Piot et al. (2012). Metallic trace elements were analyzed by Tera-environment, using inductively coupled plasma coupled with a mass spectrometer (ICP-MS) or an atomic emission spectrometer (ICP-AES) after acid digestion of a portion of the Teflon filters, following standardized protocols (Alleman et al., 2010b; CEN, 2005).

III.3. Urine samples

Urine specific gravity, a measure of urine dilution, was measured in each pool prior to storage at -80°C using a handheld digital refractometer (Atago-PAL-10S). Urinary malondialdehyde (MDA) was analyzed in sampled diluted 50 times with water before derivatization using 2,4-Dinitrophénylhydrazine (DNPH). 8-OHdG and 8-iso-PGF2α were individually isolated from 1 mL urine using solid phase extraction. The concentrated extracts as well as the MDA-DNPH derivative were analyzed by liquid chromatography mass spectrometry (Thermo Fischer Quantiva). Oxidative stress biomarkers were analyzed at CURML-CHUV (Switzerland). Concentrations below the limit of detection (LOD) or limit of quantification (LOQ) were replaced by LOD or LOQ divided by the square root of two, respectively.

III.4. Blood samples

Innate and adaptative immunity of the women were measured at baseline in plasma and in whole blood after a 24-hour *ex vivo* activation at 37°C using Resiquimod (R848) and phytohaemagglutinin (PHA) (Table 7). Cytokine were measured in the culture supernatant (for activated cells) or in plasma by cytometric bead arrays (BD™ CBA Human cytokines Flex Set that is a bead-based immunoassay capable of simultaneously measuring several cytokines in biological fluids, BD Biosciences).

Table 7. Cytokines analyzed in the blood samples, depending on the sample activation type.

R848-activated cytokines	PHA-activated cytokines	Non-activated cytokines
IFN-α, IFN-γ, IL-1b, IL-6, IL-8, IL-10, IL-12p70, TNF	IFN-γ, IL-2, IL-9, IL-10, IL-13, IL-17A, TNF	IL-8, MCP-1, RANTES

Only cytokines with at least 70% of detected values were considered in this study, and the concentrations below the limit of detection were imputed by a fill-in approach, that randomly select values between 0 and the LOD based on the underlying distribution (Helsel, 1990; Lubin et al., 2004).

IV. Statistical tools

IV.1. Personal exposure to PM and OP and biological or respiratory health endpoints

The associations of exposure to $PM_{2.5}$ and OP with oxidative stress and immunological biomarkers and with respiratory health followed a similar pathway of analysis, summarized in Figure 18.

Firstly, the data was curated by several people working on the SEPAGES database handling, or having worked on the variables before. Among the curation and correction performed, a two-step standardization method based on regression residuals (Mortamais et al., 2012) was used to correct cytokine concentrations and N_2MBW parameters related to technical between-participant variability related to the experimentations. Cytokines concentrations were corrected, when necessary on technical variables 1) for baseline cytokines: analytical batch, time between sample collection and reception, time between sample receipt and analysis; 2) for activated cytokines the same variables were used, along with the duration of the activation, R848 or PHA age at the time of sample activation, storage duration. FRC and LCI, two parameters measured by N_2MBW, were corrected for the degree of hypoventilation that may be induced by using pure oxygen during the test, and was shown to affect FRC and LCI measures (Gustafsson et al., 2017). The degree of hypoventilation was calculated for each N_2MBW test, comparing the maximum drop of tidal volume during the first 15 breaths after O_2 inhalation and the mean tidal volume before inhalation. The method employed to correct these parameters first assessed the influence of the factors introducing between-participant variability on each outcome using adjusted linear models. The model estimates of each associated variable ($p<0.10$) were then used to remove the variability in cytokines concentrations or FRC and LCI. It is particularly necessary to apply this method

when the parameters influenced by experimentation-related variability are the independent variables, which was the case in other studies in SEPAGES for cytokine concentrations, LCI and FRC. This method was not employed for urinary biomarker correction, and protocol variables were introduced as covariates in the model, when necessary.

With the available database, a descriptive analysis of the population selected for each study was conducted, by presenting the distribution of their characteristics and exposure levels. Included and excluded population were compared, to investigate any potential selection bias. Bivariate analysis was then conducted to investigate the relationship between different variables, and Spearman's correlation coefficients were calculated between the different exposure variables and between the different health endpoints.

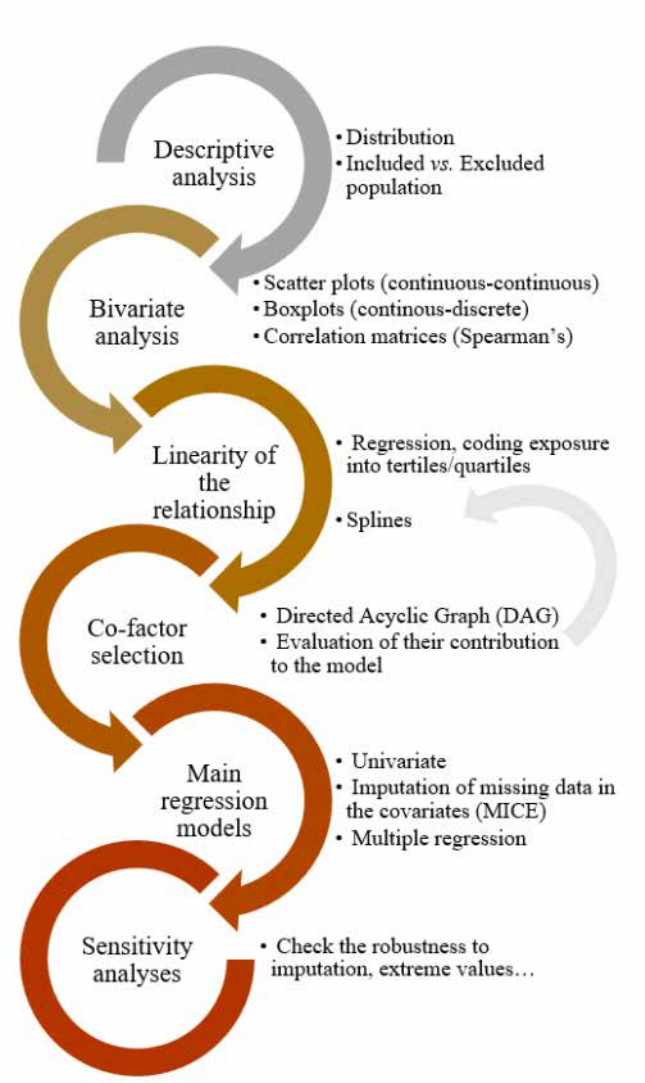


Figure 18. Summary of the statistical analysis conducted for the personal exposure to PM and OP and biological or respiratory health endpoints.

To assess the linearity of the air pollutant-health endpoint associations, univariate regression analysis was performed, coding the exposure into tertiles or quartiles. Potential confounding factors in the health or biological endpoints - air pollution exposure models were selected a priori, based on previous studies, and were further investigated: 1) by exploring the linear relationship between each outcome and each factor, 2) by adding each factor one by one in the outcome - exposure model, to examine the impacts on the effect size of the exposure, 3) by performing a "fully adjusted" model. Potential confounders in the main models were then selected based on these analyses, by selecting influencing variables in at least one of the relationships considered. A p-value of 0.4 was used as criterion to select variables to exclude from the "fully adjusted" model to create the main model. Finally, a likelihood ratio test between the adjusted main model and the adjusted model, modelling the exposure with a natural spline with 5 degrees of freedom. P-values below 0.05 for the likelihood ratio test highlighted that spline models did not perform better than the adjusted main linear models.

Missing data regarding covariates was imputed using multiple imputation by chained equation (MICE), after checking that the Missing Completely At Random (MCAR) hypothesis was not rejected, using Little's test (Little, 1988). MICE iteratively imputes missing data by taking into account the relationship between the variables of the data table. Analyses were performed separately on multiple imputed datasets, and results were combined using Rubin's rule (Rubin, 1987), i.e. averaging the estimates of the complete dataset. Each analysis comprised less than 10% of imputed data. Although post-hoc correction techniques reduce type I errors, they increase type II ones, by hypothesizing that there is no true association among all the tested exposures and outcomes, which is unlikely to be true as the study is based on strong a priori hypothesis from previous studies. For these reasons, results of this work were interpreted by looking at the consistency of association of PM and OP exposures across the health endpoints parameters, and no formal correction for multiple testing was applied.

In the analyses on prenatal exposure to OP and children lung function, associations were evaluated with personal OP_v, whereas both OP_m and OP_v were investigated in relation with biomarkers. Indeed, when considering short term exposure, and biological endpoints, OP_m could additionally provide insights into the effects of oxidative properties of $PM_{2.5}$, regardless of the inhaled air volume.

In the analyses on urinary biomarkers of oxidative stress, concentrations were corrected for specific gravity prior to analysis (MacPherson et al., 2018; van 't Erve et al., 2019) following: $[OSB]^i_{corr} = [OSB]^i * (median(SG) - 1)/(SG^i - 1)$; with [OSB]: concentration of oxidative stress biomarker, i: pool of urine, SG: specific gravity. SG-corrected concentrations had a skewed distribution and were transformed using natural logarithm.

An overview of the population selection for chapter III, IV and V is presented on Figure 19. The study on maternal exposure to $PM_{2.5}$ and OP with child's lung function (chapter III) was restricted to women with at least one OP measurement week during pregnancy and whose child had attended at least one clinical visit at 6 weeks or 3 years (n=356). The study on personal exposure to $PM_{2.5}$ and OP with oxidative stress biomarkers (chapter IV) was restricted to women with one OP measurement week for which urine samples collected at the end of the measurement week were analyzed for oxidative stress biomarkers (n=300). Finally, the study on personal exposure to NO_2, $PM_{2.5}$ and OP with immunological biomarkers was restricted to women with at least one air pollution measurement week, for which a blood sample was retrieved within 2 days of the end the measurement week, and analyzed for cytokines (n=270).

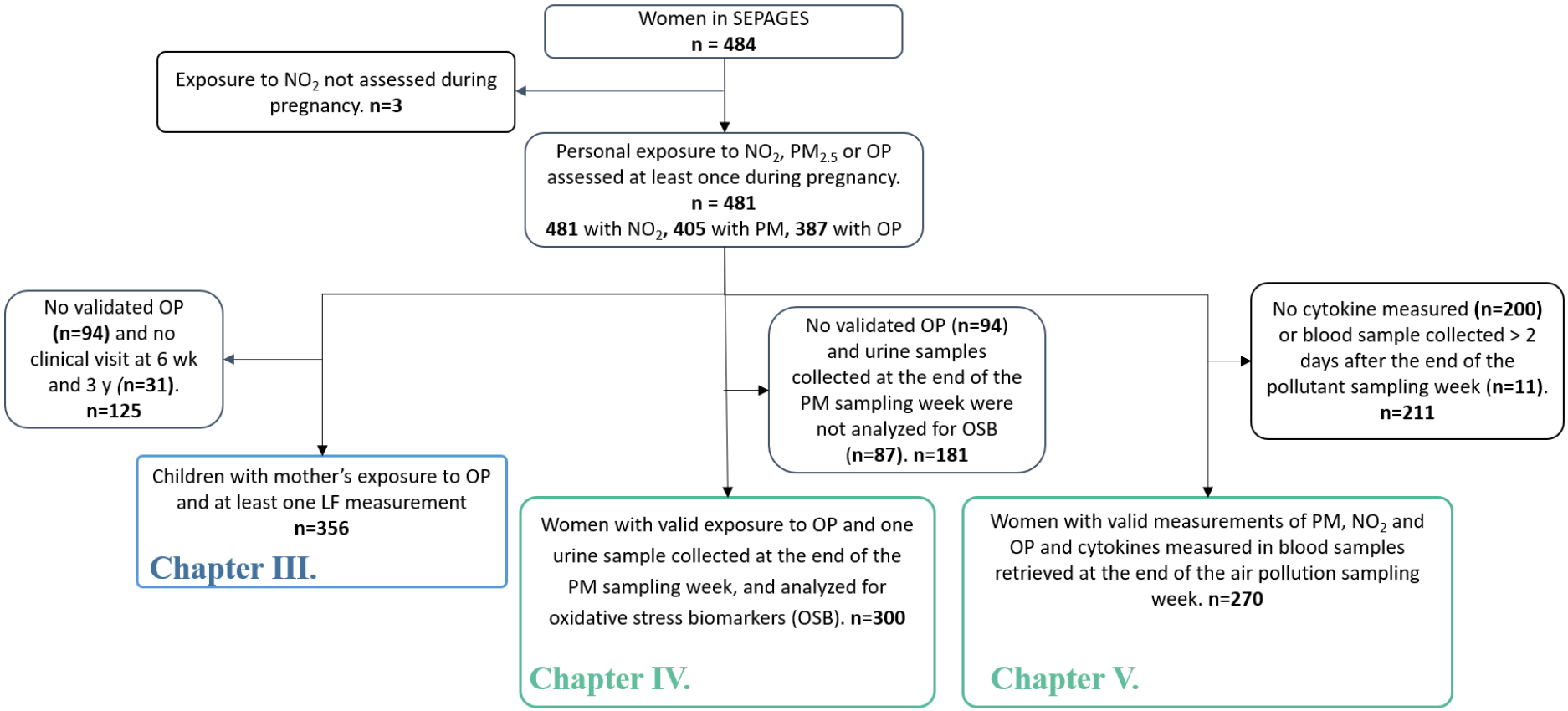


Figure 19. Overview of the population selection.

IV.2. Indoor and outdoor measurement campaign

Figure 20 summarizes the statistical workflow followed for the indoor-outdoor measurement campaign. The consistency of the concurrent samplers (respectively equipped with quartz and Teflon filters) operation conditions was assessed, including sampling duration and volume. Some samplers equipped with Teflon filters were stopped by participants while the adjacent sampler equipped with quartz filter continued running. Since most species were measured on quartz filters, reconstructed PM were more consistent than the gravimetric analysis measured on the Teflon filter. When a house had a Teflon filter but no quartz filter to reconstruct PM, then the gravimetric analysis was used (N=5).

PM mass concentration was calculated using the following equation:

$$[PM_{2.5}] = [OM] + [EC] + [nss - sulfate] + [nitrate] + [ammonium] + [seasalt] + [dust]$$

Where the organic matter (OM) was estimated with an OM to OC conversion factor of 1.8 for outdoor and ambient PM and 1.4 for indoor PM (Favez et al., 2010; Putaud et al., 2010; Tofful et al., 2021).

[nss-sulfate], corresponded to the sulfate fraction from which the marine component was subtracted (Seinfeld & Pandis, 2016) according to Eq. 4.

$$[nss - sulfate] = [SO_4^{2-}] - 0.252[Na^+] \quad (4)$$

The seasalt fraction, was calculated from sodium concentrations according to Eq. 5.

$$[seasalt] = 3.252 * Na^+] \quad (5)$$

The dust fraction, taking into account metallic elements and oxydes, was calculated following the empirical Eq. 6 (Putaud et al., 2004): $[dust] = 5.6 * [nss - Ca^{2+}]$ (6)

Where: $[nss - Ca^{2+}] = [Ca^{2+}] - [Na^+]/26$ (7)

To create a database by individual, with PM constituents' concentrations measured at two seasons in three environments, i.e. indoor, outdoor (measured outside of the house) and ambient (measured at the central monitoring station), daily ambient PM_{10} chemical species and OP was averaged over the individual period of indoor-outdoor sampling.

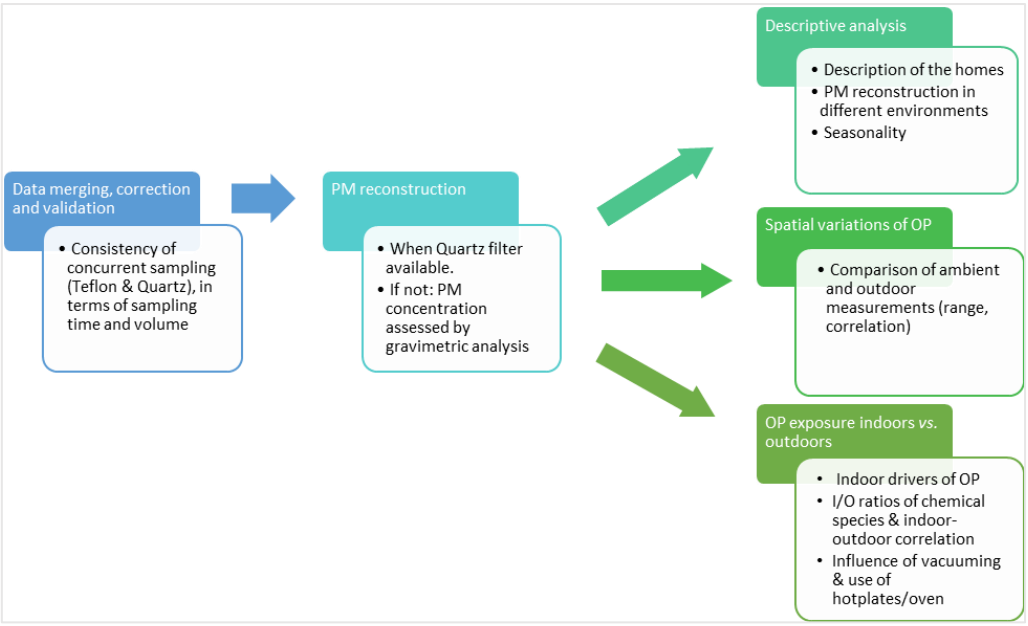


Figure 20. Summary of the statistical analysis conducted for the indoor-outdoor measurement campaign.

A descriptive analysis of the homes, their occupant's activity and the reconstructed PM in the different environments was performed. Then, spatial variations of outdoor PM and OP were assessed based on their descriptive statistics, and on the Spearman's correlation coefficients between each PM's chemical constituents and OP measured outdoors, and the corresponding weekly average at the ambient site. Finally, exposure in the indoor environment and the potential habits that could influence this exposure were characterized. This was done by identifying the main chemical drivers of PM OP using Spearman's correlation coefficient calculated between $PM_{2.5}$, OP and the chemical constituents, in each indoor and outdoor environment separately. Then, indoor-outdoor ratios of each chemical specie and OP was calculated, and the significance of the concentration difference between indoor and outdoor environments was assessed using Wilcoxon rank sum test. I/O ratios higher than 1 indicate potential indoor emission sources of chemical specie, or OP. To assess whether the specie potentially originating from indoor sources was mostly influenced by the indoor environment, I/O ratios were discussed with regards to the Spearman's correlation coefficients between each PM's chemical constituents and OP measured outdoors and indoors. Lastly, the influence of vacuuming, (binary variable, <2 *vs.* ≥ 2 times during the sampling week) and of cooking (binary: $<$ *vs.* $\geq$ median of the reported cumulated duration of oven and hotplates use) was assessed, by comparing the means of I/O ratio in each category and using Wilcoxon test for mean comparison. Smoking, candle and incense lightning were not considered in these habits due to the very limited number of cases for each.

Chapter III

Prenatal Exposure to $PM_{2.5}$ Oxidative Potential and Lung Function in Infants and Preschool-Age Children: A Prospective Study

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Contribution: *This work was published in Environmental Health Perspectives doi: 10.1289/EHP11155. I was involved in the OP data curation, performed the statistical analyses, generated the plots, and wrote the first draft of the article. OP analysis was performed by the workforce of IGE's plateau AirOSol. Among the statistical methods presented, I beneficiated from the work of other IAB's team members and former students, and was able to use readily validated $PM_{2.5}$ mass concentration exposure, and corrected lung function parameters (two-step standardization method) and covariates.*

I. French summary

Contexte. L'exposition prénatale aux PM peut influencer le développement pulmonaire du fœtus et de l'enfant, ainsi que sa santé respiratoire ultérieure (Cai et al., 2020; Carraro et al., 2014; Dutta et al., 2021; Korten et al., 2017; Latzin et al., 2009). Très peu d'études (Dutta et al., 2021; Latzin et al., 2009; Muttoo et al., 2019) ont utilisé des techniques non invasives permettant la mesure de la fonction pulmonaire chez les très jeunes enfants, pourtant, ces techniques reposent sur la respiration courante, ce qui les rend particulièrement adaptées et réalisables chez de très jeunes enfants.

La plupart des études épidémiologiques utilisent la concentration massique des PM pour évaluer l'exposition aux particules en association avec les paramètres de santé (D. Gao et al., 2020b; Pope, 2000). La capacité des PM à générer des espèces réactives de l'oxygène et ainsi à induire un stress oxydatif est mesurée par le potentiel oxydant (PO). Cette métrique est intégrative de plusieurs propriétés physico-chimiques des PM et de leurs effets sur la santé, puisqu'elle mime la voie oxydative par laquelle les PM affectent de nombreux paramètres de santé (Hellack et al., 2014). Actuellement, très peu d'études ont évalué l'exposition au PO des particules dans des cohortes, et encore moins en utilisant des mesures personnelles sur des femmes enceintes afin d'examiner les liens avec la santé ultérieure de l'enfant.

Objectifs. L'objectif de cette étude est d'évaluer si l'exposition personnelle maternelle aux $PM_{2.5}$, exprimées en concentration massique et en termes de PO, est associée à la fonction pulmonaire chez les nouveau-nés et les jeunes enfants.

Méthodes. Cette étude repose sur 356 femmes de la cohorte SEPAGES, équipées d'échantillonneurs personnels de $PM_{2.5}$ deux fois une semaine durant leur grossesse. Le PO a ensuite été analysé sur le filtre comportant les $PM_{2.5}$, en utilisant le test à l'acide ascorbique (AA) et celui au dithiothréitol (DTT), et en normalisant les activités du PO par le volume d'air prélevé, pour quantifier l'exposition de chaque femme. Par la suite, la fonction pulmonaire de leurs enfants a été évaluée à 6 semaines par la technique des rinçages à l'azote (N_2MBW) et par l'analyse des courbes débit-volume (TBFVL), et à 3 ans par la

technique de l'oscillométrie des voies aériennes (aussi appelée oscillations forcées, AOS). Les associations entre l'exposition prénatale aux $PM_{2.5}$ et à son PO et la fonction pulmonaire à 6 semaines et 3 ans ont été étudiées par des régression linéaires multiples, en ajustant sur les facteurs de confusions sélectionnés d'après la littérature et récoltés à l'aide de plusieurs questionnaires administrés aux volontaires. Les valeurs manquantes sur ces co-variables ont été imputées 10 fois en utilisant une méthode d'imputation multiple par équations chaînées (MICE).

Résultats. Chez les nouveau-nés, une augmentation d'un interquartile (IQR) de 0,89 nmol/min/m³ de PO_v^{DTT} est associée à une diminution de la capacité résiduelle fonctionnelle (CRF) mesurée par N_2MBW (β : -2,26 mL ; avec un intervalle de confiance [IC] à 95% de -4,68 à 0,15). Les $PM_{2.5}$ montrent une association similaire au PO_v^{DTT}, mais de moindre ampleur. L'indice de clairance pulmonaire (LCI) et les paramètres mesurés par le TBFVL ne montrent aucune association claire avec les expositions considérées. À l'âge de 3 ans, une augmentation de la fréquence-dépendance de la résistance des poumons (Rrs_{7-19}) mesurée par AOS montre une tendance positive en lien avec l'exposition au PO_v^{DTT} (β : 0,09 hPa×s/L ; [IC] à 95 % : -0,06 - 0,24) et au PO_v^{AA} (IQR = 1,14 nmol/min/m³ ; β : 0,12 hPa×s/L ; IC à 95 % : -0,04 - 0,27), mais pas aux $PM_{2.5}$ (IQR = 6,9 µg/m³ ; β : 0,02 hPa×s/L ; IC à 95 % : -0,13 - 0,16).

Conclusions. Cette étude identifie des associations entre l'exposition aux $PM_{2.5}$ et son PO pour la CRF, un indicateur du volume pulmonaire, et avec la Rrs_{7-19}, qui mesure l'hétérogénéité mécanique liée à l'obstruction des petites bronches, également influencée par les volumes pulmonaires. Ces résultats, pris conjointement suggèrent des effets spécifiques du PO sur la croissance pulmonaire, ce qui est également supporté par des études montrant que l'exposition prénatale aux polluants environnementaux affecte la croissance in-utero, notamment la croissance des organes (Lavigne et al., 2018; Saadeh and Klaunig, 2014) et que le stress oxydant peut endommager les tissus placentaires, et par ce biais affecter la croissance pulmonaire in-utero (Øvrevik, 2019; Veras et al., 2017).

II. Abstract

Background. Fine particulate matter ($PM_{2.5}$) has been found to be detrimental to respiratory health of children, but few studies have examined the effects of prenatal $PM_{2.5}$ oxidative potential (OP) on lung function in infants and preschool children.

Objectives. We estimated the associations of personal exposure to $PM_{2.5}$ and OP during pregnancy on offspring objective lung function parameters and compared the strengths of associations between both exposure metrics.

Methods. We used data from 356 mother–child pairs from the SEPAGES cohort. PM filters collected twice during a week were analyzed for OP, using the dithiothreitol (DTT) and the ascorbic acid (AA) assays, quantifying the exposure of each pregnant woman. Lung function was assessed with tidal breathing analysis (TBFVL) and nitrogen multiple-breath washout (N2MBW) test, performed at 6 wk, and airwave oscillometry (AOS) per- formed at 3 y. Associations of prenatal $PM_{2.5}$ mass and OP with lung function parameters were estimated using multiple linear regressions.

Results. In neonates, an interquartile (IQR) increase in OP_v^{DTT} (0.89 nmol/min/m^3) was associated with a decrease in functional residual capacity (FRC) measured by N_2MBW [β: −2.26 mL; 95% confidence interval (CI): −4.68, 0.15]. Associations with $PM_{2.5}$ showed similar patterns in comparison with OP_v^{DTT} but of smaller magnitude. Lung clearance index (LCI) and TBFVL parameters did not show any clear association with the exposures considered. At 3 y, increased frequency-dependent resistance of the lungs ($Rrs_{7–19}$) from AOS tended to be associated with higher OP_v^{DTT} (β: 0.09 hPa × s/L; 95% CI: −0.06, 0.24) and OP_v^{AA} (IQR = 1.14 nmol/min/m^3; β: 0.12 hPa × s/L; 95% CI: −0.04, 0.27) but not with $PM_{2.5}$ (IQR = 6.9 µg/m^3; β: 0.02 hPa × s/L; 95% CI: −0.13, 0.16). Results for FRC and $Rrs_{7–19}$ remained similar in OP models adjusted on $PM_{2.5}$.

Discussion. Prenatal exposure to OP_v^{DTT} was associated with several offspring lung function parameters over time, all related to lung volumes.

III. Introduction

Exposure to ambient particulate matter (PM) increases risk of chronic respiratory diseases and triggers asthma and chronic obstructive pulmonary diseases. (Janssen et al., 2003; Murray et al., 2020; WHO, 2016) Early life, including pregnancy, is a vulnerable time window for the health effects of air pollution. (Capello and Pili, 2018; Sly and Flack, 2008) Exposure to PM during pregnancy is reported to influence foetal and infant lung development and respiratory health. (Cai et al., 2020; Carraro et al., 2014; Dutta et al., 2021; Korten et al., 2017; Latzin et al., 2009)

Measures of children's respiratory health, including spirometry outcomes (Bergstra et al., 2018; Gehring et al., 2013; Schultz et al., 2016a), asthma incidence (He et al., 2019), or fraction of nitric oxide in exhaled air (Fe_{NO}) (He et al., 2020a), have been widely investigated in association with outdoor air pollution. Although studying lung function of children in early childhood is of great interest for the evaluation of their susceptibility to respiratory diseases later in life, most previous studies (Bergstra et al., 2018; Gehring et al., 2013; He et al., 2019, 2020a; Schultz et al., 2016a) were limited to children older than 5 years of age, when spirometry becomes feasible. Very few studies (Dutta et al., 2021; Latzin et al., 2009; Muttoo et al., 2019) have used non-invasive techniques that allow for the measurement of lung function in very young children, such as tidal breathing flow-volume loops analysis (TBFVL), nitrogen multiple-breath washout (N_2MBW) or airwave oscillometry (AOS). Yet, these techniques rely on tidal breathing, making them particularly suitable and feasible in population-based cohorts. Muttoo et al. (2019) and Latzin et al. (2009) found decreases in functional residual capacity (FRC) and tidal volume (V_T), respectively estimated by MBW and TBFVL, in children that had higher prenatal exposure to nitrogen oxides (NO_x) or particulate matter with diameter ≤ 10 μm (PM_{10}). Dutta et al. (2021) found higher airway reactance (Xrs_5) measured by AOS in children with higher postnatal exposures to particles with < 2.5 μm diameter ($PM_{2.5}$).

Most epidemiological studies examining the health effects of PM used the mass concentration metric in association with health parameters. (D. Gao et al., 2020b; Pope, 2000) While the biological pathways are not fully understood yet, evidence suggest that oxidative stress caused by PM is a key factor in

understanding PM-associated health effects. (Crobeddu et al., 2017; Hatzis et al., 2006; Leni et al., 2020; Riva et al., 2011) The ability of PM to generate reactive oxygen species and thereby induce oxidative stress is measured by the oxidative potential (OP), an integrative metric of several physical and chemical properties of PM and its health effects. (Hellack et al., 2014) Several recent studies have presented OP as a better predictor than concentration for assessing association with some cardio-respiratory diseases. (Bates et al., 2019; Weichenthal et al., 2021) The studies addressing the effects of OP exposure on children's lung function, although few in number, converged to a stronger detrimental effect of OP as compared to PM mass. (Delfino et al., 2013; He et al., 2021; Yang et al., 2016) These latter studies used average urban ambient OP measurements or OP estimated by land-use regression models, which could lead to measurement errors given that most people in western countries spend over 80% of their time indoors. (Avery et al., 2010) Thereby, personal sampling has been proposed to increase the accuracy in exposure assessment, but, to the best of our knowledge, no study has estimated personal prenatal exposure to OP in relation to respiratory function in the first years of life.

The aim of this study was to assess whether maternal personal exposure to $PM_{2.5}$ mass concentration and to the OP of $PM_{2.5}$ is associated with lung function in newborns and in preschool children. The effects of OP and PM were also compared, and the independency of OP effects from $PM_{2.5}$ were tested.

IV. Methods

IV.1. Study population

This study is based on the data from the French mother-child SEPAGES cohort that has been setup to describe maternal and child personal exposure to environmental pollutants and their effects on health. The study design and protocol have been previously described by Lyon-Caen et al. (2019) Briefly, pregnant women were recruited from July 2014 to July 2017 in eight obstetrical ultrasonography practices located in the Grenoble area in the French Alps. The included women had to be pregnant by < 19 gestational weeks, be older than 18 y old, to have a singleton pregnancy, to be planning to give birth in one of the four maternities clinics from Grenoble area and to live in the study area (i.e. living one

hour driving from Grenoble city centre). The volunteers were then followed during pregnancy, and their children were recruited at birth and then followed up. The mother-child pairs selected for this study had at least one period of $PM_{2.5}$ sampling during pregnancy (n=405), with validated and positive OP analysis (n=387) and the children had performed at least one lung function test at either 6 wk or 3 y (n=356) (Figure 21).

Parents signed an informed consent for themselves and their child and the study protocol was approved by the Comité de Protection des Personnes Sud-Est V (CPP) and the French data privacy institution (Commission Nationale de l'Informatique et des Libertés, CNIL).

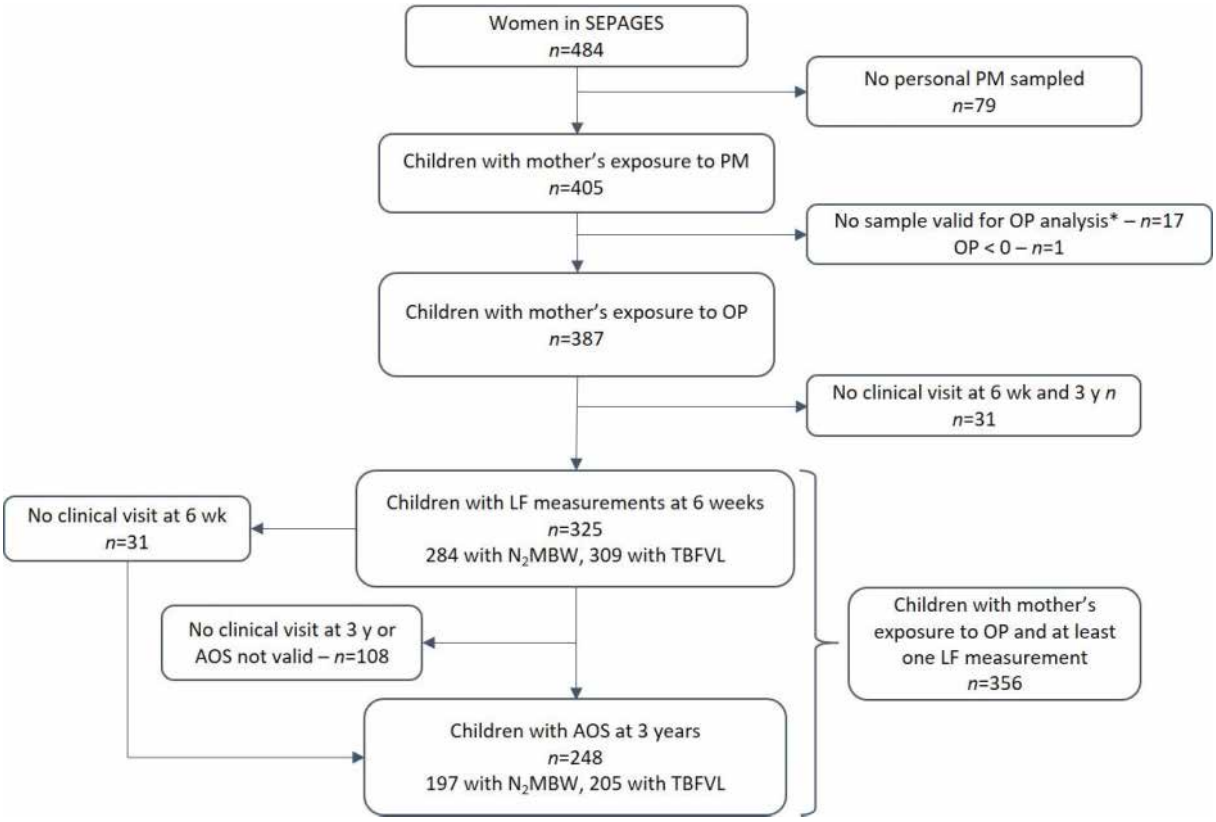

*Figure 21. Flow chart for the selection of the study population. Note: *$PM_{2.5}$ net weight < 4 µg.*
Abbreviations: AOS (Airwave oscillometry), LF (Lung function), N_2MBW (Nitrogen multiple-breath washout), OP (Oxidative potential), $PM_{2.5}$, PM with aerodynamic diameter <2.5 µm, TBFVL (Tidal breathing flow-volume loops).

IV.2. Maternal exposure

Active personal air samplers (MicroPEM™, RTI International, Research Triangle Park, NC, USA) were used to sample $PM_{2.5}$ onto Teflon filters. The participants were asked to carry the devices or keep them at close proximity during the entire sampling period (consecutive 7-8 d). The measurements took place at different periods of the pregnancy. The sample filters on which OP was measured consisted of 286

collected at a median gestational age (GA) of 18 wk (min: 12, Q1: 32, Q3: 19, max: 28) and 294 at 34 wk (min: 28, Q1: 32, Q3: 35, max: 38). Therefore, the median (IQR) of time between the first and second measurement was 16 (14, 18) wk with a minimum of 4 wk and a maximum of 23 wk, mainly due to the availability of the samplers or the volunteers. For each participant, personal exposure was estimated from one (132 out of 356, 37%) or 2 wk (224 out of 356, 63%) of sampling. An average exposure was calculated when two periods of measurements were available.

The net mass (micrograms) of $PM_{2.5}$ collected was determined by gravimetric analysis (Mettler Toledo UMX2 ultra-microbalance) before and after sampling at the same hygrometric conditions (21°C, 25% relative humidity). Following gravimetric analysis, the samples filters were cold-stored (-20°C) until OP analysis, for an average of 26 wk. OP analysis followed the protocol established by Calas et al. (2018, 2017) Briefly, a simulated lung fluid (SLF, mixture of Gamble and dipalmitoylphosphatidylcholine - DPPC) was used to extract $PM_{2.5}$ from the filters for a final concentration of 10 µg/mL, maintaining a constant amount of extracted $PM_{2.5}$ for intercomparison. The extracts were then subjected to vortex mixing at 37°C for 1.25 h. The OP was measured using the dithiothreitol (DTT) and ascorbic acid (AA) assays.

For the DTT assay, $PM_{2.5}$ extracts were mixed with a DTT solution using a 96-well plate (CELLSTAR, Greiner-Bio). Every ten minutes, the remaining DTT was titrated by dithionitrobenzoic acid (DTNB) and the formation of 2-nitro-5-thiobenzoic acid (TNB) was measured by absorbance at 412 nm (TECAN spectrophotometer Infinite M200 Pro), for a total reaction time of 30 min (e.g. 3 titrations in total). For the AA assay, a modified version of the synthetic respiratory tract lining fluid (RTLF) was used. (Kelly and Mudway, 2003) AA was mixed with the $PM_{2.5}$ extract in a 96-well plate and the AA consumption was evaluated measuring the change in absorbance at 265 nm over time. Absorbance measurements were collected at 4-minute intervals for a total reaction time of 30 min. For both assays, the consumption rate (nanomoles per minute) was then normalized by the corresponding filtrated air sample volume (cubic meter) to represent human exposure through inhalation. OP_v^{DTT} corresponds to the consumption of DTT (nmol/min/m^3) and OP_v^{AA} corresponds to the consumption of AA (nmol/min/m^3). All samples were subjected to triplicate analysis and each sample result is reported as the mean of the repeated measurements. The coefficient of variation (CV) is between 0 and 10% for each assay.

To ensure accuracy of each OP measurement, positive control tests were performed for every experiment. A 1,4-naphthoquinone (1,4-NQ) solution was used for both the DTT and AA assays. Particularly, a 40 µL of 24.7 µM stock solution was used for the DTT assay and an 80 µL of 24.7 µM 1,4-NQ solution for AA assay (Calas et al., 2018, 2017). The measurement quality, estimated by the CV of the positive control tests, were at <3.2% for both OP assays.

IV.3. Lung Function at 6 weeks

Lung function tests were performed on infants, aged 6-12 wk, using an infant face mask during natural sleep, in supine position and with the head midline, following guideline of the European Respiratory Society (ERS) and American Thoracic Society (ATS). (Bates et al., 2000) After stabilization of the breathing pattern (20-30 breaths rejected), 10 min of tidal breathing flow-volume loops (TBFVL) were recorded and three measurements of nitrogen multiple-breath washout (N_2MBW) were performed.

For TBFVL measurements, the first 30 to 50 regular breaths were used. The sighs and 10 breaths preceding and following a sigh were excluded. The following TBFVL parameters were retained in the present analysis: tidal volume (V_T) and the ratio of time to peak tidal expiratory flow (t_{PTEF}) to expiratory time (t_E). Out of the 484 mother-child pairs, 325 children performed the TBFVL test.

The N_2MBW technique measures lung volumes and ventilation heterogeneity. For this test, infants inhaled pure oxygen (O_2) and the concentration of exhaled N_2 was monitored employing the Exhalyzer© and Spiroware© equipment (Ecomedics). The main outcomes were *a*) functional residual capacity (FRC) and *b*) lung clearance index (LCI), defined as the number of respirations required to reduce the concentration of N_2 below 2.5%. Up to three valid measurements were obtained, guided by the following criteria: regular breathing during quiet sleep, tidal volume within target, no swallowing or sighs in the first five breaths, no sign of leak, and N_2 concentration below 2.5% for at least three consecutive breaths to end the test. A transient decrease in tidal volume may be induced by using pure oxygen during the test, that has been shown to affect FRC and LCI measures. (Gustafsson et al., 2017) Hence, the degree of hypoventilation was calculated for each N_2MBW test, comparing the maximum drop of tidal volume during the first 15 breaths after O_2 inhalation and the mean tidal volume before inhalation. Then, FRC and LCI values were corrected for the degree of hypoventilation using a 2-step standardization method

based on regression residuals. (Mortamais et al., 2012) First, the influence of hypoventilation was characterized using adjusted linear mixed regression models (accounting for the repeated data), and, in a second step, the model estimate was used to remove the variability in FRC (or LCI) due to hypoventilation. A total of 865 valid N_2MBW tests were retained, with a median (Q1; Q3) of 3 (2; 3) tests per child. Out of the 484 mother-child pairs, 350 children performed the N_2MBW test. For each child, both LCI and FRC corrected values were averaged.

IV.4. Lung Function at 3 years

At the age of 3 y (median: 3.1 y.), the impedance of the respiratory system was assessed based on airwave oscillometry (AOS) using commercial device (TremoFlo; Thorasys Systems) complying with current European standards. (Beydon et al., 2007) The device was calibrated daily, using a reference resistance.

For this technique, pressure waves with frequencies varying from 7 to 41 Hz are applied during tidal breaths and lung impedance is calculated from the changes in flow and pressure. To ensure the quality and reproducibility of the measurements, they were performed after at least 15 d from any respiratory infection (self-reported by the mother via a questionnaire administrated by a clinical research assistant at the clinical visit), with the child sitting, the head slightly extended, and wearing a nose clip. Children were asked to firmly close their lips around the mouthpiece while their cheeks and chins were maintained by the technician to avoid any signal damping by the mouth walls. After getting used to the device during approximately 30 s, three to five acceptable measurements were obtained and averaged. A rest interval of 1 min was respected between each 16-s-long measurement. We excluded measurements with the following artefacts: leakage, swallowing, glottis closure, vocalization or obstruction of the mouthpiece by the tongue.

The key components of impedance are the resistance and the reactance of the respiratory system. The resistance is representative of friction forces mainly in the airways and the reactance depends on the inertive and elastic behaviors of the respiratory system. (Gosselink and Stam, 2005) The parameters included in this study are raw values of resistance and reactance at a frequency of 7 Hz (Rrs_7 and Xrs_7), the area under the reactance curve (AX) and the frequency-dependence of the resistance, defined by the

resistance difference between 7 and 19 Hz (Rrs_{7-19}). Rrs_7 is a parameter that reflects large airway resistance, whereas AX and Rrs_{7-19} better characterize the peripheral airways. Rrs_{7-19} also evaluates the heterogeneous obstruction of the distal bronchi. (Lundblad et al., 2021) Increased Rrs, Rrs_{7-19} and AX, and decreased Xrs are associated with a reduced lung function.

Among the 320 children to the 3-y follow-up who performed AOS (66% follow-up rate), measurements for 306 children (96% success rate for AOS test) were retained, complying with validity and reproducibility criteria (at least 2 measurements with a coefficient of variation of <15% for Rrs_7). The mean value of the valid measurements was calculated for each parameter and used for the analyses. Out of them, 248 had personal prenatal exposure to OP, resulting in a total attrition rate of 51% for the exposure to personal prenatal OP-AOS parameters association study.

IV.5. Statistical methods

Both univariate and multiple linear regressions were used to study the associations between maternal personal exposure to $PM_{2.5}$ and OP with each lung function parameter. The three exposure metrics used in this study ($PM_{2.5}$, OP_v^{DTT}, OP_v^{AA}) were continuous and scaled by their IQR, allowing to compare their respective effects on the outcomes. The Spearman correlation coefficient (r_s) was used to calculate correlations between the exposures. Linear regressions were used after confirming linearity by a likelihood ratio test between the adjusted model, modelling the exposure with a natural spline with 5 degrees of freedom and the adjusted main model (Figures S1 to S6 in the Supplement, all p-values $\leq$ 0.05). All analyses were performed using *R* software (version 4.1; R Development Core Team).

Potential confounders were selected *a priori*, based on previous studies (Latzin et al., 2009; Schultz et al., 2016a): *a*) parental characteristics: educational level (defined as the maximum number of studying years after high-school degree between the parents and expressed in two classes: above or <5 y; self-reported through a self-administrated questionnaire), parental history of rhinitis (binary, self-reported by a questionnaire administrated by a clinical research assistant), mother's age (calculated with the date of birth self-reported by a questionnaire administrated by a clinical research assistant) and body mass index (BMI) before pregnancy (continuous; calculated based on self-reported weight before pregnancy and height measured by a clinical research assistant during a SEPAGES clinical visit); *b*) infant

characteristics: child sex (male/female), age (continuous, calculated with the date of birth collected in the child health booklet), height and weight (continuous, measured by a clinical research assistant at the clinical visit), passive smoking (yes/no, *in utero*, including maternal passive smoking or until the clinical visit; assessed by several self-administrated questionnaires during and after the pregnancy), breastfeeding (still some breastfeeding at 6 wk, yes/no, self-reported by a questionnaire administrated by a clinical research assistant); *c*) exposure characteristics: season of sampling [3-class variable: cold (all filters sampled between October and March), warm (all filters sampled between April and September), and cold+warm (one filter sampled in the cold season and one filter sampled in the warm season)], mean temperature during pregnancy (continuous, assessed at home address by Hough's model). (2020) The effects of the confounders were analyzed by looking at the effect of each confounder separately on the regression model adjusted for sex, height and weight (Figures S9, S10). Missing data regarding covariates in the main model were imputed by multiple chained equation, using the R package mice (van Buuren and Groothuis-Oudshoorn, 2011), assuming that the data was missing completely at random (MCAR), which was checked by Little's test (Little, 1988) (*p*-values of the test > 0.05). Descriptive statistics of the covariates can be found in Table S1. Ten imputed datasets were created and results from each dataset were combined using Rubin's rule. (Rubin, 1987) We did not correct for multiple tests, but results were interpreted by looking at the consistency of association of PM and OP exposures across the different lung function parameters.

Several sensitivity analyses were conducted to address the robustness of the results from the main model by assessing the impacts of: *a*) data imputation, by conducting a complete case analysis; *b*) extreme exposure and health outcome values, by excluding the lowest (below 1st percentile) and highest values (above the 99th percentile) of the outcomes and exposures, resulting in the exclusion of 4%-5% of the population of each analysis; *c*) the number of PM and OP measurement weeks, by excluding participants with only one measurement week (n=132); *d*) the independency of OP effects to PM, by adjusting OP models on $PM_{2.5}$; *e*) LCI and FRC measurement error due to the degree of hypoventilation, by adding an analysis excluding one fourth of the children that had the highest hypoventilation degree during the N_2MBW test (n=72); *f*) leverage and influencing points, by excluding points that had a Cook's distance (Cook, 1977) higher than 4/n, where n is the number of observations in the main model (exclusion of 4-

7% of the observations); *g*) the independency of OP and PM effects to personal NO_2 concentrations during the same weeks of sampling [passive sampler (Passam AG), worn simultaneously to the active PM sampler]. Multicollinearity was assessed using the variance inflation factor in the two-pollutant models (VIFs < 2).

V. Results

V.1. Description of the population

The present study was conducted with children that had at least one prenatal measurement of OP and one lung function parameter assessed, leading to 356 mother-child couples (73% of SEPAGES cohort) (Figure 21). The included children had parents with a higher educational level, had less parental history of rhinitis, higher exposure to $PM_{2.5}$, higher Rrs_7 and a lower Xrs_7 as compared to the children not included in the study (Table 8). No difference between the included and excluded population was observed for both OP and lung function at 6 wk. In the study population, 52% (*n*=185) of the children were boys, and the majority of children were born on term (96%, *n*=341) by vaginal delivery (85%, *n*=302) from mothers that were mainly nulliparous or primiparous (45%, *n*=160 and 46%, *n*=162, respectively). In infancy, most children were still breastfed at 6 weeks (86%, *n*=306) and less than 27% (*n*=95) were exposed to tobacco smoke *in utero* (including maternal passive smoking) and after birth (< 6 wk). The parental level of education is high since 72% (*n*=256) of the parents have studied 5 y or more after their French high school diploma (i.e., having at least a MSc diploma). Only 15 children were born before the 37th week, with a minimum of 34 gestational weeks. Regarding lung function tests (Figure 21), 325 children performed a valid test of the lung function at 6 wk (284 had a valid N_2MBW analysis and 309 had valid TBFVL measurements), and 248 children had valid AOS measurements. Out of these 248 children, 197 had available N_2MBW results and 205 had valid TBFVL test results.

Table 8. Characteristics of the included (N=356) and excluded (N=128) population from the cohort SEPAGES in this study. Included population corresponds to children that have at least both one prenatal oxidative potential assessment and one test of lung function.

Characteristics	Included population[a] (N=356)	Excluded population[a] (N=128)	p-value[b]
Sex of child			**0.2**
Male	185 (52%)	73 (59%)	
Female	171 (48%)	51 (41 %)	
Missing	/	4	
Birthweight (g)			**0.13**
Median (IQR)	3295 (3048, 3580)	3220 (2995, 3507)	
Missing	/	5	
Preterm birth, <37 wk			**0.2**
0 (No)	341 (96%)	115 (93%)	
1 (Yes)	15 (4%)	9 (7%)	
Missing	/	4	
Parental educational level >5 y			**0.048**
0 (No)	100 (28%)	48 (38%)	
1 (Yes)	256 (72%)	80 (62%)	
Delivery mode			**0.084**
Vaginal	302 (85%)	96 (78%)	
C-section	54 (15%)	27 (22%)	
Missing	/	5	
Still breastfed at 6 wk			**0.11**
0 (No)	49 (14%)	20 (20%)	
1 (Yes)	306 (86%)	78 (80%)	
Missing	1	30	
Parental history of rhinitis			**0.003**
0 (No)	132 (40%)	26 (24%)	
1 (Yes)	202 (60%)	83 (76%)	
Missing	22	19	
Parity			**0.6**
0 (nulliparous)	160 (45%)	62 (48%)	
1 (primiparous)	162 (46%)	52 (41%)	
2 or more (multiparous)	34 (9.6%)	14 (11%)	
ETS in utero and < 6 wk			**0.7**
0 (No)	259 (73%)	84 (75%)	
1 (Yes)	95 (27%)	28 (25%)	
Missing	2	16	
ETS < 3 yrs.			**0.7**
0 (No)	270 (79%)	70 (77%)	
1 (Yes)	73 (21%)	21 (23%)	
Missing	13	37	
Exposure to particulate air pollution[c]			
$PM_{2.5}$ ($\mu g/m^3$)	13.3 (10.6, 17.5)	12.2 (8.2, 16.6)	**0.033**
Missing	/	79	
OP_v^{DTT} ($nmol/min/m^3$)	1.49 (1.11, 2.00)	1.53 (1.05, 1.91)	**0.8**
Missing	/	97	
OP_v^{AA} ($nmol/min/m^3$)	1.56 (1.07, 2.21)	1.66 (0.93, 2.30)	**>0.9**
Missing	/	97	
Mean temperature during pregnancy (°C)			
Median (IQR)	13.0 (10.6, 14.6)	11.6 (10.1, 13.6)	**0.001**
Missing	0	4	
N_2MBW parameters[c] (6 wk)			
FRC (mL)	105 (95, 115)	108 (95, 115)	**0.6**
Missing	72	62	
LCI	7.58 (6.75, 8.47)	7.49 (6.99, 8.12)	**0.9**
Missing	72	62	
TBFVL parameters[c] (6 wk)			
V_T (mL)	34 (29, 39)	33 (29, 36)	**0.4**
Missing	47	112	
t_{PTEF}/t_E (%)	35 (29, 42)	36 (26, 45)	**0.8**
Missing	47	112	
AOS parameters[c] (3 y)			
Rrs_7 (hPa×s/L)	11.53 (10.05, 13.04)	12.67 (10.87, 14.17)	**0.021**
Missing	108	70	

Characteristics	Included population[a] (N=356)	Excluded population[a] (N=128)	p-value[b]
Rrs_{7-19} (hPa×s/L)	1.02 (0.56, 1.61)	1.18 (0.63, 1.98)	**0.2**
Missing	108	70	
Xrs_7 (hPa×s/L)	-3.88 (-4.56, -3.28)	-4.25 (-5.59, -3.51)	**0.037**
Missing	108	70	
AX (hPa/L)	68 (45, 92)	70 (53, 105)	**0.3**
Missing	108	70	

Note: AA, ascorbic acid; AOS, airwave oscillometry; DTT, dithiothreitol; AX, area under the reactance curve; ETS, environmental tobacco smoke; FRC, functional residual capacity; LCI, lung clearance index; N_2MBW, nitrogen multiple-breath washout; OP, oxidative potential; OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay; $PM_{2.5}$, PM with an aerodynamic diameter <2.5 µm; Rrs7, resistance at a frequency of 7 Hz; Rrs7–19, difference between the resistance at 7 Hz and at 19 Hz; TBFVL, tidal breathing flow-volume loops; tPTEF=tE ratio of time to peak tidal expiratory flow to expiratory time; VT, tidal volume, Xrs7, reactance at a frequency of 7 Hz.
[a]Expressed in n (%) or Median (IQR). [b]p-Value from Wilcoxon rank sum test and Pearson's chi-squared test comparing included and excluded population. [c]Variables used for population selection (selected children had prenatal exposure to PM and OP and either N_2MBW or TBFVL or AOS measures)

V.2. Exposure to $PM_{2.5}$ and its oxidative potential

The median (Q1, Q3) of average prenatal personal exposures to $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA} were 13.3 (10.6, 17.5) µg/m^3, 1.49 (1.11, 2.00) nmol/min/m^3 and 1.56 (1.07, 2.21) nmol/min/m^3. Personal $PM_{2.5}$ and OP (particularly OP_v^{AA}) presented a seasonal trend, with higher levels reached during the cold season (Figure 22, Table S2). OP_v^{DTT} was highly correlated with both $PM_{2.5}$ concentration and OP_v^{AA} (r_s=0.64 and r_s=0.72, respectively. n=356, $p<2.2\cdot10^{-16}$ for both), whereas the correlation between $PM_{2.5}$ concentration and OP_v^{AA} was moderate (r_s=0.51, $p<2.2\cdot10^{-16}$) (Figure S7). For participants with two periods of sampling, there was no differences in $PM_{2.5}$, OP_v^{AA} and OP_v^{DTT} levels at early vs. late pregnancy (Figure S8, Table S3).

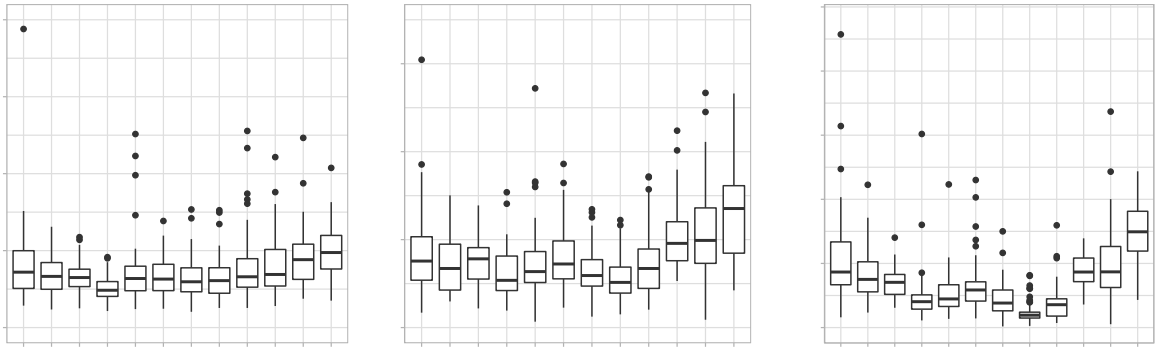

Figure 22. Monthly distribution of personal measurements of $PM_{2.5}$ (left), OP_v^{DTT} (center), and OP_v^{AA} (right).
See Table S2 for corresponding numeric data. Note: Boxes represent 25th–75th percentiles; the middle horizontal line represents the median; whiskers extend to the most extreme point within 1.5 IQRs of the box and the dots outside boxes indicate outliers. AA, ascorbic acid; DTT, dithiothreitol; IQR, interquartile range; OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay; $PM_{2.5}$, PM with an aerodynamic diameter <2.5µm.

V.3. Association between exposures to prenatal $PM_{2.5}$ and OP and lung function

Lung function at 6 weeks. In the univariate analysis, increased personal prenatal exposure to $PM_{2.5}$ and OP_v^{DTT} were associated with a lower FRC at 6 weeks (-2.16 mL; 95% CI: -4.41, 0.09 for each 6.9 µg/m^3 increase of $PM_{2.5}$, and -2.69 mL; 95% CI: -5.28, -0.11 for each 0.89 nmol/min/m^3 increase of OP_v^{DTT}). After adjusting for potential confounders (Table 9, Table S4, Figure 23), in both main and complete-case analysis, the magnitude of association between OP_v^{DTT} and FRC slightly decreased and associations were borderline significant (β: -2.26 mL; 95% CI: -4.68, 0.15 for the main model and β: -2.65 mL; 95% CI: -5.16, -0.14 for the complete-case analysis). The confounders mainly driving the differences between the univariate and the main analysis were the season of sampling and the parental history of rhinitis (Figure S9). LCI and t_{PTEF}/t_E did not show any clear association trend for all exposures considered. In general, for air pollution-lung function associations showing marginal association, the sensitivity analyses showed similar patterns of association to the ones of the main model, except for the negative OP_v^{DTT}-V_T association that disappeared when excluding extreme values. The analyses excluding leverage and influencing points (estimated by Cook's distance) overall led to similar results and resulted in statistically significant association for FRC and exposure to both $PM_{2.5}$ and OP_v^{DTT} (Table S4, Figure S11). The analyses further adjusted on personal NO_2 sampled simultaneously to $PM_{2.5}$ showed that NO_2 did not modify the estimates and 95% CI for any of the studied associations. The magnitude of the associations of OP_v^{DTT} on lung volumes, estimated by FRC, remained similar in models further adjusted on $PM_{2.5}$. The change in FRC in the two-pollutant model with OP_v^{DTT} and $PM_{2.5}$ showed a stronger effect of OP_v^{DTT} than $PM_{2.5}$ [-1.82 mL (95% CI:-5.03, 1.40) for OP_v^{DTT} vs. -0.59 mL (95% CI: -3.37, 2.19) for $PM_{2.5}$], although this association became non-significant (Figure 23, Table S6).

Table 9. Associations between prenatal exposure to air pollution and lung function at 6 weeks and 3 years. Regression coefficients are estimated from univariate and multiple linear models.

Age	Pollutants		$PM_{2.5}$ (µg/m³)		OP_v^{DTT} (nmol/min/m³)		OP_v^{AA} (nmol/min/m³)	
	Regression model		Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
6 weeks	FRC (mL)[b]	Coefficients (95% CI)	-2.16 (-4.41, 0.09)	-1.58 (-3.67, 0.5)	-2.69 (-5.28, -0.11)	-2.26 (-4.68, 0.15)	-1.05 (-3.31, 1.22)	-0.59 (-2.85, 1.68)
	LCI[b]	Coefficients (95% CI)	-0.02 (-0.17, 0.13)	-0.01 (-0.14, 0.13)	-0.05 (-0.22, 0.12)	-0.06 (-0.22, 0.09)	-0.02 (-0.17, 0.12)	-0.05 (-0.19, 0.1)
	V_T (mL)[c]	Coefficients (95% CI)	-0.52 (-1.4, 0.37)	-0.54 (-1.35, 0.28)	-0.65 (-1.67, 0.38)	-0.58 (-1.54, 0.38)	-0.11 (-1.01, 0.78)	0.13 (-0.76, 1.02)
	t_{PTEF}/t_E (%)[c]	Coefficients (95% CI)	0.34 (-0.86, 1.54)	0.25 (-1.02, 1.51)	0.8 (-0.59, 2.19)	0.69 (-0.79, 2.17)	0.42 (-0.78, 1.63)	0.14 (-1.23, 1.51)
3 years	Rrs_7 (hPa×s/L)[d]	Coefficients (95% CI)	-0.01 (-0.33, 0.32)	-0.02 (-0.33, 0.3)	0.12 (-0.21, 0.46)	0.05 (-0.28, 0.37)	-0.04 (-0.37, 0.28)	-0.08 (-0.41, 0.25)
	Rrs_{7-19} (hPa×s/L)[d]	Coefficients (95% CI)	-0.01 (-0.15, 0.14)	0.02 (-0.13, 0.16)	0.08 (-0.07, 0.23)	0.09 (-0.06, 0.24)	0.1 (-0.05, 0.24)	0.12 (-0.04, 0.27)
	Xrs_7 (hPa×s/L)[d]	Coefficients (95% CI)	-0.01 (-0.17, 0.16)	0.01 (-0.15, 0.17)	-0.09 (-0.26, 0.08)	-0.05 (-0.22, 0.11)	-0.07 (-0.23, 0.1)	-0.07 (-0.23, 0.1)
	AX (hPa/L)[d]	Coefficients (95% CI)	0.65 (-4.45, 5.74)	0.22 (-4.81, 5.25)	2.34 (-2.95, 7.64)	1.07 (-4.08, 6.22)	-1 (-6.07, 4.06)	-2.21 (-7.48, 3.07)

Note: Coefficients are calculated for an increase of one IQR for $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA}, corresponding to 6.9 µg/m³, 0.89 nmol/min/m³, and 1.14 nmol/min/m³, respectively. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; FRC, functional residual capacity; LCI, lung clearance index; V_T, tidal volume; t_{PTEF}/t_E ratio of time to peak tidal expiratory flow to expiratory time ; Rrs_7, resistance at a frequency of 7 Hz; Rrs_{7-19}, difference between the resistance at 7 Hz and at 19 Hz; Xrs_7, reactance at a frequency of 7 Hz; AX, area under the reactance curve.

[a] Model adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis and mean temperature during pregnancy.

[b] Number of observations is 284 for FRC and LCI.

[c] Number of observations is 309 for V_T and t_{PTEF}/t_E.

[d] Number of observations is 248 for Rrs_7, Rrs_{7-19}, Xrs_7 and AX.

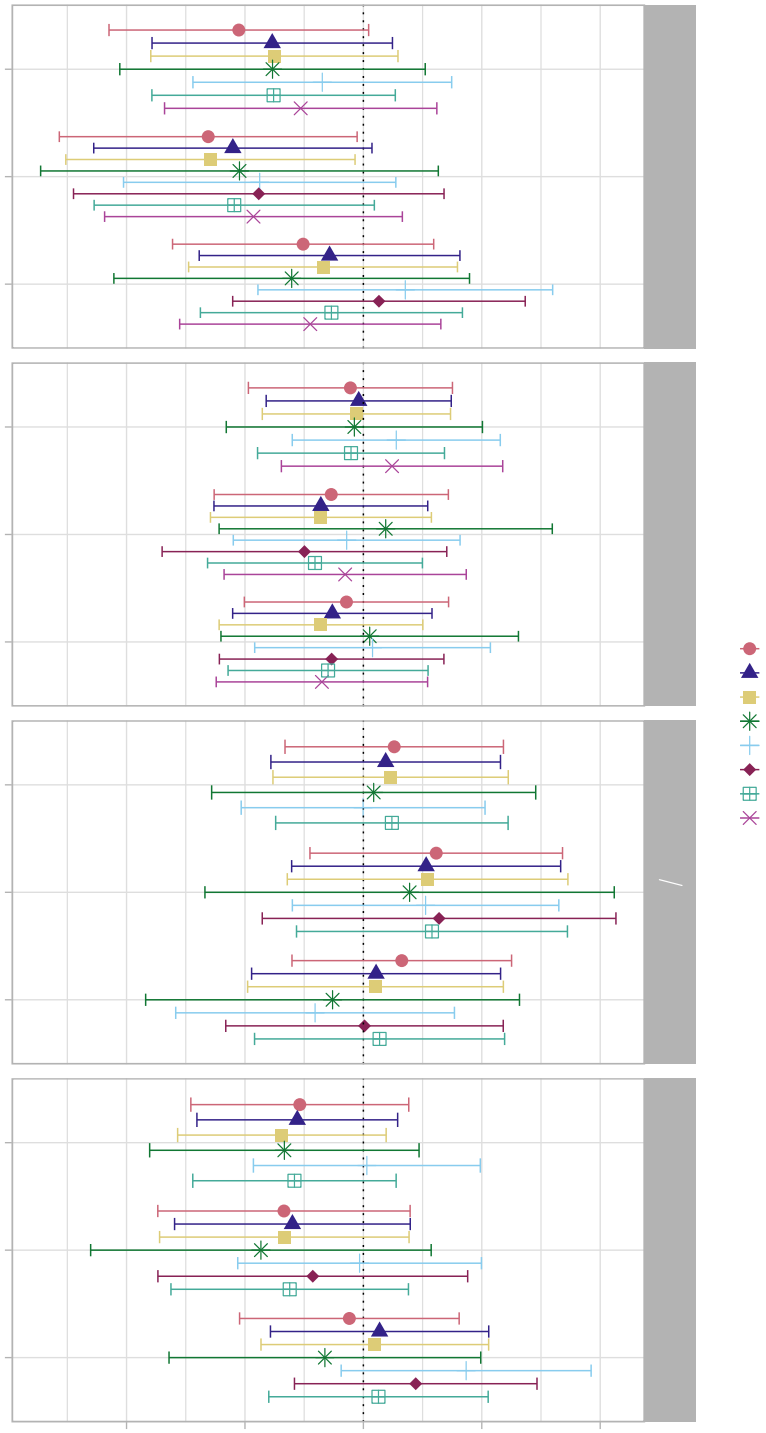

Figure 23. Association between personal exposure to $PM_{2.5}$, OP_v^{DTT}, and OP_v^{AA} during pregnancy and lung function parameters measured at 6 wk in the univariate and multiple linear models and in the sensitivity analyses.
Outcomes and exposures were scaled by their IQR. See Tables S4 and S6 for corresponding numeric data. Whiskers represent the 95% confidence interval around the estimate. The main model was adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis, and mean temperature during pregnancy. In addition, "2 sampling periods" are the analyses reduced to the children that had 2 wk of prenatal measurements of air pollution (63%–66% of the population); "Excluding extreme values" are the analyses excluding the exposures and outcomes below the first percentile and above the 99th (exclusion of approximately 5% of the population); "Adjusted on PM" corresponds to adding personal exposure to $PM_{2.5}$ in the set of confounders, "Adjusted on NO_2" corresponds to adding personal exposure to NO_2 in the set of confounders, and the last analyses were performed excluding children that had the highest hypoventilation degree during the nitrogen multiple breath washout test (excluding 25% of the population).

Lung function at 3 years. Increased personal prenatal exposures to OP_v^{DTT} and OP_v^{AA} were associated with an increase of 0.09 (95% CI: -0.06, 0.24) and 0.12 (95% CI: -0.04, 0.27) hPa×s/L in Rrs_{7-19} respectively, whereas no trend for association was found with exposure to $PM_{2.5}$ (β: 0.02 hPa×s/L; 95% CI: -0.13, 0.16) (Table 9, Figure 24). The confounders mainly driving the differences between the univariate and the adjusted model were the season of sampling, parental history of rhinitis and maternal age before pregnancy (Figure S10). The sensitivity analyses confirmed these trends of association. In particular, the analysis excluding extreme values resulted in a statistically significant positive association, with an IQR increase in OP being associated with an increase of 0.20 (95% CI: 0.04, 0.36) hPa×s/L in Rrs_{7-19} for OP_v^{DTT}. Likewise, the model excluding leverage and influencing points led to statistically significant results with Rrs_{7-19} and exposure to both OP_v, whereas the results for other outcomes were not modified, with their 95% CI largely overlapping with that of the main model (Table S5, Figure S12). The analyses further adjusted on personal NO_2 sampled simultaneously to $PM_{2.5}$ showed that NO_2 did not modify the estimates and 95% CI for any of the studied association. The two-pollutant models for Rrs_{7-19} showed that the effects of both OP_v were stronger than the effects of $PM_{2.5}$ [0.14 (-0.06, 0.34) and -0.07 (-0.27, 0.13) hPa×s/L for OP_v^{DTT} and $PM_{2.5}$; 0.15 (-0.03, 0.33) and -0.05 (-0.22, 0.12) hPa×s/L for OP_v^{AA} and $PM_{2.5}$], and other associations were not modified in this model (Figure 24, Table S7). No clear trends were observed for the other AOS parameters in the main model and this was confirmed by the sensitivity analyses.

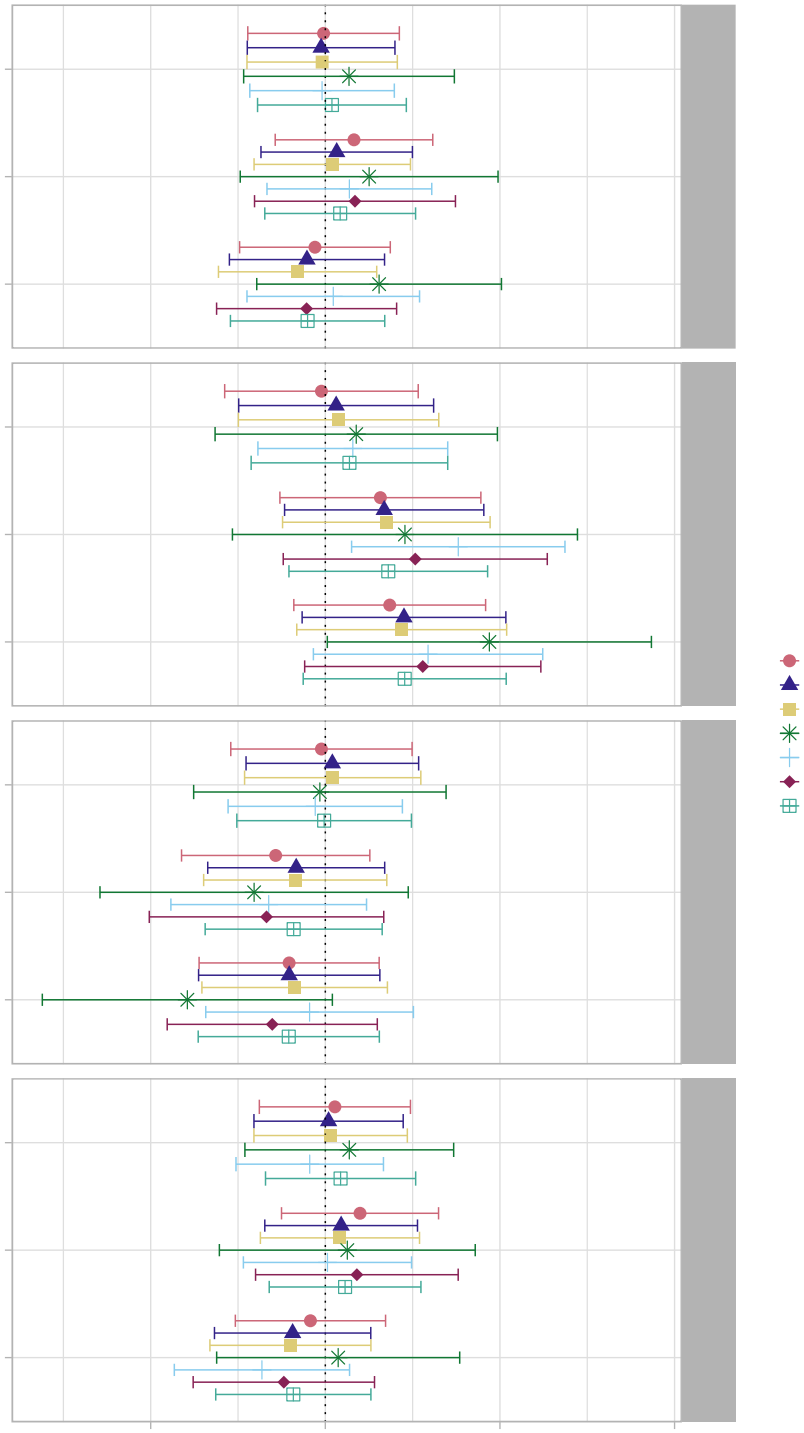

Figure 24. Association between personal exposure to $PM_{2.5}$, OP_v^{DTT}, and OP_v^{AA} during pregnancy and lung function parameters measured at 3 y in the univariate and multiple linear models and in the sensitivity analyses.
Outcomes and exposures were scaled by their IQR. See Tables S5 and S7 for corresponding numeric data. Whiskers represent the 95% confidence interval around the estimate. The main model was adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis and mean temperature during pregnancy. In addition, "2 sampling periods" are the analyses reduced to the children that had 2 wk of prenatal measurements of air pollution (61% of the population); "Excluding extreme values" are the analyses excluding the exposures and outcomes below the first percentile and above the 99th (exclusion of approx. 5% of the population); "Adjusted on PM" corresponds to adding personal exposure to $PM_{2.5}$ in the set of confounders; "Adjusted on NO_2" corresponds to adding personal exposure to NO_2 in the set of confounders.

VI. Discussion

To the best of our knowledge, this study is the first one to address the associations between maternal personal exposures to $PM_{2.5}$ and OP and children's objective lung function parameters measured as early as 6 weeks of age and at 3 years. Regarding OP_v^{DTT}, our findings showed consistency across some lung function parameters with higher prenatal exposure being associated with a lowered indicator of lung volumes (FRC) at 6 weeks, and with a trend towards reduced $Rrs_{7\text{-}19}$ at 3 years, an indicator influenced by both lung volumes and ventilation heterogeneity. Interestingly, the effects of OP_v^{DTT} exposure on FRC were stronger than those of $PM_{2.5}$ mass in the two-pollutant model.

VI.1. PM and OP exposures and lung function

Our results are in agreement with existing studies reporting a higher prevalence of reduced lung function in participants that are exposed to higher levels of $PM_{2.5}$. (Gauderman et al., 2004; Gehring et al., 2013; Guo et al., 2019; Hwang et al., 2015; Rice et al., 2016) Regarding TBFVL and N_2MBW tests, our findings are in line with the results of the South African birth cohort MACE (Muttoo et al., 2019), that investigated the effects of NO_x from LUR models and lung function of children at 1.5, 6, 12 and 24 months, and with the results of a Swiss birth cohort (Latzin et al., 2009) which examined the association between PM_{10} and NO_2 from an ambient monitoring station and lung function measured in neonates (median age of 34 days). Both studies showed decreases in FRC and V_T in infants prenatally exposed to higher concentrations of NO_x or NO_2 and PM_{10}, while no effects were found on LCI. Our results extend their findings by confirming the pattern of decreased FRC with exposure to $PM_{2.5}$ and OP, further supporting the importance of considering the oxidative stress caused by PM during pregnancy to predict lung growth restriction of children. In our study, none of the exposures considered were associated with LCI nor with t_{PTEF}/t_E, two parameters still poorly studied in association with air pollution and with conflicting results regarding LCI. (Latzin et al., 2009; Muttoo et al., 2019) The decrease in V_T with OP_v^{DTT} and $PM_{2.5}$ is not confirmed by all sensitivity analyses, indicating limited robustness of this association. $Rrs_{7\text{-}19}$ is usually used to detect the obstruction of the distal bronchi, and can be modified by both lung volumes and heterogeneity of ventilation. (Lundblad et al., 2021) The trend for an increase

of this parameter in children prenatally exposed to higher OP is in accordance with the results found at 6 weeks, as lower lung volume could lead to an increased resistance of the small airways. This partially confirms the results from previous studies indicating a detrimental effect of air pollution on respiratory mechanical parameters. In the BAMSE birth cohort, Schultz et al. (2016b) investigated the effects of early life exposure to PM_{10} on lung mechanic components measured by impulse oscillometry in 2415 adolescents and found increased frequency dependent resistance (Rrs_{5-20}) and $AX^{0.5}$ with higher PM_{10} exposure, although the associations were not statistically significant. Shao et al. (2019) found increased AX in 84 children exposed to $PM_{2.5}$ from a 6-week episode of fire during infancy. In addition, regarding acute respiratory effect of OP, He et al. (2021) found that an increase in OP measured 2 days prior to visit was significantly associated with increased Rrs_{5-20} and Rrs_5 in 43 asthmatic children aged 5-13 years. Although AOS parameters have been found associated with air pollution in previous studies, the parameter varies between studies. (Dutta et al., 2021; He et al., 2021; Schultz et al., 2016b; Shao et al., 2019) In our case, we confirmed results with Rrs_{7-19}, a parameter specific of the small airways.

VI.2. Comparison of the exposure metrics

Our study that identified associations between OP of PM with FRC, an indicator of lung volume, and with Rrs_{7-19}, an indicator accounting for lung volumes too, indicates specific effects on lung growth. These observations are supported by studies showing that prenatal exposure to environmental pollutants impacts in-utero growth, including organ growth (Lavigne et al., 2018; Saadeh and Klaunig, 2014) and that oxidative stress may cause placental tissue damage, which could in turn affect lung growth in-utero. (Øvrevik, 2019; Veras et al., 2017)

Only a few cohort studies tackled the associations of PM and OP exposure with lung function. (Delfino et al., 2013; He et al., 2021; Yang et al., 2016) The associations found with reduced lung function seemed generally clearer with OP than with $PM_{2.5}$ mass concentration, which agrees with the existing literature. (Bates et al., 2019) For example, the PIAMA birth cohort study (Yang et al., 2016) found associations between OP_v^{DTT} at home address and increased asthma and rhinitis prevalence and decreased lung function in 12-year-old children but no association with $PM_{2.5}$ mass. The effect magnitudes of OP models adjusted on other pollutants were similar, although more sensitive to NO_2 adjustment, which

was not the case in our model. In asthmatic children aged 9-18 years, Delfino et al. (2013) found significant positive associations between ambient OP_v^{DTT} and OP measured by the in-vitro ROS-macrophage assay and airway inflammation, while no association was found for $PM_{2.5}$. Conclusions were not modified in their two-pollutant model. He et al. (2021) also used the ROS-macrophage assay and found associations with Rrs_5, Rrs_{5-20} and Rrs_{20} for OP, while associations for PM were only found with Rrs_5. Overall our results add to the existing evidence indicating that the OP of PM has a stronger effect on various respiratory outcomes than PM mass and is thereby a relevant complementary health metric for air pollution. (Abrams et al., 2017; Delfino et al., 2013; Fang et al., 2016; He et al., 2021; Janssen et al., 2015; Weichenthal et al., 2016; Yang et al., 2016) The different health effects found for $PM_{2.5}$ and OP could be partially explained by the difference in sources contributing to OP and $PM_{2.5}$ concentration in the SEPAGES study area (Grenoble). In fact, previous studies showed that biomass burning and regional transport of secondary inorganic pollutants (nitrates and sulfates) were the main sources contributing to the ambient $PM_{2.5}$ mass concentration, while vehicular emissions and biomass burning were the main drivers of OP levels over the area.(2021b, 2021a) We acknowledge that by using active personal samplers, exposure measurements incorporate both indoor- and outdoor-generated pollution, which can have different composition and thus different health effects. (Evangelopoulos et al., 2020)

Our study extends the findings of others by comparing OP measured by the AA and the DTT assays. In their reviews, Bates et al. (2019) and Rao et al. (2020) showed that OP^{DTT} was a better predictor than OP^{AA} for most health outcomes. Here, we found that OP_v^{AA} had a comparable effect to OP_v^{DTT} on lung function as measured by Rrs_{7-19} at 3 years. However, results at 6 weeks were more contrasted. The effects of OP_v^{AA} on FRC seemed to be influenced by $PM_{2.5}$ mass concentration, since the OP_v^{AA} coefficients in the model adjusted on PM were pulled towards zero. Overall, although both OP assays (i.e. DTT and AA) were developed to account for the toxicity of PM components, their health impact may differ, which could be explained by their different sensitivities to chemical components (traffic-related metals, organic carbon and inorganic species for OP^{DTT} and metals only for OP^{AA}) (Fang et al., 2017; Janssen et al., 2014; Visentin et al., 2016) and their different reactivities to specific ROS. (Xiong et al., 2017)

VI.3. Strengths and limitations

One of the main strengths of this study is the assessment of maternal exposure by personal measurements, which was proven to be more representative of real exposure (Avery et al., 2010; Evangelopoulos et al., 2020; Ouidir et al., 2015) than studies using ambient measurement from monitoring stations or exposure models. It is also expected to be more accurate as compared to approaches modelling the personal exposure (He et al., 2021), combining 1) self-reported time-activity patterns in different microenvironments (at home, at work, in a car, in public transport, outdoor…) and 2) indoor-outdoor ratios estimation for each identified microenvironment, both being at risk of errors. Additionally, the use of OP in this study is a way to consider the potential oxidative stress caused by PM, which is thought to be a better predictor of PM damages than its concentration. Interestingly, the similitude of the seasonality observed in personal levels of $PM_{2.5}$ and OP in the present study with the results of a previous study that showed higher ambient $PM_{2.5}$ and OP during winter in the Grenoble area (Borlaza et al., 2021a), supports the external validity of our exposure data.

We acknowledge that a mixed influence of pre- and post-natal exposure cannot be totally ruled out, but cannot be assessed because OP of $PM_{2.5}$ was not measured in early childhood in SEPAGES. Nevertheless, other studies (Cai et al., 2020; Stapleton et al., 2022) that considered both pre- and post-natal exposure to PM found an effect of prenatal exposure on lower lung function in children. Although the design of the study enables evaluation of the effects of air pollution on child's lung function at different stages of the pregnancy, we *a priori* decided not to perform this analysis in our study in order to avoid lowering the number of participants included (224 with two measurement weeks) and increasing the number of statistical tests.

One limitation of active personal samplers is that it cannot be used by the participants during their entire pregnancy. The compromise in this study was to perform sampling for two one-week periods during the pregnancy and to use the average of the two measures in the association studies. This tended to avoid the influence of seasonality and extreme pollution events during the sampling weeks, especially for OP_v^{DTT} (Figure 22, Figure S8). However, this influence could not be avoided for individuals with only one week of measurement (N=132). To account for this limitation, models were adjusted for the season

of sampling and sensitivity analysis excluding participants with only one measurement week were conducted. Interestingly, in general, the associations between OP or $PM_{2.5}$ and FRC, and Rrs_{7-19} were stronger in this latter sensitivity analysis consisting in a restricted population with a more accurate exposure assessment, which supports our *a priori* hypothesis.

The novelty of this study also lies in the repeated assessment of lung function in early life, while most of the other studies considered children older than 5-years old, when spirometry starts to be feasible. Assessing lung function at the youngest age allows to better investigate the effects related to pregnancy and early infancy time-windows, which are believed to predict long-term respiratory morbidity. However, the use of pure oxygen during the N_2MBW test (SF_6 being forbidden in France) induced a transient decrease in tidal volume, that could affect the measurement of FRC and LCI. Although parameters were *a posteriori* corrected for the degree of hypoventilation, and sensitivity analysis excluding children with the highest hypoventilation degree showed similar patterns of association, residual errors in lung function assessment that would lead to underestimated effect estimates cannot be totally excluded. Because two different techniques of lung function measurement were used at 6 weeks and 3 years of age, the effect of prenatal exposure of air pollution on lung function growth could not be assessed.

The amount of data collected during the follow-up of the cohort allowed to adjust for a number of confounding factors. However, the residual confounding due to the observational design of this study remains a limitation. Interestingly, the analysis excluding leverage and influencing points showed that these points tended to drive some of the regression estimates towards the null hypothesis, which indicate that influencing points might partly be related to measurement errors. Although the aim of our study was based on *a priori* hypothesis derived from previous association studies and from the biological specificities of OP of $PM_{2.5}$, the number of associations tested was still relatively high (N=24) and we did not apply any formal correction for multiple comparisons. Thus, we acknowledge part of the associations observed may result from chance findings and thus should be interpreted cautiously. The attrition rate of 51% for the associations between the personal prenatal OP and lung function at three years could not be *a priori* defined as low, but given the demanding protocol and the originality of the longitudinal data collected, both for exposures (personal prenatal exposure to OP) and health outcomes

(with objective lung function measures in preschool children, which is rare in population-based cohorts), this can be considered as acceptable. However, a selection bias cannot be totally ruled out, in particular, the associations for $PM_{2.5}$ may have been underestimated because included participants tended to have both higher exposure to $PM_{2.5}$ and better lung function on two AOS parameters (lower Rrs_7, higher Xrs_7) at 3 years as compared to the non-included participants. Nevertheless, with no differences in OP between included and excluded children, the associations reported with OP are probably not driven by selection bias. Although a bigger sample would lead to more statistical power and therefore clearer conclusions, the use of objective and validated respiratory health parameters in early life and novel personal prenatal air pollution exposure metrics offers important and relevant information on PM exposure and its health effects.

In summary, our study shows consistency in the associations between personal prenatal OP_v^{DTT} and several early-life lung function parameters related to lung growth restriction, and therefore supports the detrimental health effects of $PM_{2.5}$ exposure on health through oxidative stress and the relevance of OP of $PM_{2.5}$ as a useful health-based metric. These findings, together with identifying sources of OP of PM, could help target emission sources that are critical in decreasing health effects of atmospheric pollution.

VII. Acknowledgments

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VIII. Supplemental Material

VIII.1. List of Figures

VIII.1. List of Tables

Table S1. Description of covariates and child's characteristics in the group that have lung function measurements performed at 6 weeks (by the Nitrogen Multiple Breath Washout technique or by Tidal Breathing analysis) and in the group with lung function at 3 years (Forced Oscillation Technique).

Characteristics	Children with N_2MBW at 6 weeks (median age: 47 days) N=325/356	Children with AOS at 3 years (median age: 3.07 yrs.) N=248/356
Sex of child		
Male	172 (53%)	129 (52%)
Female	153 (47%)	119 (48%)
Missing	/	/
Birthweight (g)		
Median (IQR)	3280 (3040, 3560)	3305 (3078, 3582)
Missing	/	/
Preterm birth, <37 weeks	14 (4.3%)	11 (4.4%)
Missing	/	/
Parental educational level >5 years	235 (72%)	187 (75%)
Delivery mode		
Vaginal	277 (85%)	207 (83%)
C-section	48 (15%)	41 (17%)
Missing	/	/
Child still breastfed at 6 weeks	281 (86%)	219 (88%)
Missing	1	/
Parental history of rhinitis	186 (61%)	144 (61%)
Missing	21	12
Parity		
0 (nulliparous)	145 (45%)	105 (42%)
1 (primiparous)	146 (45%)	119 (48%)
2 or more (multiparous)	34 (10%)	24 (9.7%)
ETS in utero and < 6 wks.		
0	232 (71%)	178 (72%)
1	93 (29%)	68 (28%)
Missing	0	2
ETS < 3 yrs.		
0	243 (78%)	195 (79%)
1	69 (22%)	53 (21%)
Missing	13	0
Maternal age at conception		
Median (IQR)	32 (30, 35)	32 (30, 35)
Maternal BMI at conception		
Median (IQR)	21 (20, 24)	21 (20, 23)
Season of sampling		
Warm	112	96
Warm+Cold	133	90
Cold	80	62
Mean temperature during pregnancy		
Median (IQR)	13.0 (10.5, 14.6)	12.9 (10.5, 14.7)
Season at the clinical visit		
Fall	107	94
Spring	68	28
Summer	59	57
Winter	91	69

Note: N_2MBW, nitrogen multiple breath washout; TBFVL, tidal breathing flow-volume loops; AOS, airwave oscillometry; ETS, environmental tobacco smoke.

Table S2. Monthly distribution of personal measurements of $PM_{2.5}$, OP_v^{DTT}, and OP_v^{AA}.

Exposure	Statistics	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
$PM_{2.5}$ ($\mu g/m^3$)	Minimum	5.70	4.70	5.00	4.30	4.80	4.90	4.10	5.10	5.10	5.60	7.50	7.00
	25th percentile	10.17	9.95	10.65	8.10	9.58	9.60	9.30	8.92	10.50	10.80	12.52	15.23
	Median	14.40	13.30	13.00	9.70	12.75	12.60	11.90	12.20	13.20	13.80	17.65	19.50
	75th percentile	19.97	16.90	15.17	11.95	15.95	16.45	15.52	15.55	17.90	20.30	21.67	23.95
	Maximum	77.60	26.20	23.50	18.30	50.30	27.70	30.70	30.50	51.10	44.30	49.30	41.50
OP_v^{DTT} (nmol/min/m^3)	Minimum	0.34	0.59	0.44	0.39	0.13	0.45	0.25	0.30	0.41	1.06	0.18	0.85
	25th percentile	1.08	0.85	1.10	0.84	1.02	1.11	0.94	0.78	0.89	1.52	1.46	1.69
	Median	1.51	1.34	1.56	1.07	1.27	1.45	1.18	1.03	1.34	1.91	1.98	2.71
	75th percentile	2.07	1.90	1.82	1.63	1.73	1.97	1.55	1.38	1.79	2.41	2.72	3.23
	Maximum	6.09	3.01	2.78	3.08	5.44	3.72	2.69	2.44	3.43	4.48	5.34	5.32
OP_v^{AA} (nmol/min/m^3)	Minimum	0.40	0.58	0.77	0.28	0.34	0.36	0.04	0.07	0.18	0.90	0.13	1.08
	25th percentile	1.67	1.39	1.29	0.72	0.82	1.03	0.65	0.37	0.45	1.79	1.56	2.98
	Median	2.16	1.88	1.76	1.01	1.12	1.47	0.96	0.48	0.89	2.16	2.17	3.73
	75th percentile	3.34	2.56	2.07	1.27	1.67	1.78	1.46	0.60	1.12	2.71	3.16	4.53
	Maximum	11.43	5.57	3.51	7.55	5.58	5.75	3.75	2.04	3.99	3.48	8.42	6.09

Note: $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 μm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay.

Table S3. Comparison of the distribution of personal measurements of $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA} during each week of sampling, and their average.

Exposure	Trimester	Minimum	25th percentile	Median	75th percentile	Maximum
$PM_{2.5}$ ($\mu g/m^3$)	early pregnancy	4.30	9.70	13.85	18.85	51.10
	late pregnancy	4.20	9.78	12.75	17.32	77.60
	average	5.25	11.36	13.75	17.46	46.70
OP_v^{DTT} (nmol/min/m^3)	early pregnancy	0.18	1.05	1.46	1.97	4.22
	late pregnancy	0.13	1.01	1.44	2.08	6.09
	average	0.57	1.17	1.52	2.00	3.79
OP_v^{AA} (nmol/min/m^3)	early pregnancy	0.04	0.83	1.52	2.19	11.43
	late pregnancy	0.07	0.87	1.48	2.33	7.86
	average	0.27	1.17	1.61	2.22	6.60

Note: $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 μm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay.

Table S4. Sensitivity analyses of the associations between prenatal exposure to air pollution and lung function at 6 weeks. Regression coefficients are estimated from multiple linear models.

Pollutants	Regression model	FRC (mL)		LCI		V_T (mL)		t_{PTEF}/t_E (%)	
		Coefficients (95% CI)	N	Coefficients (95% CI)	N	Coefficients (95% CI)	N	Coefficients (95% CI)	N
$PM_{2.5}$ (µg/m³)	Complete Cases	-1.54 (-3.69, 0.60)	262	-0.01 (-0.15, 0.13)	262	-0.66 (-1.51, 0.19)	285	0.30 (-0.99, 1.59)	285
	Main model	-1.58 (-3.67, 0.50)	284	-0.01 (-0.14, 0.13)	284	-0.54 (-1.35, 0.28)	309	0.25 (-1.02, 1.51)	309
	2 sampling periods	-1.58 (-4.23, 1.07)	178	-0.01 (-0.20, 0.17)	178	-0.64 (-1.74, 0.45)	204	0.11 (-1.67, 1.89)	204
	Excluding extreme values	-0.71 (-2.96, 1.53)	270	0.05 (-0.10, 0.20)	271	0.03 (-0.89, 0.95)	293	0.00 (-1.34, 1.34)	293
	Adjusted on NO_2	-1.56 (-3.67, 0.55)	284	-0.02 (-0.15, 0.12)	284	0.31 (-0.96, 1.59)	309	-0.56 (-1.39, 0.27)	309
	Cook's distance	-1.97 (-3.56, -0.38)	266	0.04 (-0.08, 0.16)	262	-0.22 (-0.97, 0.53)	290	-0.5 (-1.6, 0.59)	295
	Excluding high degrees of hypoventilation	-1.09 (-3.45, 1.27)	212	0.04 (-0.12, 0.20)	212	/	/	/	/
OP_v^{DTT} (nmol/min/m³)	Complete Cases	-2.65 (-5.16, -0.14)	262	-0.06 (-0.22, 0.10)	262	-0.64 (-1.66, 0.37)	285	0.71 (-0.84, 2.25)	285
	Main model	-2.26 (-4.68, 0.15)	284	-0.06 (-0.22, 0.09)	284	-0.58 (-1.54, 0.38)	309	0.69 (-0.79, 2.17)	309
	2 sampling periods	-2.15 (-5.60, 1.30)	178	0.03 (-0.21, 0.27)	178	-0.83 (-2.22, 0.55)	204	0.51 (-1.74, 2.76)	204
	Excluding extreme values	-1.8 (-4.16, 0.56)	272	-0.02 (-0.19, 0.14)	272	-0.03 (-1.02, 0.96)	296	0.68 (-0.78, 2.15)	296
	Adjusted on NO_2	-2.24 (-4.67, 0.19)	284	-0.07 (-0.23, 0.09)	284	-0.60 (-1.57, 0.37)	309	0.75 (-0.74, 2.24)	309
	Cook's distance	-2.19 (-4.11, -0.27)	263	-0.06 (-0.20, 0.08)	263	-0.37 (-1.24, 0.49)	291	0.61 (-0.66, 1.87)	296
	Excluding high degrees of hypoventilation	-1.91 (-4.49, 0.68)	212	-0.03 (-0.20, 0.15)	212	/	/	/	/
OP_v^{AA} (nmol/min/m³)	Complete Cases	-0.70 (-3.03, 1.63)	262	-0.06 (-0.21, 0.09)	262	0.09 (-0.83, 1.02)	285	0.13 (-1.27, 1.54)	285
	Main model	-0.59 (-2.85, 1.68)	284	-0.05 (-0.19, 0.10)	284	0.13 (-0.76, 1.02)	309	0.14 (-1.23, 1.51)	309
	2 sampling periods	-1.24 (-4.33, 1.84)	178	0.01 (-0.21, 0.23)	178	-0.31 (-1.58, 0.95)	204	-0.34 (-2.39, 1.72)	204
	Excluding extreme values	0.73 (-1.83, 3.28)	271	0.01 (-0.16, 0.18)	271	0.84 (-0.18, 1.85)	295	-0.53 (-2.06, 1.00)	294
	Adjusted on NO_2	-0.55 (-2.83, 1.72)	284	-0.05 (-0.20, 0.09)	284	0.12 (-0.77, 1.01)	309	0.18 (-1.20, 1.55)	309
	Cook's distance	-0.43 (-2.35, 1.48)	266	-0.03 (-0.17, 0.10)	265	0.59 (-0.24, 1.43)	292	0.25 (-0.97, 1.46)	296
	Excluding high degrees of hypoventilation	-0.92 (-3.19, 1.34)	212	-0.06 (-0.21, 0.09)	212	/	/	/	/

Note: Coefficients are calculated for an increase of one IQR for $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA}, corresponding to 6.9 µg/m³, 0.89 nmol/min/m³, and 1.14 nmol/min/m³, respectively. FRC, functional residual capacity; LCI, lung clearance index; V_T, tidal volume; t_{PTEF}/t_E ratio of time to peak tidal expiratory flow to expiratory time; $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay.

Table S5. Sensitivity analyses of the associations between prenatal exposure to air pollution and lung function at 3 years. Regression coefficients are estimated from multiple linear models.

Pollutants	Regression model	Rrs_7 (hPa×s/L) Coefficients (95% CI)	N	Rrs_{7-19} (hPa×s/L) Coefficients (95% CI)	N	Xrs_7 (hPa×s/L) Coefficients (95% CI)	N	AX (hPa/L) Coefficients (95% CI)	N
$PM_{2.5}$ (µg/m³)	Complete Cases	-0.01 (-0.33, 0.31)	235	0.02 (-0.13, 0.17)	235	0.01 (-0.15, 0.18)	235	0.36 (-4.82, 5.53)	235
	Main model	-0.02 (-0.33, 0.30)	248	0.02 (-0.13, 0.16)	248	0.01 (-0.15, 0.17)	248	0.22 (-4.81, 5.25)	248
	2 sampling periods	0.1 (-0.35, 0.55)	151	0.05 (-0.17, 0.26)	151	-0.01 (-0.24, 0.22)	151	1.62 (-5.42, 8.66)	151
	Excluding extreme values	-0.01 (-0.32, 0.29)	238	0.04 (-0.1, 0.18)	239	-0.02 (-0.18, 0.14)	238	-1.05 (-6.03, 3.92)	238
	Adjusted on NO_2	0.03 (-0.29, 0.35)	248	0.04 (-0.11, 0.18)	248	0.00 (-0.16, 0.16)	248	1.03 (-4.03, 6.09)	248
	Cook's distance	-0.07 (-0.35, 0.21)	232	0.04 (-0.07, 0.16)	233	-0.05 (-0.17, 0.08)	235	-0.50 (-4.81, 3.80)	237
OP_v^{DTT} (nmol/min/m³)	Complete Cases	0.03 (-0.3, 0.36)	235	0.09 (-0.06, 0.25)	235	-0.06 (-0.22, 0.11)	235	0.99 (-4.38, 6.35)	235
	Main model	0.05 (-0.28, 0.37)	248	0.09 (-0.06, 0.24)	248	-0.05 (-0.22, 0.11)	248	1.07 (-4.08, 6.22)	248
	2 sampling periods	0.19 (-0.36, 0.74)	151	0.12 (-0.14, 0.38)	151	-0.13 (-0.41, 0.15)	151	1.48 (-7.15, 10.11)	151
	Excluding extreme values	0.1 (-0.25, 0.45)	235	0.20 (0.04, 0.36)	235	-0.10 (-0.28, 0.08)	236	0.14 (-5.53, 5.81)	235
	Adjusted on NO_2	0.06 (-0.26, 0.39)	248	0.10 (-0.05, 0.25)	248	-0.06 (-0.22, 0.10)	248	1.33 (-3.78, 6.45)	248
	Cook's distance	0.15 (-0.13, 0.43)	233	0.16 (0.04, 0.29)	233	-0.09 (-0.21, 0.04)	235	0.38 (-4.04, 4.8)	237
OP_v^{AA} (nmol/min/m³)	Complete Cases	-0.12 (-0.46, 0.22)	235	0.12 (-0.04, 0.27)	235	-0.06 (-0.23, 0.11)	235	-2.35 (-7.78, 3.07)	235
	Main model	-0.08 (-0.41, 0.25)	248	0.12 (-0.04, 0.27)	248	-0.07 (-0.23, 0.10)	248	-2.21 (-7.48, 3.07)	248
	2 sampling periods	0.23 (-0.29, 0.75)	151	0.25 (0, 0.49)	151	-0.25 (-0.52, 0.01)	151	0.87 (-7.33, 9.06)	151
	Excluding extreme values	0.03 (-0.33, 0.4)	238	0.16 (-0.02, 0.33)	237	-0.03 (-0.22, 0.16)	237	-4.28 (-10.19, 1.63)	237
	Adjusted on NO_2	-0.08 (-0.41, 0.25)	248	0.12 (-0.03, 0.27)	248	-0.07 (-0.23, 0.10)	248	-2.16 (-7.39, 3.07)	248
	Cook's distance	0.01 (-0.28, 0.29)	233	0.17 (0.05, 0.29)	234	-0.1 (-0.23, 0.03)	235	-3.55 (-8.54, 1.44)	236

Note: Coefficients are calculated for an increase of one IQR for $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA}, corresponding to 6.9 µg/m³, 0.89 nmol/min/m³, and 1.14 nmol/min/m³, respectively. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay; Rrs_7, resistance at a frequency of 7 Hz; Rrs_{7-19}, difference between the resistance at 7 Hz and at 19 Hz; Xrs_7, reactance at a frequency of 7 Hz; AX, area under the reactance curve.

Table S6. Results of the two-pollutant models for exposure to air pollution and lung function at 6 weeks.

	Single-pollutant model Coefficients (95% CI)[1]	Two-pollutant model (adjusted for exposures below)		
		$PM_{2.5}$ Coefficients (95% CI)[1]	OP_v^{DTT} Coefficients (95% CI)[1]	OP_v^{AA} Coefficients (95% CI)[1]
FRC (mL)				
$PM_{2.5}$	-1.58 (-3.67, 0.50)	--	-0.59 (-3.37, 2.19)	-1.74 (-4.10, 0.62)
OP_v^{DTT}	-2.26 (-4.68, 0.15)	-1.82 (-5.03, 1.40)	--	--
OP_v^{AA}	-0.59 (-2.85, 1.68)	0.27 (-2.27, 2.81)	--	--
LCI				
$PM_{2.5}$	-0.01 (-0.14, 0.13)	--	0.03 (-0.15, 0.21)	0.00 (-0.15, 0.15)
OP_v^{DTT}	-0.06 (-0.22, 0.09)	-0.09 (-0.29, 0.12)	--	--
OP_v^{AA}	-0.05 (-0.19, 0.10)	-0.05 (-0.21, 0.12)	--	--
V_T (mL)				
$PM_{2.5}$	-0.54 (-1.35, 0.28)	--	-0.22 (-1.29, 0.86)	-0.62 (-1.53, 0.29)
OP_v^{DTT}	-0.58 (-1.54, 0.38)	-0.41 (-1.67, 0.85)	--	--
OP_v^{AA}	0.13 (-0.76, 1.02)	0.43 (-0.56, 1.41)	--	--
t_{PTEF}/t_E (%)				
$PM_{2.5}$	0.25 (-1.02, 1.51)	--	-0.19 (-1.85, 1.47)	0.27 (-1.14, 1.68)
OP_v^{DTT}	0.69 (-0.79, 2.17)	0.83 (-1.11, 2.78)	--	--
OP_v^{AA}	0.14 (-1.23, 1.51)	0.01 (-1.51, 1.54)	--	--

Note: Coefficients are calculated for an increase of one IQR for $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA}, corresponding to 6.9 µg/m³, 0.89 nmol/min/m³, and 1.14 nmol/min/m³, respectively. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay; FRC, functional residual capacity; LCI, lung clearance index; V_T, tidal volume; t_{PTEF}/t_E ratio of time to peak tidal expiratory flow to expiratory time.
[1]adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis and mean temperature during pregnancy.

Table S7. Results of the two-pollutant models for exposure to air pollution and lung function at 3 years.

	Single-pollutant model Coefficients (95% CI)[1]	Two-pollutant model (adjusted for exposures below)		
		$PM_{2.5}$ Coefficients (95% CI)[1]	OP_v^{DTT} Coefficients (95% CI)[1]	OP_v^{AA} Coefficients (95% CI)[1]
Rrs_7 (hPa×s/L)				
$PM_{2.5}$	-0.02 (-0.33, 0.30)	--	-0.12 (-0.54, 0.30)	0.00 (-0.36, 0.37)
OP_v^{DTT}	0.05 (-0.28, 0.37)	0.13 (-0.3, 0.56)	--	--
OP_v^{AA}	-0.08 (-0.41, 0.25)	-0.08 (-0.46, 0.30)	--	--
Rrs_{7-19} (hPa×s/L)				
$PM_{2.5}$	0.02 (-0.13, 0.16)	--	-0.07 (-0.27, 0.13)	-0.05 (-0.22, 0.12)
OP_v^{DTT}	0.09 (-0.06, 0.24)	0.14 (-0.06, 0.34)	--	--
OP_v^{AA}	0.12 (-0.04, 0.27)	0.15 (-0.03, 0.33)	--	--
Xrs_7 (hPa×s/L)				
$PM_{2.5}$	0.01 (-0.15, 0.17)	--	0.08 (-0.13, 0.29)	0.06 (-0.13, 0.24)
OP_v^{DTT}	-0.05 (-0.22, 0.11)	-0.11 (-0.32, 0.11)	--	--
OP_v^{AA}	-0.07 (-0.23, 0.10)	-0.10 (-0.29, 0.10)	--	--
AX (hPa/L)				
$PM_{2.5}$	0.22 (-4.81, 5.25)	--	-1.58 (-8.27, 5.10)	1.12 (-4.72, 6.96)
OP_v^{DTT}	1.07 (-4.08, 6.22)	2.13 (-4.71, 8.96)	--	--
OP_v^{AA}	-2.21 (-7.48, 3.07)	-2.80 (-8.91, 3.32)	--	--

Note: Coefficients are calculated for an increase of one IQR for $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA}, corresponding to 6.9 µg/m³, 0.89 nmol/min/m³, and 1.14 nmol/min/m³, respectively. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay; Rrs_7, resistance at a frequency of 7 Hz; Rrs_{7-19}, difference between the resistance at 7 Hz and at 19 Hz; Xrs_7, reactance at a frequency of 7 Hz; AX, area under the reactance curve.
[1]adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis and mean temperature during pregnancy.

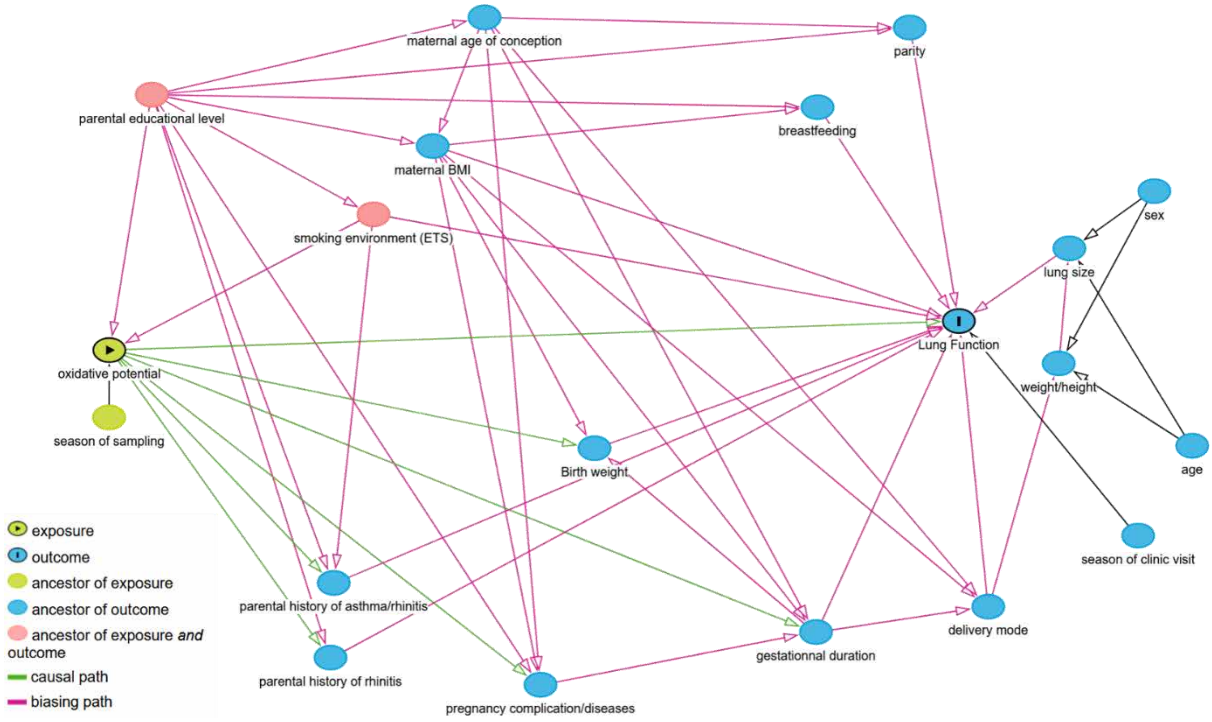

Figure S1. Directed acyclic graph (DAG) mapping causal relationships between maternal OP of PM exposure and child lung function.

Please note that at 6 weeks, there is an additional link between birth weight and weight/height at the clinical visit.

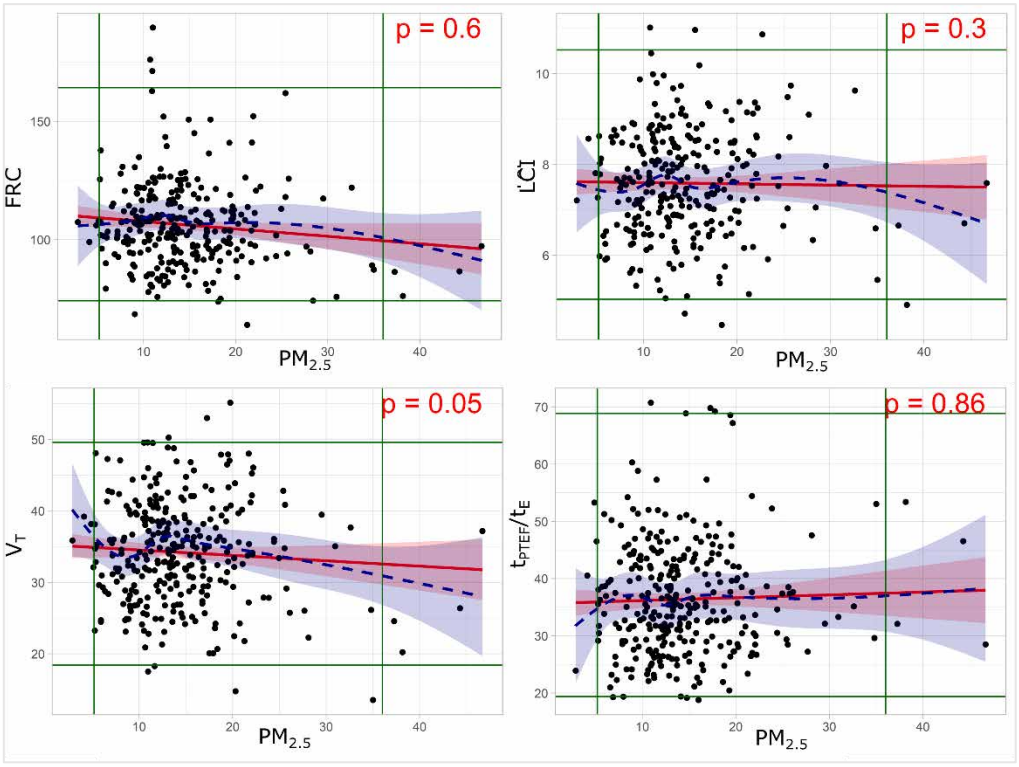

Figure S2. Test of linearity of the $PM_{2.5}$-lung function parameters at 6 weeks.
Comparison of the adjusted linear model (red) and the adjusted model, modelling exposure as a natural spline with 5 degrees of freedom (blue dashed line), for exposure to $PM_{2.5}$ and lung function at 6 weeks. P-value is from the likelihood-ratio test. Summary table available in the Excel supplemental file. The green lines represent the thresholds for 1st and 99th percentile of outcome and exposure.

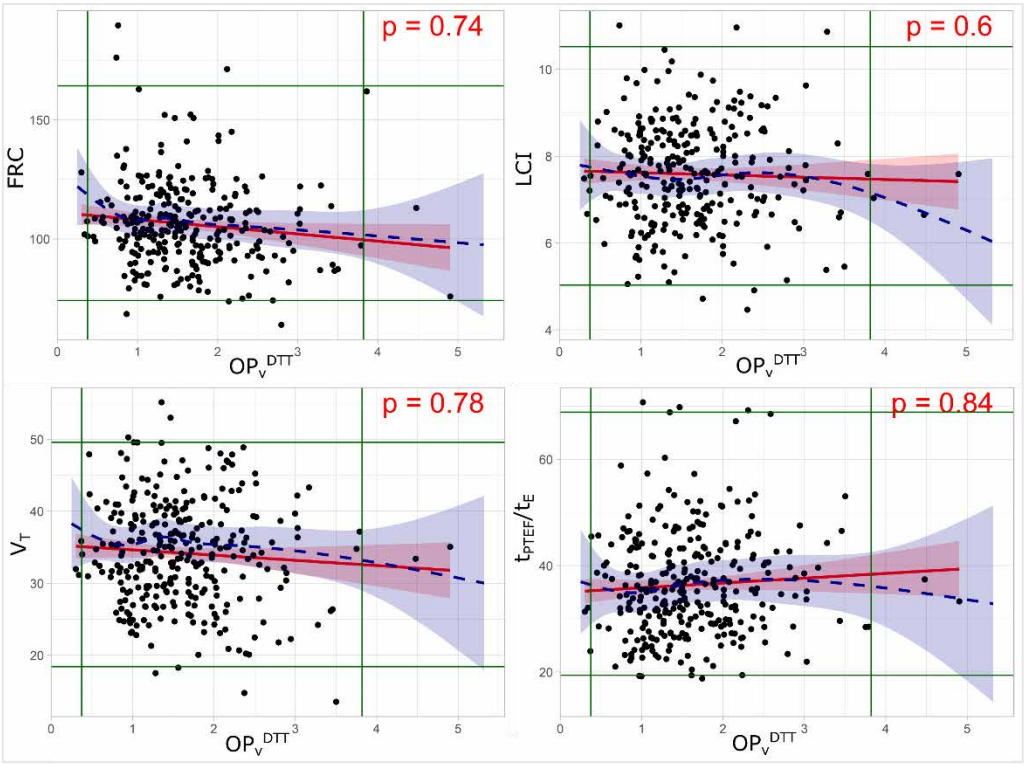

Figure S3. Test of linearity of the OP_v^{DTT}-lung function parameters at 6 weeks.
Comparison of the adjusted linear model (red) and the adjusted model, modelling exposure as a natural spline with 5 degrees of freedom (blue dashed line), for exposure to OP_v^{DTT} and lung function at 6 weeks. P-value is from the likelihood-ratio test. Summary table available in the Excel supplemental file. The green lines represent the thresholds for 1st and 99th percentile of outcome and exposure.

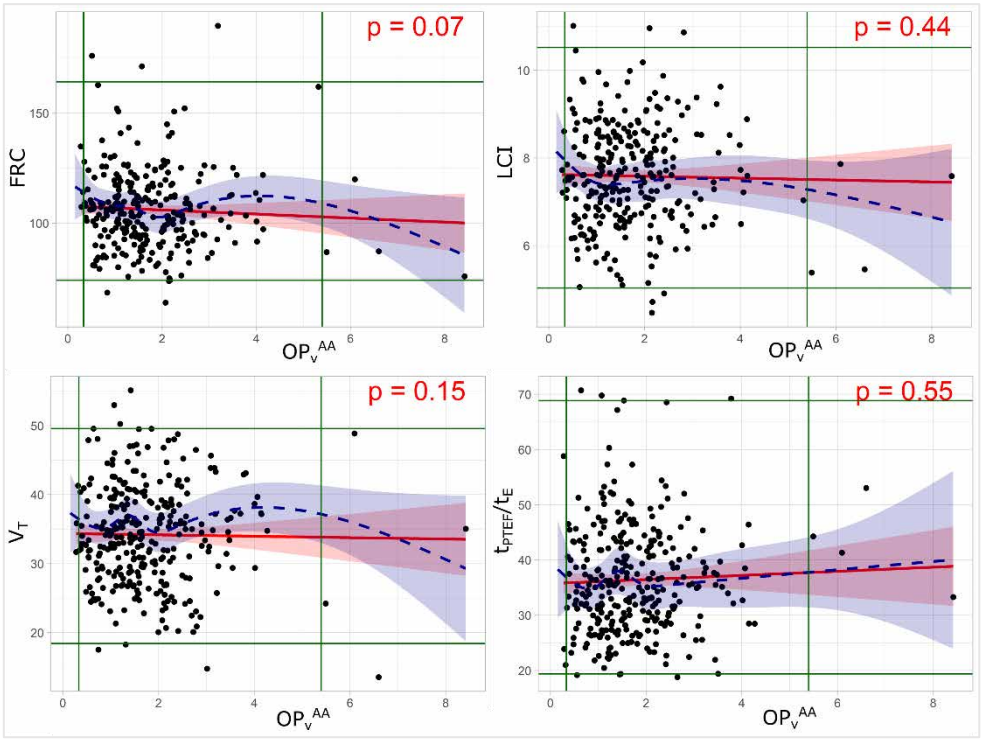

Figure S4. Test of linearity of the OP_v^{AA}-lung function parameters at 6 weeks.
Comparison of the adjusted linear model (red) and the adjusted model, modelling exposure as a natural spline with 5 degrees of freedom (blue dashed line), for exposure to OP_v^{AA} and lung function at 6 weeks. P-value is from the likelihood-ratio test. Summary table available in the Excel supplemental file. The green lines represent the thresholds for 1st and 99th percentile of outcome and exposure.

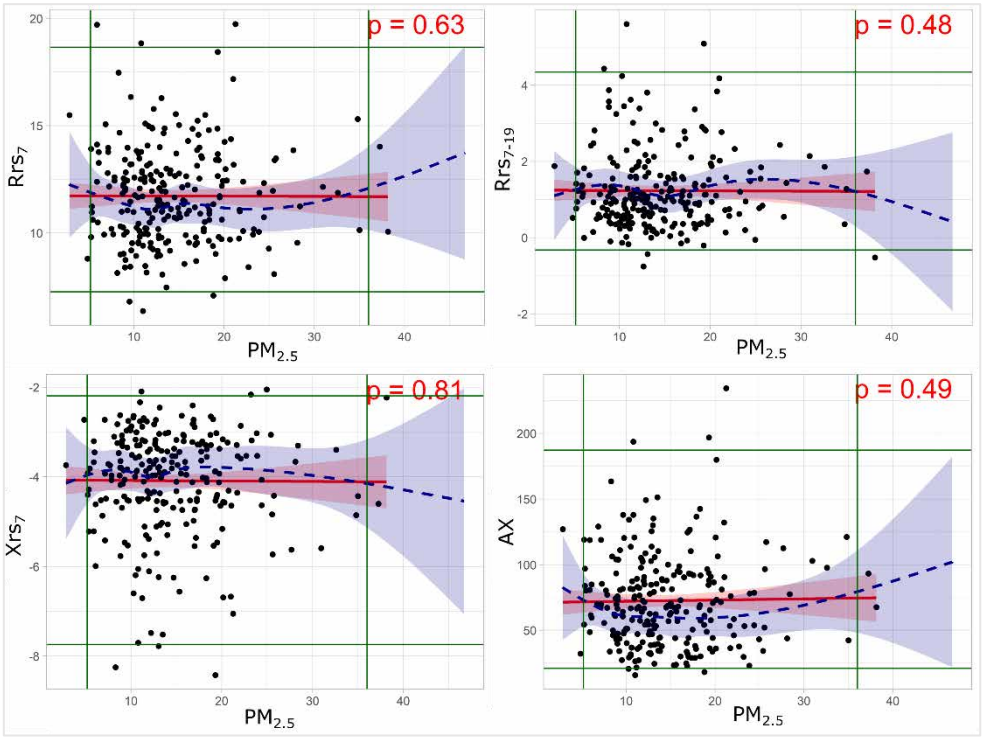

Figure S5. Test of linearity of the $PM_{2.5}$-lung function parameters at 3 years.
Comparison of the adjusted linear model (red) and the adjusted model, modelling exposure as a natural spline with 5 degrees of freedom (blue dashed line), for exposure to $PM_{2.5}$ and lung function at 3 years. P-value is from the likelihood-ratio test. Summary table available in the Excel supplemental file. The green lines represent the thresholds for 1st and 99th percentile of outcome and exposure.

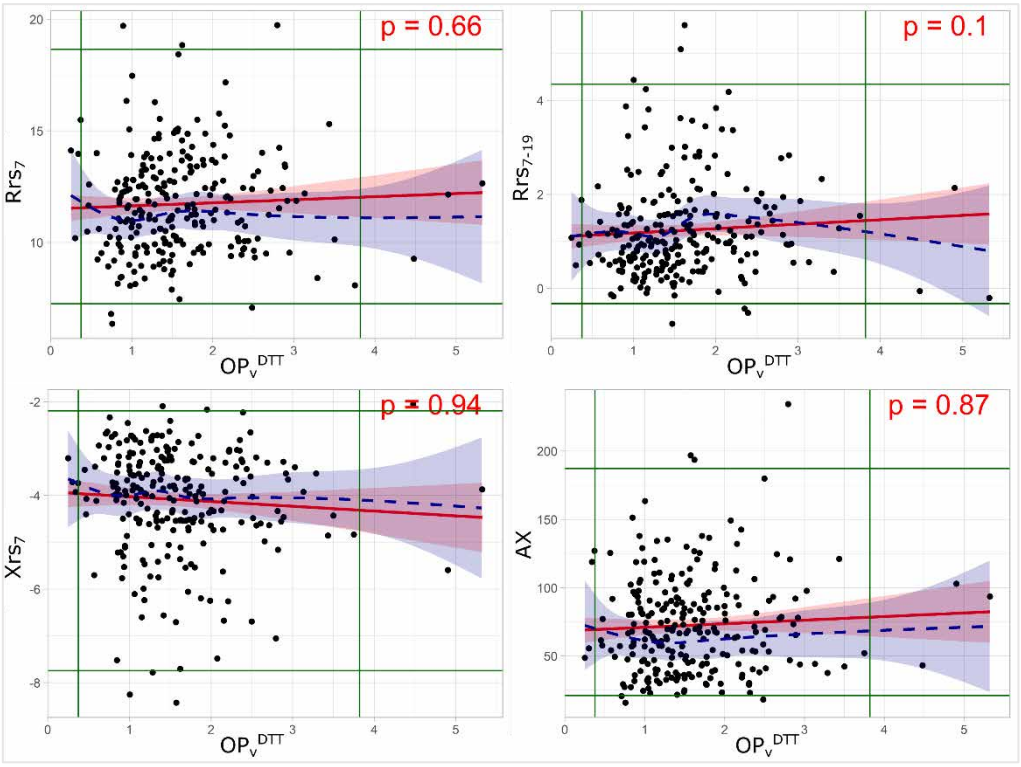

Figure S6. Test of linearity of the OP_v^{DTT}-lung function parameters at 3 years.
Comparison of the adjusted linear model (red) and the adjusted model, modelling exposure as a natural spline with 5 degrees of freedom (blue dashed line), for exposure to OP_v^{DTT} and lung function at 3 years. P-value is from the likelihood-ratio test. Summary table available in the Excel supplemental file. The green lines represent the thresholds for 1st and 99th percentile of outcome and exposure.

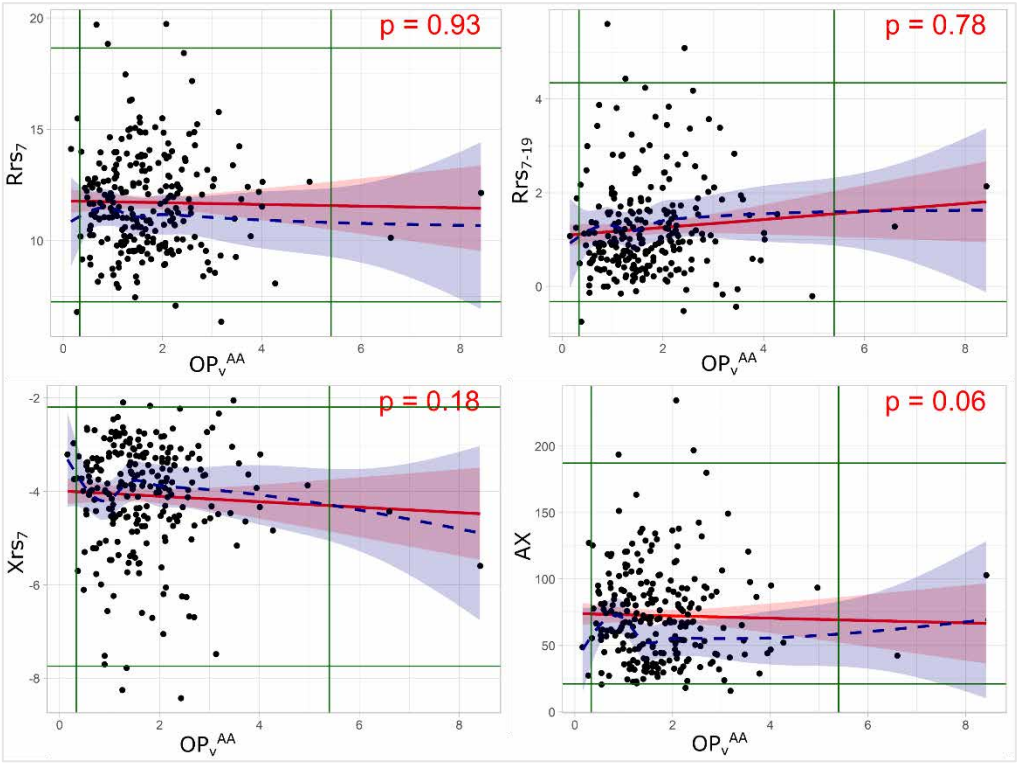

Figure S7. Test of linearity of the OP_v^{AA}-lung function parameters at 3 years.
Comparison of the adjusted linear model (red) and the adjusted model, modelling exposure as a natural spline with 5 degrees of freedom (blue dashed line), for exposure to OP_v^{AA} and lung function at 3 years. P-value is from the likelihood-ratio test. Summary table available in the Excel supplemental file. The green lines represent the thresholds for 1st and 99th percentile of outcome and exposure.

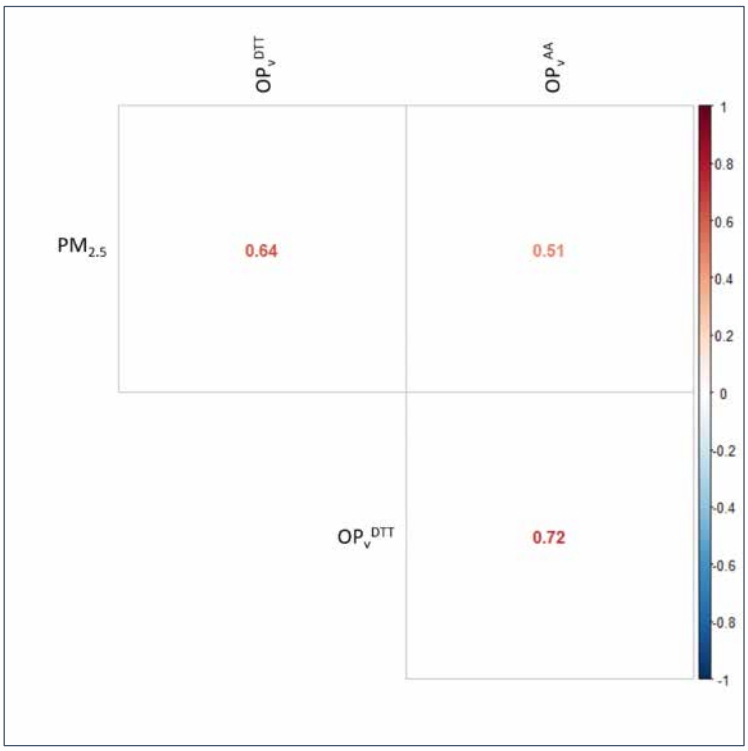

Figure S8. Spearman correlation coefficients between the exposures.
$PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay

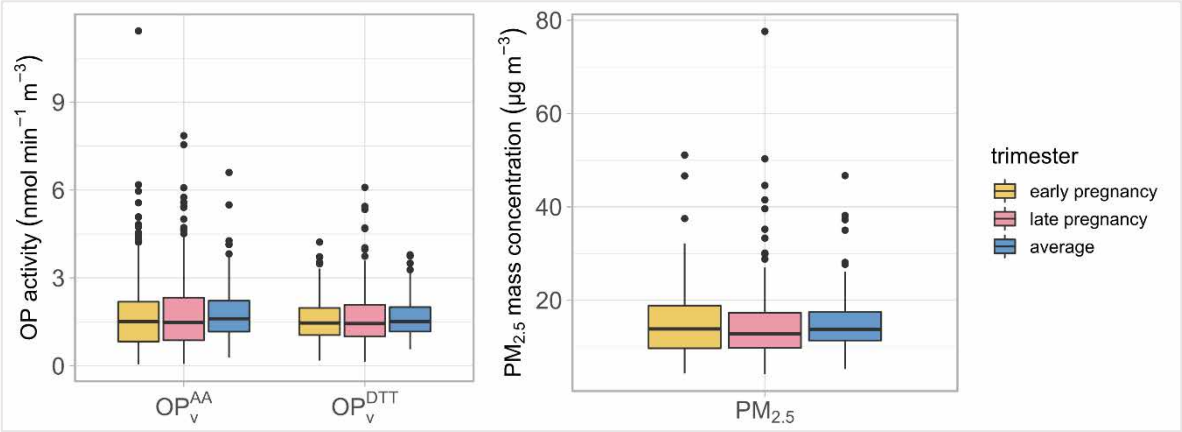

Figure S9. Comparison of the distribution of personal measurements of OP_v^{AA} (left), OP_v^{DTT} (center) and $PM_{2.5}$ (right) during each week of sampling, and their average. See Table S3 for corresponding numeric data.
Note: Boxes represent 25th to 75th percentiles, the middle horizontal line represents the median, whiskers extend to the most extreme point within 1.5 interquartile ranges of the box and the dots outside boxes indicate outliers. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay.

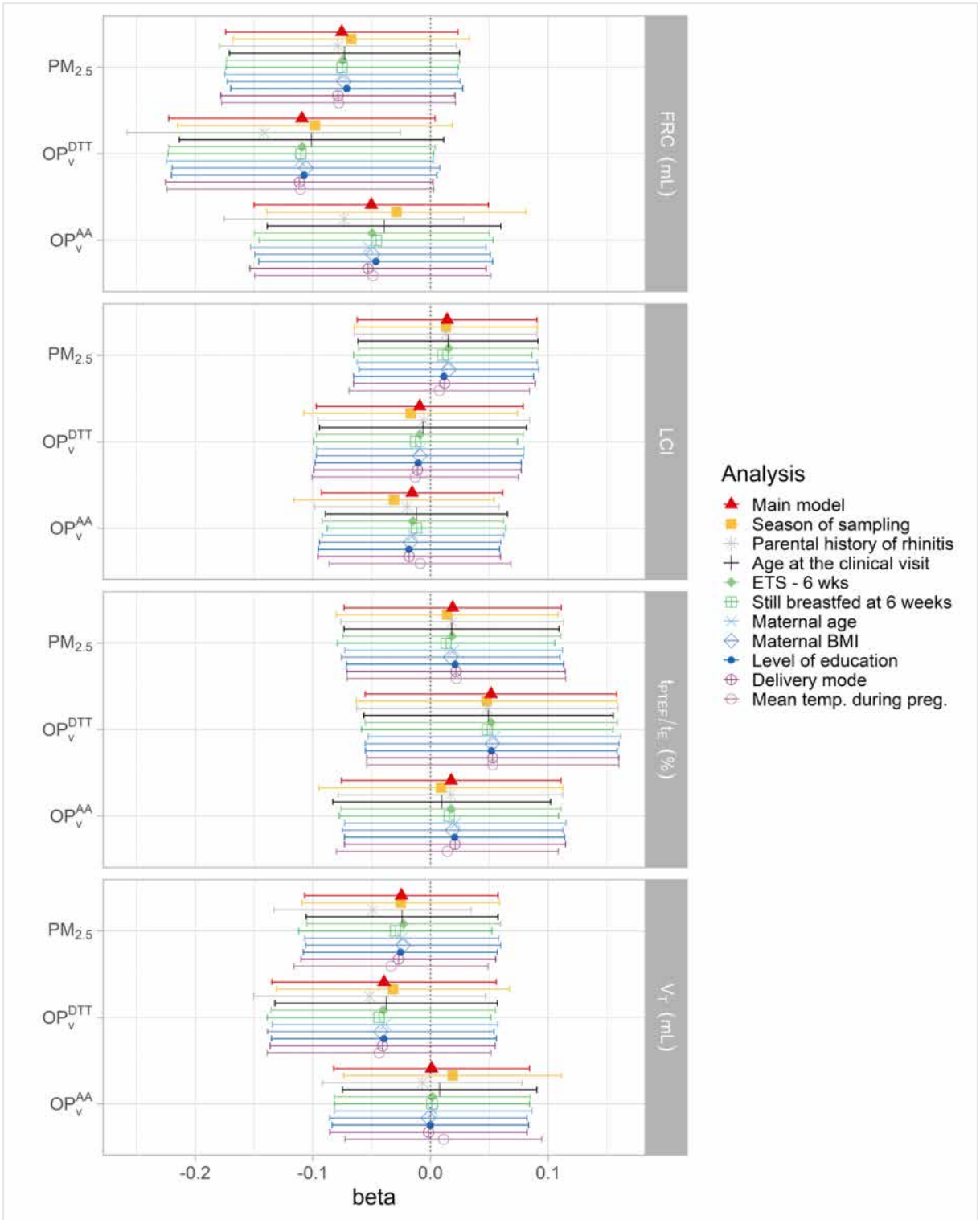

Figure S10. Effect of each confounder separately on the regression models at 6 weeks, adjusted for sex, height and weight, and comparison to the main model, adjusted on all the confounders listed.
Outcomes and exposures were scaled by their IQR. See Excel supplemental file for corresponding numeric data. Whiskers represent the 95% confidence interval around the estimate. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay (nmol/min/m³); OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay (nmol/min/m³); FRC, functional residual capacity; LCI, lung clearance index; V_T, tidal volume; t_{PTEF}/t_E ratio of time to peak tidal expiratory flow to expiratory time.

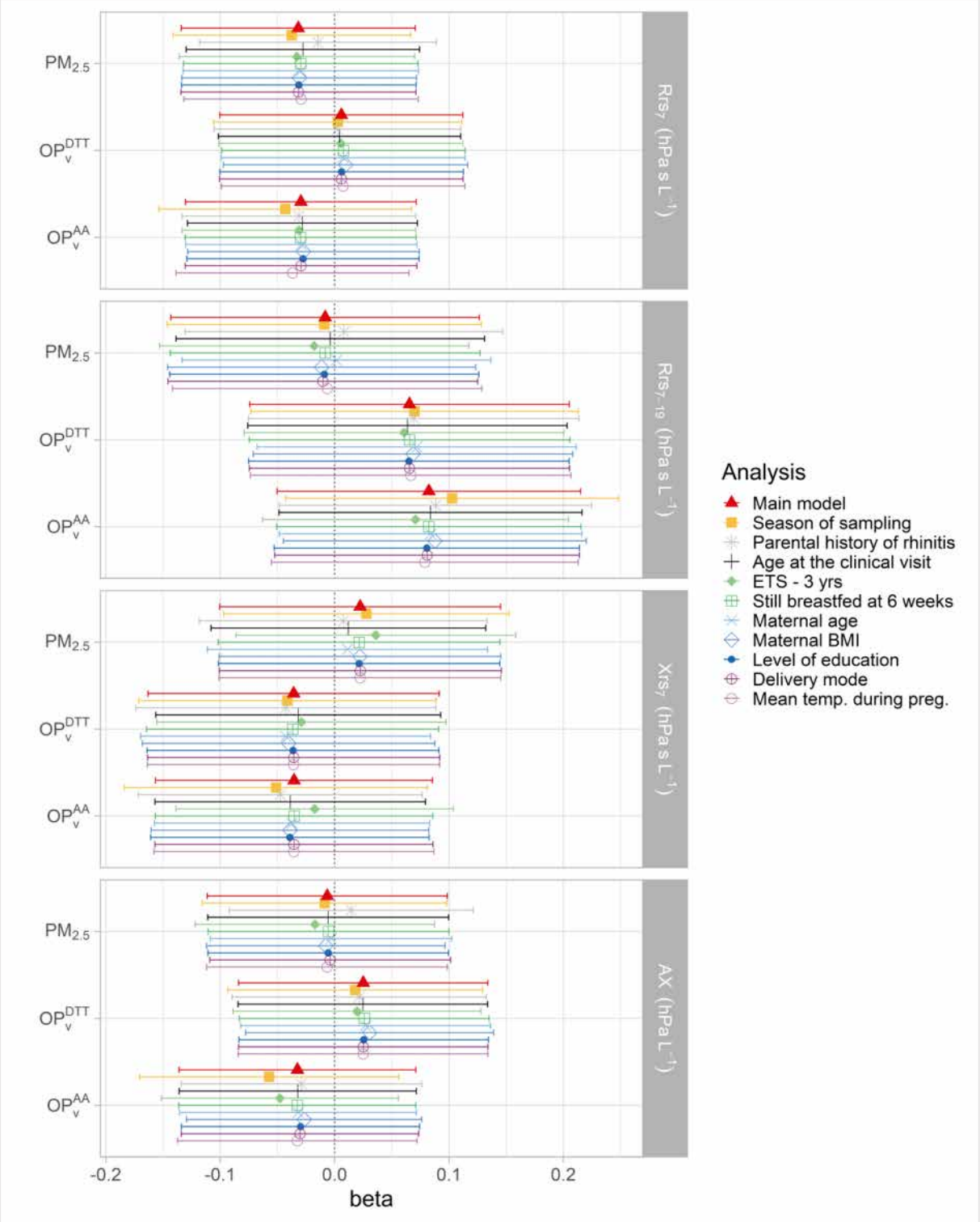

Figure S11. Effect of each confounder separately on the regression models at 3 years, adjusted for sex, height and weight, and comparison to the main model, adjusted on all the confounders listed.
Outcomes and exposures were scaled by their IQR. See Table S3 for corresponding numeric data. Whiskers represent the 95% confidence interval around the estimate. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay (nmol/min/m³); OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay (nmol/min/m³); Rrs_7, resistance at a frequency of 7 Hz; Rrs_{7-19}, difference between the resistance at 7 Hz and at 19 Hz; Xrs_7, reactance at a frequency of 7 Hz; AX, area under the reactance curve.

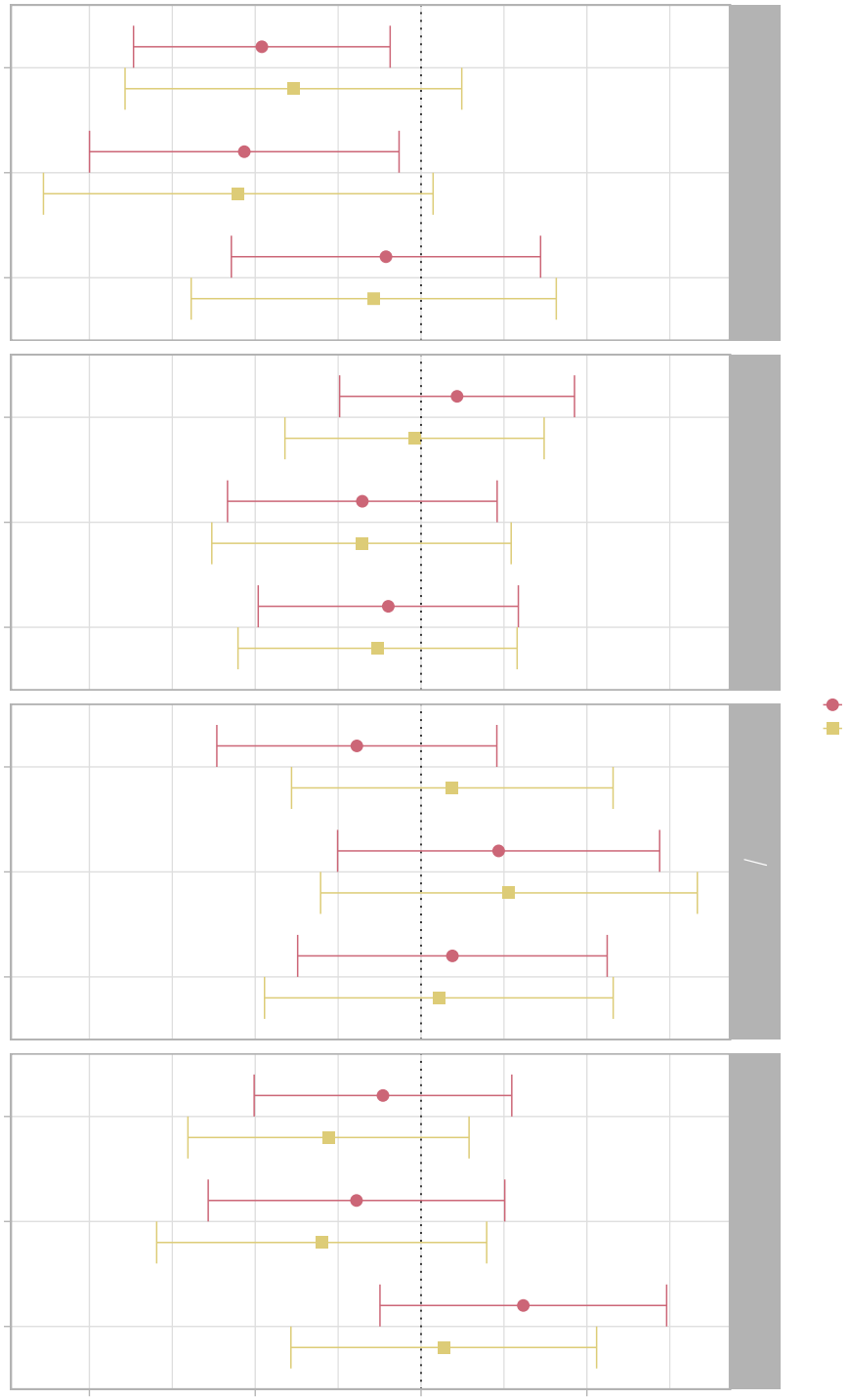

Figure S12. Association between personal exposure to $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA} during pregnancy and lung function parameters measured at 6 weeks in the multiple linear models and in the sensitivity analysis excluding leverage and influencing points, estimated by Cook's distance.
Outcomes and exposures were scaled by their IQR. See Table S4 for corresponding numeric data. Whiskers represent the 95% confidence interval around the estimate. Models were adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis and mean temperature during pregnancy. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay (nmol/min/m³); OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay (nmol/min/m³); FRC, functional residual capacity; LCI, lung clearance index; V_T, tidal volume; t_{PTEF}/t_E ratio of time to peak tidal expiratory flow to expiratory time.

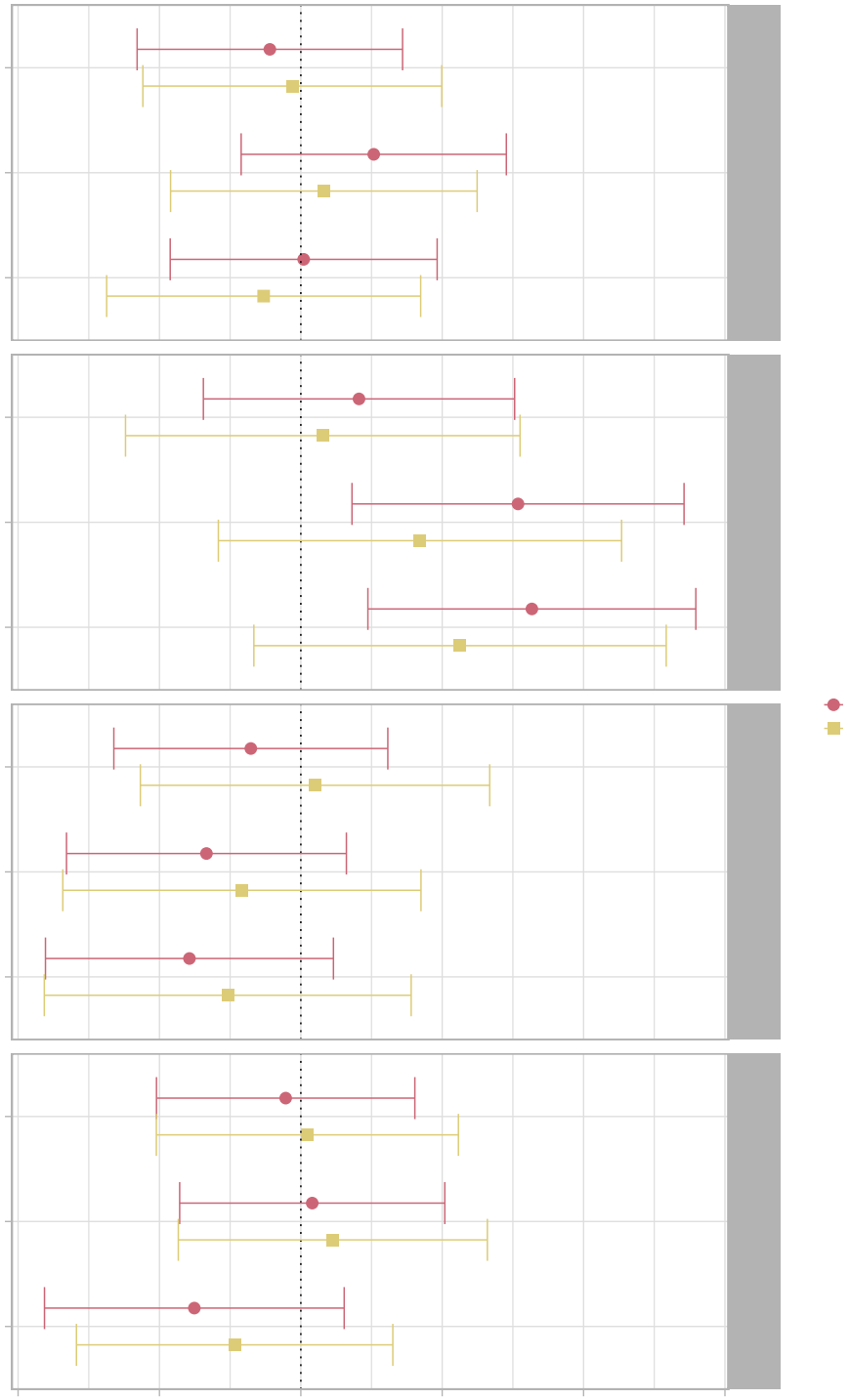

Figure S13. Association between personal exposure to $PM_{2.5}$, OP_v^{DTT} and OP_v^{AA} during pregnancy and lung function parameters measured at 3 years in the multiple linear models and in the sensitivity analysis excluding leverage and influencing points, estimated by Cook's distance.
Outcomes and exposures were scaled by their IQR. See Table S5 for corresponding numeric data. Whiskers represent the 95% confidence interval around the estimate. Model were adjusted on child's height, weight, sex, age, season of sampling, breastfeeding, environmental tobacco smoke, maternal age and BMI before pregnancy, parental level of education, parental history of rhinitis and mean temperature during pregnancy. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay (nmol/min/m³); OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay (nmol/min/m³); Rrs_7, resistance at a frequency of 7 Hz; $Rrs_{7\text{-}19}$, difference between the resistance at 7 Hz and at 19 Hz; Xrs_7, reactance at a frequency of 7 Hz; AX, area under the reactance curve.

Chapter IV

Effects of personal exposure to the oxidative potential of $PM_{2.5}$ on oxidative stress biomarkers in pregnant women

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*** Contributed equally**

Contribution: *This work was published by Science of The Total Environment. I was involved in the OP data curation and the investigation of biomarkers' variability regarding protocol variables. I performed the statistical analyses, generated the plots and wrote the first draft of the article. OP analysis was performed by the workforce of IGE's plateau AirOSol. Among the statistical methods presented, I beneficiated from the work of other IAB's team members and former students, and was able to use readily validated $PM_{2.5}$ mass concentration exposure.*

I. French summary

Contexte. Le stress oxydant est une des voies principales responsables des effets de l'exposition aux particules fines ($PM_{2.5}$) sur la santé. Le potentiel oxydant (PO) des PM est associé à plusieurs indicateurs de santé, mais son impact sur les biomarqueurs du stress oxydant reste insuffisamment étudié. L'exposition aux PM des femmes durant leur grossesse a de nombreux effets délétères sur la santé ultérieure de l'enfant, ce qui rend cette période particulièrement intéressante pour étudier les mécanismes biologiques sous-jacent. Par ailleurs, des études récentes ont examiné l'interaction entre la concentration massique de $PM_{2.5}$, et son potentiel oxydant intrinsèque (exprimé par microgramme de PM). Les résultats indiquent des effets plus forts des PM sur les issues de naissance et les hospitalisations pédiatriques pour des issues respiratoires, lorsque la capacité intrinsèque des PM à générer du stress oxydant était élevé. Il est nécessaire d'examiner si des résultats similaires sont obtenus au niveau moléculaire, et particulièrement sur le stress oxydant systémique de femmes enceintes.

Objectifs. Le principal objectif de cette étude est d'explorer l'exposition personnelle aux $PM_{2.5}$ et son potentiel oxydant en lien avec les niveaux de trois biomarqueurs urinaires du stress oxydant chez les femmes enceintes. L'objectif secondaire est d'évaluer si la concentration de $PM_{2.5}$ modifie les associations entre le PO et les biomarqueurs sélectionnés.

Méthodes. Trois cents femmes enceintes de la cohorte SEPAGES (Grenoble, France) ont porté des échantillonneurs personnels de $PM_{2.5}$ pendant une semaine, et le PO des PM a été mesuré à l'aide des tests de l'acide ascorbique (AA) et du dithiothréitol (DTT), puis normalisé par 1) la masse de $PM_{2.5}$ (PO_m), et 2) le volume d'air échantillonné (PO_v). Un pool urinaire composé de trois prélèvements

effectués le 7e jour de l'échantillonnage des PM a été analysé pour trois biomarqueurs du stress oxydant, à savoir la 8-hydroxy-2-désoxyguanosine (8-OHdG), le malondialdéhyde (MDA) et la 8-iso-prostaglandine-F2α (8-iso-PGF2α). La gravité spécifique urinaire a été mesurée dans le pool, et les niveaux de biomarqueurs ont été corrigés pour ce facteur, représentant le taux de dilution des échantillons, puis ln-transformés. Les associations ont été étudiées à l'aide de régressions linéaires multiples ajustées sur des caractéristiques des volontaires (niveau d'éducation, antécédents d'asthme et de rhinite, indice de masse corporelle avant la grossesse, âge, parité, tabagisme actif ou passif), des caractéristiques d'exposition (température moyenne durant la semaine de mesure des PM), des variables concernant les échantillons urinaires (âge gestationnel lors de la récolte d'urine, nombre d'échantillons dans le pool urinaire, temps de stockage à -80°C avant l'analyse). Les effets du PO des PM ont été également explorés en stratifiant par la concentration médiane de $PM_{2.5}$ (14 µg/m³).

Résultats. Dans les modèles principaux, aucune association n'a été observée avec la 8-iso-PGF2α, ni le MDA. Une augmentation d'un écart interquartile (IQR) de PO_m^{AA} a été associée à une augmentation de 6,2 % de la 8-OHdG (effet : 6,2 % ; intervalle de confiance à 95 % : 0,2 % à 13 %). Dans l'analyse stratifiée, l'exposition au PO_m^{AA} est associée à la 8-OHdG chez les participantes exposées à de faibles niveaux de $PM_{2.5}$ (effet : 11 % ; IC à 95 % : 3,3 % à 20 %), mais pas chez celles exposées à des niveaux élevés (effet : -1,0 % ; IC à 95 % : -11 % à 9,6 %). Les associations pour le PO_m^{DTT} présentent des résultats similaires (*p*-valeurs pour les termes d'interaction PO_m^{AA}-PM et PO_m^{DTT}-PM de 0,10 et 0,08, respectivement).

Conclusions. En résumé, nos résultats suggèrent que le PO_m^{AA} serait associé à des dommages oxydatifs à l'ADN. Cette association n'a pas été observée avec l'exposition à la concentration massique des $PM_{2.5}$. Les effets du PO_m^{AA} sur la 8-OHdG avaient tendance à être plus forts aux concentrations plus faibles de PM (inférieures à la médiane) par rapport aux concentrations plus élevées. Plus de recherches épidémiologiques, toxicologiques et en science des aérosols sont nécessaires pour approfondir la compréhension des effets du PO_m^{AA} sur la 8-OHdG, et de l'effet potentiel de la concentration massique des PM sur cette association.

II. Abstract

Oxidative stress is a prominent pathway for the health effects associated with fine particulate matter ($PM_{2.5}$) exposure. Oxidative potential (OP) of PM has been associated to several health endpoints, but its impact on biomarkers of oxidative stress remains insufficient. 300 pregnant women from the SEPAGES cohort (France) carried personal $PM_{2.5}$ samplers for a week and OP was measured using ascorbic acid (AA) and dithiothreitol (DTT) assays, and normalized by 1) $PM_{2.5}$ mass (OP_m) and 2) sampled air volume (OP_v). A pool of three urine spots collected on the 7th day of PM sampling was analyzed for biomarkers, namely 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA) and 8-iso-prostaglandin-F2α (8-iso-PGF2α). Associations were investigated using adjusted multiple linear regressions. OP effects were additionally investigated by stratifying by median $PM_{2.5}$ concentration (14 µg/m^3). In the main models, no association was observed with 8-iso-PGF2α, nor MDA. An interquartile range (IQR) increase in OP_m^{AA} exposure was associated with increased 8-OHdG (percent change: 6.2%; 95% CI: 0.2% to 12.6%). In the stratified analysis, exposure to OP_m^{AA} was associated to 8-OHdG for participants exposed to low levels of $PM_{2.5}$ (percent change: 11.4%; 95% CI: 3.3% to 20.1%), but not for those exposed to high levels (percent change: -1.0%; 95% CI: -10.6% to 9.6%). Associations for OP_m^{DTT} also followed a similar pattern (*p*-values for OP_m^{AA}-PM and OP_m^{DTT}-PM interaction terms were 0.12 and 0.11, respectively). Overall, our findings suggest that OP_m^{AA} may be associated with increased DNA oxidative damage. This association was not observed with $PM_{2.5}$ mass concentration exposure. The effects of OP_m^{AA} in 8-OHdG tended to be stronger at lower (below median) *vs.* higher concentrations of $PM_{2.5}$. Further epidemiological, toxicological and aerosol research are needed to further investigate the OP_m^{AA} effects on 8-OHdG and the potential modifying effect of PM mass concentration on this association.

III. Introduction

Exposure to particulate matter (PM) has been targeted as a major global public health risk factor for several years (Health Effects Institute, 2020; Murray et al., 2020; WHO, 2016), and pregnancy represents a specific window of susceptibility for the future child. One of the main suggested pathways for PM adverse health effects is oxidative stress, caused by a redox imbalance, triggering an inflammatory cascade (Crobeddu et al., 2017; Delfino et al., 2011; Kelly, 2003; Lodovici and Bigagli, 2011). Oxidative stress is caused by the PM capacity to carry or induce the formation of reactive oxygen species (ROS) *in vivo*, and can be measured by several cellular and acellular tests, called the oxidative potential (OP) of PM (Calas et al., 2017; Hellack et al., 2014). The intrinsic mass-normalized OP (OP_m) quantifies the OP of 1 µg of PM and is therefore representative of the reactivity of PM, whereas the volume-normalized OP_v (OP_m multiplied by $PM_{2.5}$ mass concentration), is a proxy for exposure levels to humans (Weichenthal et al., 2016).

Maternal exposure to PM during pregnancy has deleterious effects on the child's health (Bush, 2021), and recent studies provided first evidences of these effects, including adverse birth outcomes (Lavigne et al., 2018), altered fetal growth (Borlaza et al., 2022a), and decreased lung function (Marsal et al., 2023). The interaction between OP_m and $PM_{2.5}$ was investigated in few recent papers, and results indicated that $PM_{2.5}$ had stronger effects on adverse birth outcomes (Lavigne et al., 2018) and pediatric respiratory hospitalization (Korsiak et al., 2022) when its intrinsic potential to produce ROS was higher. Because redox-active species in $PM_{2.5}$ vary based on chemical composition and sources (Bates et al., 2019, 2015; Borlaza et al., 2021a), additional investigation on the combined effect of PM concentration and its OP on human health parameters, especially at the molecular level such as oxidative stress biomarkers, are required.

PM-induced ROS, by oxidizing cellular components, can trigger the release of oxidation products of both lipids and DNA. 8-hydroxy-2-deoxyguanosine (8-OHdG) is a biomarker of oxidative damage to DNA and can be used to quantify the fraction of DNA oxidized by reactive oxygen species (ROS). Its urinary concentration has been previously associated with exposure to PM (Ambroz et al., 2016; Hu et al., 2021; Li et al., 2020), to OP of concentrated ambient PM (Liu et al., 2018), and to OP of indoor dusts (Zhang et al., 2021). Malondialdehyde (MDA) and 8-isoprostane-F2α (8-iso-PGF2α, also known as 15-F2t-IsoP) are

products of lipid peroxidation caused by the oxidation of polyunsaturated fats by ROS. The oxidation of several polyunsaturated fatty acids can result in the formation of MDA, making it abundant in human urine, but arachidonic acid is the only precursor of isoprostanes. Previous studies showed that both 8-iso-PGF2α and MDA were significantly associated with exposure to PM (Bin et al. 2016; Hashemzadeh et al. 2019; He et al. 2020; Hu et al. 2021) and to the OP of PM (Liu et al., 2018). Using a controlled-exposure study design, one study found that higher OP_m^{AA} and OP_m^{GSH} (i.e. measured by the ascorbic acid or the glutathione assays) were respectively associated with increased urinary MDA and urinary 8-OHdG, (Liu et al., 2018). 8-OHdG, MDA, and 8-iso-PGF2α could mediate the health effects of PM exposures, since previous studies suggested that their levels in pregnant women were associated with both air pollution exposure (Ambroz et al., 2016; Nagiah et al., 2015) and adverse birth outcomes (Kim et al., 2005). However, to the best of our knowledge no study has specifically addressed the relative effect of both PM and its OP, assessed using personal dosimeter, on several biomarkers of oxidative stress in pregnant women.

The primary aim of the current study was to investigate the associations between personal exposure to $PM_{2.5}$ and its OP and levels of three urinary oxidative stress biomarkers in pregnant women. Our secondary aim was to evaluate the potential effect modification of $PM_{2.5}$ levels on the associations between OP and the selected oxidative stress biomarkers (OSB).

IV. Materials and methods

IV.1. Study design and population

The study participants were volunteers of the French SEPAGES (Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé; Assessment of air pollution exposure during pregnancy and effect on health, in English) mother-child cohort living within one hour driving distance from Grenoble city center. Details on the cohort design and protocol were made available previously (Lyon-Caen et al., 2019). Briefly, women were recruited in eight obstetrical ultrasonography practices in the Grenoble area (France), from July 2014 to July 2017. To be enrolled participants had to be pregnant with singleton pregnancies by <19 gestational weeks, aged >18, intending to deliver at one of the four maternity clinics in

the Grenoble area, and residing within a one-hour drive from Grenoble city center. In this study, the included women had one PM sampling weekly period during pregnancy, including a valid OP measurement and one pooled urine sample collected on the seventh day of the measurement week, resulting in 300 included pregnant women out of the 484 women enrolled in the cohort (Figure S1).

Participants signed an informed consent and the study protocol received approval from the French data privacy institution (Commission Nationale de l'Informatique et des Libertés, CNIL - n°914138) and the Comité de Protection des Personnes Sud-Est V (CPP - 2013-A01491-44).

IV.2. Personal exposure

Participants were asked to wear or keep at close proximity the active personal $PM_{2.5}$ samplers (MicroPEM, RTI International, Research Triangle Park, NC, USA) during 7 to 8 consecutive days. In this study, 300 samples were collected at a median gestational age of 19 weeks (min: 13, Q1: 17, Q3: 20, max: 29). Personal exposure to $PM_{2.5}$ mass concentration and OP was evaluated following the same methodology as previously published in the frame of SEPAGES (Borlaza et al., 2022a; Marsal et al., 2023). Briefly, $PM_{2.5}$ net mass (µg) was quantified via gravimetric analysis of the MicroPEM's internal filter before and after the sampling week (Mettler Toledo UMX2 ultramicrobalance), at the same hygrometric conditions (21°C, 25% relative humidity). Loaded Teflon® filters were cold-stored (-20°C) until OP analysis. OP was measured following the protocol established by Calas et al. (2018, 2017). Extracts at 10 µg/mL of $PM_{2.5}$ in a simulated lung fluid (Gamble, a mixture of salts, and 1,2-dipalmitoylphosphatidylcholine, pH=7.4) were subjected to vortex mixing at 37°C for 85 minutes prior to analysis using the dithiothreitol (DTT) and ascorbic acid (AA) assays. DTT is not a constituent of the lung lining fluid, but it is used as a surrogate for biological reducing agents, and it is sensitive to organic species and transition metals (Calas et al., 2018; Cho et al., 2005), while AA is one of the main antioxidants of the lung, and is mainly sensitive to transition metals (Ayres et al., 2008). All samples underwent triplicate analysis, and the results represent the mean of repeated measurements. To validate measurement accuracy, positive control tests were conducted, employing a 1,4-naphthoquinone (1,4-NQ) solution for both DTT and AA assays. Specifically, the DTT assay utilized a 40 µL solution of 24.7 µM stock, while the AA assay employed an 80 µL solution of 24.7 µM 1,4-NQ. Measurement quality, assessed by CV of positive control tests, yielded values below 4% for both OP assays.

For both assays, the consumption rate (nmol/min) was normalized by 1) the corresponding $PM_{2.5}$ mass (µg, OP_m), corresponding to the intrinsic oxidative potential of 1 µg $PM_{2.5}$ and 2) the air volume sampled for each filter (m^3, OP_v) to represent human exposure through inhalation. OP_v^{DTT} and OP_m^{DTT} correspond to the consumption of DTT (nmol/min/m^3 and nmol/min/µg respectively); OP_v^{AA} and OP_m^{AA} correspond to the consumption of AA (nmol/min/m^3 and nmol/min/µg respectively).

IV.3. Biomarkers of oxidative stress

Three non-fasting urine samples (morning, midday and evening) were collected by the participants on the 6th (2%) or 7th (98%) day of the PM sampling week and stored in their freezer (-20°C) (more information regarding urine collection is available in the supplement). Urines from the 6th day were used for the 6 participants (2%) who did not collect their urine on the 7th day. Some women collected only one (n=6, 2%) or two (n=29, 10%) urine samples. These women were included in the analyses and the statistical model accounted for this difference in urine sample between women by including a variable (2-class variable: <3 samples or 3 samples in the pool) as a cofactor. At the end of the air pollution measurement week, samples were picked up by the study field worker and transported to the certified biobank (bb-0033-00069) of the Grenoble University Hospital (CHU-GA), and were stored for a median (IQR) of 10.5 (6.6, 15.5) days at -20°C until pooled. Equal volumes of the three spot samples were used to get one equal-volume pool for each participant (Philippat and Calafat, 2021). For the pooling procedure, single urine samples were thawed overnight at 4°C. After pooling, aliquots of the pool were stored at -80°C for a median (IQR) of 5.9 (5.5, 6.3) years prior to biomarker analysis, during which they underwent one other thawing/freezing cycle.

Specific gravity (SG) of each sample was measured after pooling using a handheld digital refractometer (Atago-PAL-10S). Urinary malondialdehyde (MDA) was analyzed after diluting 20 µL urine in 980 µL water. 50 µL of 2,4-Dinitrophenylhydrazine (DNPH) derivatization solution and 20 µL of internal standard (MDA-d_2, 0.2 µg/mL) were added to 125 µL of the water-diluted urine. Calibration solutions (0.075-10 ng mL) were prepared in water, using MDA-tetrabutylammonium salt (Sigma-Aldrich, analytical standard) for making the mother solution. The MDA-DNPH derivative was analyzed by ultra-performance liquid chromatography mass spectrometry (UPLC-MS/MS Thermo Fischer Quantiva) by injecting 20 µL of each

sample on a Zorbax C18 Eclipse plus 100 x 2.1mm x 1.8µm (Agilent). Three replicates of one quality control (QC, standard at 1 ng/mL in water) were inserted in each series, containing about 80 samples.

8-OHdG and 8-iso-PGF2α were analyzed following a previously validated method (Sambiagio et al., 2021) with minor modifications. Both biomarkers were individually isolated using solid phase extraction (SPE HLB 200 mg/3 mL, Macherey Nagel). The SPE was loaded with 1 mL urine diluted with 1 mL of internal standard (8-OHdG ^{15}N, 2.5 ng/mL, Cambridge Isotope Laboratories; 8-iso-PGF2α-d_4, 5 ng/mL, Cayman Chemical). Calibration solutions (8-OHdG: 0.2-20 ng/mL; 8-iso-PGF2α: 0.05-4 ng/mL) were prepared in urine, previously cleaned through the SPE HLB. The extracts were analyzed by ultra-performance liquid chromatography mass spectrometry (UPLC-MS/MS Thermo Fischer Quantiva) by injecting 20 µL of each sample on an Acquity HSST3 150 x 2.1mm x 1.8µm (Waters). Two replicates of low concentration QC (calibration solution at 1.05 ng/mL for 8-OHdG and 0.17 ng/mL for 8-iso-PGF2α) and two replicates of high concentration QC (calibration solution at 10.42 ng/mL for 8-OHdG and 1.08 ng/mL for 8-iso-PGF2α) were added in each series, containing about 40 samples. Additional details regarding the sample preparation, instrumental conditions, limit of quantification (LOQ), limit of detection (LOD), repeatability, recovery and quality control are given in Supplementary material. All analyses were done at CURML-CHUV (Switzerland). For the statistical treatment, concentrations below the LOD or LOQ were replaced by LOD or LOQ divided by the square root of two, respectively.

Biomarker concentrations were corrected for specific gravity prior to analysis (MacPherson et al., 2018; van 't Erve et al., 2019) following: $[OSB]^i_{corr} = [OSB]^i * (median(SG) - 1)/(SG^i - 1)$; with [OSB]: concentration of oxidative stress biomarker, i: pool of urine, SG: specific gravity. SG-corrected concentrations had a skewed distribution and were transformed using natural logarithm.

IV.4. Statistical methods

Correlations between the exposure variables and between the different biomarker concentrations were investigated using Spearman's correlation coefficient. Univariate and multiple linear regression models were used to estimate the associations of personal exposure to $PM_{2.5}$ and OP (OP_v^{DTT}, OP_m^{DTT}, OP_v^{AA}, OP_m^{AA}) with SG-corrected and log-transformed concentrations of oxidative stress biomarkers. The percent change

and 95% confidence interval (CI) of the biomarker associated with one interquartile range (IQR) increase in personal exposure was retrieved from the model outputs.

Potential confounders were selected *a priori*, based on previous studies (Ambroz et al., 2016; Liu et al., 2018): 1) volunteer's characteristics: educational level (defined as the maximum number of studying years after high-school degree and expressed in three classes: up to 3 years, 4 years, 5 years or more;), history of asthma and history of rhinitis (both binary), body mass index (BMI) before pregnancy (continuous) and age (continuous), parity (3-class variable: nulliparous, primiparous, multiparous); active or passive smoking (yes/no at any time of the pregnancy); 2) exposure characteristics: mean temperature during the week of sampling (Hough et al., 2020) (continuous); 3) variables regarding the urine sample: gestational age at the urine collection (continuous); number of samples in the pool (binary: less than 3 *vs.* 3); storage time at -80°C before analysis (continuous) (see Table S6 for details regarding information collection in the covariates). In a fully adjusted model, we also considered the mean relative humidity during the week of sampling and the individual technician performing the pooling procedure, but neither were included in the final main model because the *p*-values were above 0.4 in all models. The set of confounders was reduced to the abovementioned list after analyzing the effect of each confounder separately on the association between exposure and outcome. The effects of the confounders were analysed by looking at the effect of each confounder separately (Figures S2, S3). Variance inflation factors (VIFs) were used to investigate multicollinearity in the model (all VIFs < 2, after choosing (1) mean temperature during the week of exposure over season of sampling, and (2) storage time over batch).

Multiple chained equation was used to impute missing covariate data (10% missing data) using the *R* package mice (van Buuren and Groothuis-Oudshoorn, 2011). This algorithm iteratively estimates missing values in a dataset, by creating a series of predictive models that account for the relationships between variables. Results of 10 imputed datasets were combined using Rubin's rule (Rubin, 1987). The missing completely at random (MCAR) hypothesis was checked by Little's test (Little, 1988) (*p*-values of the test > 0.05).

Several sensitivity analyses were performed, applying small modifications to the main model to test if results were robust: 1) the quality of the imputation was tested by performing a complete case analysis (i.e. excluding the 10% with missing data regarding covariates), 2) the influence of extreme exposure and

biomarker levels, that is common in driving linear regressions, was evaluated by excluding values below 1st percentile or above 99th percentile of the OSB and exposures (exclusion of 4% of the population in each analysis), 3) because smoking is a major source of oxidative stress, an analysis was conducted in non-smokers only (excluding current smokers, representing 6 to 7% of the population), and 4) the quality of the SG correction was tested by using raw concentrations, while SG was added in the co-factors, 5) because pool samples based on less than three urine samples could be affected by circadian variations of biomarkers, an analysis was conducted excluding women with pools of one or two urine samples (exclusion of 12% of the population).

To assess a potential effect modification of $PM_{2.5}$ levels on the associations between intrinsic OP and levels of oxidative stress biomarkers, we first conducted a stratified analysis, estimating OP effects separately in those with PM levels above and below the median. Then, we added an interaction term in the model between OP and categorical PM (below *vs.* above the median of 14 $\mu g/m^3$), to calculate the p-value for interaction.

All analyses were performed using *R* software (version 4.1.0).

V. Results

V.1. Description of the population

In this study, 300 pregnant women with one measurement of weekly personal exposure to $PM_{2.5}$ and OP and one urine pool sample collected at the end of the measurement week were included (62% of the enrolled women in the SEPAGES cohort). The median (IQR) age of the population was 32 (30, 35) and most of participants studied for at least 3 years after obtaining their French high school diploma (i.e., having at least an equivalent to BSc diploma). The PM personal sampling week took place equally in both cold (N=153, 51%) and warm (N=147, 49%) seasons. The included participants were mostly non-smokers (N=258, 94%), were in a healthy weight range (i.e. median (IQR) BMI of 21.6 (19.8, 24.1)) and had their urine samples collected at a median (IQR) gestational age of 19 (17, 20) weeks. The majority of participants (N=277, 92%) took supplemental minerals or vitamins at least once during pregnancy. As compared to the non-included women, those included had higher exposure to $PM_{2.5}$, higher levels of raw 8-OHdG and higher levels of 8-

iso-PGF2α corrected for specific gravity (The median (IQR) personal exposure was 14 (10, 18) µg/m^3, 1.41 (1.03, 1.97) nmol/min/m^3, 1.45 (0.90, 2.13) nmol/min/m^3, 0.11 (0.09, 0.14) nmol/min/µg and 0.11 (0.07, 0.16) nmol/min/µg for $PM_{2.5}$, OP_v^{DTT}, OP_v^{AA}, OP_m^{DTT} and OP_m^{AA}, respectively. Spearman's correlation coefficients between exposure variables are presented in Table S7. High correlations were found between $PM_{2.5}$ and OP_v^{DTT} (r_s=0.68, p<0.01), and between OP_m^{AA} and OP_m^{DTT} (r_s=0.65, p<0.01), but more moderate correlations were found between $PM_{2.5}$ and OP_v^{AA} (r_s=0.47, p<0.01). For both assays, OP_v was significantly higher for above median compared to below median $PM_{2.5}$ concentrations (Table S8, median $PM_{2.5}$ concentration of 14 µg/m^3), with a median of 1.91 *vs.* 1.10 nmol/min/m^3 and 1.90 *vs.* 1.20 nmol/min/m^3 for OP_v^{DTT} and OP_v^{AA} respectively (associated p-values<0.001). Intrinsic OP followed an inverse pattern, with a median of 0.10 *vs.* 0.12 nmol/min/µg for both OP_m (p<0.01 and p=0.04 for OP_m^{DTT} and OP_m^{AA}, respectively).

Table 10).

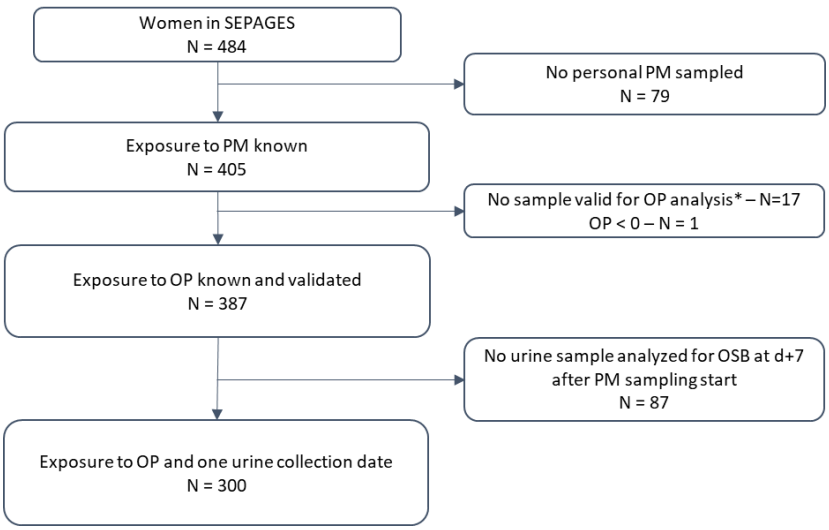

*Figure 25. Flow chart for the selection of the study population. Note: *$PM_{2.5}$ net weight < 4 µg.*

V.2. Description of exposure

The median (IQR) personal exposure was 14 (10, 18) µg/m^3, 1.41 (1.03, 1.97) nmol/min/m^3, 1.45 (0.90, 2.13) nmol/min/m^3, 0.11 (0.09, 0.14) nmol/min/µg and 0.11 (0.07, 0.16) nmol/min/µg for $PM_{2.5}$, OP_v^{DTT}, OP_v^{AA}, OP_m^{DTT} and OP_m^{AA}, respectively. Spearman's correlation coefficients between exposure variables are presented in Table S7. High correlations were found between $PM_{2.5}$ and OP_v^{DTT} (r_s=0.68, p<0.01), and between OP_m^{AA} and OP_m^{DTT} (r_s=0.65, p<0.01), but more moderate correlations were found between $PM_{2.5}$

and OP_v^{AA} (r_s=0.47, p<0.01). For both assays, OP_v was significantly higher for above median compared to below median $PM_{2.5}$ concentrations (Table S8, median $PM_{2.5}$ concentration of 14 µg/m^3), with a median of 1.91 *vs.* 1.10 nmol/min/m^3 and 1.90 *vs.* 1.20 nmol/min/m^3 for OP_v^{DTT} and OP_v^{AA} respectively (associated *p*-values<0.001). Intrinsic OP followed an inverse pattern, with a median of 0.10 *vs.* 0.12 nmol/min/µg for both OP_m (p<0.01 and p=0.04 for OP_m^{DTT} and OP_m^{AA}, respectively).

Table 10. Population description.

Characteristic	N	Excluded, N = 184	Included, N = 300	p-value[a]
$PM_{2.5}$ (µg/m^3)	**345**	**45**	**300**	**<0.001**
Median (IQR)		8 (3, 14)	14 (10, 18)	
Missing		139	0	
OP_v^{DTT} (nmol/min/m^3)	**313**	**13**	**300**	**0.4**
Median (IQR)		1.43 (0.81, 1.70)	1.41 (1.03, 1.97)	
Missing		171	0	
OP_v^{AA} (nmol/min/m^3)	**313**	**13**	**300**	**0.2**
Median (IQR)		1.26 (0.53, 1.84)	1.45 (0.90, 2.13)	
Missing		171	0	
OP_m^{DTT} (nmol/min/µg)	**313**	**13**	**300**	**0.2**
Median (IQR)		0.09 (0.08, 0.11)	0.11 (0.09, 0.14)	
Missing		171	0	
OP_m^{AA} (nmol/min/µg)	**313**	**13**	**300**	**0.3**
Median (IQR)		0.08 (0.06, 0.15)	0.11 (0.07, 0.16)	
Missing		171	0	
MDA_{raw} (ng/mL)	**471**	**171**	**300**	**>0.9**
Median (IQR)		55.7 (39.8, 77.4)	54.5 (33.9, 86.9)	
Geometric means (geometric SD)		53.7 (1.75)	54.8 (1.99)	
Missing		13	0	
$8\text{-}OHdG_{raw}$ (ng/mL)	**471**	**171**	**300**	**0.02**
Median (IQR)		3.20 (2.41, 4.27)	3.58 (2.49, 4.97)	
Geometric means (geometric SD)		3.16 (1.66)	3.52 (1.79)	
Missing		13	0	
8-iso-PGF2α_{raw} (ng/mL)	**471**	**171**	**300**	**0.7**
Median (IQR)		0.32 (0.22, 0.42)	0.31 (0.20, 0.44)	
Geometric means (geometric SD)		0.30 (1.65)	0.29 (1.79)	
Missing		13	0	
MDA_{corr} (ng/mL)[b]	**471**	**171**	**300**	**0.11**
Median (IQR)		60.2 (45.7, 75.0)	54.8 (39.8, 74.2)	
Geometric means (geometric SD)		59.4 (1.53)	57.8 (1.76)	
Missing		13	0	
$8\text{-}OHdG_{corr}$ (ng/mL)[b]	**471**	**171**	**300**	**0.10**
Median (IQR)		3.43 (2.73, 4.46)	3.75 (2.87, 4.83)	
Geometric means (geometric SD)		3.49 (1.49)	3.71 (1.53)	
Missing		13	0	
8-iso-PGF2α_{corr} (ng/mL)[b]	**471**	**171**	**300**	**0.01**
Median (IQR)		0.33 (0.26, 0.44)	0.31 (0.24, 0.39)	
Geometric means (geometric SD)		0.33 (1.46)	0.31 (1.49)	
Missing		13	0	
Gestational age at urine collection (weeks)	**343**	**44**	**299**	**<0.001**
Median (IQR)		20 (18, 21)	19 (17, 20)	
Missing		140	1	
Level of education	**482**	**184**	**298**	**0.07**
up to 3 years		36 (20%)	47 (16%)	
4 years		38 (21%)	89 (30%)	
5 years or more		110 (60%)	162 (54%)	
Missing		0	2	

Characteristic	N	Excluded, N = 184	Included, N = 300	p-value[a]
Active or passive smoking at any time of the pregnancy	**440**	**158**	**282**	**0.5**
No		129 (82%)	223 (96%)	
Yes		29 (18%)	59 (21%)	
Missing		26	18	
Parity	**484**	**184**	**300**	**0.07**
0 (nulliparous)		94 (51%)	128 (43%)	
1 (primiparous)		69 (38%)	145 (48%)	
2 or more (multiparous)		21 (11%)	27 (9%)	
Age	**484**	**184**	**300**	**0.9**
Median (IQR)		32.4 (29.5, 35.3)	32.1 (30.0, 35.1)	
BMI before pregnancy (kg m^{-2})	**480**	**183**	**297**	**0.4**
Median (IQR)		21.3 (19.7, 23.6)	21.6 (19.8, 24.1)	
Missing		1	3	
History of rhinitis	**484**	**184**	**300**	**0.2**
No		98 (53%)	177 (59%)	
Yes		86 (47%)	123 (41%)	
History of asthma	**484**	**184**	**300**	**0.2**
No		146 (79%)	252 (84%)	
Yes		38 (21%)	48 (16%)	
Mean temperature during the week of sampling (°C)	**331**	**31**	**300**	**0.4**
Median (IQR)		13 (8,18)	11 (6, 19)	
Missing		153	0	
Season of sampling	**313**	**13**	**300**	**0.05**
Cold		3 (23%)	153 (51%)	
Warm		10 (77%)	147 (49%)	
Missing		171	0	
Sample storage time at -80°C (yrs)	**401**	**170**	**300**	**<0.001**
Median (IQR)		7.00 (6.63, 7.31)	5.95 (5.50, 6.35)	
Missing		14	0	
Number of samples in the pool	**449**	**174**	**300**	**0.2**
3		162 (93%)	265 (88%)	
<3		12 (7%)	35 (12%)	
Missing		17	0	
Pool specific gravity	**474**	**174**	**300**	**0.15**
Median (IQR)		1.017 (1.013, 1.021)	1.018 (1.014, 1.023)	
Missing		10	0	
Vitamins/minerals consumption at any trimester of the pregnancy	**466**	**173**	**293**	**0.3**
Never		9 (5%)	16 (6%)	
At least during 1 trimester		164 (95%)	277 (94%)	
Missing		11	7	
Type of stove	**421**	**148**	**273**	**0.6**
Gas stove		66 (45%)	114 (42%)	
Electric stove		78 (53%)	154 (56%)	
Other		4 (2.7%)	5 (1.8%)	
Missing		36	27	

Note: $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_m^{DTT}, mass-normalized oxidative potential measured by the DTT assay; OP_m^{AA}, mass-normalized oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay; OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay.

[a]*p-value from Wilcoxon rank sum test, Pearson's Chi-squared test or Fisher's exact test comparing included and excluded population.*

[b]*biomarker concentration corrected for specific gravity*

V.3. Description of biomarkers

Raw OSB and SG-corrected OSB were highly correlated (Table 2, all $r_s \geq 0.75$, $p<0.01$). There was no correlation between the three OSB corrected for specific gravity ($r_s < 0.5$). MDA_{corr} was the most abundant biomarker, ranging from 7.4 ng/mL to 537 ng/mL with a median (IQR) of 55 (40, 74) ng/mL. 8-$OHdG_{corr}$ ranged from 0.77 ng/mL to 13.5 ng/mL with a median (IQR) of 3.75 (2.87, 4.83) ng/mL, while 8-iso-$PGF2\alpha_{corr}$ ranged from 0.06 ng/mL to 1.24 ng/mL with a median (IQR) of 0.31 (0.24, 0.39) ng/mL.

Table 11. Spearman correlation coefficients between the oxidative stress biomarkers (raw, and corrected for specific gravity).

	8-OHdG	**8-iso-PGF2α**	**MDA_{corr} [a]**	**8-$OHdG_{corr}$ [a]**	**8-iso-$PGF2\alpha_{corr}$ [a]**
MDA	0.43**	0.49**	0.80**	0.09	0.15
8-OHdG		0.64**	0.09	0.75**	0.32**
8-iso-PGF2α			0.12	0.28**	0.75**
MDA_{corr} [a]				0.10	0.10*
8-$OHdG_{corr}$ [a]					0.37**

*Note: **: p-value<0.01; *: p-value<0.05. [a]biomarker concentration corrected for specific gravity.*

V.4. Associations of $PM_{2.5}$ and OP with OSB

In the main models, we did not observe associations between exposure to $PM_{2.5}$ mass concentration or its OP and 8-iso-PGF2α or MDA. However, an IQR increase in exposure to OP_m^{AA} was significantly associated with increased levels of urinary 8-OHdG (percent change: 6.2%; 95% CI: 0.2% to 12.6%). The confounders mainly driving the differences between the univariate and the main analysis were the mean temperature during the sampling week (Figure S2, S3). The sensitivity analyses suggest that the results are overall robust to the influence of data imputation, active smoking, and are not influenced by the number of samples in the pool (Figure 26). The analysis excluding extreme values, the analysis testing SG correction show similar trends to the main model, but with slightly weaker effects that became not statistically significant. This could imply that few participants with extreme values (in the 1st percentile and 99th percentile of both 8-OHdG and OP_m^{AA}) partly drove the main model's effect size (percent change: 3.2%; 95% CI: -3.0% to 9.9%). A weak positive OP_v^{AA}-8-OHdG association can also be observed and was robust to the different sensitivity analyses (percent change: 3.7%, 95% CI: -1.7% to 9.4%). OP_m^{DTT} also presents a positive trend with 8-OHdG, that disappeared in the model excluding extreme values in exposure and outcomes.

The analyses stratified by $PM_{2.5}$ levels (below *vs.* above the median value of 14 $\mu g/m^3$) indicate that exposure to OP_m^{AA} was associated to 8-OHdG for participants exposed to levels of $PM_{2.5}$ below the median of 14 $\mu g/m^3$ (percent change: 11.4%; 95% CI: 3.3% to 20.1%), although no association was observed for participants exposed to above median levels of $PM_{2.5}$ (percent change: -1.0%; 95% CI: -10.6% to 9.6%, with OP_m^{AA}-PM interaction term *p*-value=0.12) (Figure 27, Tables S9, S10). Interestingly, a similar pattern was observed for OP_m^{DTT} (percent change: 7.1%; 95% CI: 0.7% to 14.2% *vs.* percent change: -4.9% ; 95% CI: -15.5% to 7.0% for low *vs.* high $PM_{2.5}$ strata, *p*-value for interaction = 0.11). There was a trend for positive association between 8-iso-PGF2α and exposure to OP_m^{AA} at below median levels of $PM_{2.5}$, and an inverse trend at above median levels of $PM_{2.5}$ was observed (*p*-value for interaction = 0.06). There was no evidence of an effect modification of PM level on the OP-MDA associations, regardless of the type of OP assay.

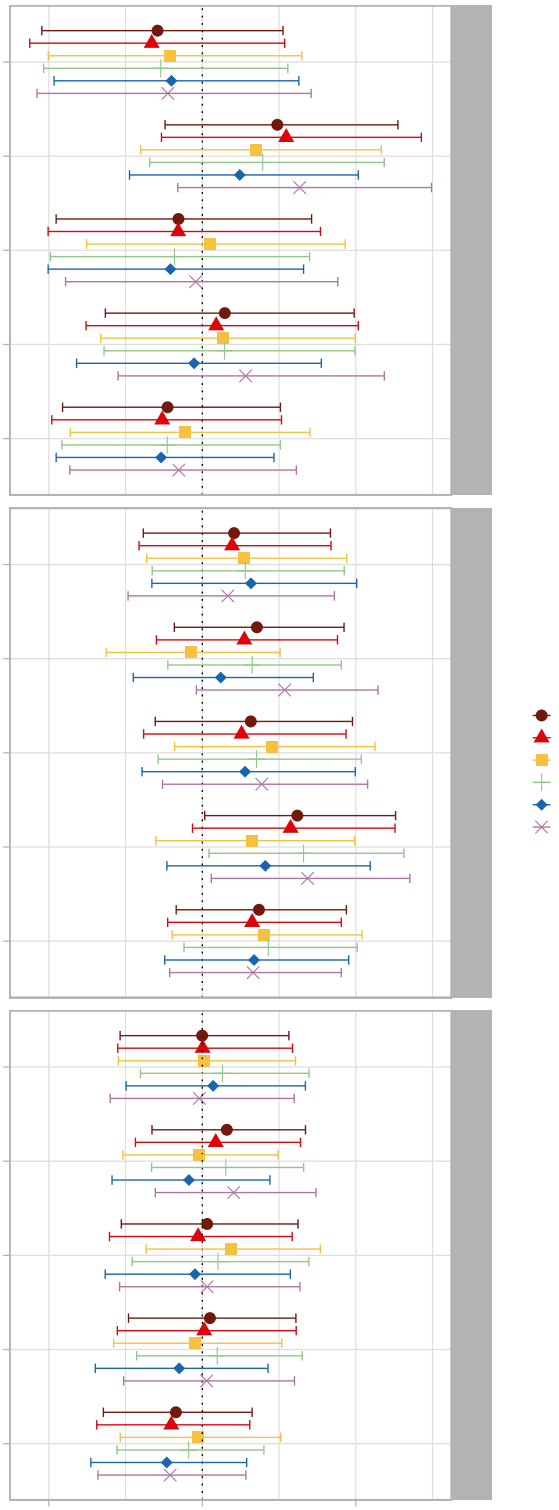

Figure 26. Association between personal exposure to $PM_{2.5}$, OP_m^{DTT}, OP_v^{DTT}, OP_m^{AA} and OP_v^{AA} and oxidative stress biomarkers in multiple linear models and in the sensitivity analyses.
Exposures were scaled by their IQR. See corresponding numeric data in Table S5. Whiskers represent the 95% confidence interval around the estimate. The main model was adjusted on educational level, history of rhinitis, history of asthma, age, BMI, parity, active or passive smoking, mean temperature during the sampling week, gestational age at urine collection, number of samples in the pool, storage time at -80°C before analysis. "Complete Cases" is an analysis excluding the 10% with missing data regarding covariates; "Excluding extreme values" are the analyses excluding the outcomes and exposures below the 1st percentile and above the 99th (excluding approx. 4% of the population); "Excluding smokers" is an analysis excluding approx. 6-7% of the population; "Not corrected for SG" is an analysis using raw concentrations of OSB, adding specific gravity in the confounders.

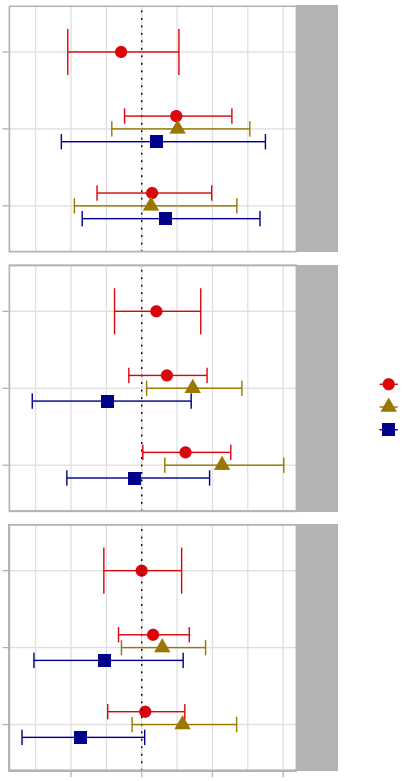

Figure 27. Association between personal exposure to $PM_{2.5}$, OP_m^{DTT} and OP_m^{AA} and oxidative stress biomarkers in multiple linear models and in the stratified analyses.
Exposures were scaled by their global IQR. Corresponding numeric data was made available in Table S6. Whiskers represent the 95% confidence interval around the estimate. Models were adjusted on educational level, history of rhinitis, history of asthma, age, BMI, parity, smoking, mean temperature during the sampling week, gestational age at urine collection, number of samples in the pool, storage time at -80°C before analysis. "Low PM concentration" are the analyses stratified on $PM_{2.5}$ concentrations below or equal to the median (14 µg/m³) and "High PM concentration" are the analyses stratified on $PM_{2.5}$ concentrations above the median.

VI. Discussion

To our knowledge, this study is the first to investigate the associations between personal exposure to the OP of $PM_{2.5}$ and three urinary biomarkers of oxidative stress in pregnant women (MDA, 8-OHdG and 8-iso-PGF2α). Overall, our findings suggest that OP_m^{AA} may be associated with higher 8-OHdG, whereas $PM_{2.5}$ mass concentration is not, and that the effects of OP_m^{AA} on 8-OHdG tend to be stronger at below compared to above median concentrations of $PM_{2.5}$.

VI.1. PM effects on OSB

In this study, exposure to $PM_{2.5}$ mass concentration was not associated with any of the considered OSB, which is partly in line with previous studies that have often yielded contrasting results. Ambroz et al. (2016)

compared urinary 8-OHdG and plasma 8-iso-PGF2α in pregnant women living in two cities with different ambient $PM_{2.5}$ levels. They found higher urinary 8-OHdG levels in women living in the most polluted city compared to the women living in the other city, but they did not identify any effect on 8-iso-PGF2α . In a population of 43 asthmatic children, He et al. (2020a) identified an association between 24-h personal $PM_{2.5}$ and airway oxidative stress, measured by MDA levels in nasal fluid, but not with systemic oxidative stress, measured by urinary MDA and 8-OHdG. In their meta-analysis, Li et al. (2020) reported overall heterogenous associations. They highlighted a positive, borderline significant pooled association between the increase in short-term exposure to ambient $PM_{2.5}$ and 8-OHdG levels, and a positive and statistically significant pooled association with increased MDA levels. These contrasting results could be attributable to differences in exposure assessment (ambient *vs.* personal), and to the duration of exposure (7-day average *vs.* 24-h). Another difference between studies lies in the sources contributing to PM, that most probably have various physicochemical properties in the different studies, since they were performed in different regions of the world. Some PM chemical constituents would induce more oxidative stress than others, which is supported by our findings showing associations for OP of PM exposure but not for $PM_{2.5}$ exposure. Sources with a larger proportion of redox-active species, that do not necessarily contribute to $PM_{2.5}$ mass concentration, would induce more systemic oxidative stress. By assessing personal exposure of 300 pregnant women, indoor sources are also included in the current study.

VI.2. OP effects on OSB

Our findings, showing positive association between OP_m^{AA} and urinary 8-OHdG levels, are partly in line with a controlled human exposure study using concentrated ambient particles (Liu et al., 2018). They found an association between 21-h post-exposure change in urinary 8-OHdG and the intrinsic OP levels, using the glutathione assay (OP_m^{GSH}). This association was also observed for $PM_{2.5}$ exposure, with weaker effect than for OP_m^{GSH}, but was not found for OP_m^{AA} exposure. Our study extends their findings by identifying an association between personal OP_m^{AA} and urinary 8-OHdG, in a population of 300 pregnant women exposed to realistic levels of $PM_{2.5}$. The association of OP_m^{AA} with 8-OHdG reflects an effect of the intrinsic toxicity of $PM_{2.5}$ on DNA damage. We do not have a clear explanation for this result, but a hypothesis is that the oxidant capacity of $PM_{2.5}$ is dominant compared to the amount of $PM_{2.5}$ inhaled for the negative effects on

DNA damage. Some specific $PM_{2.5}$ may act as catalysts, inducing physical damage to cellular and mitochondrial structures (Li et al., 2015), thus facilitating or enhancing oxidative stress on DNA.

In the current study's area (Grenoble), anthropogenic sources, such as biomass burning, primary traffic and industrial emissions constantly account for more than 70% of total PM_{10} OP_m^{AA} (Borlaza et al., 2021a). This contribution is expected to be similar or higher for OP measured in the $PM_{2.5}$ fraction since the natural sources contribute mostly to the coarser size fraction ($PM_{2.5}$-PM_{10}), while anthropogenic sources are mainly responsible of species emitted in the finer fractions of PM (Clements et al., 2014; Karagulian et al., 2015). The chemical composition of these sources is often rich in transition metals, which are species with low mass and size but with high specific surface area and therefore high reactivity (Borlaza et al., 2018; Charrier and Anastasio, 2012). These sources have been found to cause oxidative damage in DNA in a Chinese cohort of people working in a coking plant (Hu et al., 2021).

VI.3. OP^{AA} in epidemiological studies

Very few epidemiological studies have found significant associations using the OP^{AA} assay, while there is more epidemiological evidence for associations with the DTT and the GSH assays (Bates et al., 2019; He and Zhang, 2023). Contrary to these previous studies, we found that exposure-response associations were stronger with the AA assay, and the main models did not show any association with OP^{DTT}. Our results on OP^{AA} are consistent with our previous studies on lung function in children in the same cohort, which also reported a trend for decreased lung volumes with personal prenatal exposure to OP_v^{AA} (Marsal et al., 2023). In another study, exposure to OP^{AA} was found associated to increased markers of airway inflammation (Janssen et al., 2015).

While OP^{DTT} is sensitive to several organic species and metals, OP^{AA} is more sensitive to transition metals (Fe, Cu, Zn) (Calas et al., 2018; Grange et al., 2022; Pietrogrande et al., 2022). The use of both DTT and AA assays was shown to be complementary in several studies aiming to understand PM sources in different environments, since they were also sensitive to different PM sources (Daellenbach et al., 2020; Pietrogrande et al., 2019; Visentin et al., 2016; Weber et al., 2021). While it might be tempting to reduce investigations on OP on the assays for which a large amount of evidence on health effects can be found in the literature, this area of research is still relatively new and more studies are needed to fully understand the differences

between both assays in terms of health effects, and at the molecular level. Moreover, it should be noted that there is currently no standardized protocol to measure OP, regardless of the assay used. It is therefore more complex to compare studies between each other. Finally, this study is based on personal exposure, which is rarely the case in other OP-health studies; different sources and chemical species could therefore be potentially captured indoors by OP^{AA} compared to OP^{DTT}. However, to the best of our knowledge, no study specifically compared OP^{AA} and OP^{DTT} in indoor environments.

VI.4. Effect modification by $PM_{2.5}$

Our study indicates a potential modifying role of $PM_{2.5}$ concentration on associations between OP and OSB, characterized by stronger effects of OP_m^{AA} and OP_m^{DTT} in 8-OHdG and 8-iso-PGF2α in the context of low (lower than 14 µg/m^3) *vs.* high (higher than 14 µg/m^3) $PM_{2.5}$ mass concentration. Previous studies inversely investigated $PM_{2.5}$ effects in OP strata, and were able to show that $PM_{2.5}$ effects on several endpoints were stronger at higher OP_m levels (Korsiak et al., 2022; Lavigne et al., 2018; Tonne et al., 2012). Our findings suggest stronger effects of OP_m^{AA} on 8-OHdG for $PM_{2.5}$ at low $PM_{2.5}$ levels, contradicting some of the previous studies. However, our results support recent findings reporting a steeper slope of $PM_{2.5}$ effects on mortality (Stafoggia et al., 2022; Weichenthal et al., 2022) and asthma onset (Liu et al., 2021) at low levels of $PM_{2.5}$ exposure. These studies aimed at investigating $PM_{2.5}$ effects at ambient levels lower than the WHO 2005 and 2021 guidelines, i.e. 10 µg/m^3 and 5 µg/m^3 respectively. Here, we considered levels below the median $PM_{2.5}$ personal exposure of the study participants, i.e. 14 µg/m^3, but the slopes of the concentration-response curve proposed by Stafoggia et al. (2022), Weichenthal et al. (2022), and Liu et al. (2021) seem to remain steeper than high concentrations of $PM_{2.5}$ up to 15 to 20 µg/m^3. The higher intrinsic OP activity at lower $PM_{2.5}$ mass concentration is not easy to explain without an exhaustive chemical characterization of PM, but such result was already highlighted in previous studies in ambient PM (Campbell et al., 2021; Wang et al., 2020). A possible explanation would be that participants with below-median $PM_{2.5}$ levels are exposed to finer particles with little contribution to PM mass concentration but with high OP. A greater portion of redox-inactive species may contribute to $PM_{2.5}$ of women with above-median $PM_{2.5}$ levels. Another hypothesis involves antagonist chemical reactions between PM constituents, such as organic species and metals (Borlaza et al., 2021a; Pietrogrande et al., 2022; Samake et al., 2017). Participants with above-median

PM levels would be exposed to particles that would react either with each other or with assay constituents, reducing the measured OP. In such cases, particles contributing mainly to PM mass would cause additional chelation phenomena, altering the redox properties of chelated species. This, in turn, would result in lower OP levels in the higher stratum of PM levels compared to the lower stratum.

VI.5. Strengths & Limitations

This study is unique because it benefits from measurements of personal exposure to $PM_{2.5}$ and two OP assays on pregnant volunteers, with subsequent analysis of OSB levels in urine.

In this study, associations between $PM_{2.5}$ and its OP and oxidative stress biomarkers were estimated using a personal integrated 7-day sample for $PM_{2.5}$ and OP, and a pooled urine sample collected on the last day of the measurement week. The major strength of this study relies in the assessment of exposure and OSB. Using personal exposure integrates the variety of environments to which participants were exposed, and is therefore more representative of their true exposure than exposure assessments relying on ambient levels only, particularly when considering short-term effects. As for OSB, the use of a pooled urine sample with the 3 urine samples collected on the 7th day reduces intra-day variability of biomarkers, that was reported in some studies for 8-iso-PGF2α, with higher levels in the first urine void (Pelletier et al., 2017) and for 8-OHdG (Kanabrocki et al., 2002) that has a marked circadian cycle. Although pooling procedure aims to reduce biomarker measurement error, we cannot guaranty that all participants collected their urine at the same time of the day as requested by the protocol, which could have introduced measurement error. The heterogeneity in the findings from the few studies assessing exposure to $PM_{2.5}$ and its OP can be due to the differences in exposure assessment, duration and concentrations, as well as the biofluids used to measure biomarkers. Previous studies considered different lags in the effects of $PM_{2.5}$ on OSB levels, and there is no consensus on a gold-standard duration to consider between exposure assessment and urine collection. Associations between exposure to $PM_{2.5}$ and urinary 8-OHdG at lags 0, 1, 2, 3 days and at lags 0, 1, 2 days for urinary MDA were reported (Gong et al., 2014). However, Pelletier et al. (2017) did not find significant associations between $PM_{2.5}$ and urinary 8-OHdG, MDA and 8-iso-PGF2α, at lags 0, 1 and 2 days in sites with $PM_{2.5}$ levels below the personal exposure levels of the present study. Effect dilution by considering a 7-day

integrated sample cannot be totally ruled out and could potentially have resulted in underestimating the associations reported in this study.

In this study, we analyzed OSB in urine, but previous studies found different association patterns with MDA in nasal fluids or exhaled breath condensate and with urinary MDA (Gong et al. 2014; He et al. 2020). Using biofluids from the upper-respiratory tract could lead to clearer associations, because the OSB levels in these biofluids would most likely be caused by air pollutant exposure, however urinary OSB levels are more representative of the systemic effect of exposure to $PM_{2.5}$ and OP. Previous studies investigated the stability over storage of urinary MDA, 8-OHdG and 8-iso-PGF2α at -20°C, and found no effect after 3 months for 8-OHdG and 8-iso-PGF2α (Martinez and Kannan, 2018), and mixed results for MDA depending on the study, but with a potential decrease (Martinez and Kannan, 2018; Weitner et al., 2016). No study specifically addressed this point over several years at -80°C, and to account for a potential effect of storage duration in the estimated associations, we included this variable as a confounding factor.

In the SEPAGES cohort, urine dilution was measured relying on SG, and creatinine was not measured because it can be affected by individuals' characteristics (Barr et al., 2005). SG-correction is traditionally applied in spot urine samples, but Philippat et al. (2021) showed that this method was not necessarily relevant in pool samples, since it lowered correlation coefficients with the gold-standard method for some chemical species. Unfortunately, to the best of our knowledge, no study particularly investigated the relevance of SG-correction in pooled urine samples for OSB. The sensitivity analysis using raw outcomes showed a trend for OP_m^{AA}-8-OHdG association, that was not significant. This correction should therefore be considered cautiously.

Although the design of the study allowed to adjust for a number of confounding factors, residual confounding is a potential limitation of the study. Moreover, studies report contradictory influence of vitamin and minerals supplementation on oxidative stress (Moller and Loft, 2006; Sley et al., 2020; van 't Erve, 2018), but we could not perform an analysis taking this factor into account, since only 16 participants were not supplemented. In fact, in France, most pregnant women take some supplementation in vitamins and minerals. Similarly, a potential confounding by endogenous antioxidant levels cannot be totally ruled out. Our results were not adjusted on the natural variation of melatonin levels (measures not available in SEPAGES), which

might be a confounder in the associations between short-term air pollution exposure and urinary 8-OHdG and MDA (He et al., 2020).

A selection bias cannot be totally ruled out. Indeed, included participants tended to have higher exposure to $PM_{2.5}$ and higher levels of SG-corrected 8-iso-PGF2α, potentially leading to the overestimation of the $PM_{2.5}$-8-iso-PGF2α association. But we did not observe this association. Nevertheless, since there was no significant difference in OP levels between the included and excluded participants, it is unlikely that the reported association with OP were influenced by selection bias.

VII. Conclusion

In conclusion, this study highlighted that the capacity of $PM_{2.5}$ to generate ROS in a simulated lung fluid, measured by the AA assay was associated with DNA damage in pregnant women. These results are important because future child's health could potentially be affected. Although the clear mechanisms remain unknown, this study further underlines the importance of the OP of $PM_{2.5}$ in epidemiological studies and we recommend future studies in epidemiology, toxicology and aerosol sciences to investigate the role of key chemical tracers in OP. This will aid in better understanding the underlying biological mechanisms, particularly in the context of low $PM_{2.5}$ mass concentrations.

VIII. Acknowledgment

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IX. Supplemental Material

IX.1. List of Figures

IX.2. List of Tables

Urine sampling and handling

In addition to the effects of pregnancy exposure to air pollution on mother and child health, the SEPAGES cohort aims to assess the effect of exposure to chemical pollution, mainly phenols and phthalates. Because urine phenols and phthalate metabolites have short half-lives (thus important intra-day variability), the design of urine sampling consisted in 3 urine samples per day during 2 weeks (one in trimester 2 and one in trimester 3) and the pooling of daily samples to limit measurement errors related to the metabolites (Perrier et al., 2016; Vernet et al., 2019).

Urine samples were collected by the women at their homes in polypropylene tubes, and were stored in their freezers until the end of the measurement week. Trained field workers retrieved and transported the samples to the certified biobank (bb-0033-00069) of the Grenoble University Hospital, where they were stored at -20°C until pooling analysis. Since measurement weeks were not simultaneous, urine samples were

systematically treated individually (i.e. all samples of one participant each time) during the pooling procedure, therefore being at very low risk of cross-contamination.

Quality assurance/quality control for biomarkers analysis

MDA analysis

The DNPH derivatization solution was prepared using a commercially available cartridge (LpDNPH S10, Supelco) eluted with 3.8 mL acetonitrile. This solution was then acidified with 200 µL acetic acid 25% (LCMS grade) and diluted to a total volume of 10 mL with H_2O (LCMS grade). 50 µL of this reactive was added in each urine sample to derivatize the MDA at ambient temperature for 2 hours. This procedure allowed to get blank chromatograms with the lowest signal compared to derivatization solutions prepared with solid DNPH.

MDA-DNPH derivatives were separated using a Zorbax C18 Eclipse plus 100 x 2.1mm x 1.8µm and analyzed in positive electrospray ionization (ESI+) mode, optimized at 3700 V between 2-8.5 min. The temperature of the vaporizer and of the ion transfer tube were maintained at 350°C and 390°C, respectively. The mobile phases were MilliQ water (A) and methanol/acetonitrile (70/30, v/v) (B), each with 0.1% acetic acid. The LC flow was set at 0.25 mL/min, and the column temperature at 30°C. The elution gradient was set: 0% B at 0-1.1 min, 55% B at 1.1 min, 65% B at 5 min, 90% B at 5.5 min, and 0% B at 8 min. In these conditions, the retention time of the MDA-DNPH is about 7.3 min. Ion detection conditions are described in Table S8. For each analytical series, 3 replicates of the QC sample (1.0 ng/mL MDA in water) were inserted. The averaged measured QC concentrations was 1.01 ± 0.04 ng/mL over the 22 analytical series. For each analytical series, 3 replicates of the QC sample (1.0 ng/mL MDA in water) were inserted. The averaged measured QC concentrations was 1.01 ± 0.04 ng/mL over the 22 analytical series.

Table S8. Ion detection conditions for MDA analysis.

	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
MDA	235.03	143.07	23.6
	235.03	159.07	20.5
	235.03	189.0	16.3
MDA-d_2	237.03	145.07	23.6
	237.03	161.07	20.5
	237.03	191.0	16.3

8-OHdG and 8-isoprostane analysis

Both biomarkers were isolated sequentially from urine using a conditioned SPE (HLB 200 mg/3mL) with 2 mL methanol (MeOH) and 2 mL H_2O. After loading the urine sample on the SPE, a washing step with 2 x 2 mL H_2O/MeOH 90/10 was done. Then, the 8-OHdG was eluted using 2 x 1 mL H_2O 0.1% acetic acid/MeOH 75/25 and filtrated through a PTFE filter with pores of 0.45 µm. This extract, containing the 8-OHdG, was directly analyzed without any additional treatment. The SPE was further washed with 2 x 2 mL H_2O/MeOH 50/50 and dried under nitrogen. The 8-iso-PGF2α was then eluted using 2 x 2 mL of acetone and filtrated through a PTFE filter with pores of 0.45 µm. This extract was concentrated to dryness (40°C, 3 psi under nitrogen) and redissolved in 250 µL H_2O containing 0.1% acetic acid (LCMS grade). Finally, both extracts were independently analyzed on the UPLC-MSMS system as described here under. As matrix effects might be important for the analysis of these two biomarkers in urine, the use of internal standard is mandatory (Sambiagio et al., 2021). In addition, the calibration standards were prepared in real urine which was passed through the SPE HLB cartridge before use, to ensure the absence of both biomarkers and other potential interferents. The blank corresponded to this cleaned urine, not spiked with standards.

8-OHdG was separated on the Acquity HSST3 150 x 2.1mm x 1.8µm, and analyzed in positive electrospray ionization (ESI+) mode, optimized at 3700 V between 0.1-10 min. The temperature of the vaporizer and of the ion transfer tube were both maintained at 350°C. The mobile phases were MilliQ water 0.1% acetic acid (A) and MilliQ water (B), and acetonitrile (C). The LC flow was set at 0.25 mL/min, and the column temperature at 35°C. The elution gradient was set: 90% B, 0% C at 0 min, 10% B, 80% C at 5.5 min, and 90% B, 0% C at 7.6 min. In these conditions, the retention time of the 8-OHdG is about 6.8 min. Ion detection conditions are described in Table S9.

For each analytical series, 3 replicates of the low and high QC samples (1.05 ng/mL and 10.42 ng/mL 8-OHdG in urine, respectively) were inserted. The averaged measured low QC concentration was 1.05 ± 0.05 ng/mL (n=24), and the high QC concentrations 10.52 ± 0.33 ng/mL (n=24).

Table S9. Ion detection conditions for 8-OHdG analysis.

	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)
8-OHdG	284.1	140.0	28.8	37
	284.1	168.1	10.0	37
	284.1	243.0	10.2	37
8-OHdG ^{15}N	289.1	173.1	10.0	40
	289.1	248.0	10.2	40

8-iso-PGF2α was separated on the Acquity HSST3 150 x 2.1mm x 1.8µm, and analyzed in negative electrospray ionization (ESI-) mode, optimized at 3400 V between 7-16 min. The temperature of the vaporizer and of the ion transfer tube were both maintained at 350°C. The mobile phases were MilliQ water with 0.1 acetic acid (A) and acetonitrile (B). The LC flow was set at 0.30 mL/min, and the column temperature at 35°C. The elution gradient was set: 25% B at 0 min, 40% B at 20 min, 90% B at 26 min, and 20% B at 27 min. In these conditions the retention time of the 8-iso-PGF2α is about 14.6 min. Ion detection conditions are described in Table S10.

Table S10. Ion detection conditions for 8-iso-PGF2α analysis.

	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)
8-iso-PGF2α	353.2	193.11	25.0	80
	353.2	290.97	20.0	80
	353.2	309.24	20.0	80
8-iso-PGF2α-d_4	357.2	197.11	25.0	78
	357.2	295.11	20.0	78
	357.2	313.17	19.0	78

For each analytical series, 3 replicates of the low and high QC samples (0.17 ng/mL and 1.08 ng/mL 8-iso-PGF2α in urine, respectively) were inserted. The averaged measured low QC concentration was 0.167 ± 0.017 ng/mL (n=24), and the high QC concentrations 1.063 ± 0.057 ng/mL (n=24).

Based on the low and high QC, several analytical parameters were determined (Table S11).

Table S11. Analytical parameters based on the low- and high-quality controls.

	MDA (ng/mL)	8-OHdG (ng/mL)	8-iso-PGF2α (ng/mL)
LOD	0.025	0.2	0.05
LOQ	0.075	0.5	0.10
Repeatability low QC [%]	3.8	4.9	10.2
Repeatability high QC [%]	-	3.1	5.3
Recovery low QC [%]	101.2	100.5	98.3
Recovery high QC [%]	-	101.0	98.4

The first standard solution used for the calibration corresponded to the LOQ. This level presented a variability lower than 30% in all cases. The LOD was calculated based on the LOQ and corresponds to 1/3 LOQ.

Table S12. Number and percentage of samples detected (≥ LOD) and quantified (≥ LOQ) for each oxidative stress biomarkers.

	MDA	8-OHdG	8-iso-PGF2α
≥ LOD	300 (100%)	300 (100%)	299 (99.6%)
≥ LOQ	300 (100%)	299 (99.6%)	294 (98%)

Description of population and covariates

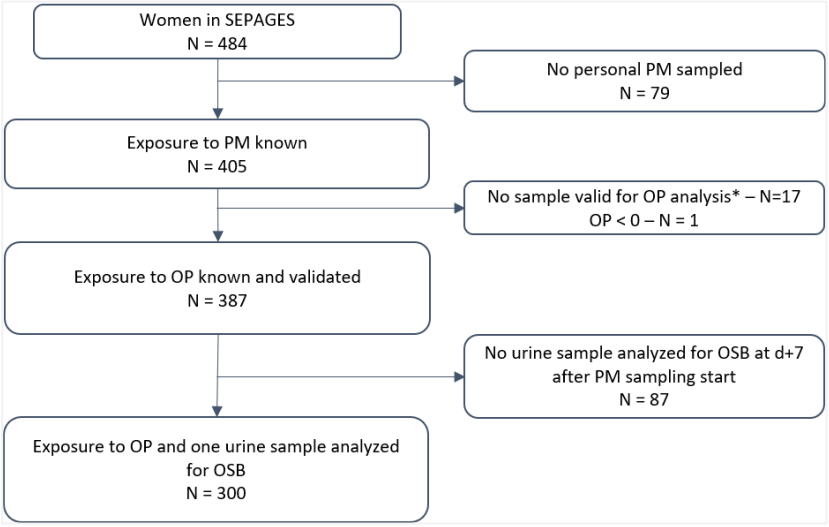

Figure S14. Flowchart of the population selection.

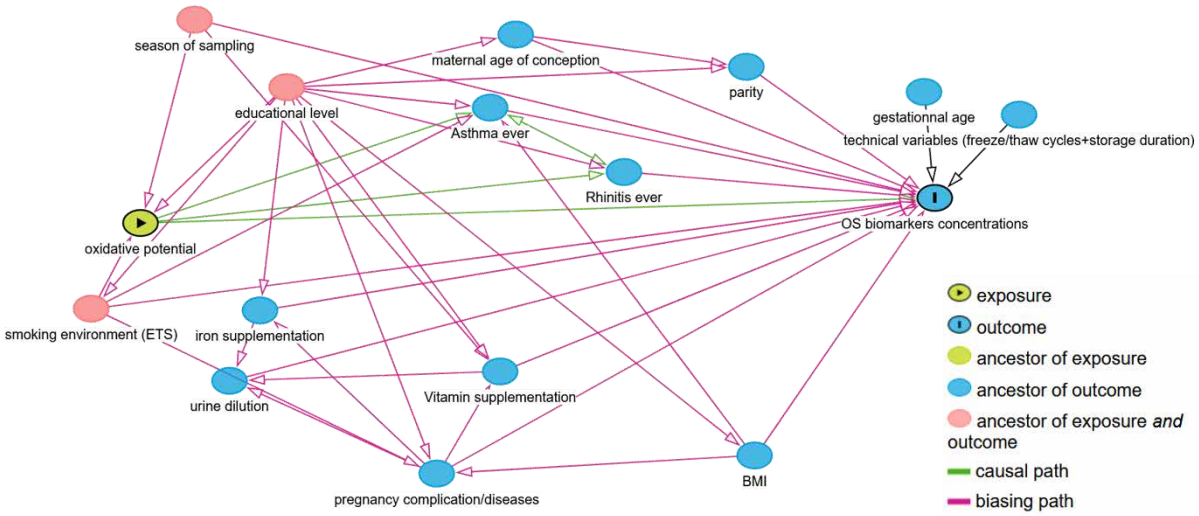

Figure S15. Directed Acyclic Graph for the potential covariates.

Table S13. Description of covariates in the multiple regression models.

Potential confounders in the main model	Variable type	Details regarding information collection
educational level - defined as the maximum number of studying years after high-school degree	3-class variable: up to 3 years, 4 years, 5 years or more	self-reported through a self-administrated questionnaire
history of asthma	binary	self-reported through a self-administrated questionnaire
history of rhinitis	binary	self-reported through a self-administrated questionnaire
body mass index (BMI) before pregnancy	continuous	calculated based on self-reported weight before pregnancy and height measured by a clinical research assistant during a SEPAGES clinical visit
age	continuous	calculated with the date of birth self-reported by a questionnaire administrated by a clinical research assistant
parity	3-class variable: 0, nulliparous/1, primiparous/2, multiparous;	reported by a self-administrated questionnaire
Active or passive smoking	Binary	assessed by several self-administrated questionnaires during the pregnancy
mean temperature during the week of sampling	continuous	assessed at home address by a hybrid model (Hough et al., 2020)
gestational age at the urine collection	continuous	calculated based on the difference in weeks between gestational duration and date of the urine collection
number of samples in the pool	binary: less than 3 *vs.* 3	reported by the laboratory technician performing the pooling
storage time at -80°C before analysis	continuous	calculated from the date the samples were sent for analysis and the date the storage started

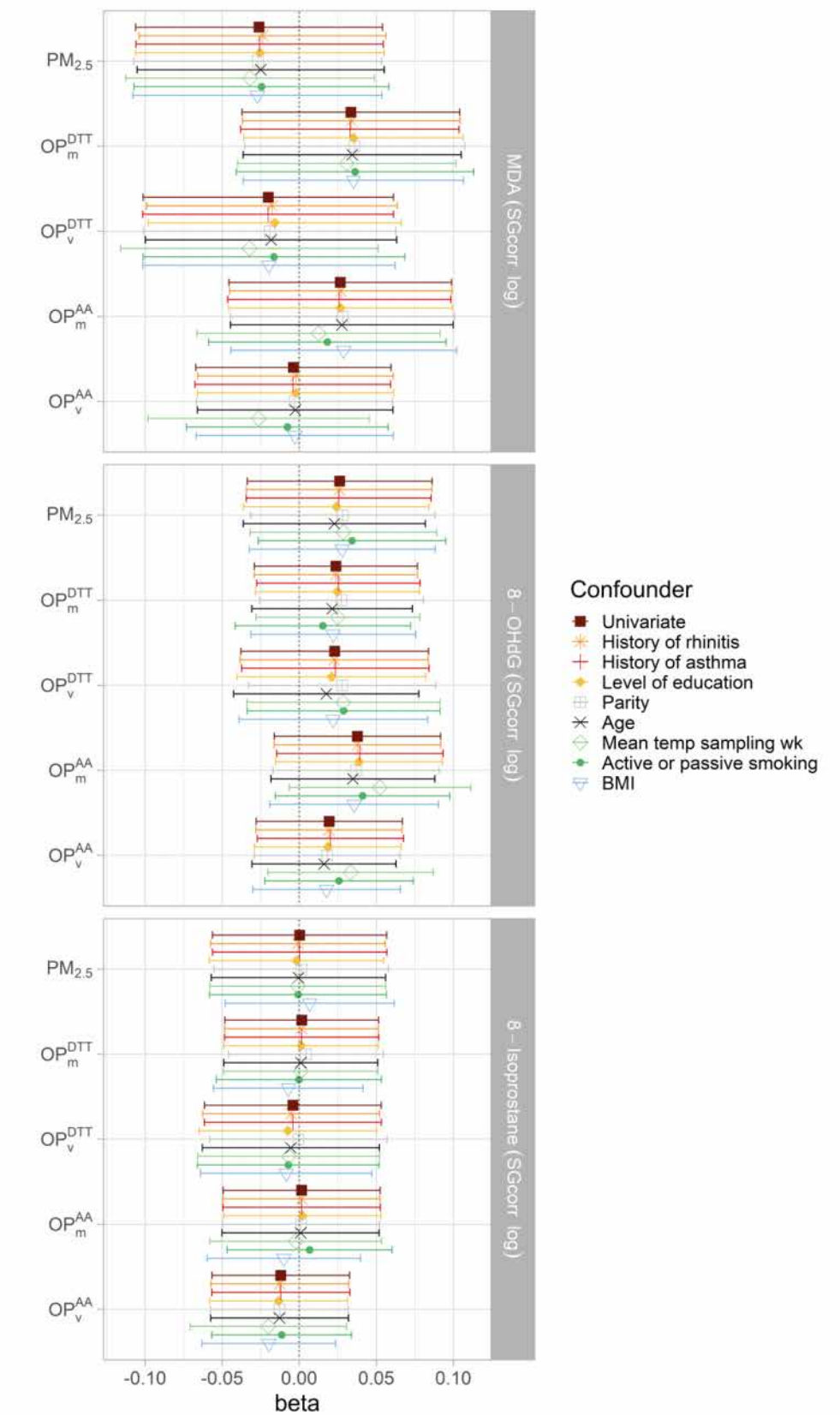

Figure S16. Effect of each confounder related to participants' characteristics separately on the regression models.
Outcomes and exposures were scaled by their IQR. Whiskers represent the 95% confidence interval around the estimate. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 μm (μg/m³); OP_m^{DTT}, mass-normalized oxidative potential measured by the DTT assay (nmol/min/μg); OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay (nmol/min/m³);); OP_m^{AA}, mass-normalized oxidative potential measured by the AA assay (nmol/min/μg); OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay (nmol/min/m³).

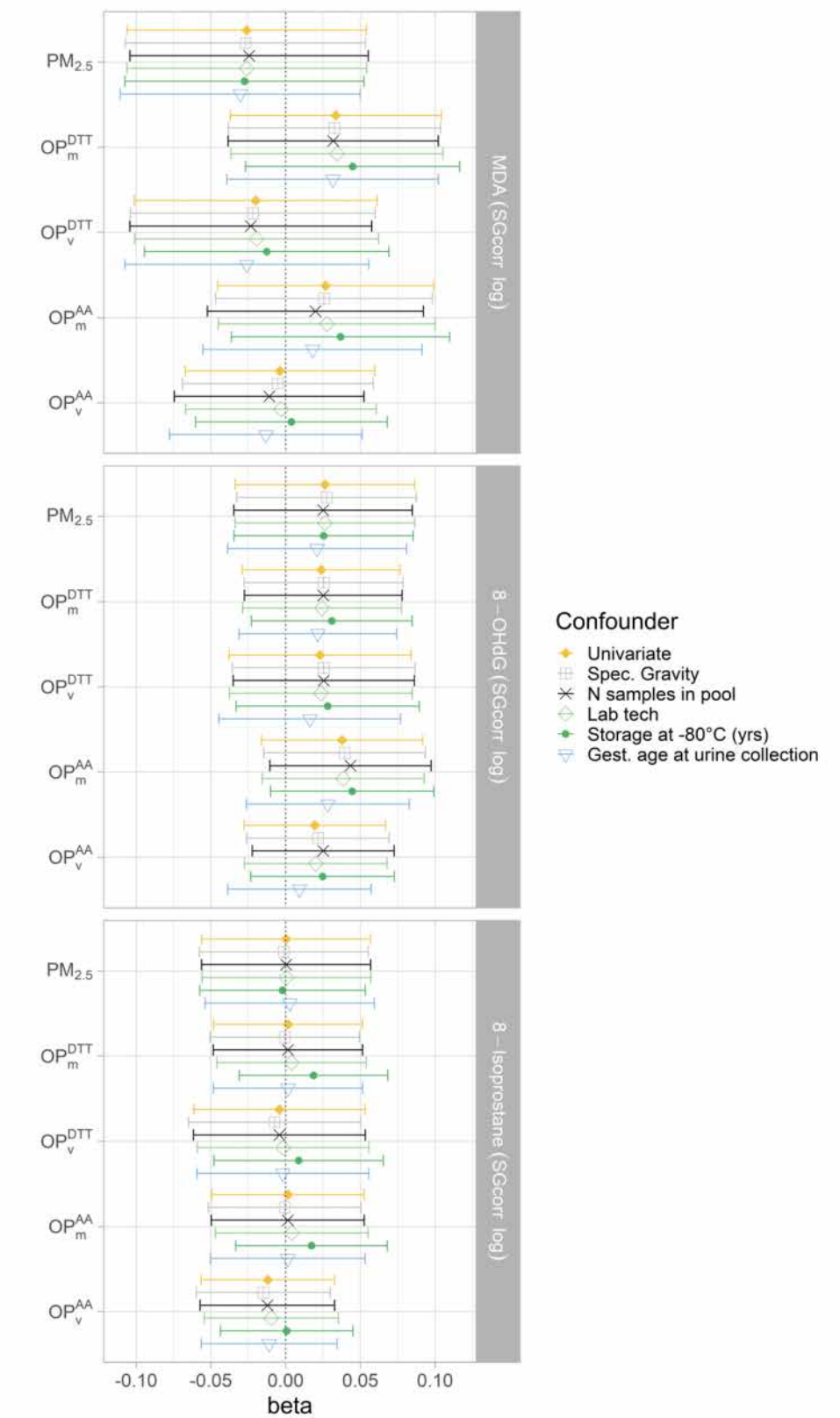

Figure S17. Effect of each confounder related to technical variables separately on the regression models.
Outcomes and exposures were scaled by their IQR. Whiskers represent the 95% confidence interval around the estimate. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_m^{DTT}, mass-normalized oxidative potential measured by the DTT assay (nmol/min/µg); OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay (nmol/min/m³);); OP_m^{AA}, mass-normalized oxidative potential measured by the AA assay (nmol/min/µg); OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay (nmol/min/m³).

Results

Table S14. Spearman correlation coefficients between exposures and oxidative stress biomarkers (raw, and corrected for specific gravity).

	OP_v^{DTT}	OP_v^{AA}	OP_m^{DTT}	OP_m^{AA}
$PM_{2.5}$	0.68**	0.47**	-0.25**	-0.14**
OP_v^{DTT}		0.71**	0.47**	0.35**
OP_v^{AA}			0.40**	0.77**
OP_m^{DTT}				0.65**
OP_m^{AA}				

*Note: **: p-value<0.01; *: p-value<0.05. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm; OP_m^{DTT}, mass-normalized oxidative potential measured by the DTT assay; OP_m^{AA}, mass-normalized oxidative potential measured by the AA assay; OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay; OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay.*

Table S15. Description of $PM_{2.5}$, OP_v^{DTT}, OP_v^{AA}, OP_m^{DTT} and OP_m^{AA} in the low vs. high concentrations of $PM_{2.5}$ (using the median exposure of 14 µg/m³ as threshold).

Characteristic	**N**	**$PM_{2.5}$ ≤ 14 µg/m³**, N = 151	**$PM_{2.5}$ > 14 µg/m³**, N = 149	**p-value**[1]
$PM_{2.5}$	300			<0.001
Median (IQR)		10 (8, 11)	18 (15, 22)	
Range		4, 14	14, 51	
OP_v^{DTT}	300			<0.001
Median (IQR)		1.10 (0.83, 1.35)	1.91 (1.47, 2.41)	
Range		0.18, 2.74	0.52, 4.56	
OP_v^{AA}	300			<0.001
Median (IQR)		1.20 (0.68, 1.65)	1.90 (1.17, 2.78)	
Range		0.08, 3.51	0.04, 11.43	
OP_m^{DTT}	300			0.009
Median (IQR)		0.12 (0.09, 0.15)	0.10 (0.08, 0.12)	
Range		0.02, 0.35	0.03, 0.18	
OP_m^{AA}	300			0.037
Median (IQR)		0.12 (0.08, 0.17)	0.10 (0.06, 0.15)	
Range		0.01, 0.47	0.00, 0.45	

[1]Wilcoxon rank sum test

Note: $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_m^{DTT}, mass-normalised oxidative potential measured by the DTT assay (nmol/min/µg); OP_v^{DTT}, volume-normalised oxidative potential measured by the DTT assay (nmol/min/m³); OP_v^{AA}, volume-normalised oxidative potential measured by the AA assay (nmol/min/m³); OP_m^{AA}, mass-normalised oxidative potential measured by the AA assay (nmol/min/µg).

Table S16. Estimated associations between exposure to OP_m^{AA} and OP_m^{DTT} and oxidative stress biomarkers in pregnant women, after stratification on $PM_{2.5}$ levels (using the median exposure of 14 µg/m³ as threshold).

OSB and exposure	**$PM_{2.5}$ ≤ 14 µg/m³**, N = 151 **percent change (95% CI)**	**$PM_{2.5}$ > 14 µg/m³**, N = 149 **percent change (95% CI)**	**interaction *p*-value**
MDA			
OP_m^{AA}	1.3 (-9.5, 13.5)	3.4% (-8.4, 16.7)	0.99
OP_m^{DTT}	5.1 (-4.2, 15.3)	2.1% (-11.4, 17.5)	0.63
8-OHdG			
OP_m^{AA}	11.4% (3.3, 20.1)	-1.0% (-10.6, 9.6)	0.12
OP_m^{DTT}	7.1% (0.70, 14.2)	-4.9% (-15.5, 7.0)	0.11
8-iso-PGF2α			
OP_m^{AA}	5.8% (-1.4, 13.4)	-8.7% (-16.9, 0.4)	0.06
OP_m^{DTT}	2.9% (-2.9, 9.0)	-5.3% (-15.2, 5.9)	0.31

Note: percent change (95% CI) correspond to the stratified analysis, adjusted on educational level, history of rhinitis, history of asthma, age, BMI, parity, active or passive, mean temperature during the sampling week, gestational age at urine collection, number of samples in the pool and storage time at -80°C before analysis. The interaction p-value corresponds to the $OP_m^{AA} \times PM_{2.5}$ or $OP_m^{DTT} \times PM_{2.5}$ term, after binarizing the $PM_{2.5}$ variable using the median exposure of 14 µg/m³ as threshold.

Table S17. Numerical data for figures 1 and 2 of the main text: Association between personal exposure to $PM_{2.5}$, OP_m^{DTT}, OP_v^{DTT}, OP_m^{AA} and OP_v^{AA} and oxidative stress biomarkers in multiple linear models and in the sensitivity analyses.

	Biomarker	8-iso-PGF2α		MDA		8-OHdG	
Pollutant	**Analysis**	**percent change (95% CI)**	**N**	**percent change (95% CI)**	**N**	**percent change (95% CI)**	**N**
$PM_{2.5}$	Univariate	0.02 (-5.47, 5.84)	300	-2.57 (-10.07, 5.55)	300	2.66 (-3.31, 9.00)	300
	Complete Cases	0.02 (-5.51, 5.88)	273	-3.3 (-11.25, 5.36)	273	1.94 (-4.12, 8.39)	273
	Main model	-0.01 (-5.36, 5.64)	300	-2.92 (-10.46, 5.26)	300	2.07 (-3.85, 8.35)	300
	Excluding extreme values	0.13 (-5.48, 6.07)	288	-2.12 (-10.04, 6.49)	288	2.69 (-3.62, 9.41)	289
	Excluding active smokers	1.31 (-4.03, 6.95)	283	-2.71 (-10.34, 5.57)	283	2.8 (-3.27, 9.25)	283
	Not corrected for SG	0.71 (-4.97, 6.72)	300	-2.01 (-9.66, 6.29)	300	3.17 (-3.29, 10.06)	300
	3 samples in pool	-0.19 (-6, 5.99)	265	-2.25 (-10.77, 7.09)	265	1.66 (-4.84, 8.6)	265
OP_m^{DTT}	Univariate	0.16 (-4.71, 5.28)	300	3.41 (-3.65, 10.98)	300	2.41 (-2.87, 7.98)	300
	Complete Cases	0.88 (-4.36, 6.40)	273	5.47 (-2.66, 14.27)	273	2.74 (-2.99, 8.81)	273
	Main model	1.6 (-3.28, 6.72)	300	4.89 (-2.43, 12.75)	300	3.56 (-1.82, 9.23)	300
	Excluding extreme values	-0.25 (-5.17, 4.93)	288	3.52 (-4.02, 11.66)	288	-0.76 (-6.27, 5.07)	289
	Excluding active smokers	1.53 (-3.30, 6.6)	283	3.93 (-3.43, 11.85)	283	3.25 (-2.25, 9.06)	283
	Not corrected for SG	-0.87 (-5.88, 4.41)	300	2.44 (-4.74, 10.16)	300	1.2 (-4.50, 7.23)	300
	3 samples in pool	2.04 (-3.06, 7.41)	265	6.35 (-1.6, 14.93)	265	5.37 (-0.39, 11.45)	265
OP_v^{DTT}	Univariate	-0.41 (-5.96, 5.46)	300	-1.98 (-9.63, 6.31)	300	2.33 (-3.71, 8.75)	300
	Complete Cases	-0.27 (-6.05, 5.86)	273	-1.57 (-10.05, 7.7)	273	2.56 (-3.83, 9.37)	273
	Main model	0.31 (-5.28, 6.24)	300	-1.55 (-9.53, 7.12)	300	3.16 (-3.07, 9.79)	300
	Excluding extreme values	1.86 (-3.66, 7.69)	288	0.53 (-7.54, 9.31)	288	4.51 (-1.81, 11.25)	289
	Excluding active smokers	1.02 (-4.57, 6.94)	283	-1.82 (-9.9, 6.99)	283	3.53 (-2.88, 10.36)	283
	Not corrected for SG	-0.48 (-6.33, 5.73)	300	-2.08 (-10.05, 6.6)	300	2.78 (-3.93, 9.97)	300
	3 samples in pool	0.31 (-5.39, 6.36)	265	-0.43 (-8.91, 8.83)	265	3.87 (-2.59, 10.77)	265
OP_m^{AA}	Univariate	0.16 (-4.82, 5.39)	300	2.70 (-4.45, 10.39)	300	3.85 (-1.60, 9.60)	300
	Complete Cases	0.12 (-5.54, 6.12)	273	0.9 (-7.58, 10.16)	273	5.75 (-0.64, 12.56)	273
	Main model	0.5 (-4.81, 6.1)	300	1.47 (-6.32, 9.9)	300	6.19 (0.16, 12.59)	300
	Excluding extreme values	-0.45 (-5.78, 5.18)	289	1.34 (-6.62, 9.97)	288	3.24 (-3.03, 9.93)	288
	Excluding active smokers	0.97 (-4.28, 6.51)	283	1.44 (-6.41, 9.95)	283	6.6 (0.44, 13.13)	283
	Not corrected for SG	-1.51 (-6.97, 4.28)	300	-0.54 (-8.19, 7.75)	300	4.11 (-2.31, 10.94)	300
	3 samples in pool	0.28 (-5.13, 6.01)	265	2.82 (-5.49, 11.86)	265	6.86 (0.58, 13.53)	265
OP_v^{AA}	Univariate	-1.19 (-5.50, 3.32)	300	-0.38 (-6.49, 6.14)	300	1.97 (-2.75, 6.92)	300
	Complete Cases	-2.02 (-6.88, 3.09)	273	-2.61 (-9.81, 5.16)	273	3.25 (-2.26, 9.06)	273
	Main model	-1.72 (-6.45, 3.24)	300	-2.27 (-9.11, 5.09)	300	3.69 (-1.7, 9.39)	300
	Excluding extreme values	-0.26 (-5.35, 5.11)	289	-1.11 (-8.61, 7.01)	288	4.04 (-1.97, 10.4)	288
	Excluding active smokers	-0.89 (-5.56, 4.01)	283	-2.29 (-9.15, 5.09)	283	4.3 (-1.19, 10.09)	283
	Not corrected for SG	-2.32 (-7.26, 2.89)	300	-2.69 (-9.52, 4.67)	300	3.37 (-2.45, 9.54)	300
	3 samples in pool	-2.1 (-6.8, 2.84)	265	-1.53 (-8.63, 6.13)	265	3.31 (-2.13, 9.05)	265

Pollutants were scaled by their IQR. The main model was adjusted on educational level, history of rhinitis, history of asthma, age, body mass index, parity, active or passive smoking, mean temperature during the sampling week, gestational age at urine collection, number of samples in the pool, storage time at -80°C before analysis. "Complete Cases" is an analysis excluding pregnant women with missing data for at least one covariate; "Excluding extreme values" are the analyses excluding the exposures and outcomes below the 1st percentile and above the 99th; "Excluding active smokers" is an analysis excluding women that actively smoked during their pregnancy; "Not corrected for SG" is an analysis using raw concentrations of OSB, adding SG in the confounders; "3 samples in pool" is an analysis excluding women for which the pooled urine sample was based on less than 3 urine samples. $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 µm (µg/m³); OP_m^{DTT}, mass-normalized oxidative potential measured by the DTT assay (nmol/min/µg); OP_v^{DTT}, volume-normalized oxidative potential measured by the DTT assay (nmol/min/m³); OP_v^{AA}, volume-normalized oxidative potential measured by the AA assay (nmol/min/m³); OP_m^{AA}, mass-normalized oxidative potential measured by the AA assay (nmol/min/µg); SG: specific gravity.

Chapter V

Personal exposure to air pollutants and immune system biomarkers in pregnant women

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***Contribution*:** *While Chapter IV was focused on the mechanical pathway that led to oxidative stress, this work more globally concerns systemic modifications of the immune function in pregnant women. Therefore, another air pollutant, namely NO_2, was included.*
The manuscript related to this work is under review by the co-authors and should be submitted soon. I was involved in the OP data curation and initiated the first steps of the statistical analyses during my second year of PhD. We decided to entrust this work to Laurène Frau during the summer 2023. She performed the rest of the statistical analyses, and we wrote the first draft of the article together. This work validated her internship and her first year of Masters. OP analysis was performed by the workforce of IGE's plateau AirOSol. Among the statistical methods presented, we beneficiated from the work of other IAB's team members and former students, and were able to use readily validated $PM_{2.5}$ mass concentration exposure, and corrected cytokines concentrations (two-step standardization method and fill-in approach for values <LOD).

I. French summary

Contexte. Le stress oxydant et la modulation du système immunitaire sont deux mécanismes cycliques, s'influant l'un et l'autre, qui sont principalement responsable des effets sanitaires de la pollution atmosphérique. L'exposition aux polluants de l'air a été associée à une réponse pro-inflammatoire, mais seulement très peu d'études se sont intéressées à l'exposition au potentiel oxydant des PM, alors même que cette métrique a été développée pour simuler la capacité des PM à générer du stress oxydant. Seulement quelques études de faible effectif ont étudié les effets de l'exposition au PO des PM sur des marqueurs de la fonction immunitaire, et ces études sont limitées à quelques marqueurs. Par ailleurs, alors que les femmes enceintes représentent une population particulièrement vulnérable du fait des modifications de leur système immunitaire, peu d'études ont spécifiquement porté sur cette population.

Objectifs. Ce travail vise à évaluer les associations entre l'exposition personnelle aux NO_2, $PM_{2.5}$, et PO des $PM_{2.5}$ et la fonction immunitaire dans une population de femmes enceintes.

Méthodes. Cette étude repose sur 270 femmes enceintes appartenant à la cohorte couple-enfant française SEPAGES. Des échantillonneurs passifs et actifs ont été portés durant une semaine pour calculer l'exposition au NO_2 et $PM_{2.5}$, respectivement. Le potentiel oxydant des $PM_{2.5}$ prélevées sur le filtre de l'échantillonneur personnel a été mesuré par les méthodes du dithiothréitol (DTT) et de l'acide ascorbique (AA). Les niveaux de cytokines sécrétées par les monocytes, les cellules dendritiques, les granulocytes et les lymphocytes T ont été analysés dans des échantillons sanguins prélevés à la fin de la semaine de mesure d'exposition aux polluants de l'air. Les niveaux de 29 cytokines et chimiokines ont été mesurés à l'état basal et après activation des lymphocytes T et des cellules dendritiques avec de la phytohémagglutinine (PHA) et du résiquimod (R848), respectivement. Des modèles de régression linéaire multiple ont été réalisés pour évaluer l'association entre chaque polluant de l'air et chaque cytokine en ajustant pour les facteurs de confusion potentiels.

Résultats. Aucune association significative n'a été mise en évidence pour l'exposition aux $PM_{2.5}$. Une augmentation d'un écart interquartile (IQR) de l'exposition au NO_2 (10 µg/m^3) était associée à une augmentation de l'IL-10 et à une diminution du TNF activé par la PHA (β [intervalle de confiance à 95 %] : 0,18 [0,03 ; 0,32], *p*=0,02 ; β [IC à 95 %] = -0,18 [-0,32 ; -0,02], *p*=0,03, respectivement). Une augmentation de l'exposition au PO_v^{AA} (IQR = 1,65 nmol/min/m^3) était associée à une diminution de l'IL-8 activée par le R848 (β [IC à 95 %] = -0,17 [-0,33 ; 0,00], *p*=0,05), et une tendance similaire était observée avec l'IL-8 à l'état basal (β [IC à 95 %] = -0,18 [-0,41 ; 0,06], *p*=0,14. Une augmentation de l'exposition au PO_m^{AA} (IQR = 0,08 nmol/min/µg) était associée à une diminution de l'IL-8 activée par le R848 (β [IC à 95 %] = -0,12 [-0,24 ; 0,00], *p*=0,05). Enfin, le PO_m^{DTT} était associé à une augmentation de l'IL-17A activée par la PHA (β [IC à 95 %] = 0,11 [0,00 ; 0,22], *p*=0,04), et une tendance similaire était observée avec le PO_v^{DTT} et le PO_m^{AA} (β [IC à 95 %] = 0,08 [-0,04 ; 0,20], *p*=0,18 ; β [IC à 95 %] = 0,10 [-0,01 ; 0,22], *p*=0,07, respectivement).

Conclusions. Cette étude a mis en évidence un effet de l'exposition des polluants de l'air sur la fonction immunitaire de la femme enceinte. Les effets sont observés avec l'exposition au NO_2 et au PO des $PM_{2.5}$, mais pas avec la concentration massique des $PM_{2.5.}$ Cela suggère que les métriques spécifiques à l'exposition aux traceurs liés au trafic (NO_2), et aux espèces redox-actives (PO des PM) sont plus spécifiques que la simple concentration en masse pour les effets biologiques sur les voies oxydantes et inflammatoire. Cela apporte des preuves biologiques aux études épidémiologiques récentes suggérant que la concentration massique des PM n'est pas la métrique la plus adéquate pour l'estimation des effets délétères de l'exposition à la pollution atmosphérique.

II. Abstract

Context: The immune function is suspected to play an important role in the health effects of air pollution but it remains poorly investigated in pregnant women.

Objectives: Associations between personal exposure to air pollutants and the immune function of pregnant women were investigated.

Methods: One-week personal measurements of exposure to nitrogen dioxide (NO_2), particulate matter with an aerodynamic diameter of ≤2.5µm mass concentration ($PM_{2.5}$) and $PM_{2.5}$ oxidative potential (OP) were assessed in 270 pregnant women from the French cohort SEPAGES. PM filters collected by an active personal sampler were analyzed for $PM_{2.5}$ OP, using the dithiothreitol (DTT) and the ascorbic acid (AA) assays. Immune function biomarkers were assessed in a blood sample withdrawn at the end of the exposure measurement week. Levels of 29 cytokines and chemokines were measured at baseline and after T cell and dendritic cell activation with phytohemagglutinin (PHA) and resiquimod (R848), respectively. Adjusted linear regression models were performed to assess the association between each air pollutant and each cytokine.

Results: No significant associations with $PM_{2.5}$ were found. An increase in NO_2 exposure was associated with higher interleukin 10 (IL-10) and lower PHA-activated tumor necrosis factor (TNF). Increased exposure to OP^{AA} was associated with lower IL-8 measured upon R848 activation and at baseline. Finally, OP^{DTT} was associated with higher PHA-activated IL-17A.

Conclusions: Our study provides significant insights into the relationships between air pollution exposure and immune function among pregnant women. OP of PM is a useful metric to highlight biological pathways induced by atmospheric pollution exposure.

III. Introduction

Exposure to atmospheric pollutants, such as particulate matter (PM) and nitrogen dioxide (NO_2), affects a number of human systems and organs, and contributes to a significant part of premature death worldwide

(Fuller et al., 2022). Oxidative stress and the modulation of the immune system are two inter-related mechanisms through which air pollutants exert their deleterious effects on health (Bernstein et al., 2004; Li et al., 2003; Mudway et al., 2020). Oxidative stress, by an excess of reactive oxygen species can damage lipoproteins or lipids in membranes, leading to the formation of oxidation-specific epitopes recognized by pattern recognition receptors of the innate immune system (Binder et al., 2016). This can enhance inflammatory mechanisms, thereby leading to a cyclic generation of oxidative stress and inflammation (Kelly and Fussell, 2015).

Most of the research carried out on immune system has focused on the assessment of ambient particles, notably PM_{10} and $PM_{2.5}$ and gases (NO_2). Epidemiological studies showed that exposure to NO_2 was associated with increased pro-inflammatory cytokines, such as IL-6 and TNF-α (Lim et al., 2022; Xu et al., 2022). Previous studies highlighted that ambient exposure to fine particles was associated with an intensification of inflammatory responses and an increase in B lymphocytes (Glencross et al., 2020; Zhao et al., 2019). The oxidative potential (OP) of PM, a metric developed to mimic oxidative stress generation in the lung fluid after PM inhalation, was assessed in very few epidemiological studies addressing its relationship with human immune system. OP^{AA}, OP^{DTT} and OP^{DCFH} from ambient PM were found associated with elevated expression of pro-inflammatory biomarkers in lung epithelial cells (Leni et al., 2020; Liu et al., 2014). To the best of our knowledge, four studies estimated associations between OP of PM and immune system biomarkers of human and showed that OP of PM was positively associated with plasma IL-6 (Delfino et al., 2010), blood IL-6 expression (Liu et al. (2018) and IL-6 levels in nasal fluids (Janssen et al. (2015), while Steenhof et al. (2013) did not find any association with blood IL-6, nor with nasal IL-6 and IL-8. Overall, these studies tend to converge towards a pro-inflammatory effect of OP exposure, but they mostly relied on the expression of IL-6 and a relatively small sample size.

Pregnant women constitute a sensitive population to air pollution, primarily due to the modifications that occur in their immune systems during pregnancy, including a modification of cytokine production (Mor and Cardenas, 2010). However, there is a notable lack of available data regarding effects of exposure to air pollution on immune system in this particular population. Furthermore, it is especially relevant to delve into the mechanisms involved in the health effects of air pollution in pregnant women, as prenatal exposure to

air pollution influences the development of child health and immune function (Baïz et al., 2011; García-Serna et al., 2021; Manches et al., 2023).

The aim of this study was to assess the associations between personal exposure to air pollutants (NO_2, $PM_{2.5}$ and PM OP) during pregnancy and immune function measured both at the baseline state and after T cell and dendritic cell activation.

IV. Materials and methods

IV.1. Study population

This study is based on data from the SEPAGES French parent-child cohort. The study population lives within 80km around the center of Grenoble in the Alpes. Briefly, 484 pregnant women, with pregnancy duration less than 19 weeks and with a singleton pregnancy, older than 18 years old and affiliated to the French national security system, were recruited between 2015-2017 (Lyon-Caen et al., 2019). Their partner and children were also recruited. Exposure information were collected using personal samplers and immunological information were collected using blood samples. Sociodemographic and medical information were collected using a combination of questionnaires, interviews, and clinical examinations during and after pregnancy. The present analysis is based on 270 mothers with exposure assessed to at least one of the measured air pollutants during pregnancy and immunological measurements at the end of the exposure assessment week (Figure 28). To be included in the analysis, mothers had to have the blood samples collected within 2 days after the pollutant sampling period to assess the immune system's status in relation to the exposure measurements.

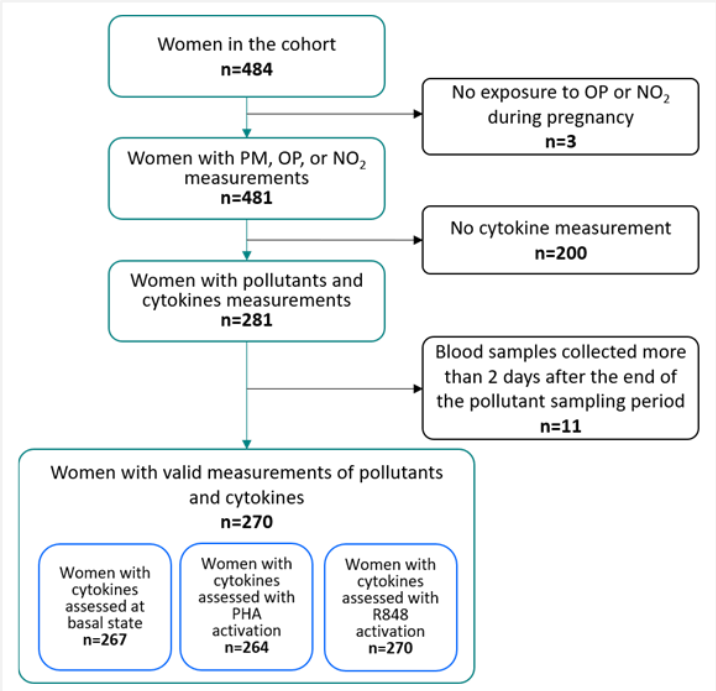

Figure 28. Flow chart for the selection of the study population

Abbreviations: $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm; NO_2 nitrogen dioxide; OP: oxidative potential; PHA: phytohemagglutinin; R848 resiquimod.

IV.2. Personal exposure assessment to air pollutants

Women in the SEPAGES cohort wore or kept nearby personal active air samplers placed in a wearable backpack to measure their personal exposures to $PM_{2.5}$ (MicroPEM™ active air sampler; RTI International, USA) and NO_2 (Passam AG passive air sampler, Switzerland) for 7 (13%) to 8 (87%) consecutive days (Borlaza et al., 2022a; Lepeule et al., 2023; Marsal et al., 2023). The measurement week took place during the second (81%) or the third trimester (19%) of pregnancy (median [min-max] gestational age = 19 weeks [14-36]).

NO_2 concentration was measured using spectrophotometry following established methods (Hafkenscheid et al., 2009). $PM_{2.5}$ filters from the MicroPEM were weighed before and after sampling at RTI International (USA), using a microbalance (Mettler Toledo UMX2) placed in an environmental chamber maintained at a temperature of 21°C and 35% relative humidity. $PM_{2.5}$ mass concentration was calculated by dividing the mass of collected PM, measured by gravimetric analysis, by the air volume sampled during the measurement week (µg/m^3). PM filters were cold-stored until OP analysis. Protocol for OP measurement was previously published (Borlaza et al., 2022a; Marsal et al., 2023), based on the protocol established by Calas et al. (2018, 2017). Briefly, $PM_{2.5}$ were extracted in a simulated lung fluid consisting in a mixture of 1,2-

dipalmitoylphosphatidylcholine (DPPC), to reach a final concentration of 10 µg/mL. Extracts were incubated at 37°C for 75 min under vortex agitation prior to analysis using the dithiothretitol (DTT) and the ascorbic acid (AA) assays. A 96-well plate (CELLSTRAR, Greiner-Bio) was used to mix the extracts with DTT or AA solutions. For the AA assay, the absorbance at 265 nm (TECAN spectrophotometer Infinite M200 Pro) is measured over time to evaluate AA consumption by $PM_{2.5}$ extract, for a total reaction time of 30 min. For the DTT assay, the absorbance at 412 nm measured the formation of the 2-nitro-5-thiobenzoic acid (TNB), which is the reaction product of the remaining DTT and dithionitrobenzoic acid (DTNB), for a total reaction time of 30 min. Samples were analyzed in triplicates, and the mean was calculated for each sample. For both assays, consumption rates were then normalized by the mass of PM of the extract (OP_m, in nmol/min/µg), or by the corresponding air volume sampled (OP_v, nmol/min/m^3).

IV.3. Maternal immune function

Blood samples were collected by trained field workers, within a maximum of 48 hours after the end of the exposure measurement week, following the procedure published by Manches et al. (2023). Briefly, blood was collected in BD Medical 368886 vacutainer tube (lithium heparin) for immunological analyses (cell culture and plasma separation), and in BD Medical 368861 vacutainer tube (EDTA) for cell counting. They were transported to the Etablissement Français du Sang (EFS) in coolers, placed on a rotating device for at least 5 min to ensure homogeneous cell content, and were then processed within 24 hours after collection.

Innate and adaptative immunity of the women were measured at baseline in plasma and in whole blood after a 24-hour *ex vivo* activation of the whole blood at 37°C using Resiquimod (R848) and phytohaemagglutinin (PHA), as previously described by Manches et al. (2023).

Briefly, cytokines were measured in the culture supernatant (for activated cells) or in plasma by cytometric bead arrays (BD™ CBA Human cytokines Flex Set, BD Biosciences).

Among the 29 cytokines that were measured (12 at baseline, 9 after PHA-activation and 8 after R848 activation) only those with at least 70% of detected values were considered (Manches et al., 2023). Hence, for the samples activated with PHA, the overall activity of T lymphocytes (T helpers Th1, Th2, Th9, Th17, and regulatory Treg) was assessed by quantifying the levels of IL-2, TNF-α, interferon (IFN) γ, IL-13, IL-17a, IL-9 and IL-10. For the samples activated with R848, the overall activity of dendritic cells was evaluated

by quantifying TNF-α, IL-10, IL-6, IL-8, IFN-α, IFN-γ, IL-1β, and IL-12p70. For the non-activated sample, the basal state of the immune system was quantified by IL-8, monocyte chemoattractant protein-1 (MCP1) and regulated on activation, normal T cell expressed and secreted (RANTES). The concentrations below the limit of detection were imputed by a fill-in approach, that randomly selects values between 0 and the LOD based on the underlying distribution (2023)(Helsel, 1990; Lubin et al., 2004). Due to their skewed distribution, cytokine concentrations were log10 transformed.

Since technical between-participant variability related to the experimentation can lead to measurement error, a two-step standardization method based on regression residuals (Mortamais et al., 2012) was used to correct, when necessary, cytokine concentrations. The same standardized variables as previously described by Manches et al. (2023) were used. Briefly, the technical variables considered were: 1) for baseline cytokines: analytical batch, time between sample collection and reception, time between sample reception and analysis; 2) for activated cytokines the same variables were used, together with the duration of the activation, R848 or PHA age at the time of sample activation, and storage duration.

IV.4. Statistical analysis

Summary statistics (mean [standard deviation] or median [Q1-Q3]) were calculated for air pollutant exposure assessments, cytokine levels, and covariates. A correlation matrix (Pearson's *r*) was calculated between the cytokine's levels and between the air pollutant concentrations. Univariate and adjusted linear regressions were conducted to estimate the associations between each air pollutant exposure and each cytokine levels. Each exposure and log-transformed cytokine variables were divided by the interquartile range (IQR), to facilitate comparison of the beta estimates. The included covariates were age of the women (continuous), BMI before pregnancy (continuous), active or passive smoking (active smoking in the 12 months prior to pregnancy, or active or passive smoking during pregnancy; binary: yes/no), educational level (binary: <master's degree, ≥ master's degree), leukocyte count (continuous), gestational age at sampling (continuous), and sampling season (4 categories with winter corresponding to January-March, spring to April-June, summer to July-September, and fall to October-December). To avoid reduction of the sample size due to missing data in cofactors (overall 29 missing values), multiple imputations (n=20 datasets) were performed using Multivariate Imputation by Chained Equations (package mice, R).

In addition, sensitivity analyses were carried out to assess the robustness of the results to 1) extreme values (after exclusion of 1% lowest and 1% highest exposure and cytokine concentrations), 2) influential values (after exclusion of values with a Cook's distance exceeding 4/n, with n being the number of participants in the main analysis), and 3) the set of confounders, with models excluding the leucocyte counts among cofactors and models including history of asthma and rhinitis which could lie in the causal path between air pollution and cytokine levels. Results with p-value < 0.05 were considered statistically significant, and results with $0.05 \leq p \leq 0.10$, indicative of a trend. All analyses were conducted using the statistical software R (version 4.2).

V. Results

V.1. Population characteristics

The population studied included 270 pregnant women with a median age of 32.1, a median BMI of 21.6 and a high educational level (58% of them had a diploma equivalent to or higher than a master's degree) (Table 12). Among these women, 9.5% were active smokers before or during pregnancy. Regarding respiratory health, 16% reported asthma symptoms before or during pregnancy, and 40% reported rhinitis.

Table 12. Description of women in the study population

Characteristics	**N = 270**[1]
Age (years)	32.1 (29.9 - 35.1)
Age (categories)	
<30	69 (25.5%)
30-35	133 (49.3%)
≥ 35	68 (25.2%)
BMI before pregnancy	21.6 (19.8 – 24.1)
BMI before pregnancy (categories)	
<18.5 kg/m^2	15 (5.6%)
18.5-25 kg/m^2	197 (73.8%)
25-30 kg/m^2	43 (16.1%)
≥ 30 kg/m^2	12 (4.5%)
Missing	3
Education level	
High school	13 (4.8%)
Bachelor's degree	29 (11%)
Master's degree	70 (26%)
≥ Postgraduate	157 (58%)
Missing	1
Active smoking before or during pregnancy	
No	228 (90%)

Characteristics	N = 270[1]
Yes	24 (9.5%)
Missing	18
Passive smoking during pregnancy	
No	204 (80%)
Yes	50 (20%)
Missing	16
Symptoms of asthma before or during pregnancy	
No	228 (84%)
Yes	42 (16%)
Symptoms of rhinitis before or during pregnancy	
No	162 (60%)
Yes	108 (40%)
Season of sampling	
Autumn	55 (21%)
Spring	79 (30%)
Summer	69 (26%)
Winter	64 (24%)
Gestational age at sampling (weeks)	19.0 (18.0- 21.0)

[1] Median (Q1-Q3); n (%)

V.2. Exposure to NO_2 , $PM_{2.5}$ and OP

Median (Q1-Q3) exposure to NO_2, $PM_{2.5}$, OP_m^{DTT}, OP_v^{DTT}, OP_m^{AA} and OP_v^{AA} were 20.2 (16.2 - 25.8) µg/m^3, 13.8 (9.9-18.5) µg/m^3, 0.11 (0.09-0.14) nmol/min/µg, 1.48 (1.06-2.00) nmol/min/m^3, 0.12 (0.08-0.16) nmol/min/µg and 1.65 (0.98-2.63) nmol/min/m^3, respectively (Table 13). For $PM_{2.5}$ and NO_2, personal exposure levels are in the range of typical ambient exposure levels, as reported in the ELAPSE project, pooling eight European cohorts (Strak et al., 2021). A seasonal trend was observed during the cold season with higher concentrations of all studied pollutants. OP_v^{AA} presents a strong Pearson correlation coefficient ($r > 0.5$) with OP_v^{DTT} and with OP_m^{AA}, whereas OP_v^{DTT} is strongly correlated with $PM_{2.5}$; and OP_m^{AA} with OP_m^{DTT} (Figure 29). Moderate Pearson correlation coefficients ($0.30 < r < 0.50$) were observed between $OP_v^{DTT}$and OP_m^{DTT} or OP_m^{AA}, and between OP_v^{AA} and $PM_{2.5}$. Remaining correlations were considered weak ($r < 0.3$).

Table 13. Description of air pollutant characteristics

Air pollutant	N	Median (Q1-Q3)	IQR
NO_2 (µg/m^3)	270	20.2 (16.2 – 25.8)	9.5
$PM_{2.5}$ (µg/m^3)	210	13.8 (9.9 – 18.5)	8.60
OP_m^{DTT} (nmol/min/µg)	194	0.11 (0.09 - 0.14)	0.05

OP_v^{DTT} (nmol/min/m³)	194	1.48 (1.06 - 2.00)	0.93
OP_m^{AA} (nmol/min/µg)	194	0.12 (0.08 - 0.16)	0.09
OP_v^{AA} (nmol/min/m³)	194	1.65 (0.98 - 2.63)	1.65

Abbreviations: IQR interquartile range; $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm; NO_2 nitrogen dioxide; OP: oxidative potential; DTT: dithiothreitol; AA: ascorbic acid.

V.3. Cytokines measurements

Regarding raw concentrations of cytokines, women exhibited high concentrations of RANTES (median [Q1-Q3] = 14026 pg/mL [9873 - 17511]) at the basal state, high levels of IL-2, IL-10, TNF-α and IFN-γ (median [Q1-Q3]= 2665 pg/mL [1702 - 4249], 904 pg/mL [609 - 1370]), 760 pg/mL [248 - 1468] and 758 pg/mL [430 - 1384]), after activation with PHA, and high levels of IL-1β, IL-6, and IL-8 (median [Q1-Q3]= 9157 pg/mL [6482 - 13545]; 44668 pg/mL [32069 - 60509] and 39811 pg/mL [20896 - 66940], respectively) after activation with R848. Regarding imputed, corrected and log-10 transformed cytokine concentrations, the intra-group (basal, PHA activated, R848 activated) correlations at the basal level displayed a low correlation (-0.1 < Pearson r < 0.2), correlations after PHA activation were moderate-to-strong ($r > 0.4$) and correlations after R848 activation were weak-to-moderate ($0.2 < r < 0.4$) (Figure 29). The inter-group correlation was very weak ($r < 0.1$), except for IL-8 between basal state and R848-activated measures ($r = 0.37$), IFN-γ between PHA- and R848- activated measures ($r = 0.44$) and between PHA-activated IL-2 and R848-activated IL-6, IL-1β, and IL-12p70 ($0.22 < r < 0.27$). A negative correlation was also observed between IL-10 secretion after PHA activation and IL-8 secretion at basal state and after R848 activation ($r = -0.15$ and -0.16, respectively).

Table 14. Description of biomarkers characteristics

Cytokine levels	**Percentile of raw values**						**Percentile of imputed, corrected and log10 transformed values**					
	N	**10%**	**25%**	**50%**	**75%**	**90%**	**10%**	**25%**	**50%**	**75%**	**90 %**	**IQR**
Concentration of leucocytes (G/L)	265	6.8	7.6	8.9	10.2	11.6						
At basal state												
IL-8 (basal, pg/mL)	266	5.5	8.9	17.8	94.3	283.1	0.50	0.84	1.07	1.36	1.86	0.52
MCP1 (basal, pg/mL)	266	19.0	25.9	38.4	56.2	116.0	1.22	1.38	1.51	1.64	1.77	0.26
RANTES (basal, pg/mL)	266	7374.1	9873.3	14026.3	17511.4	23935.5	3.84	3.96	4.08	4.16	4.24	0.2
After PHA activation												

IFN-γ (pg/mL)	264	300.0	429.7	758.4	1383.8	3121.9	2.60	2.75	2.94	3.14	3.30	0.39
IL-2 (pg/mL)	264	1067.1	1701.6	2664.7	4245.8	7877.3	3.13	3.30	3.47	3.65	3.78	0.35
IL-9 (pg/mL)	264	13.8	20.7	35.8	59.7	86.8	1.29	1.45	1.64	1.85	2.05	0.4
IL-10 (pg/mL)	264	428.4	609.1	904.3	1369.6	2428.1	2.76	2.91	3.04	3.18	3.30	0.27
IL-13 (pg/mL)	264	57.1	83.9	138.1	205.8	339.4	1.75	1.93	2.11	2.26	2.40	0.33
IL-17A (pg/mL)	259	94.0	156.6	282.0	518.6	1151.4	2.15	2.32	2.55	2.78	2.97	0.43
TNF-α (pg/mL)	264	12.8	247.9	760.2	1468.2	2489.4	1.38	2.22	2.70	2.97	3.22	0.75
After R848 activation												
IFN-α (pg/mL)	270	41.6	116.7	259.2	573.1	976.9	2.20	2.39	2.68	2.92	3.12	0.53
IFN-γ (pg/mL)	270	82.3	173.0	366.8	738.5	1211.7	1.93	2.24	2.60	2.89	3.16	0.65
IL-1β (pg/mL)	270	4503.4	6481.8	9157.4	13545.0	19700.1	3.60	3.73	3.88	4.02	4.15	0.29
IL-6 (pg/mL)	270	21720.3	32069.2	44667.9	60509.2	87701.3	4.26	4.40	4.54	4.66	4.79	0.26
IL-8 (pg/mL)	270	12534.4	20895.5	39810.9	66939.8	112322.1	3.93	4.10	4.35	4.54	4.76	0.44
IL-10 (pg/mL)	270	348.4	530.7	782.9	1090.5	1348.7	2.51	2.66	2.79	2.91	3.05	0.25
IL-12p70 (pg/mL)	270	7.1	11.7	22.5	37.5	61.0	0.87	1.11	1.35	1.59	1.79	0.48
TNF-α (pg/mL)	270	116.3	2643.8	6382.1	11480.7	15127.2	1.84	2.82	3.21	3.44	3.66	0.62

Abbreviations: IL, interleukin; IFN interferon; TNF tumor necrosis factor; RANTES regulated on activation, normal T cell expressed and secreted; MCP monocyte chemoattractant protein; PHA phytohemagglutinin; R848 resiquimod.

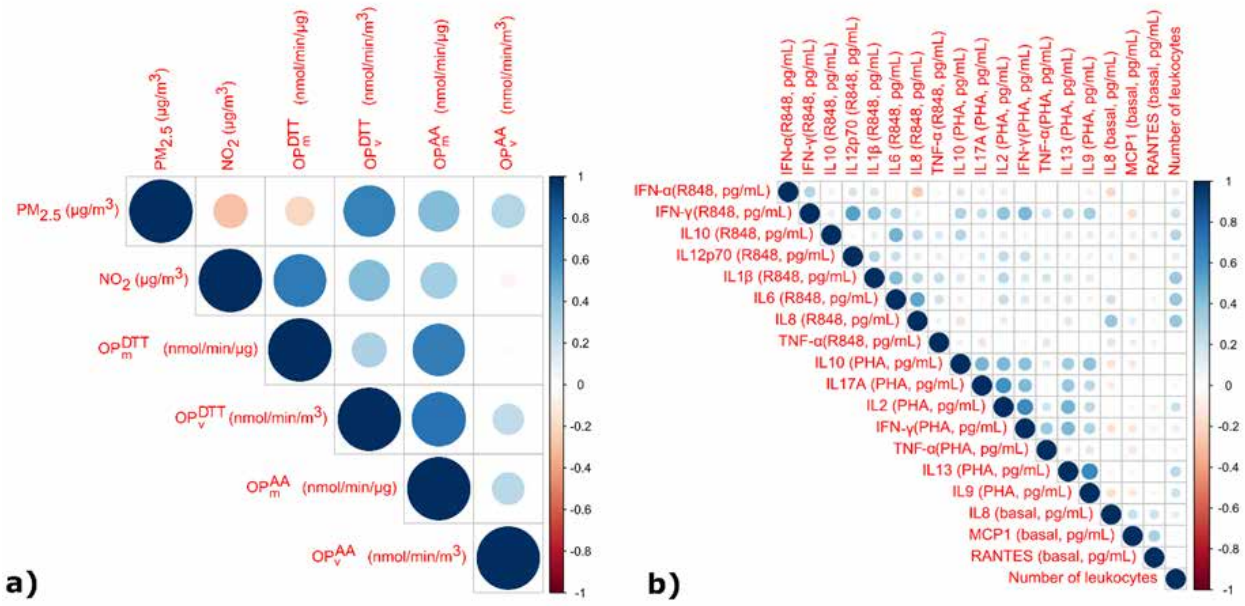

Figure 29. Pairwise Pearson's correlations between pollutants (a). Pairwise Pearson's correlations between cytokine and number of leukocyte (b).
Pearson correlation coefficients are indicated using a scale of size and color only when the p-values were less than 0.05. Abbreviations: $PM_{2.5}$ particulate matter with diameter ≤ 2.5 μm; NO_2 nitrogen dioxide; OP: oxidative potential; DTT: dithiothreitol; AA: ascorbic acid; IL interleukin; IFN interferon; TNF tumor necrosis factor; RANTES regulated on activation, normal T cell expressed and secreted; MCP monocyte chemoattractant protein; PHA phytohemagglutinin; R848 resiquimod.

V.4. Association between personal exposures to air pollutants and immune function parameters

Univariate analyses are presented in Table S1. In multivariate analyses, no significant association with $PM_{2.5}$ was found (Figure 30). A 10 µg/m3 (IQR) increase in NO_2 exposure was associated with higher basal IL-10 and lower PHA-activated TNF-α (β [95%CI] = 0.18 [0.03, 0.32], p=0.02; β [95%CI] = -0.18 [-0.32, -0.02], p=0.03, respectively). Increased exposure to OP_v^{AA} (IQR=1.65 nmol/min/m^3) was associated with lower IL-8 measured upon R848 activation (β [95%CI] = -0.17 [-0.33, 0.00], p=0.05), and a similar trend, although not significant, was observed for basal IL-8 levels (β [95%CI] = -0.18 [-0.41, 0.06], p=0.14). An IQR-increase in exposure to OP_m^{AA} (IQR = 0.08 nmol/min/µg) was statistically significantly associated with lower R848-activated IL-8 (β [95%CI] = -0.12 [-0.24, 0.00], p=0.05).

Finally, OP_m^{DTT} was associated with higher PHA-activated IL-17A (β [95%CI] = 0.11 [0.00, 0.22], p=0.04), and a similar trend of association was observed for OP_v^{DTT} and OP_m^{AA} (β [95%CI] = 0.08 [-0.04, 0.20], p=0.18; β [95%CI] = 0.10 [-0.01, 0.22], p=0.07, respectively) (Table S2).

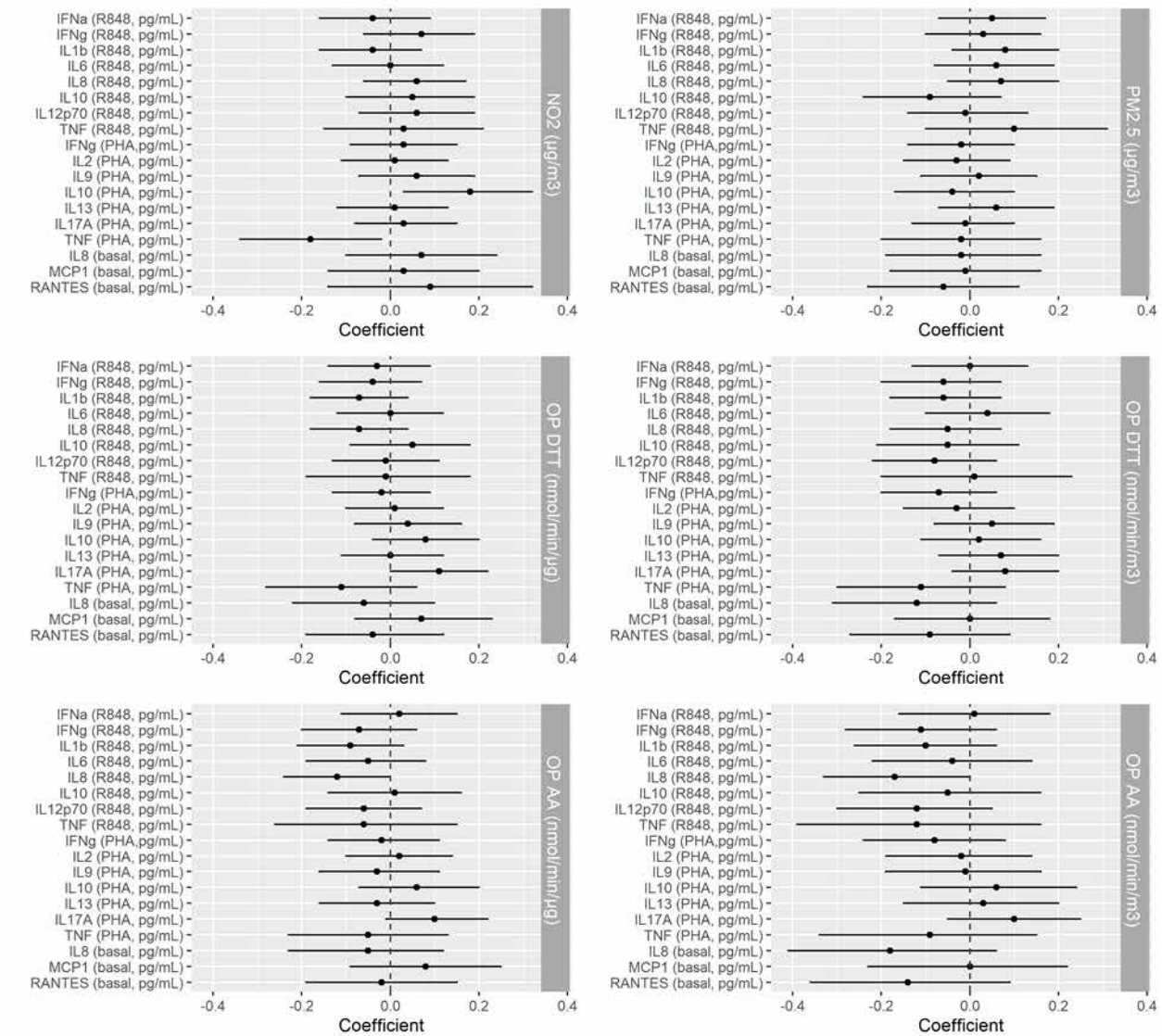

Figure 30. Adjusted association between each immunological parameter and each personal exposure to NO_2, $PM_{2.5}$, OP^{DTT} and OP^{AA} during pregnancy.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. Models were adjusted on women age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season. Abbreviations: $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm; NO_2 nitrogen dioxide; OP oxidative potential; DTT, dithiothreitol; AA, ascorbic acid; CI, confidence interval; IQR, interquartile; BMI, body mass index; Il, interleukin; IFN interferon; TNF tumor necrosis factor; RANTES regulated on activation, normal T cell expressed and secreted; MCP monocyte chemoattractant protein; PHA phytohemagglutinin; R848 resiquimod

Overall, results were robust, as displayed by the sensitivity analyses. Only the association between NO_2 and PHA activated IL-10 disappeared with the exclusion of extreme and influential values. Other findings were either similar, or stronger, as for the NO_2-TNF association (Figure S2). The other models excluding the leucocyte counts among cofactors, and including history of asthma and rhinitis, have the same results as the main models.

VI. Discussion

VI.1. Comparison with others studies

To our knowledge, this is the first study investigating the association between personal exposures to $PM_{2.5}$, OP of $PM_{2.5}$, and NO_2, with basal and activated immune function parameters in pregnant women. Our results show that increase in NO_2 exposure was associated with higher PHA-activated TNF−α. Similarly, an increase in OP_m^{AA}, as well as in OP_v^{AA}, led to increase in R848-activated IL-8. Finally, a positive association was suggested between PHA-activated IL-17A with the three OP measurements. Interestingly, the study did not find any significant association with $PM_{2.5}$, but it did reveal significant associations with the capacity of PM to induce oxidative stress, as measured by OP.

The mechanisms involved in the health impacts of air pollutants include impairment of the innate and adaptative immunity, and the activation of oxidative stress and reactive oxygen species (Leikauf et al., 2020). Adaptive immunity and oxidative stress closely interact with each other, these two mechanisms being triggered independently but having effects on each other. By investigating the effects of NO_2, $PM_{2.5}$ and OP of $PM_{2.5}$ exposure on immunological parameters, our study was able to provide a complete picture of the cross-interaction of oxidative stress and adaptative immunity pathways.

The results observed in the current study, showing decreased TNF-α levels upon NO_2 exposure, are in line with previous research in non-pregnant adults (Hu et al., 2020). However, positive associations between $PM_{2.5}$ and TNF-α reported in previous studies (Chen et al., 2018; Friedman et al., 2021; Gong et al., 2022; Zhang et al., 2022) were not observed in our study. Potential differences in study populations, measurement methodologies and analysis models could play a role in these disparities. More specifically, Chen et al. (2018) investigated these associations in students and the three other studies (Gong et al. (2022), Friedman et al. (2021) and Zhang et al. (2022)) were based on a population of pregnant women but used ambient exposure (not personal exposure assessment), and Gong et al. (2022) examined cytokines levels in the placenta, which could lead to different results compared to blood.

Very few studies have addressed the effects of OP exposure on immune response in human blood, and when they did, IL-6 levels were mostly investigated (Liu et al., 2018; Steenhof et al., 2013). In the RAPTES project (Steenhof et al., 2013), blood and nasal lavage from 31 healthy student volunteers were retrieved after 5

hours of exposure in different ambient settings, with contrasted pollution levels. No effect was observed with any of the analyzed OP tests (AA and Glutathione assays). The observed negative OP-IL-8 association is consistent with some previous findings among non-pregnant individuals that considered $PM_{2.5}$ exposure from one week (Hu et al., 2020) to three months (Audi et al., 2017) and from both indoor (Audi et al., 2017) and ambient environments (Fiorito et al., 2018; Hu et al., 2020; Parenteau et al., 2022). Contradictory, positive IL-8-$PM_{2.5}$ mass and IL-8-NO_2 relationships were reported by some experimental studies (Cachon et al., 2014; Jeong et al., 2017; Longhin et al., 2018), which may differ from real environmental contexts in human studies.

The results of our study indicate a positive association between OP exposure and IL-17A. To the best of our knowledge, no prior research has specifically examined this relationship. Previous studies used NO_2 or $PM_{2.5}$ exposure to examine effects on IL-17A in non-pregnant participants, and similar positive associations were reported with $PM_{2.5}$ (N. Gao et al., 2020) and NO_2 exposure (Fiorito et al., 2018; N. Gao et al., 2020; Hu et al., 2020). The study conducted by Hu et al. (2020) addressed these associations by varying the durations of exposure to $PM_{2.5}$ and NO_2, which led to contrasting observations. Specifically, a short-term exposure to NO_2 (between 12 and 24 hours) was significantly correlated with high levels of IL-17A, whereas prolonged exposure (two weeks) was statistically associated with reduced levels of IL-17A. Our study extends these findings that relied on ambient $PM_{2.5}$ and NO_2 exposure, by using personal exposure assessment, by measuring $PM_{2.5}$ OP exposure, and by activating the cells.

Our study presents the specificity of analyzing cytokines in pregnant women, who present several variations of immunity compared to non-pregnant women. In a recent study (Jarmund et al., 2021), at basal state, most inflammatory cytokines among which TNF-α and IL-8 decreased in the second trimester. We observe here at the same period of pregnancy a global trend towards a decrease of these cytokines at basal state and after activation of immune cells, suggesting that there might be a cumulative negative effect of pregnancy and exposure to air pollutants on inflammatory cytokines secretions that could impair maternal health and capacity to respond to pathogens. Moreover, we observed a positive association between NO_2 exposure and IL-10 secretion upon activation with PHA, which could participate to the reduction of inflammatory cytokines that was detected here. Indeed, IL-10 has broad regulatory effects on several immune cells, and is involved in normal pregnancy processes of tolerance (Thaxton and Sharma, 2010). In our experimental

settings where whole blood cells are activated by PHA, IL-10 might be produced by regulatory T cells that are also involved in tolerance mechanisms towards fetuses during pregnancy (Tsuda et al., 2019).

Overall, our results on the impacts of air pollution exposure on the inflammatory function of the immune system may provide insights into the mechanisms underlying the adverse health effects of air pollution. In particular, IL-17A secretion is strongly linked to severe forms of asthma (Brandt et al., 2013; Weng et al., 2018), hypertension during pregnancy (Dhillion et al., 2012) and changes in birth weight (Laine et al., 2020). T lymphocytes, producers of IL-17A, are also pathogenic cellular components of autoimmune diseases, such as multiple sclerosis or psoriasis (Dhillion et al., 2012). Circulating IL-17A decreases during pregnancy (Jarmund et al., 2021), and our results suggest that air pollution could interfere with this regulation of Th17 cells, with potential consequences on maternal health. Studies have suggested that air pollution can affect the risk of multiple sclerosis and its severity, with a suggested link between air pollution-induced oxidative stress and proinflammatory cytokines (Abbaszadeh et al., 2021; Noorimotlagh et al., 2021). In addition, TNF-α has also been identified as playing a role in the inflammatory response in allergies, which are related to air pollution (Melén et al., 2008).

VI.2. Strengths and Limitations

One of the primary strengths of this study lies in its meticulous exposure measurements. Personal exposure measurements provided accurate assessment, tailored to the individual, in contrast to conventional assessments based on ambient exposure data obtained from monitoring stations or exposure models. Further, pregnant women are inclined to spend a greater portion of their time indoors, where air pollutants sources and chemical components differ from the ambient environment. Another asset is that the study focuses on cytokines produced not only at basal level, but also after activation of innate and T cells. Consequently, the obtained results closely approximate real immune cell functionality, reflecting the actual immune system response to aggression in the context of potential damages induced by air pollution exposure. Activation could potentially reduce confusion bias compared to basal state, by activating participants' cells using the same procedure. The use of OP measurements aims to account for the detrimental impacts of $PM_{2.5}$ through the oxidative stress pathway, and this led to clearer associations compared to the mass concentration metric. Furthermore, since the main sources contributing to PM and to the OP of PM were already reported in the

Grenoble area (Borlaza et al., 2021a), it is particularly relevant to examine both parameters in the current Grenoble-based study.

Although the study is based on an *a priori* hypothesis, the number of associations tested is relatively high and we have not applied a formal correction for multiple comparisons. It should therefore be recognized that some of the associations identified could result from chance finding and should therefore be interpreted with caution. The relatively small sample size of this study limits the statistical power, and a larger population would potentially lead to more robust conclusions. However, this is counterbalanced by the accuracy of the measurements, by the use of personal samplers, that significantly decreases measurement error. A further constraint of this study relates to the recruited population in SEPAGES, which does not reflect the overall diversity of the general population. This study is geographically restricted to the Grenoble region with a specific semi-continental climate and orography that lead to important thermal-inversions in the winter season, thereby increasing ground concentrations of pollutants. In addition, the included participants had higher levels of education and were more often non-smoker, as compared to pregnant women in France. Nevertheless, analyses in this homogeneous population are less prone to confounding biases related to social environment. Lastly, the conducted study specifically focuses on the independent effects of each pollutant on each included cytokine. This might be considered as simplistic, because interaction and cumulative effect of various air pollutants are expected, and cytokines do not operate in isolation within the immune system but are involved in a complex network of regulations and interactions of the body. However, studying the isolated effects provides a solid grasp of the underlying mechanisms, even though all intricacies of the system are not captured.

VII. Conclusion

In conclusion, our study provides significant insights into the relationships between exposure to air pollutants and immune function among pregnant women. These findings offer a convincing perspective on the association between specific air pollutants beyond PM mass concentration as oxidative potential and alterations of the immune system.This crucial data can be instrumental in creating strategies to reduce the oxidative potential of $PM_{2.5}$ and mitigate its adverse effects on health.

VIII. Supplemental Material

VIII.1. List of Figures

VIII.2. List of Tables

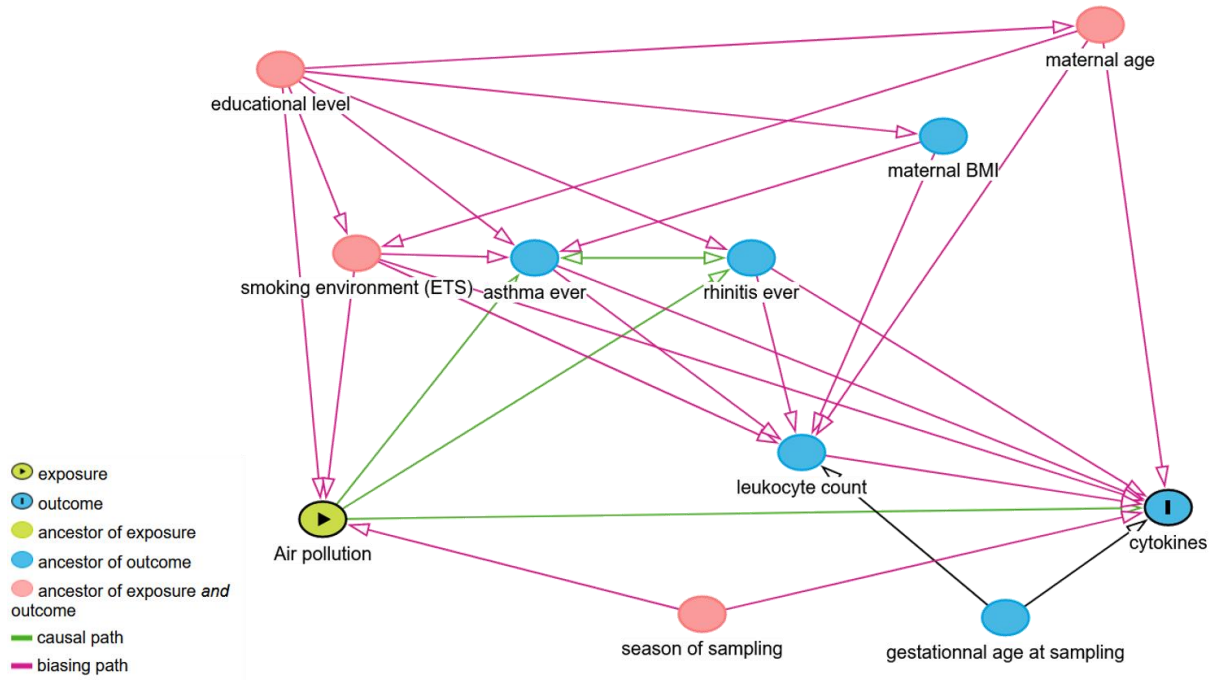

Figure S18. Directed acyclic graph (DAG) mapping causal relationships

Table S18. Unadjusted association between each air pollutant and each cytokine levels.

	NO_2 (µg/m³)			$PM_{2.5}$ (µg/m³)			OP_m^{DTT} (nmol/min/µg)			OP_v^{DTT} (nmol/min/m³)			OP_m^{AA} (nmol/min/µg)			OP_v^{AA} (nmol/min/m³)		
	N	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**
IL-8 (basal, pg/mL)	266	0.03	[-0.14, 0.2]	209	-0.05	[-0.23, 0.12]	193	-0.07	[-0.23, 0.09]	193	-0.18	[-0.36, -0.01]	193	-0.13	[-0.29, 0.04]	193	-0.28	[-0.48, -0.07]
MCP1 (basal, pg/mL)	266	0.03	[-0.13, 0.19]	209	-0.03	[-0.19, 0.13]	193	0.08	[-0.07, 0.23]	193	0.00	[-0.17, 0.17]	193	0.06	[-0.09, 0.22]	193	-0.02	[-0.21, 0.18]
RANTES (basal, pg/mL)	266	0.09	[-0.14, 0.32]	209	-0.07	[-0.23, 0.1]	193	-0.01	[-0.17, 0.14]	193	-0.07	[-0.24, 0.1]	193	-0.01	[-0.16, 0.15]	193	-0.09	[-0.29, 0.1]
IFN-γ (PHA, pg/mL)	264	0.04	[-0.08, 0.16]	204	-0.02	[-0.14, 0.09]	188	-0.01	[-0.12, 0.1]	188	-0.05	[-0.17, 0.07]	188	0.01	[-0.1, 0.13]	188	-0.03	[-0.17, 0.11]
IL-2 (PHA, pg/mL)	264	0.03	[-0.09, 0.16]	204	0.00	[-0.12, 0.13]	188	0.02	[-0.1, 0.13]	188	0.04	[-0.09, 0.16]	188	0.07	[-0.05, 0.18]	188	0.07	[-0.07, 0.22]
IL-9 (PHA, pg/mL)	264	0.09	[-0.04, 0.21]	204	0.04	[-0.09, 0.17]	188	0.06	[-0.06, 0.18]	188	0.10	[-0.04, 0.23]	188	0.03	[-0.09, 0.16]	188	0.06	[-0.09, 0.22]
IL-10 (PHA, pg/mL)	264	0.19	[0.04, 0.33]	204	0.00	[-0.13, 0.13]	188	0.09	[-0.03, 0.21]	188	0.08	[-0.05, 0.21]	188	0.12	[-0.01, 0.24]	188	0.15	[0, 0.31]
IL-13 (PHA, pg/mL)	264	0.04	[-0.09, 0.17]	204	0.08	[-0.05, 0.21]	188	0.02	[-0.11, 0.14]	188	0.12	[-0.02, 0.25]	188	0.02	[-0.1, 0.15]	188	0.09	[-0.06, 0.25]
IL-17A (PHA, pg/mL)	259	0.06	[-0.06, 0.18]	199	0.04	[-0.08, 0.16]	183	0.10	[-0.01, 0.21]	183	0.14	[0.02, 0.26]	183	0.15	[0.04, 0.26]	183	0.20	[0.06, 0.33]
TNF-α (PHA, pg/mL)	264	-0.18	[-0.34, -0.03]	204	-0.05	[-0.22, 0.13]	188	-0.11	[-0.27, 0.05]	188	-0.13	[-0.31, 0.04]	188	-0.08	[-0.25, 0.08]	188	-0.14	[-0.35, 0.06]
IFN-α (R848, pg/mL)	270	0.00	[-0.13, 0.12]	210	0.06	[-0.06, 0.19]	194	-0.01	[-0.12, 0.11]	194	0.05	[-0.08, 0.18]	194	0.08	[-0.04, 0.2]	194	0.10	[-0.05, 0.25]
IFN-γ (R848, pg/mL)	270	0.08	[-0.04, 0.2]	210	0.01	[-0.12, 0.14]	194	-0.04	[-0.15, 0.08]	194	-0.06	[-0.19, 0.07]	194	-0.04	[-0.16, 0.08]	194	-0.07	[-0.22, 0.08]
IL-1β (R848, pg/mL)	270	-0.03	[-0.15, 0.09]	210	0.07	[-0.05, 0.2]	194	-0.05	[-0.17, 0.07]	194	-0.02	[-0.15, 0.11]	194	-0.06	[-0.18, 0.06]	194	-0.06	[-0.21, 0.09]
IL-6 (R848, pg/mL)	270	-0.01	[-0.15, 0.12]	210	0.03	[-0.11, 0.17]	194	0.00	[-0.13, 0.13]	194	0.02	[-0.13, 0.16]	194	-0.09	[-0.22, 0.04]	194	-0.10	[-0.27, 0.06]
IL-8 (R848, pg/mL)	270	0.04	[-0.08, 0.17]	210	0.03	[-0.1, 0.16]	194	-0.05	[-0.17, 0.08]	194	-0.06	[-0.2, 0.07]	194	-0.14	[-0.27, -0.02]	194	-0.21	[-0.36, -0.05]
IL-10 (R848, pg/mL)	270	0.05	[-0.1, 0.21]	210	-0.09	[-0.25, 0.07]	194	0.06	[-0.08, 0.21]	194	-0.02	[-0.19, 0.14]	194	0.01	[-0.14, 0.17]	194	-0.04	[-0.23, 0.15]
IL-12p70 (R848, pg/mL)	270	0.07	[-0.05, 0.19]	210	0.01	[-0.12, 0.13]	194	-0.01	[-0.13, 0.11]	194	-0.06	[-0.19, 0.07]	194	-0.04	[-0.15, 0.08]	194	-0.07	[-0.22, 0.08]
TNF-α (R848, pg/mL)	270	0.01	[-0.16, 0.19]	210	0.09	[-0.1, 0.28]	194	0.00	[-0.19, 0.18]	194	0.00	[-0.2, 0.2]	194	-0.08	[-0.27, 0.11]	194	-0.13	[-0.36, 0.1]

Pollutants and cytokines variables were standardized by IQR.
Abbreviations: NO_2 nitrogen dioxide, $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm, OP: oxidative potential, DTT: dithiothreitol, AA: ascorbic acid, CI, confidence interval, IL, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

Table S19. Adjusted associations between each air pollutant and each cytokine levels.

	NO_2 (µg/m³)			$PM_{2.5}$ (µg/m³)			OP_m^{DTT} (nmol/min/µg)			OP_v^{DTT} (nmol/min/m³)			OP_m^{AA} (nmol/min/µg)			OP_v^{AA} (nmol/min/m³)		
	N	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**	**N**	**β**	**95% CI**
IFN-α R848 (pg/mL)	270	-0.04	[-0.16, 0.09]	210	0.05	[-0.07, 0.17]	194	-0.03	[-0.14, 0.09]	194	0	[-0.13, 0.13]	194	0.02	[-0.11, 0.15]	194	0.01	[-0.16, 0.18]
IFN-γ (R848, pg/mL)	270	0.07	[-0.06, 0.19]	210	0.03	[-0.1, 0.16]	194	-0.04	[-0.16, 0.07]	194	-0.06	[-0.2, 0.07]	194	-0.07	[-0.2, 0.06]	194	-0.11	[-0.28, 0.06]
IL-1β (R848, pg/mL)	270	-0.04	[-0.16, 0.07]	210	0.08	[-0.04, 0.2]	194	-0.07	[-0.18, 0.04]	194	-0.06	[-0.18, 0.07]	194	-0.09	[-0.21, 0.03]	194	-0.1	[-0.26, 0.06]
IL-6 (R848, pg/mL)	270	0.00	[-0.13, 0.12]	210	0.06	[-0.08, 0.19]	194	0.00	[-0.12, 0.12]	194	0.04	[-0.1, 0.18]	194	-0.05	[-0.19, 0.08]	194	-0.04	[-0.22, 0.14]
IL-8 (R848, pg/mL)	270	0.06	[-0.06, 0.17]	210	0.07	[-0.05, 0.2]	194	-0.07	[-0.18, 0.04]	194	-0.05	[-0.18, 0.07]	194	-0.12	[-0.24, 0]	194	-0.17	[-0.33, 0]
IL-10 (R848, pg/mL)	270	0.05	[-0.1, 0.19]	210	-0.09	[-0.24, 0.07]	194	0.05	[-0.09, 0.18]	194	-0.05	[-0.21, 0.11]	194	0.01	[-0.14, 0.16]	194	-0.05	[-0.25, 0.16]
IL-12p70 (R848, pg/mL)	270	0.06	[-0.07, 0.19]	210	-0.01	[-0.14, 0.13]	194	-0.01	[-0.13, 0.11]	194	-0.08	[-0.22, 0.06]	194	-0.06	[-0.19, 0.07]	194	-0.12	[-0.3, 0.05]
TNF-α (R848, pg/mL)	270	0.03	[-0.15, 0.21]	210	0.1	[-0.1, 0.31]	194	-0.01	[-0.19, 0.18]	194	0.01	[-0.2, 0.23]	194	-0.06	[-0.26, 0.15]	194	-0.12	[-0.39, 0.16]
IFN-γ (PHA, pg/mL)	264	0.03	[-0.09, 0.15]	204	-0.02	[-0.14, 0.1]	188	-0.02	[-0.13, 0.09]	188	-0.07	[-0.2, 0.06]	188	-0.02	[-0.14, 0.11]	188	-0.08	[-0.24, 0.08]
IL-2 (PHA, pg/mL)	264	0.01	[-0.11, 0.13]	204	-0.03	[-0.15, 0.09]	188	0.01	[-0.1, 0.12]	188	-0.03	[-0.15, 0.1]	188	0.02	[-0.1, 0.14]	188	-0.02	[-0.19, 0.14]
IL-9 (PHA, pg/mL)	264	0.06	[-0.07, 0.19]	204	0.02	[-0.11, 0.15]	188	0.04	[-0.08, 0.16]	188	0.05	[-0.08, 0.19]	188	-0.03	[-0.16, 0.11]	188	-0.01	[-0.19, 0.16]
IL-10 (PHA, pg/mL)	264	0.18	[0.03, 0.32]	204	-0.04	[-0.17, 0.1]	188	0.08	[-0.04, 0.2]	188	0.02	[-0.11, 0.16]	188	0.06	[-0.07, 0.2]	188	0.06	[-0.11, 0.24]
IL-13 (PHA, pg/mL)	264	0.01	[-0.12, 0.13]	204	0.06	[-0.07, 0.19]	188	0	[-0.11, 0.12]	188	0.07	[-0.07, 0.2]	188	-0.03	[-0.16, 0.1]	188	0.03	[-0.15, 0.2]
IL-17A (PHA, pg/mL)	259	0.03	[-0.08, 0.15]	199	-0.01	[-0.13, 0.1]	183	0.11	[0, 0.22]	183	0.08	[-0.04, 0.2]	183	0.1	[-0.01, 0.22]	183	0.1	[-0.05, 0.25]
TNF-α (PHA, pg/mL)	264	-0.18	[-0.34, -0.02]	204	-0.02	[-0.2, 0.16]	188	-0.11	[-0.28, 0.06]	188	-0.11	[-0.3, 0.08]	188	-0.05	[-0.23, 0.13]	188	-0.09	[-0.34, 0.15]
IL-8 (basal, pg/mL)	266	0.07	[-0.1, 0.24]	209	-0.02	[-0.19, 0.16]	193	-0.06	[-0.22, 0.1]	193	-0.12	[-0.31, 0.06]	193	-0.05	[-0.23, 0.12]	193	-0.18	[-0.41, 0.06]
MCP-1 (basal, pg/mL)	266	0.03	[-0.14, 0.2]	209	-0.01	[-0.18, 0.16]	193	0.07	[-0.08, 0.23]	193	0.00	[-0.17, 0.18]	193	0.08	[-0.09, 0.25]	193	0.00	[-0.23, 0.22]
RANTES (basal, pg/mL)	266	0.09	[-0.14, 0.32]	209	-0.06	[-0.23, 0.11]	193	-0.04	[-0.19, 0.12]	193	-0.09	[-0.27, 0.09]	193	-0.02	[-0.19, 0.15]	193	-0.14	[-0.36, 0.09]

Pollutants and cytokines variables were standardized by IQR. Models were adjusted on mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.

Abbreviations: NO_2 nitrogen dioxide, $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm, OP: oxidative potential, DTT: dithiothreitol, AA: ascorbic acid, CI, confidence interval, BMI, body mass index, IL, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein..

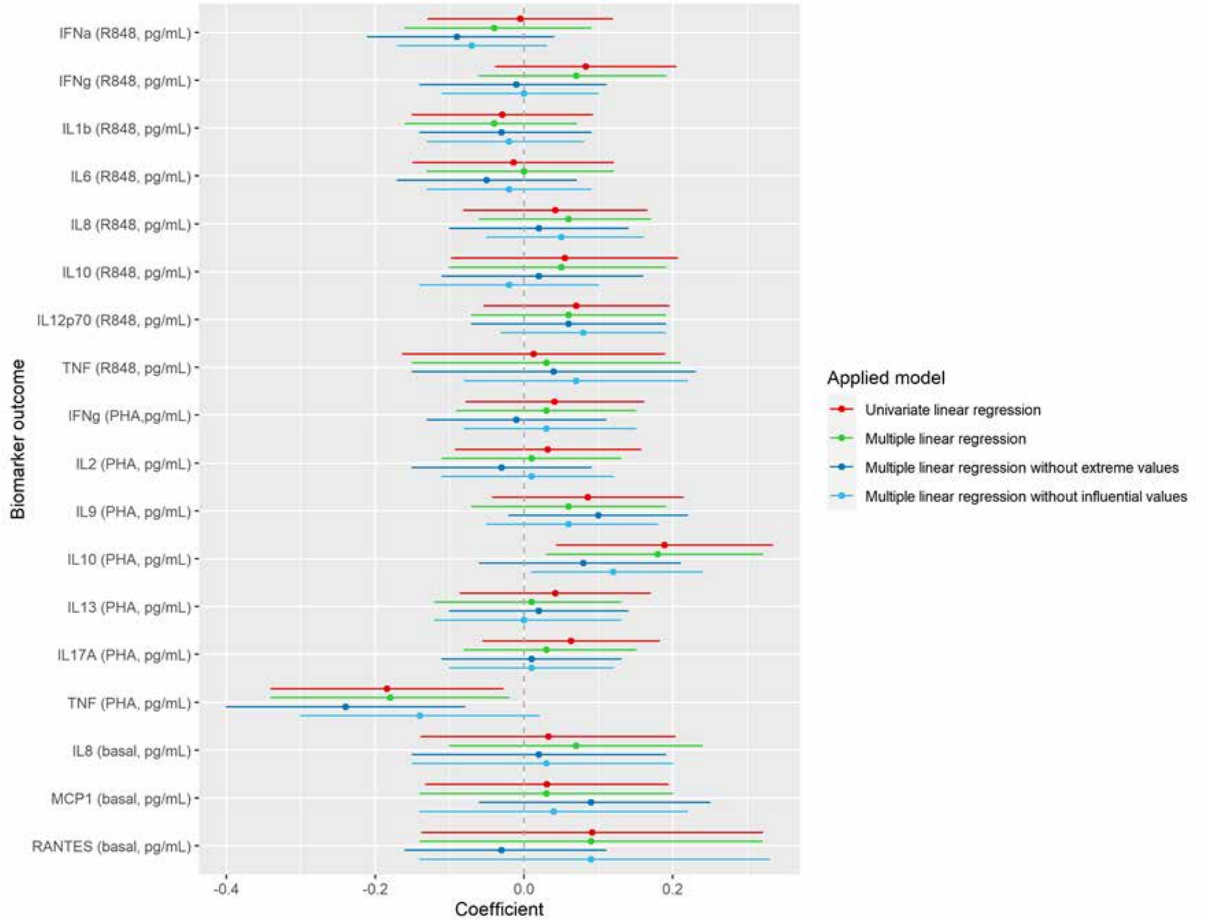

Figure S19. Sensitivity analyses of the associations between NO_2 and cytokine levels.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. ***Univariate****: regression model included air pollutant exposure.* ***Multivariate****: Regression model was adjusted for mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.* ***Regression without extreme values:*** *Exclusions from the multivariate regression models were made for participants whose exposures or outcomes were outside the 1st and 99th percentiles. This exclusion accounted for approximately 2.5% of the total population.* ***Regression without influential values:*** *Multivariate regression with a Cook's distance above 4/n, where n represents the length of the regression population, were excluded from the analysis. This exclusion accounted for approximately 7% of the total population.*
Abbreviations: NO_2 nitrogen dioxide, CI, confidence interval, IQR, interquartile, BMI, body mass index, IL, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

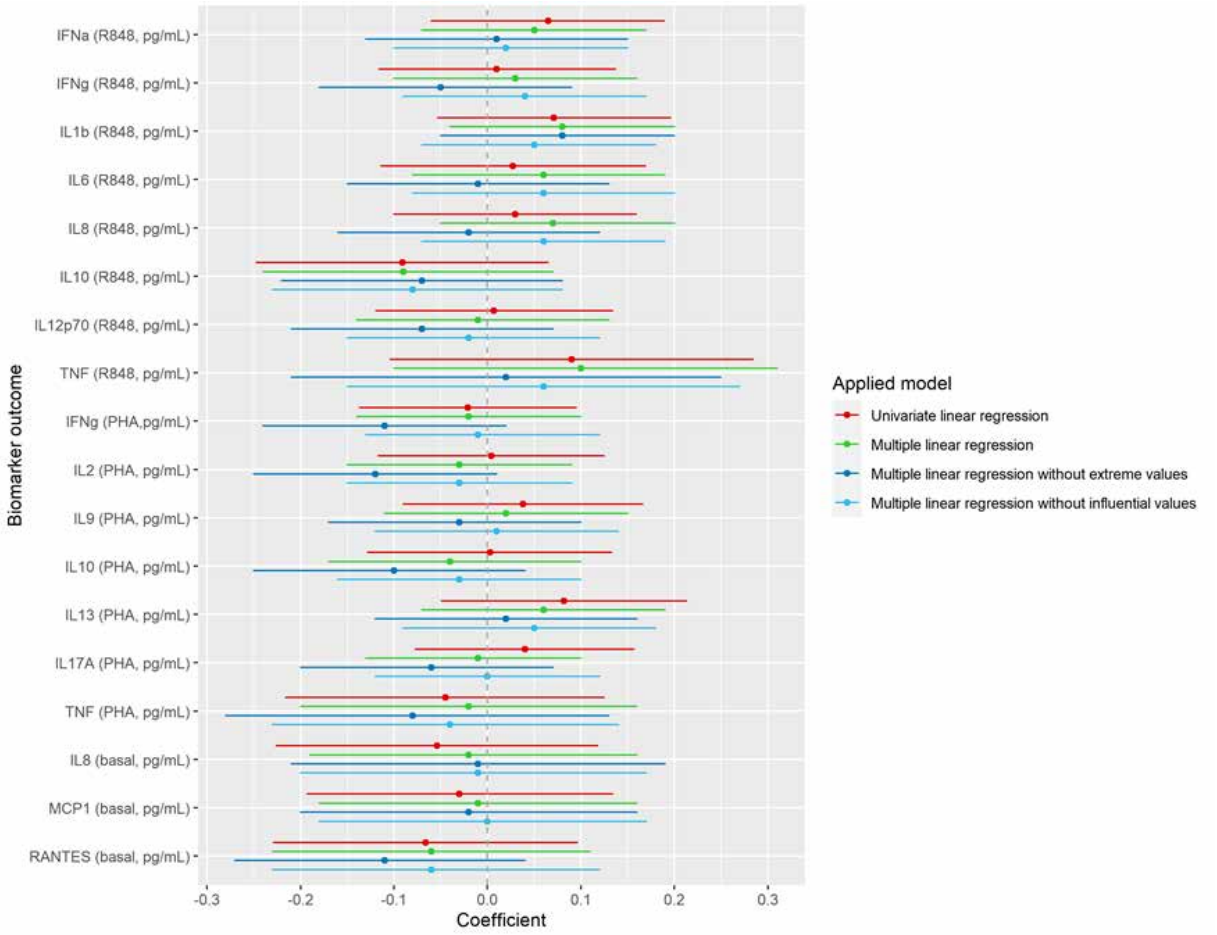

Figure S20. Sensitivity analyses of the associations between $PM_{2.5}$ and cytokine levels.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. ***Univariate****: regression model included air pollutant exposure.* ***Multivariate****: Regression model was adjusted for mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.* ***Regression without extreme values****: Exclusions from the multivariate regression models were made for participants whose exposures or outcomes were outside the 1st and 99th percentiles. This exclusion accounted for approximately 2.5% of the total population.* ***Regression without influential values****: Multivariate regression with a Cook's distance above 4/n, where n represents the length of the regression population, were excluded from the analysis. This exclusion accounted for approximately 7% of the total population.*
Abbreviations: $PM_{2.5}$ particulate matter with diameter ≤ 2.5 µm, CI, confidence interval, IQR, interquartile, BMI, body mass index, IL, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

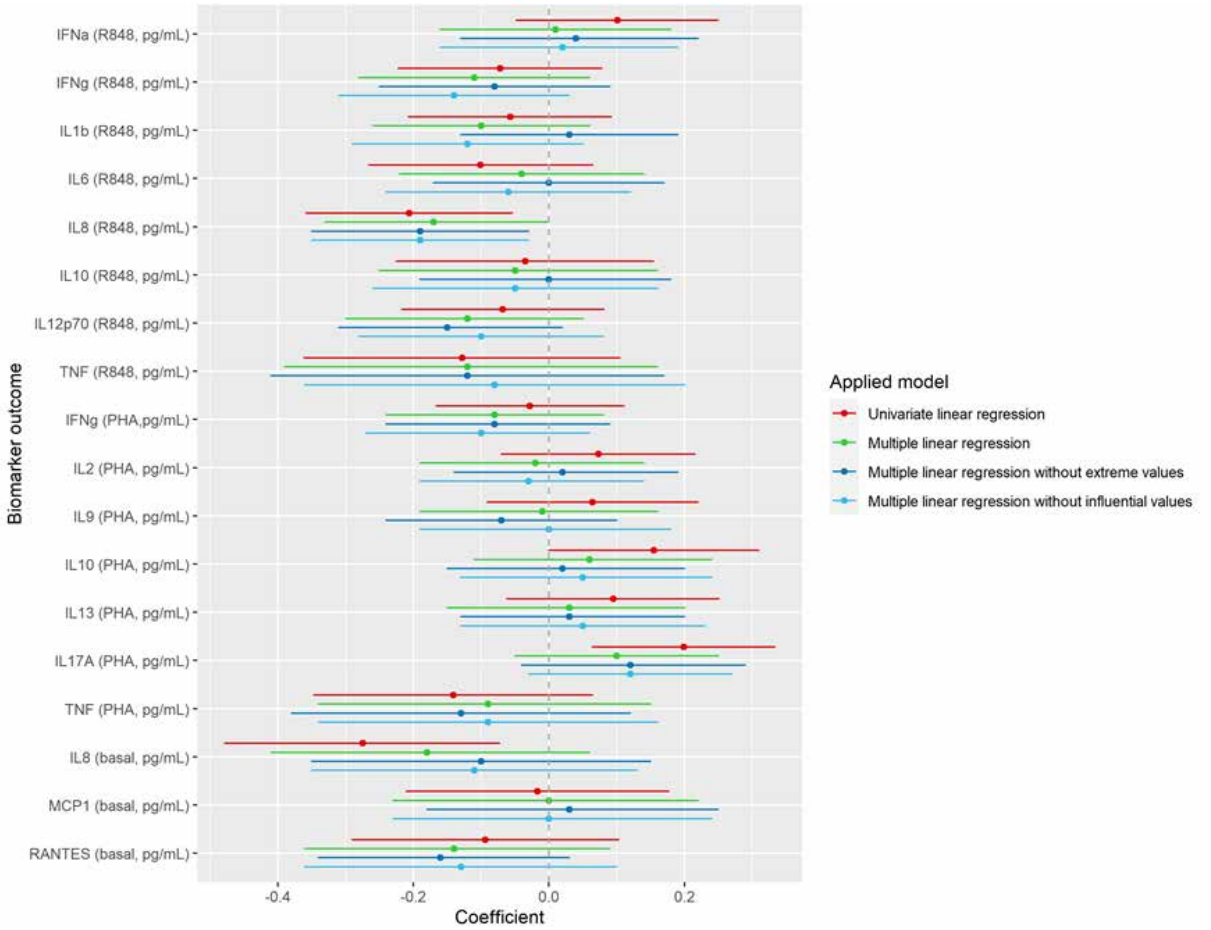

Figure S21. Sensitivity analyses of the associations between OP_v^{AA} and cytokine levels.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. ***Univariate****: regression model included air pollutant exposure.* ***Multivariate****: Regression model was adjusted for mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.* ***Regression without extreme values****: Exclusions from the multivariate regression models were made for participants whose exposures or outcomes were outside the 1st and 99th percentiles. This exclusion accounted for approximately 2.5% of the total population.* ***Regression without influential values:*** *Multivariate regression with a Cook's distance above 4/n, where n represents the length of the regression population, were excluded from the analysis. This exclusion accounted for approximately 7% of the total population.*
Abbreviations: OP: oxidative potential, AA: ascorbic acid, CI, confidence interval, IQR, interquartile, BMI, body mass index, IL, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

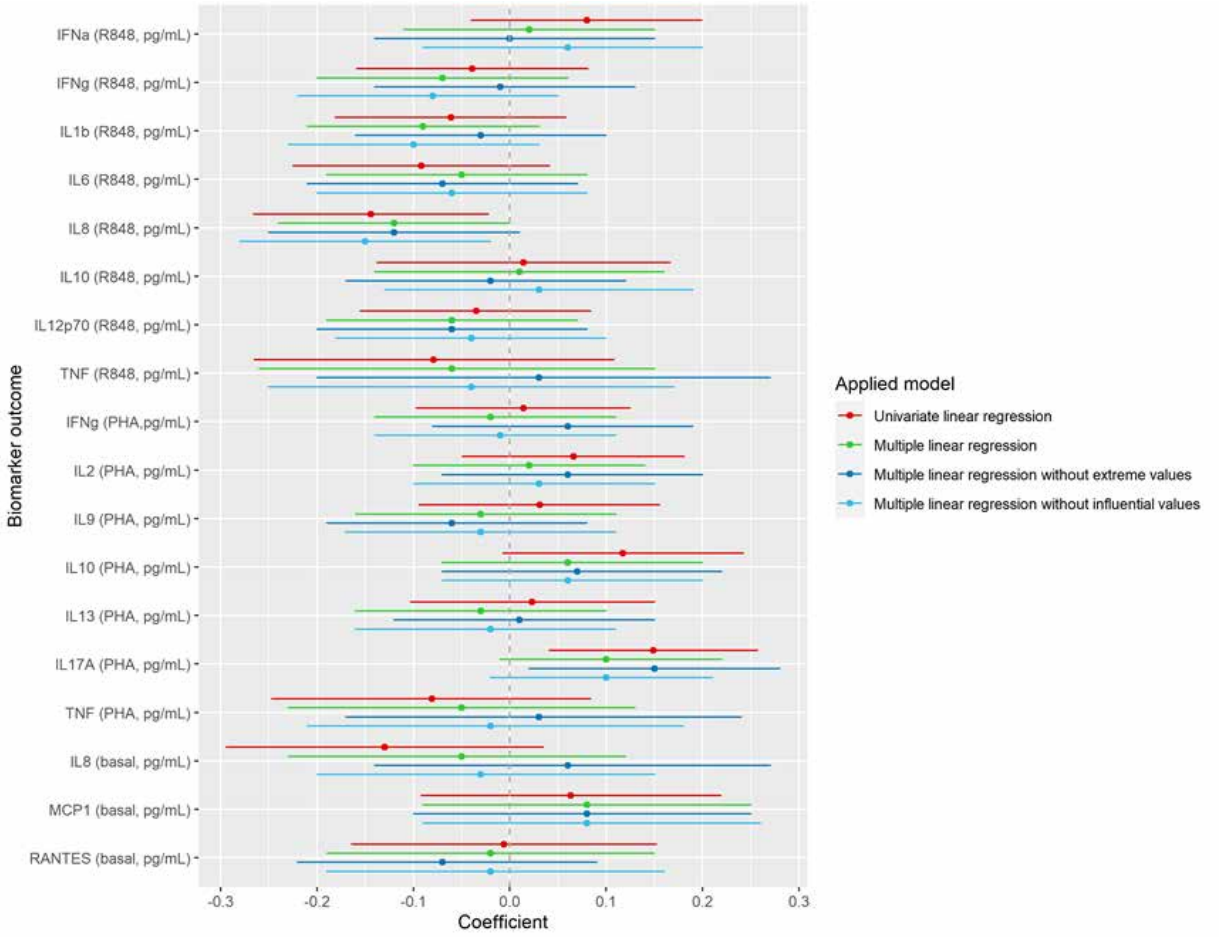

Figure S22. Sensitivity analyses of the associations between OP_m^{AA} and cytokine levels.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. ***Univariate****: regression model included air pollutant exposure.* ***Multivariate****: Regression model was adjusted for mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.* ***Regression without extreme values:*** *Exclusions from the multivariate regression models were made for participants whose exposures or outcomes were outside the 1st and 99th percentiles. This exclusion accounted for approximately 2.5% of the total population.* ***Regression without influential values****: Multivariate regression with a Cook's distance above 4/n, where n represents the length of the regression population, were excluded from the analysis. This exclusion accounted for approximately 7% of the total population.*
Abbreviations: OP: oxidative potential, AA: ascorbic acid, CI, confidence interval, IQR, interquartile, BMI, body mass index, Il, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

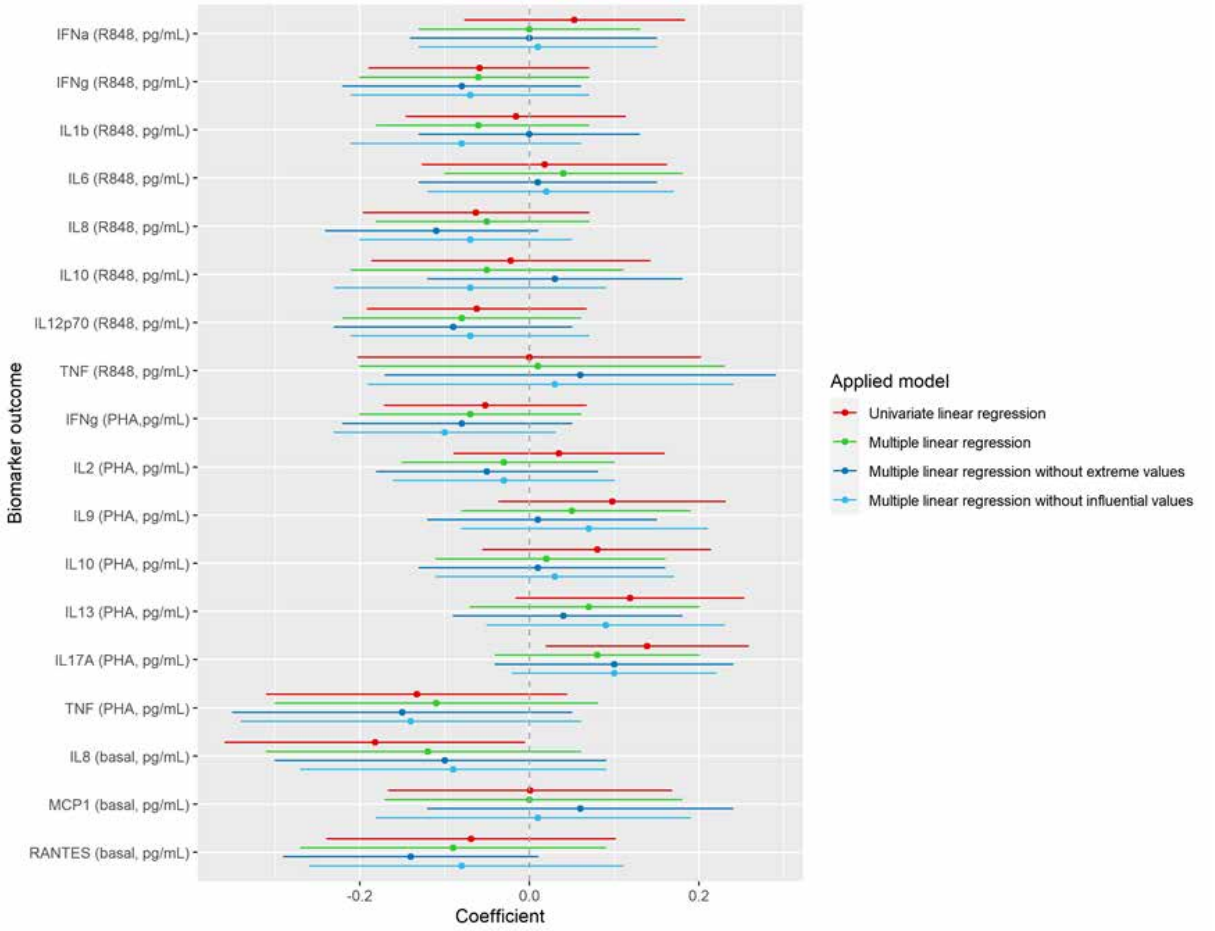

Figure S23. Sensitivity analyses of the associations between OP_v^{DTT} and cytokine levels.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. ***Univariate****: regression model included air pollutant exposure.* ***Multivariate****: Regression model was adjusted for mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.* ***Regression without extreme values:*** *Exclusions from the multivariate regression models were made for participants whose exposures or outcomes were outside the 1st and 99th percentiles. This exclusion accounted for approximately 2.5% of the total population.* ***Regression without influential values****: Multivariate regression with a Cook's distance above 4/n, where n represents the length of the regression population, were excluded from the analysis. This exclusion accounted for approximately 7% of the total population.*
Abbreviations: OP: oxidative potential, AA: ascorbic acid, CI, confidence interval, IQR, interquartile, BMI, body mass index, Il, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

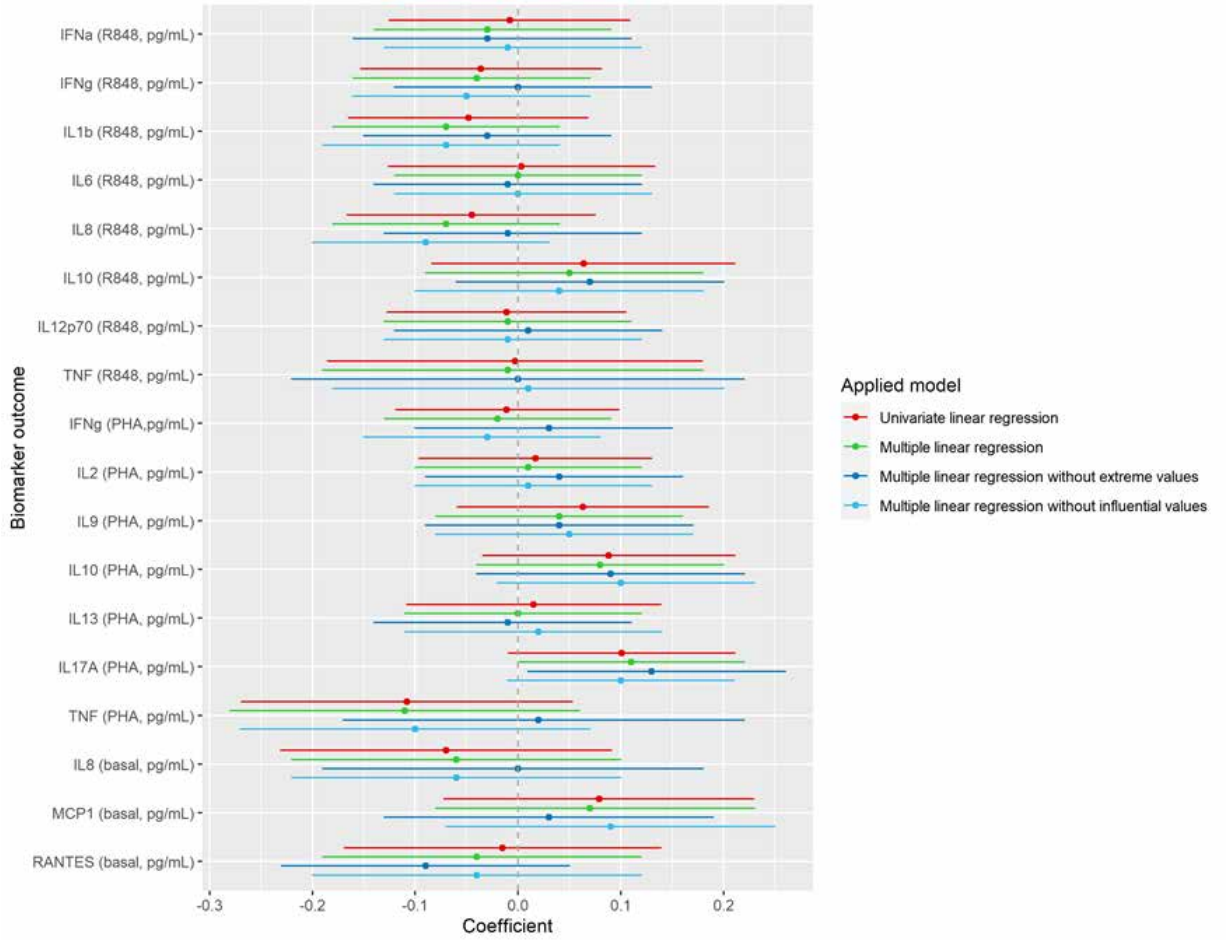

Figure S24. Sensitivity analyses of the associations between OP_m^{DTT} and cytokine levels.
Pollutants and cytokines variables were standardized by their IQR. Beta values and their 95% CI were estimated by multiple linear regression models. ***Univariate****: regression model included air pollutant exposure.* ***Multivariate****: Regression model was adjusted for mother age, BMI, active or passive smoking, educational level, white blood cell count, gestational age at sampling, and sampling season.* ***Regression without extreme values:*** *Exclusions from the multivariate regression models were made for participants whose exposures or outcomes were outside the 1st and 99th percentiles. This exclusion accounted for approximately 2.5% of the total population.* ***Regression without influential values****: Multivariate regression with a Cook's distance above 4/n, where n represents the length of the regression population, were excluded from the analysis. This exclusion accounted for approximately 7% of the total population.*
Abbreviations: OP: oxidative potential, AA: ascorbic acid, CI, confidence interval, IQR, interquartile, BMI, body mass index, Il, interleukin, IFN interferon, TNF tumor necrosis factor, RANTES regulated on activation, normal T cell expressed and secreted, MCP monocyte chemoattractant protein.

Chapter VI

Characteristics of $PM_{2.5}$ and OP in indoor and outdoor environments

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***Contribution*:** *The manuscript related to this work will be improved prior to submission, notably by trying to gather. Chemical analyses were performed by the workforce of IGE's plateau AirOSol. I was involved in part of the OP analysis, I performed the data curation and statistical analyses, generated the plots, and wrote the first draft of the manuscript. Although this did not lead to conclusive results, we spent a significant amount of time with Lucille J. S. Borlaza investigating source apportionment possibilities, and I would like to thank her for that.*

I. French summary

Contexte. Le potentiel oxydant (PO) des PM mesure la capacité des PM à générer du stress oxydant in vivo mais la variabilité spatiale de ce paramètre a été peu étudiée à fine échelle au sein de villes de tailles moyennes. Par ailleurs, la majorité du temps est passée dans des environnements intérieurs, pour lesquels le PO des PM a été très peu étudié. L'intérieur des logements est particulièrement crucial pour une partie de la population, et notamment les personnes âgées, les femmes enceintes et les jeunes enfants, pour lesquels l'intérieur du domicile est l'environnement principalement fréquenté. Les PM à l'intérieur des domiciles peuvent provenir de l'extérieur, mais peuvent également être générées à l'intérieur. Très peu d'études ont effectué une caractérisation chimique des particules dans l'intérieur de domiciles européens, et aucune n'a mesuré le PO.

Objectifs. Cette étude vise à décrire la variabilité spatial et saisonnière du PO en air extérieur à Grenoble, puis à caractériser les traceurs du PO dans l'air intérieur de domiciles et de les comparer à l'air extérieur.

Méthodes. Cette campagne de mesure a été réalisée dans un sous-échantillon de la cohorte SEPAGES, dans laquelle 41 familles se sont portées volontaires. Chaque domicile a été équipé pendant 7 jours de deux préleveurs bas volume à l'intérieur, équipés d'un filtre quartz et d'un filtre Teflon, respectivement, et, de la même manière, deux préleveurs étaient placés à l'extérieur. Les espèces chimiques analysées comportaient la fraction carbonée, ionique, métallique et le PO mesuré par l'acide ascorbique (AA) et le dithiothréitol (DTT).

Résultats. La quasi-totalité des participants n'ont ni fumé, ni utilisé d'encens et de bougies durant la semaine de prélèvement. Les médianes (Q1-Q3) de $PM_{2.5}$ sont très similaires à l'intérieur et à l'extérieur (10.3 [7.8, 14.9] µg/m^3 et 10.3 [7.4, 13.7] µg/m^3, respectivement. Le PO des PM présente également des niveaux proches à l'intérieur et à l'extérieur des logements. Le PO présente une forte variabilité spatiale,

particulièrement en hiver, alors que ces contrastes sont plus importants en été pour les $PM_{2.5}$. Comparé à l'extérieur des domiciles, le PO^{AA} en intérieur était beaucoup plus corrélé aux concentrations de cuivre, et légèrement plus corrélé aux concentrations de carbone organique. En outre, ces deux espèces chimiques ont des concentrations supérieures à l'intérieur par rapport à l'extérieur, et leurs faibles corrélations entre ces deux environnements suggèrent des sources intérieures de ces éléments, probablement liées aux activités des occupants. Le PO^{DTT} était très peu corrélé aux espèces chimiques mesurées, autant à l'intérieur qu'à l'extérieur. Enfin, les habitants passant l'aspirateur plus de deux fois par semaine présentent des niveaux de particules, d'espèces issues de la remise en suspension de PM, de cuivre et de PO^{AA} significativement supérieurs aux concentrations obtenues dans les logements dont les occupants ont passé l'aspirateur moins de deux fois dans la semaine.

Conclusions. Dans l'ensemble, nos résultats suggèrent une variation spatiale importante des $PM_{2.5}$, et particulièrement du PO des PM sur la ville de Grenoble. Les schémas d'exposition sont différents à l'intérieur des domiciles par rapport à l'extérieur, avec une forte influence de l'utilisation de l'aspirateur. Bien que le PO des PM ne soit pas significativement plus élevé à l'intérieur des logements par rapport à l'extérieur, les concentrations élevées de cuivre impactent fortement le PO^{AA} à l'intérieur, ce qui souligne la nécessité de prendre en compte les environnements intérieurs pour l'exposition personnelle au PO des PM.

II. Abstract

Context. The oxidative potential (OP) of PM measures the ability of PM to generate oxidative stress in vivo, but the spatial variability of this parameter remains poorly studied within medium-sized cities. Moreover, people spend the majority of time indoors, but there are very few studies reporting OP of PM in indoor residential environments. PM indoors can originate from the outdoors but can also be generated by indoor sources. Very few studies have conducted chemical characterization of particles within European households, and none have measured the OP.

Objectives. This study aims to describe the spatial and seasonal variability of OP in outdoor air in Grenoble, and then to characterize OP chemical drivers in indoor household air, in comparison with outdoor air.

Methods. This measurement campaign was conducted in a subsample of the SEPAGES cohort, in which 41 families volunteered. Two low-volume samplers were placed in the main living area of each home, and equipped with a quartz and a Teflon filter, respectively. Similarly, two samplers were placed outdoors. The chemical species analyzed included the carbonaceous, ionic, metallic fraction, and the OP measured by ascorbic acid (AA) and dithiothreitol (DTT).

Results. Most participants neither smoked nor used incense and candles during the sampling week. $PM_{2.5}$ median (Q1-Q3) concentrations are very similar indoors and outdoors (10.3 [7.8, 14.9] µg/m^3 and 10.3 [7.4, 13.7] µg/m^3, respectively). OP of PM also shows similar levels indoors and outdoors of homes. OP exhibits strong spatial variability, especially in winter, while these contrasts are more significant in summer for $PM_{2.5}$. Compared to the outdoors of homes, indoor OP^{AA} was much more correlated with copper concentrations and slightly more correlated with organic carbon concentrations. Furthermore, these two chemical species have higher concentrations indoors than outdoors, and their low correlations between these two environments suggest indoor origins, likely related to occupant activities. OP^{DTT} was

very weakly correlated with the measured chemical species, both indoors and outdoors. Finally, residents vacuuming more than twice a week had significantly higher levels of $PM_{2.5}$, of species resulting from PM resuspension, of copper, and of OP^{AA} concentrations compared to homes where occupants vacuumed less than twice a week.

Conclusions. Overall, our results suggest significant spatial variation in $PM_{2.5}$, especially in the OP of PM across the city of Grenoble. Exposure patterns differ indoors compared to outdoors, with a strong influence of vacuum cleaner use. Although the OP of PM is not significantly higher indoors compared to outdoors, elevated copper concentrations strongly impact indoor OP^{AA}, highlighting the need to consider indoor environments for personal exposure to PM OP.

III. Introduction

Particulate matter (PM) is estimated to be responsible for 7 to 8.8 million deaths per year worldwide (Lelieveld et al., 2019; WHO, 2016) and 2.3 to 3.8 million deaths are attributable to indoor air pollution specifically. The role of the oxidative stress pathway in PM's adverse health effects has been underlined in many studies (Gangwar et al., 2020; Kelly, 2003; Mudway et al., 2020). The oxidative potential (OP) of PM is an indicator integrative of PM's physicochemical complexity, developed to quantify PM's capacity to carry or generate reactive oxygen species in vivo (Ayres et al., 2008; Cho et al., 2005; Hellack et al., 2014). There are currently several acellular tests used, including the dithiothreitol (DTT) assay, ascorbic acid (AA) assay, glutathione (GSH) assay, electron paramagnetic resonance (EPR) spectroscopy, among others. The different assays were developed to capture different pathways for oxidative stress generation, such as direct PM-bound reactive oxygen species measurement (EPR spectroscopy) or indirect measurement, by measuring the depletion of lung antioxidant (AA, GSH) or reductant (DTT) (Bates et al., 2019; Shahpoury et al., 2022).

PM can penetrate indoor environment through building cracks, mechanical or natural ventilation (Bo et al., 2017), but can also be generated indoors, by synthetic building materials, or indoor activities such

as cooking, cleaning or smoking (Liu and Zhang, 2019; Tofful et al., 2021). While many studies compared indoor and outdoor environments in terms of mass concentration of PM, fewer studies additionally relied on a chemical and OP characterization of PM. The OP of PM and its chemical tracers have been studied during outdoor to indoor transport in environmental chambers or research house for OP^{DTT}, a cellular OP assay and OP^{EPR} (Hu 2023, Niu, 2021, Kurshid 2019), in a large-scale study on office buildings across Europe for OP^{GSH} and OP^{AA} (Szigeti et al., 2016), in Chinese student dormitories for OP^{DTT} (Yang et al., 2021) and in rural and urban Chinese homes for OP^{DTT} and a cellular test (Brehmer et al., 2020; Secrest et al., 2016). Results show high variability in OP during outdoor-indoor transport, due to the change in water-soluble iron and sulphate concentrations, that modifies particles' pH and therefore metals solubilities (Yang et al., 2021). The role of transition metals, mainly copper and iron, and of sulfates, and PAHs were pointed in most studies. Few studies have concurrently measured OP and PM chemical constituents, and most of them are not performed in residences (Hu et al., 2023; Niu et al., 2021; Sauvain et al., 2015; Szigeti et al., 2016, 2014), or are located in Asia (Anand et al., 2022; Guo et al., 2019; Secrest et al., 2016; Yang et al., 2021; Zhan et al., 2018; Zhang et al., 2021), with specific indoor sources .

To the best of our knowledge, a fine description of the residential indoor and outdoor $PM_{2.5}$ chemical composition and OP has not been performed in Europe to this date. We address this gap by measuring concurrent OP^{AA}, OP^{DTT} and $PM_{2.5}$ chemical species in the indoor and outdoor environments of 41 homes in Grenoble, France. The objectives of our study are: (i) to provide a description of $PM_{2.5}$ and its main chemical constituents indoors and outdoors, ii) to investigate the spatial variations of PM constituents and OP across the city of Grenoble (France), and (iii) to investigate OP specific indoor tracers, and its potential sources.

IV. Material and methods

IV.1. Site description

The present study was conducted in France, for a sub-sample of the SEPAGES cohort whose families live within a 80 km radius of Grenoble (Lyon-Caen et al., 2019). The local meteorology that can be encountered in Grenoble are described by Bessagnet et al. (2020) and Borlaza et al. (2021b). Briefly, the Grenoble basin is surrounded by three mountain ranges, which leads to winter thermal inversion phenomena, favouring the ground concentration of pollutants.

In total, 41 families volunteered for this campaign. The spatial repartition of the homes is representative of the typologies encountered around an urban area, namely, peri-urban, urban background, urban hyper-centre and traffic sites. In each home, indoor and outdoor sampling were carried out simultaneously. Two indoor samplers were placed in the main living area while two outdoor samplers were placed on the adjacent balcony, terrace or garden when available. If there was none available, sampling was performed indoors only (N=12).

IV.2. Sampling procedure

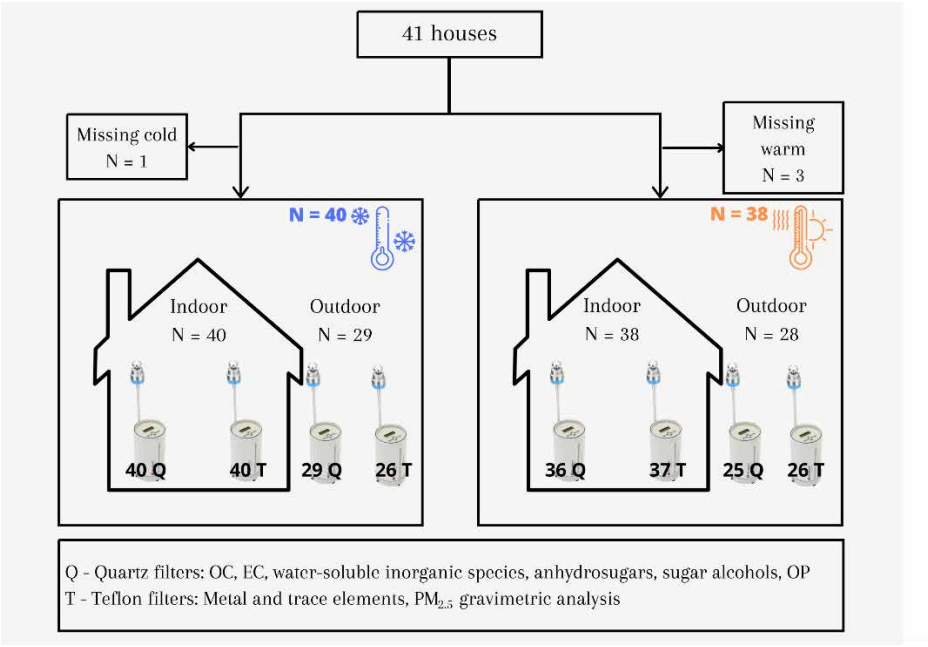

Figure 31. Schematic of the study protocol.

Low-volume samplers (Ecotech, MicroVol 1100) equipped with a $PM_{2.5}$ sampling head were operated a flow rate of 3 m^3/h during 7 ± 1 days. Sampling inlets of the instruments were set up in a height of 50-150 cm to sample in the breathing zone of the kids. In each measuring environment (i.e. indoor or outdoor), two such samplers were installed side by side, one equipped with a quartz filter (Tissuquartz 47 mm) and one with a Teflon filter (PTFE 47 mm). The four samplers were started simultaneously. 37 out of 41 homes were equipped twice: once during a cold period (2018-11-26 to 2019-04-18) and once during a warm period (2019-05-09 to 2019-10-31). At the end of the sampling week, filters were transported on ice and cold-stored (-20°C) until gravimetric and chemical analyses.

Teflon filters were subjected to double weighing prior and after sampling, in accordance with standard NF EN 12341, with a time between weighing of 12h and 24-72h, respectively. This was performed in ATMO SUD's gravimetric laboratory, where strict control of the laboratory environment is applied (grey clean room, relative humidity in the 45%-50% range, temperature of 19°C to 21°C, 1 µg resolution) and with filter conditioning in the gravimetry laboratory for at least 48 h prior to weighing. The total operating volume recorded by the samplers was used to calculate $PM_{2.5}$ mass concentration. The time and volume of sampling recorded by the samplers is calculated only when the pump is operating, accounting for periods during which the sampler could have been recorded unplugged by the volunteer. At the end of the sampling week, smoking, the use of candles and incense, vacuuming habits (number of times and duration), and the use of the hotplates/oven (number of times and duration) were assessed through a questionnaire performed by the field workers.

During the overall period of the measurement campaign, daily PM_{10} filters were collected at the central monitoring site "Les Frênes" of the regional monitoring Air Quality agency (Atmo Aura) using a Digitel DA-80 (30 m^3/h). Quartz fiber filters were analyzed for the same set of chemical species and OP and PM_{10} mass was also monitored continuously at this site. In the rest of this article, "outdoor" will refer to the measurements outside of the house, and "ambient" will refer to the measurements at the central monitoring station.

IV.3. Chemical analyses

Filter samples were subjected to chemical analysis to measure the carbonaceous fraction, water-soluble ions, anhydrosugars and polyols, on the quartz filter; trace elements analysis was performed on the Teflon filter.

Organic and elemental carbon were analyzed on a 1.5 cm^2 punch of the quartz filter , by thermo-optical analysis following the EUSAAR2 protocol, using the Sunset Lab EC/OC analyzer (Birch and Cary, 1996; Cavalli et al., 2010). Briefly, the sample is placed in a quartz furnace and subjected to a prescribed temperature protocol, under a more or less oxidizing atmosphere. A gas stream carries the volatilized carbon through several steps, converting them into methane, analyzed by flame ionization detection. The measured organic carbon (OC) is converted into matter (OM) using a conversion factor of 1.8, as measured in ambient environment in Grenoble by Favez et al. (2010), and 1.4 for indoor environment, as measured by Tofful et al. (2021).

Water-soluble ions, anhydrosugars, and polyols were analyzed on the same water extract of the sample. Briefly, 10 mL ultrapure water is used for the solid/liquid extraction of the filter during 20 minutes under vortex agitation, prior to filtration using a 0.25 µm Acrodisc filter (Milipore Millex-EIMF).

Ionic fraction is measured by ion chromatography (IC, Thermo Fisher ICS 3000), following a standard protocol previously described (CEN, 2017; Jaffrezo et al., 2005), with a CS16 column for cations analysis (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) and an AS11HC column for anions (SO_4^{2-}, NO_3^-, Cl^-).

The analysis of anhydrosugars (levoglucosan, mannosan, galactosan), polyols (arabitol, sorbitol, mannitol) is performed on the water extract, using high-performance liquid chromatography (HPLC), with pulsed amperometric detection (PAD) (model Thermo Fisher 5000+) with Metrosep columns (Carb 1 – Guard+A Supp 15 – 150+Carb 1 – 150), following the procedure described by Piot et al. (2012).

Metallic trace elements were analyzed by Tera-environment, using inductively coupled plasma coupled with a mass spectrometer (ICP-MS) or an atomic emission spectrometer (ICP-AES) after acid digestion of a portion of the Teflon filters, following standardized protocols (Alleman et al., 2010b; CEN, 2005).

IV.4. OP analysis

Following Calas et al. (2018, 2017), $PM_{2.5}$ from quartz filter and PM_{10} was extracted in a simulated lung fluid (SLF, a mixture of 1,2-dipalmitoylphosphatidylcholine - DPPC and Gamble), reaching a final extraction concentration of 10 µg/mL for $PM_{2.5}$ filters and 25 µg/mL for PM_{10} filters, in order to maintain the amount of extracted PM constant between each sample. The extracts were then placed at 37°C under vortex agitation for 75 min. OP was measured using the dithiothreitol (DTT) and ascorbic acid (AA) assays. Extracts are placed in a 96-well plate (CELLSTAR, Greiner-Bio), and DTT or AA solutions are added, and their consumption is followed over time. For the DTT assay, a titration of the remaining DTT is performed every 10 min for a total reaction time of 30 min, by adding dithionitrobenzoic acid (DTNB), that forms the 2-nitro-5-thiobenzoic acid chromophore, measured by absorbance at 412 nm (TECAN spectrophotometer Infinite M200 Pro). For the AA assay, AA consumption can be measured directly by absorbance at 265 nm, and measurements are performed every 4min for a total reaction time of 30 min. Positive control tests were performed for every experiment, using a 1,4-naphthoquinone (1,4-NQ) solution for both assays. Particularly, 40µL and 80 µL of a 24.7µM 1,4-NQ solution were used for the DTT and the AA assays, respectively, and the measurement quality, estimated by the coefficient of variation of the positive control tests were at <3.2%.

IV.5. Data validation and statistical analysis

Due to volunteer's activities, some samplers were stopped during the measurement week (mostly unplugging due to the noise). Samples with less than 1 day (N=2) of sampling were excluded from the analysis. Although gravimetric measurements of $PM_{2.5}$ were performed on Teflon filters, some samplers equipped with Teflon filters were stopped while the adjacent sampler equipped with Quartz filter continued running. Since most species were measured on Quartz filters, reconstructed PM using chemistry were more consistent than the gravimetric analysis (formula in the SI). When a house had a Teflon filter but no Quartz filter to reconstruct PM, then the gravimetric analysis was used (N=5). Chemical species for which more than 75% of the samples were below the limit of detection (table S1) were not considered.

Spearman's correlation coefficients were calculated 1) by pairs of outdoor-ambient PM's chemical constituents and OP; 2) by pairs of indoor chemical species; 3) by pairs of outdoor chemical species; 4) by pairs of indoor-outdoor PM's chemical constituents and OP.

Indoor-outdoor ratios of each chemical specie and OP were calculated, and the significance of the concentration difference between indoor and outdoor environments was assessed using Wilcoxon rank sum test. I/O ratios higher than 1 indicate potential indoor emission sources of chemical specie, or OP. Lastly, the influence of vacuuming, (binary variable, <2 *vs.* ≥2 times during the sampling week) and of cooking (binary: < *vs.* ≥ median of the reported cumulated duration of oven and hotplates use) was assessed, by comparing the means of I/O ratio in each category and using Wilcoxon test for mean comparison. Smoking, candle and incense lightning were not considered in these habits due to the very limited number of cases for each.

V. Results and discussion

V.1. General description of the homes

Table 15. Description of the lifestyle habits reported by the participants of the indoor-outdoor campaign.

Lifestyle habits	Cold season (N = 40)	Warm season (N=38)
Tobacco consumption in the sampler room	1	0
Candle lighting in the sampler room	3	0
Incense use in the sampler room	0	0
Missing	/	1
Oven or hotplates use during the week (minutes)		
Q1	230	225
Median	285	285
Q3	380	359
Maximum	715	905
Vacuum cleaning use during the week (minutes)		
Q1	20	20
Median	30	33
Q3	47.5	60
Maximum	135	180
Number of vacuuming events during the week		
0	4	3
1	13	17
2	10	6
3	7	6
4	3	1
> 4 (max = 8)	3	5

The majority of inhabitants did not smoke or use candles or incense in the sampled room during the time of the measurement campaign. The median vacuuming duration was 30 minutes per week, and the maximum was 135 min and 180 min in the cold and hot seasons, respectively. Vacuuming was performed more than twice during the sampling week in 13 residences in the cold season, against 12 in the warm season. The maximum duration of total use of oven or hotplates was reached in summer, with 905 min of use, but the median duration was the same (285 min) for both seasons.

Out of the 41 homes of the study, 27 were located within a 10km radius of the local air quality monitoring station, of which 16 were located within 5km.

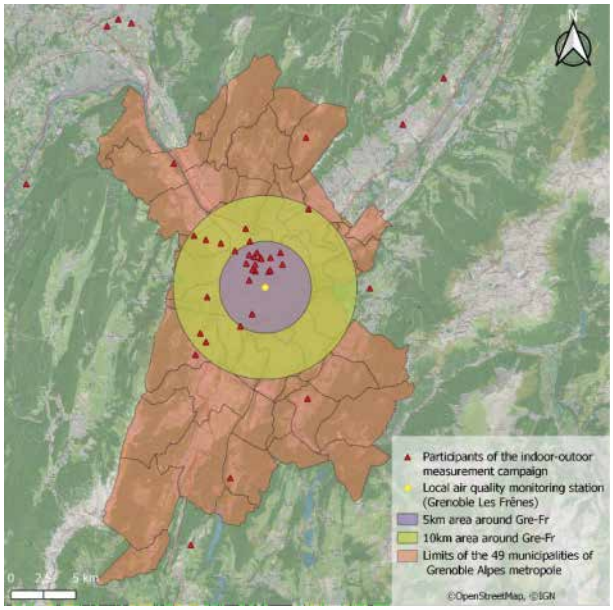

Figure 32. Geographical repartition of the residences in the Grenoble basin, and localization of the central air quality monitoring station.

V.2. Characteristics of concurrent indoor and outdoor measurements

V.2.1. PM reconstruction

The median (IQR) $PM_{2.5}$ concentration of the overall set of measurements was very similar indoors and outdoors, with 10.3 (7.8, 14.9) µg/m^3 and 10.3 (7.4, 13.7) µg/m^3, respectively (Table S2). $PM_{2.5}$ mass concentration ranged from 2.5 to 39.1 µg/m^3 indoors and from 2.1 to 35.3 µg/m^3 outdoors. The 25 µg/m^3 European air quality yearly threshold was exceeded only in one outdoor situation, during the cold season, while the average ambient PM_{10} concentration did not exceed 10 µg/m^3 over the same period of

time. Indoors, this threshold was exceeded six times mostly during the cold season (5 out of 6 times), and 2 of these 6 occurrences were in homes without concurrent outdoor sample to be compared with. Organic matter (OM) was the largest contributor to PM, accounting for more than 70% indoors and 60% outdoors for $PM_{2.5}$, and for 50% of ambient PM_{10} at both seasons (figure 1). For indoors samples during the cold season, the second largest contributor was EC, with a median (IQR) of 7.5% [5.6%, 8.4%] *vs.* 6.0 [4.5%, 6.2%] in the warm season. The second main contributor indoors in the warm season was non-sea-salt sulfate (7.3% [5.9%, 9.1%]). Outdoors, the second largest contribution is found for inorganic species like nitrate (12.4% [9.1%, 17.0%]) and non-sea-salt sulfate (11.8% [8.5%, 14.2%]) in the cold and warm seasons, respectively. The pattern of ambient PM_{10} chemical components is similar to outdoor $PM_{2.5}$ except for the larger dust contribution, which is the second largest contributor to PM_{10} in the warm season. PM concentrations, in all environments present a strong seasonality, with higher levels in the cold than in the warm season, which is mostly driven by the differences for OM and inorganic species, and which is consistent with a one-year study on PM_{10} sources at three sites in Grenoble (Borlaza et al., 2021).

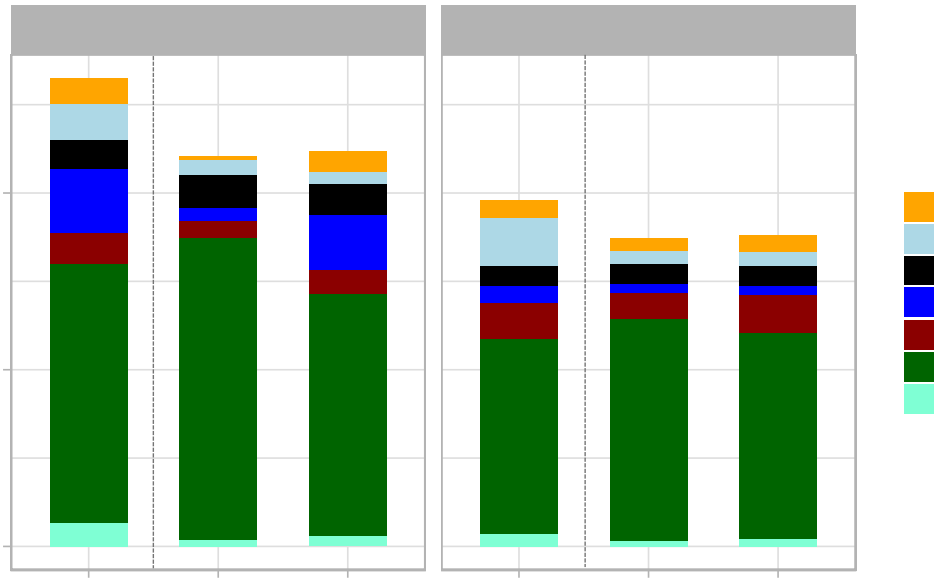

Figure 33. Median PM mass concentration its reconstructed contributors.

Many studies have investigated PM mass concentration in indoor residential environments, and they are mostly located in Asia (Anand et al., 2022; Brehmer et al., 2020; Li et al., 2017) or the USA (Li et al., 2017). Their findings show indoor $PM_{2.5}$ concentrations much higher than the concentrations in the 41 houses of this study, i.e. above 30 µg/m^3 and up to 630 µg/m^3 in a residence with smokers. To the best

of our knowledge, 6 studies had indoor residential $PM_{2.5}$ levels in the same range as ours, in the UK (7.9 ± 5.2 µg/m^3) (Jones et al., 2000), in Finland (9.5 ± 6.1µg/m^3) (Götschi et al., 2002), in Sweden (7.5 ± 6.0 µg/m^3), in Australia (15.5 ± 7.9 µg/m^3 with human activity) (Morawska, 2003), in the USA (7.0 µg/m^3 and 14.3 µg/m^3 in non-smoking houses and smoking houses, respectively) (Russo et al., 2015) and in China, when a filtration device was used (8.50 µg/m^3) (Zhan et al., 2018). Tofful et al. (2021) conducted an 18-day indoor-outdoor measurement campaign in a house located in a peri-urban site in Italy. They reported higher indoor and outdoor $PM_{2.5}$ concentrations than the concentrations found in this study, with higher average OC and EC concentration. However, the maxima were close (OC, 17 µg/m^3 indoors and 28 µg/m^3 outdoors *vs.* 19 and 24 µg/m^3 in our study) or lower (EC, 2.3 µg/m^3 *vs.* 3.5 µg/m^3) than the values found in the 41 houses of this study. It seems therefore that our study is in the low end of the results obtained in similar conditions in Europe.

V.2.2. Seasonality of the OP of PM

OP^{AA} presents a strong seasonality in all environments, with higher values in the cold than in the warm season (Figure 34). For example, indoor OP_m^{AA} activity has a median (IQR) of 0.13 (0.09, 0.17) *vs.*0.06 (0.04, 0.11) nmol/min/µg, in the cold and warm season, respectively. OP^{DTT} does not present any seasonality indoors. Outdoors, only OP_v^{DTT} has higher activities in the cold season than in the warm season, and this is influenced by the marked seasonality of $PM_{2.5}$ mass concentration. The pattern for outdoor OP_m^{DTT} differs from that of ambient PM_{10} available in this study, that has higher activities in the cold season than in the warm season for both OP_m^{DTT} and OP_v^{DTT}. Other studies reported different seasonal patterns of OP, depending on the assay and on the region of the world considered. Yang et al. (2021) investigated the seasonality of OP^{DTT} of $PM_{2.5}$ in six unoccupied offices and student dormitories in China, with one concurrent outdoor sampling on the outside of the building. They did not find seasonal patterns for OP_v^{DTT} neither indoors nor outdoors, but OP_m^{DTT} values were ranked as autumn > spring > summer > winter, with similar patterns indoors and outdoors. In a study of indoor and outdoor OP in European offices, Szigeti et al. (2016) found variable seasonality, with higher OP_m^{AA} in summer than in winter in Hungary and in the Netherlands, but results for OP_v^{AA} tended to be opposite, with higher values in winter than in summer. The seasonal variations in OP are strongly influenced by PM chemical compounds and the season at which they are emitted. This was underlined in a study covering France,

that found high seasonality for OP_v^{AA} and OP_v^{DTT} in some sites, including in Alpine valleys, while other sites (traffic, industrial and urban traffic) did not exhibit such seasonality (Weber et al., 2021). In the present study, the variability between OP assays shows that sources of OP^{AA} are predominant in winter, while the sources of OP^{DTT} vary throughout the year.

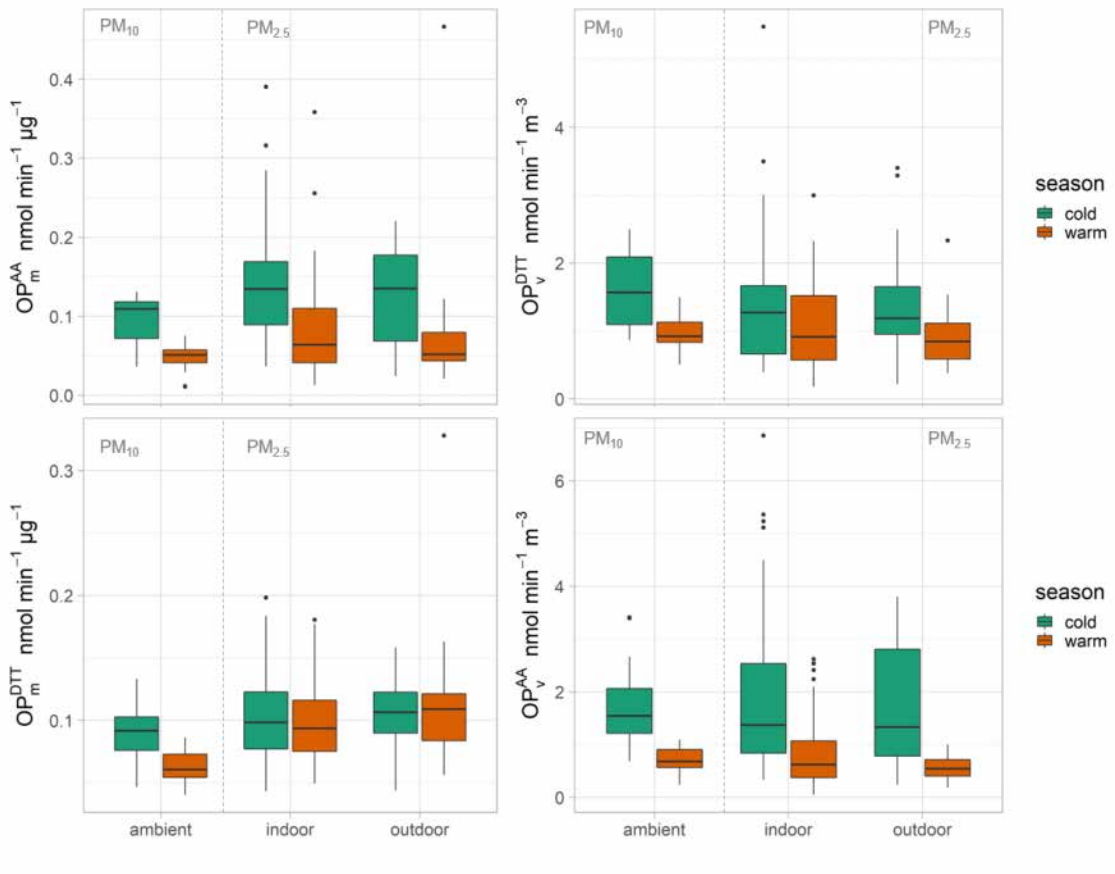

Figure 34. Seasonality of OP of $PM_{2.5}$ in the residential indoor and outdoor environments and of OP of PM_{10} in the ambient environment.

V.3. Spatial variations of PM and OP over Grenoble

In the cold season, outdoor $PM_{2.5}$ varies by a factor 7 among the measurement sites, ranging from 5 to 35 µg/m^3, whereas OP_v^{AA} and OP_v^{DTT} vary by a factor 16, respectively ranging from 0.24 to 3.81 nmol/min/m^3 and from 0.21 to 3.40 nmol/min/m^3. In the warm season, $PM_{2.5}$ vary by a factor 12, ranging from 2.1, 24.3 µg/m^3, whereas OP_v^{AA} and OP_v^{DTT} vary by a factor 5, respectively ranging from 0.19 to 1.00 nmol/min/m^3 and from 0.38 to 2.33 nmol/min/m^3. These results indicate important spatial contrasts in OP_v, particularly in winter. On the contrary, in the warm season, heterogeneities are more marked for PM mass concentrations compared to OP, which highlights the dominant variations of PM constituents that do not contribute to OP. This suggests that assessing exposure using the central monitoring station

in Grenoble would be associated with significant uncertainties, which would be greater in winter for OP, and in summer for $PM_{2.5}$.

To further characterize spatial variations of OP over the area, the outdoor-ambient correlation coefficients help identify species that have more spatial variability. Major inorganic ions (NO_3^-, NH_4^+, SO_4^{2-}), but also Na^+, Cl^- and levoglucosan present high correlation coefficients ($\rho_{out\text{-}amb}$>0.75) between ambient and outdoor environments, indicating a rather homogenous behavior of these species over the area of the study. Greater spatial homogeneity of secondary inorganic aerosols rich in nitrate and sulfate is consistent with their origin from long-range transport of air masses, as highlighted by previous studies in France (Borlaza et al., 2022; Favez et al., 2021). For levoglucosan, a tracer for biomass burning emissions, the high outdoor-ambient correlation suggests uniform distribution of the emission sources over the Grenoble basin, together with a well-mixed atmosphere. This is in line with a previous study on PM_{10} sources in Grenoble, that found strong correlations of the biomass burning source across three measurement sites located within 15 km of the city center (Borlaza et al., 2021). Moderate to low correlation coefficients are found between outdoor and ambient samples for Mg^{2+}, Ca^{2+}, EC and most metals (except As). This result is quite surprising for Mg^{2+}, for which a similar pattern to Na^+ is expected, considering that both species are tracers of aged seasalts aerosols. Therefore, this suggests a local dust origin for Mg^{2+}. For Ca^{2+}, EC, Cu and Sb, these moderate correlations suggest spatially variable patterns for sources linked to dust and road traffic (both exhaust and non-exhaust), which is consistent with the previous study that observed stronger similarity in the dust and traffic sources between two urban sites compared to the peri-urban one (Borlaza et al., 2021). This is also in line with another study conducted in Grenoble (Ouidir et al., 2015), that estimated exposure to atmospheric pollutants using GPS-data, that highlighted stronger variability of NO_2, which is a traffic tracer, than $PM_{2.5}$.

Both OP_m^{AA} and OP_v^{AA} have a higher correlation coefficient (ρ>0.7) between ambient and outdoor environments as compared to OP^{DTT} (ρ<0.36), indicating a spatially more consistent pattern of the OP measured by the AA assay, compared to the DTT assay. A previous study on the spatiotemporal variation of several OP assays in the Midwestern US (Yu et al., 2021) found an opposite trend, with more spatial variation of OP_v^{DTT} compared to OP_v^{AA}.

Interestingly, most correlation coefficients are higher for homes located within 5km of the local air quality monitoring station compared to the coefficients for all houses (Table S5). Correlation coefficients for Ca^{2+} and V remain similar to the results obtained without filtering the homes. OP_v^{DTT} and OP_m^{DTT} are correlated (ρ>0.6) between the outdoor (<5km) and ambient environments, which was not the case when considering all houses (ρ<0.3), which further highlight the spatial variability of OP^{DTT}.

Table 16. Spearman's pairwise outdoor-ambient correlation coefficient for each species.

Species	$\rho_{out\text{-}amb}$
$PM_{2.5}$	**0.61**
OC	**0.62**
EC	0.49
Cl^-	**0.76**
NO_3^-	**0.81**
SO_4^{2-}	**0.81**
Na^+	**0.81**
NH_4^+	**0.80**
K^+	**0.66**
Mg^{2+}	0.08
Ca^{2+}	0.46
Levoglucosan	**0.89**
As	**0.61**
Cu	0.17
Mn	0.35
Pb	**0.60**
Sb	0.34
V	0.41
OP_v^{AA}	**0.71**
OP_v^{DTT}	0.36
OP_m^{AA}	**0.76**
OP_m^{DTT}	0.30

V.4. Comparison of PM exposures in the indoor and outdoor environments

V.4.1. Chemical drivers of $PM_{2.5}$ and OP

Table 17 shows the Spearman's correlation coefficients of $PM_{2.5}$ mass concentration and OP with each $PM_{2.5}$ chemical constituent analyzed. Regardless of the environment considered, $PM_{2.5}$ is highly correlated to OP_v^{DTT} (ρ > 0.7), but more moderately to OP_v^{AA} and is not correlated with both mass-normalized OP. Compared to outdoor, indoor $PM_{2.5}$ mass concentration is highly correlated to calcium

concentration (ρ=0.71 vs. 0.29 outdoors), presents weaker correlation with levoglucosan and ammonium, and stronger correlations with OC, EC, and trace elements such as Cu, Mn, Pb, and Sb, and particularly with SO_4^{2-} (ρ=0.56 vs. 0.28 outdoors). This suggests that indoor dusts, identified with calcium concentrations, are important drivers for $PM_{2.5}$, as well as EC and OC.

Indoors OP_v^{AA} has a similar correlation with K^+ compared to outdoors, is a lot more correlated with Cu (ρ=0.75 vs. 0.57 outdoors), and slightly more with the other trace elements. Correlation with EC was still high, although less than outdoors, and the OP_v^{AA}-levoglucosan correlation tended to be more moderate (ρ=0.56 vs. 0.67 outdoors). The OP_v^{DTT}-OC correlation was slightly stronger indoors compared to outdoors, and there was a weaker correlation with levoglucosan (ρ=0.36 vs. 0.52 outdoors) and K^+. The correlation pattern with metals was similar indoors and outdoors. Together, this suggests that indoor OP_v^{AA} is mainly driven by copper and EC concentrations, whereas OP_v^{DTT} is mainly driven by OC concentrations.

In both indoors and outdoors $PM_{2.5}$, OP_m^{DTT} does not show any correlation with any of the chemical components of $PM_{2.5}$. On the other hand, indoor OP_m^{AA} shows moderate correlations with EC, K^+ and Cu, while outdoor OP_m^{AA} additionally presents a high correlation to EC and levoglucosan. Although mass-normalized OP is often poorly correlated to the concentration of PM chemical species, the correlation of OP_m^{DTT} is surprising because it strongly differs from ambient PM_{10}, for which OP_m^{DTT} is highly correlated to levoglucosan, moderately to K^+, EC, Cl^- NO_3^-, and negatively to SO_4^{2-}. Since these species are not expected to be in the coarser fraction of PM, the same pattern could be expected, at least with outdoor OP_m^{DTT}. This suggests that unmeasured species dominate the response of OP_m^{DTT}, while OP_m^{AA} is probably driven by combustion sources. While the result with OP_m^{DTT} indoors could be explained by sources rich in organic species specific to the indoor environment, that we did not measure (PAHs for example), this absence of correlation is quite unexpected for outdoors PM, since OP_m^{DTT} was found sensitive to metals and organics species.

Table 17. Spearman's correlation coefficients in the indoor and outdoor environments, between $PM_{2.5}$, OP_v^{AA}, OP_v^{DTT}, OP_m^{AA} and OP_m^{DTT} and the set of $PM_{2.5}$ chemical constituents.

Species	indoor					outdoor				
	$PM_{2.5}$	OP_v^{AA}	OP_v^{DTT}	OP_m^{AA}	OP_m^{DTT}	$PM_{2.5}$	OP_v^{AA}	OP_v^{DTT}	OP_m^{AA}	OP_m^{DTT}
$PM_{2.5}$	**1.00**	**0.63**	**0.83**	0.18	0.14	**1.00**	**0.63**	**0.79**	0.22	-0.14
OP_v^{AA}	**0.63**	**1.00**	**0.69**	**0.85**	0.44	**0.63**	**1.00**	**0.55**	**0.86**	0.12
OP_v^{DTT}	**0.83**	**0.69**	**1.00**	0.36	**0.62**	**0.79**	**0.55**	**1.00**	0.25	0.43
OP_m^{AA}	0.18	**0.85**	0.36	**1.00**	0.49	0.22	**0.86**	0.25	**1.00**	0.27
OP_m^{DTT}	0.14	0.44	**0.62**	0.49	**1.00**	-0.14	0.12	0.43	0.27	**1.00**
OC	**0.96**	**0.64**	**0.80**	0.24	0.17	**0.91**	**0.66**	**0.75**	0.34	-0.03
EC	**0.71**	**0.75**	**0.65**	**0.51**	0.23	**0.64**	**0.86**	**0.63**	**0.72**	0.20
Cl^-	**0.57**	0.49	0.39	0.24	-0.09	**0.52**	**0.66**	0.48	**0.56**	0.07
NO_3^-	**0.63**	**0.60**	0.50	0.35	0.07	**0.60**	**0.59**	0.48	0.46	0.01
SO_4^{2-}	**0.56**	0.17	0.50	-0.12	0.15	0.28	-0.22	0.17	-0.47	-0.16
Na^+	0.37	0.35	0.29	0.18	-0.02	0.26	0.40	0.22	0.37	0.04
NH_4^+	0.09	-0.02	0.11	-0.03	0.21	0.49	0.15	0.36	-0.07	-0.06
K^+	**0.57**	**0.69**	0.49	**0.52**	0.15	**0.63**	**0.67**	**0.57**	**0.57**	0.14
Mg^{2+}	**0.67**	0.32	0.49	0.01	-0.02	**0.52**	0.10	0.35	-0.20	-0.15
Ca^{2+}	**0.71**	0.32	**0.53**	-0.03	0.01	0.29	-0.05	0.11	-0.32	-0.24
Levoglucosan	0.39	**0.56**	0.36	0.44	0.11	**0.57**	**0.77**	**0.52**	**0.72**	0.14
As	0.42	0.36	0.41	0.22	0.19	**0.55**	0.49	0.46	0.35	-0.07
Cu	**0.51**	**0.75**	**0.57**	**0.64**	0.39	0.45	**0.57**	**0.54**	0.41	0.07
Mn	**0.52**	**0.53**	0.40	0.35	0.06	0.42	0.47	0.39	0.38	-0.02
Pb	**0.56**	**0.55**	0.45	0.34	0.08	0.47	0.48	0.42	0.38	-0.03
Sb	**0.54**	**0.51**	0.42	0.30	0.03	0.43	0.43	0.40	0.32	-0.08
V	0.27	0.22	0.22	0.15	0.09	0.14	-0.13	0.03	-0.20	-0.23

V.4.2. Indoor sources of PM et PO

Indoor to outdoor ratios

Indoor to outdoor ratios of $PM_{2.5}$ main chemical constituents and OP were calculated and are presented in figure 4. This figure shows that few species present average indoor concentrations higher than the outdoor ones. Only organic carbon and copper concentrations are found significantly ($p<0.05$, see Table S2) higher indoors than outdoors, with median (Q1-Q3) I/O ratio of 1.6 (1.1, 2.3) and 1.6 (0.8, 1.9), for OC and Cu, respectively. Both additionally present low indoor-outdoor correlations (≤0.20, Table 18), indicating that indoor levels of OC and Cu are mostly influenced by indoor sources and processes instead of those of outdoors. The OC I/O ratio is consistent with the chemistry of indoor sources, since synthetic building materials, paints, plastics, cooking activities can emit semi-volatile organic compounds that sorb onto $PM_{2.5}$, thereby enriching its carbonaceous fraction (Abbatt and Wang, 2020; Maung et al., 2022). Concerning Cu I/O ratios, previous studies also found higher indoor than outdoors concentrations of Cu and suggested several sources such as certain electrical appliances that have a rotating motor such as vacuum cleaners or electric fans (Tofful et al., 2021; Yli-Tuomi et al., 2008; Zhao et al., 2006), as well as cooking and frying, depending on the utensils used (Molnár et al., 2007). Previous studies showed that these two compounds were of great concern for human health, since Chen et al. (2020) highlighted the role of OC in inducing increased levels of pro-inflammatory cytokines and lipid peroxidation biomarker in cells exposed to personal and indoor $PM_{2.5}$, and both biomarkers were associated with increased airway inflammation biomarker in the study participants. In the HELIX (Human Early Life Exposome) project, based on six longitudinal population-based birth cohorts in Europe, copper exposure during childhood was found to influence behavioral problems in children (Maitre et al., 2021).

Figure 35 indicates that a few other variables (As, PM mass, most OP) have median I/O ratios below or equal to 1, while most inorganic species such as SO_4^{2-}, Na^+, NO_3^- and NH_4^+ are significantly lower indoors than outdoors. This is consistent with previous findings that suggested to use SO_4^{2-} as a tracer for the infiltration of non-volatile $PM_{2.5}$ species, because of the lack of indoor sources for sulfates (Diapouli et al., 2013; Liu and Zhang, 2019; Sarnat et al., 2002). The I/O ratios of 0.5 (0.2, 0.9) and 0.4 (0.1, 0.9) for nitrates and ammonium respectively, are also consistent with previous findings

highlighting the frequent desorption of nitrates from the particulate to the gaseous phase when penetrating indoors (Liu and Zhang, 2019). Levoglucosan presented higher concentrations outdoors, with a median (IQR) I/O ratio of 0.88 (0.29, 1.12), which could be explained by the high contribution of the biomass burning source in winter, that would be higher outdoors than indoors. This is consistent with the correlation coefficients between indoor and outdoor, which are highest for potassium and levoglucosan (ρ=0.67 and 0.65, respectively, Table 18). Together, this suggests that biomass burning emissions outdoors are able to penetrate the indoor environment, which is consistent with previous studies that observed this penetration in houses with low air-exchanges (Tofful et al., 2021), or that some residences have indoor biomass burning sources. In fact, in 13 cases, indoor concentrations of levoglucosan were higher than outdoors, indicating that these participants could have been exposed to indoor biomass burning emissions.

Cl^- reached very high I/O ratio, particularly in the warm season with a median (Q1-Q3) ratio of 1.2 (1.0, 176), due to values below the quantification limit outdoors. This is probably caused by an important volatilization of Cl^- in the outdoor environment with a concurrent indoor environment presenting lower temperatures, leading to a more limited volatilization and therefore higher concentrations indoors. The relatively high indoor-outdoor correlation coefficient (ρ=0.61, Table 18) suggests that outdoor Cl^- partly penetrates indoor (Table 18). Outdoor Cl^- can originate from sea- and road salts and industry, and is mostly in the coarse PM fraction (Seinfeld and Pandis, 2016). Higher values indoors can also be caused by indoor sources of Cl^- such as chlorine-based cleaning agents or from the chlorinated municipal tap water, as suggested in previous studies (Habre et al., 2014; Zhao et al., 2006).

Interestingly, $PM_{2.5}$, intrinsic and volume-normalized OP^{AA} and OP^{DTT} do not present any statistically different activities indoors compared to outdoors, even when splitting by season, and have median I/O ratios between 0.88 (OP_v^{AA}) and 0.99 ($PM_{2.5}$). However, maxima for volume-normalized OP were reached in the same household, that had no concurrent outdoor sampling. Noteworthy, OP_v activities reached levels almost twice as much as the maximum OP_v reached outdoors during the entire measuring campaign (6.86 *vs.* 3.81 and 5.48 *vs.* 3.40 for OP_v^{AA} and OP_v^{DTT}, respectively). The high OP_v were probably caused by the very high metal concentrations (Cu, Pb, Sb) reached in that house, and were associated with high concentrations of EC and inorganic species (NO_3^-, NH_4^+, SO_4^{2-}). The impact of

outdoor air cannot be disentangled in this residence, since it was not measured concurrently, but such observation highlights the importance of considering indoor air when estimating exposure to OP.

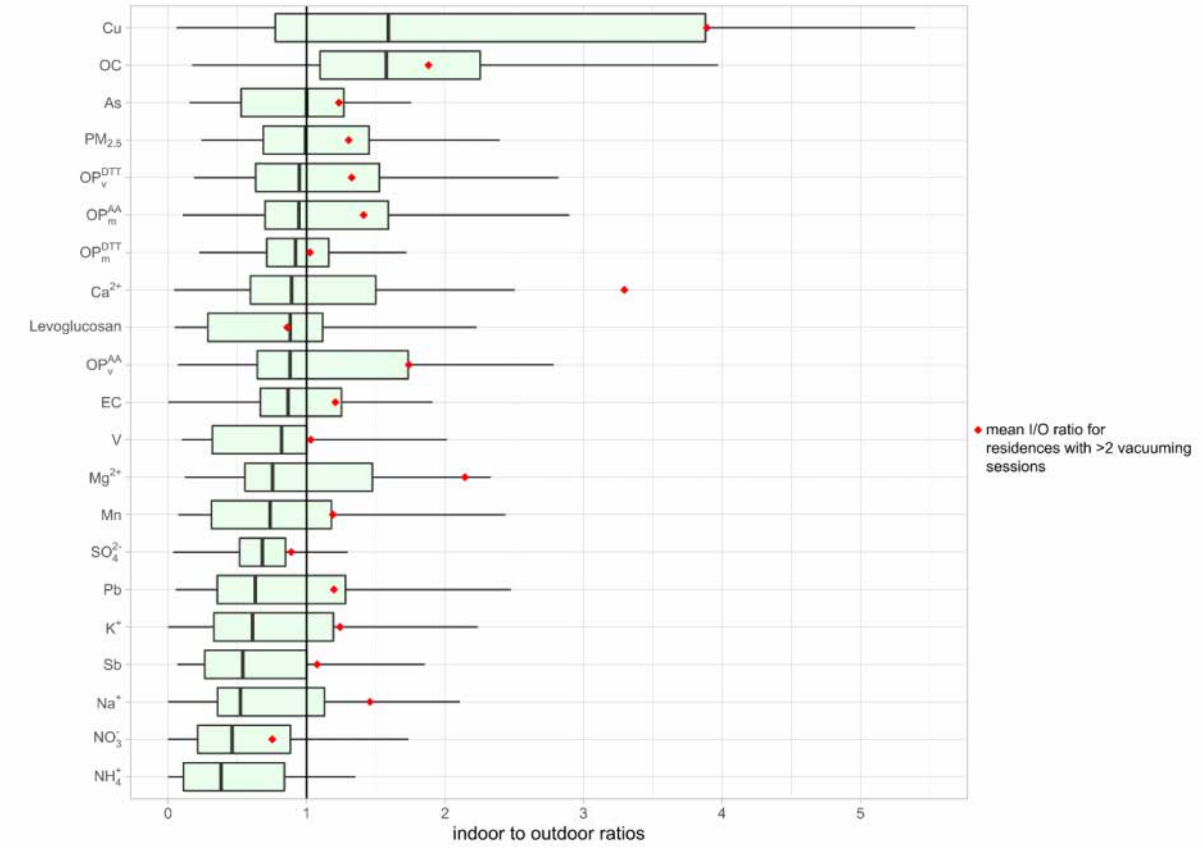

Figure 35. Indoor to outdoor ratios of concentrations of $PM_{2.5}$, its chemical constituents and OP.
Note: mean I/O ratio for NH_4^+ was not shown for residences with >2 vacuuming sessions because it reached a very high value, due to outliers.

Influence of habits on the OP of $PM_{2.5}$

The influence of vacuuming frequency and of the total duration of hotplates and oven use was investigated on OP and $PM_{2.5}$ mass concentration, since it was previously identified to influence both dust resuspension and copper emission (Vicente et al., 2020). The mean I/O ratios for people that vacuumed more than twice during the week is shown on Figure 35. Resuspension of settled dust during vacuuming is suggested by the higher average I/O ratios compared to those of all participants for most chemical species, and particularly OC, Ca^{2+}, Mg^{2+} and Na^+, and Cu. Cu could also originate from emissions of the vacuum cleaner itself. Significantly higher OP_v^{AA} is also obtained in houses with more than 2 vacuuming sessions compared to the other houses, with median (IQR) OP activity of 1.21 (0.67, 2.46) vs. 0.84 (0.40, 1.42) nmol/min/m^3 (p-value of the Wilcoxon test for mean comparison = 0.02).

$PM_{2.5}$ mass concentration presented a similar trend [12.2 (9.0, 15.6) vs. 9.9 (7.1, 11.3) p=0.06], but not OP_v^{DTT}. The total duration of hotplates and oven use did not lead to significantly different results for OP and $PM_{2.5}$, when splitting by the median duration. However, considering the reported impact of cooking on PM (Abdullahi et al., 2013; Li et al., 2016), this is a surprising result.

Table 18. Spearman's pairwise outdoor-indoor correlation coefficient for each species.

Species	$\rho_{ind\text{-}out}$
$PM_{2.5}$	0.30
OC	0.18
EC	0.48
Cl^-	**0.61**
NO_3^-	**0.57**
SO_4^{2-}	**0.57**
Na^+	0.38
NH_4^+	0.26
K^+	**0.67**
Mg^{2+}	0.14
Ca^{2+}	0.26
Levoglucosan	**0.65**
As	0.35
Cu	0.20
Mn	0.43
Pb	**0.58**
Sb	0.40
V	0.16
OP_v^{AA}	**0.52**
OP_v^{DTT}	0.27
OP_m^{AA}	0.36
OP_m^{DTT}	0.06

V.5. Strengths and limitations

To the best of our knowledge, this study is the first to concurrently assess residential indoor and outdoor OP of $PM_{2.5}$, using two different assays, along with an extensive chemical characterization and at two seasons. We acknowledge that this study has several limitations. Typical indoor sources such as cooking or cleaning emit semi-volatile organic compounds (Abdullahi et al., 2013; Maung et al., 2022), that could influence the OP of PM. 7-day sampling was performed in order to have sufficient mass for the chemical and OP analysis, these short lifetime compounds would therefore have reacted. Additional information on the type of fuel used for cooking and on the presence of a wood stove would have led to

clearer conclusions regarding the impact of these activities on the two OP assays. However, we were able to show that vacuum cleaning had a significant impact on some species and on OP activities.

We considered developing a land use regression model to predict OP's spatial variability, but this model had several limitations, namely: sampling weeks were not simultaneous in all houses and only one central monitoring station measuring PM_{10} was available to temporally adjust the measurements. We considered that these factors were too prone to induce uncertainties in a land use regression model, especially considering that previous studies with this aim highlighted the lack of specific geographic predictors for OP (Gulliver et al., 2018; Jedynska et al., 2017; Weichenthal et al., 2019).

We also investigated the possibility to apportion PM and OP sources, both outdoors and indoors. Although 38 houses had indoor sampling at 2 different seasons the Positive Matrix Factorization applied to this indoor dataset did not lead to stable results. To stabilize the solution, we tried to model outdoor PM sources, with the aim to reproduce published data of our team (Borlaza et al., 2021), but, again, this did not lead to a stable solution. These instabilities are probably due to the large variations of chemical concentration, particularly during the summer season, that led to individual homes with very high contributions to some potential sources. Reducing the database did not improve the results, and this is why we do not present source apportionment for indoor $PM_{2.5}$ and OP sources, although this would be a great tool to target specific indoor sources. Despite this, our conclusions highlight the spatial variability of OP, particularly during the cold season. We were also able to show the importance of several species for OP, namely OC, EC and copper for OP. These species are probably emitted by indoor activities such as cooking, cleaning, and we were able to highlight the role of vacuuming for their resuspension and emission. Therefore, we recommend paying particular attention to the house ventilation during these activities.

VI. Conclusion

Overall, our findings suggest important spatial variation of OP and $PM_{2.5}$ over the city of Grenoble, and different exposure patterns in the inside of residences compared to the outside, with an important

influence of vacuum cleaning, that led to increased I/O ratios of OC, Cu and OP^{AA} in house with more than 2 vacuuming session compared to the others. Although OP activities are not significantly higher indoors compared to outdoors, the important concentrations of copper greatly impact OP^{AA} indoors, which highlights the need to take indoor environments into account for OP exposure.

VII. Supplemental Material

VII.1. List of Tables

PM mass reconstruction

PM mass concentration was calculated using the following equation:

$$[PM_{2.5}] = [OM] + [EC] + [nss - sulfate] + [nitrate] + [ammonium] + [seasalt] + [dust]$$

Where the organic matter (OM) was estimated with an OM to OC conversion factor of 1.8 for outdoor and ambient PM and 1.4 for indoor PM (Favez et al., 2010; Putaud et al., 2010; Tofful et al., 2021). [nss-sulfate], corresponded to the sulfate fraction from which the marine component was subtracted (Seinfeld & Pandis, 2016) according to Eq. 4.

$$[nss - sulfate] = [SO_4^{2-}] - 0.252[Na^+] \quad (4)$$

The seasalt fraction, was calculated from sodium concentrations according to Eq. 5.

$$[seasalt] = 3.252 * Na^+] \quad (5)$$

The dust fraction, taking into account metallic elements and oxydes, was calculated following the empirical Eq. 6 (Putaud et al., 2004): $[dust] = 5.6 * [nss - Ca^{2+}]$ (6)

Where: $[nss - Ca^{2+}] = [Ca^{2+}] - [Na^+]/26$ (7)

Table S20 Summary of detection (DL) or quantification (QL) limits used to replace values below the QL, and percentage of values below the QL.

Chemical constituent	QL or DL used to replace values <QL	< QL
PM		0%
OC (µg/m^3)	0.089025596	0%
EC (µg/m^3)	2.82026E-05	0%
MSA (ng/m^3)	0.003	0%
Cl^- (ng/m^3)	0.123	17% (N=23)
NO_3^- (ng/m^3)	0.186	0.7 (N=1)
SO_4^{2-} (ng/m^3)	0.185	0%
Oxalate (ng/m^3)	0.022	0%
Na^+ (ng/m^3)	0.121	1.5% (N=2)
NH_4^+ (ng/m^3)	0.100	3.7% (N=5)
K^+ (ng/m^3)	0.022	1.5% (N=2)
Mg^{2+} (ng/m^3)	0.007	0%
Ca^{2+} (ng/m^3)	0.076	0%
Inositol (ng/m^3)	1.43	81% (N=108)
Glycerol (ng/m^3)	39.05	81% (N=109)
Erythritol (ng/m^3)	1.07	81% (N=108)
Xylitol (ng/m^3)	3.55	96% (N=128)
Arabitol (ng/m^3)	3.55	67% (N=90)
Sorbitol (ng/m^3)	1.41	90% (N=121)
Mannitol (ng/m^3)	3.52	34% (N=45)
Threalose (ng/m^3)	1.76	84% (N=112)
Levoglucosan (ng/m^3)	17.82	25% (N=34)
Mannosan (ng/m^3)	3.54	59% (N=79)
Galactosan (ng/m^3)	14.73	94% (N=126)
L-Rhamnose (ng/m^3)	3.57	95% (N=127)
Glucose (ng/m^3)	3.53	57% (N=76)
Al (ng/m^3)	236.46	93% (N=125)
As (ng/m^3)	0.18	47% (N=63)
Ba (ng/m^3)	3.68	86% (N=115)
Cd (ng/m^3)	0.18	93% (N=124)
Co (ng/m^3)	0.18	92% (N=123)
Cr (ng/m^3)	1.35	91% (N=122)
Cu (ng/m^3)	1.17	21% (N=28)
Fe (ng/m^3)	32.42	12% (N=16)
Mn (ng/m^3)	1.24	45% (N=60)
Mo (ng/m^3)	0.22	40% (N=54)
Ni (ng/m^3)	0.76	75% (N=101)

Chemical constituent	QL or DL used to replace values <QL	< QL
Pb (ng/m^3)	0.18	6.7% (N=9)
Rb (ng/m^3)	0.42	80% (N=107)
Sb (ng/m^3)	0.18	22% (N=30)
Sn (ng/m^3)	46.32	67% (N=90)
Ti (ng/m^3)	1.01	16% (N=22)
V (ng/m^3)	0.18	50% (N=67)
Zn (ng/m^3)	18.94	89% (N=120)

Table S21. Descriptive statistic of $PM_{2.5}$ constituents and OP indoors and outdoors.

Characteristic	N	Indoor, N = 78	Outdoor, N = 56	p-value[1]
Season	**134**			**>0.9**
cold		40 (51%)	29 (52%)	
warm		38 (49%)	27 (48%)	
$PM_{2.5}$ (µg/m^3)	**134**			**0.6**
Median (IQR)		10.3 (7.8, 14.9)	10.3 (7.4, 13.7)	
Range		2.5, 39.1	2.1, 35.3	
OC (µg/m^3)	**128**			**<0.001**
Median (IQR)		5.52 (3.95, 7.34)	3.58 (2.67, 4.42)	
Range		1.34, 24.16	1.43, 18.67	
Missing		3	3	
EC (µg/m^3)	**128**			**0.7**
Median (IQR)		0.69 (0.51, 1.11)	0.69 (0.57, 1.00)	
Range		0.00, 3.21	0.00, 3.54	
Missing		3	3	
Cl^- (ng/m^3)	**128**			**0.094**
Median (IQR)		25 (12, 73)	15 (0, 76)	
Range		0, 749	0, 472	
Missing		2	4	
NO_3^- (ng/m^3)	**128**			**0.002**
Median (IQR)		315 (221, 444)	465 (247, 1,596)	
Range		0, 1,409	97, 4,570	
Missing		2	4	
SO_4^{2-} (ng/m^3)	**128**			**0.017**
Median (IQR)		745 (479, 1,175)	1,026 (721, 1,563)	
Range		37, 5,143	175, 2,827	
Missing		2	4	
Na^+ (ng/m^3)	**128**			**0.01**
Median (IQR)		51 (26, 80)	75 (35, 121)	
Range		0, 336	7, 346	
Missing		2	4	
NH_4^+ (ng/m^3)	**128**			**<0.001**
Median (IQR)		182 (77, 427)	562 (324, 865)	
Range		0, 1,468	0, 1,912	
Missing		2	4	
K^+ (ng/m^3)	**128**			**0.2**
Median (IQR)		28 (13, 98)	40 (17, 109)	
Range		0, 466	3, 273	
Missing		2	4	
Mg^{2+} (ng/m^3)	**128**			**0.2**
Median (IQR)		7 (6, 11)	9 (7, 13)	
Range		2, 392	2, 38	
Missing		2	4	
Ca^{2+} (ng/m^3)	**128**			**0.3**
Median (IQR)		75 (49, 109)	66 (43, 105)	
Range		15, 3,883	11, 2,335	
Missing		2	4	
Levoglucosan (ng/m^3)	**128**			**0.3**
Median (IQR)		55 (9, 181)	72 (19, 314)	
Range		9, 793	9, 1,013	
Missing		2	4	

Characteristic	N	Indoor, N = 78	Outdoor, N = 56	p-value[1]
As (ng/m^3)	**129**			**0.7**
Median (IQR)		0.09 (0.09, 0.39)	0.23 (0.09, 0.31)	
Range		0.09, 2.32	0.09, 1.24	
Missing		1	4	
Cu (ng/m^3)	**129**			**0.019**
Median (IQR)		5.14 (1.95, 13.76)	3.05 (1.94, 7.21)	
Range		0.58, 134.2	0.58, 44.6	
Missing		1	4	
Mn (ng/m^3)	**129**			**0.071**
Median (IQR)		0.62 (0.62, 2.57)	1.93 (0.62, 3.69)	
Range		0.62, 25.42	0.62, 13.19	
Missing		1	4	
Pb (ng/m^3)	**129**			**0.3**
Median (IQR)		1.81 (0.78, 3.52)	2.31 (1.28, 3.27)	
Range		0.09, 19.79	0.09, 10.75	
Missing		1	4	
Sb (ng/m^3)	**129**			**0.007**
Median (IQR)		0.34 (0.09, 0.62)	0.50 (0.34, 0.90)	
Range		0.09, 2.59	0.09, 3.76	
Missing		1	4	
V (ng/m^3)	**129**			**0.001**
Median (IQR)		0.09 (0.09, 0.23)	0.24 (0.09, 0.40)	
Range		0.09, 1.29	0.09, 1.64	
Missing		1	4	
OP_v^{AA} (nmol/min/m^3)	**130**			**0.3**
Median (IQR)		0.93 (0.54, 1.98)	0.76 (0.48, 1.67)	
Range		0.05, 6.86	0.19, 3.81	
Missing		2	2	
OP_v^{DTT} (nmol/min/m^3)	**130**			**0.9**
Median (IQR)		1.13 (0.64, 1.66)	1.02 (0.79, 1.44)	
Range		0.18, 5.48	0.21, 3.40	
Missing		2	2	
OP_m^{AA} (nmol/min/µg)	**130**			**0.6**
Median (IQR)		0.10 (0.05, 0.15)	0.08 (0.05, 0.14)	
Range		0.01, 0.39	0.02, 0.47	
Missing		2	2	
OP_m^{DTT} (nmol/min/µg)	**130**			**0.3**
Median (IQR)		0.10 (0.08, 0.12)	0.11 (0.09, 0.12)	
Range		0.04, 0.20	0.04, 0.33	
Missing		2	2	

[1]*Pearson's Chi-squared test; Wilcoxon rank sum test*

Table S22. Spearman's correlation coefficients in the ambient environments, between PM_{10}, OP_v^{AA}, OP_v^{DTT}, OP_m^{AA} and OP_m^{DTT} and the set of PM_{10} chemical constituents.

Species	PM_{10}	OP_v^{AA}	OP_v^{DTT}	OP_m^{AA}	OP_m^{DTT}
PM_{10}	1.00	0.54	0.67	0.17	0.16
OP_v^{AA}	0.54	1.00	0.76	0.89	0.72
OP_v^{DTT}	0.67	0.76	1.00	0.54	0.73
OP_m^{AA}	0.17	0.89	0.54	1.00	0.79
OP_m^{DTT}	0.16	0.72	0.73	0.79	1.00
OC	0.91	0.56	0.67	0.22	0.19
EC	0.46	0.89	0.69	0.77	0.59
Cl^-	0.21	0.66	0.39	0.74	0.58
NO_3^-	0.57	0.77	0.63	0.65	0.60
SO_4^{2-}	0.42	-0.42	-0.09	-0.65	-0.54
Na^+	-0.09	0.24	0.01	0.34	0.13
NH_4^+	0.76	0.15	0.41	-0.12	0.07
K^+	0.70	0.84	0.78	0.64	0.60
Mg^{2+}	0.21	0.48	0.24	0.50	0.27
Ca^{2+}	0.32	-0.22	0.00	-0.47	-0.49
Levoglucosan	0.41	0.86	0.66	0.83	0.79
As	0.74	0.65	0.72	0.37	0.38
Cu	0.38	0.75	0.61	0.60	0.46
Mn	0.55	0.64	0.72	0.44	0.46
Pb	0.61	0.80	0.79	0.66	0.71
Sb	0.43	0.63	0.54	0.46	0.39
V	0.24	-0.11	0.00	-0.25	-0.37

Table S 23. Outdoor-ambient spearman correlation coefficients for homes located within 5km of the local air quality monitoring station.

Species	$\rho_{out\text{-}amb}$ (total)	$\rho_{out\text{-}amb}$ (5km)
$PM_{2.5}$	0.61	0.81
OC	0.62	0.80
EC	0.49	0.65
Cl^-	0.76	0.83
NO_3^-	0.81	0.90
SO_4^{2-}	0.81	0.81
Na^+	0.81	0.84
NH_4^+	0.80	0.86
K^+	0.66	0.83
Mg^{2+}	0.08	0.26
Ca^{2+}	0.46	0.47
Levoglucosan	0.89	0.93
As	0.61	0.73
Cu	0.17	0.28
Mn	0.35	0.60
Pb	0.60	0.74
Sb	0.34	0.42
V	0.41	0.37
OP_v^{AA}	0.71	0.91
OP_v^{DTT}	0.36	0.61
OP_m^{AA}	0.76	0.87
OP_m^{DTT}	0.30	0.65

Chapter VII

Discussion and Perspectives

I. Discussion

I.1. Summary of the main findings

The overall objective of this research was to document the health impacts of exposure to particulate pollutants, in terms of its mass concentration and its oxidative potential, in order to assess the relevance of OP as a health metric with respect to the current regulated metric. OP measurements aim to be more specific than the mass concentration metric to adverse PM health effects caused by oxidative stress. Unfortunately, there are few studies assessing precise measurements at the personal level to characterize the associations of OP exposure with different health parameters. In this work, a comprehensive approach was adopted by addressing the relationship of personal OP with various health and biological endpoints, and the variations in indoor and outdoor PM OP and chemical constituents. After summarizing the main findings of the thesis, a discussion follows on whether OP would be a relevant additional metric for air quality management.

The etiologic axis of the thesis work showed associations for prenatal exposure to OP_v^{DTT} with decreased lung growth in children, assessed by a decreased functional residual capacity at 6 weeks, and increased $Rrs_{7\text{-}19}$ at 3 years. Interestingly, prenatal exposure to $PM_{2.5}$ mass concentration was only associated with decreased FRC, but with smaller effects than OP_v^{DTT}. This study assessed personal exposure of the pregnant women at two time points, and measured objective lung function parameters as early as 6 weeks. To the best of our knowledge, this is the first study addressing the associations between prenatal exposure to $PM_{2.5}$ OP and children's lung function by using such precise techniques.

The mechanistic axis focused on the effects of OP of $PM_{2.5}$ exposure on biomarkers related to two main pathways involved in the PM adverse health effects: the oxidative stress and the immune system. While

OP measurements were included in many toxicological studies, to assess whether cellular responses indeed corresponded to oxidative stress and inflammation, it was more rarely tested in epidemiological studies. Investigating the responses of systemic oxidation and inflammation in a cohort, following PM and OP exposure explores the biological plausibility of other epidemiological studies investigating the associations of exposure to PM and OP with health outcomes. Particularly, in the context of this work, the possibility to assess biomarkers in pregnant women, and to test the relationship of OP with their concentrations is a great added value in regards of the other results on the prenatal exposure to PM and OP and their effects on children lung function. Indeed, biological plausibility is a critical criterion required to support a causal interpretation of statistical links between exposure and health endpoints. No associations of $PM_{2.5}$ mass concentration was revealed with biomarkers of oxidative stress, nor with immune function parameters. Our findings suggest a deleterious effect of mass-normalized OP^{AA} on oxidative damage to DNA (8-OHdG), and a potential effect modification of $PM_{2.5}$ levels was identified, with stronger effects of OP_m^{AA} and OP_m^{DTT} on 8-OHdG and 8-iso-PGF2α in the context of low (< median; 14 $\mu g/m^3$), vs. high (≥ median) exposure to $PM_{2.5}$ level. Regarding blood immunological biomarkers, OP was associated with a decrease in R848-activated IL-8 (OP^{AA}), and an increase in IL-17A (for all OP tests, standardized by mass and volume). Together, results on oxidative DNA damage and on the immune function are in line with the proposed hierarchical response model for oxidative stress induced by pollutant exposure, that identified inflammation in the pathway between antioxidant defense and cell. They also underline the specificity of OP compared to $PM_{2.5}$ mass concentration as exposure metric able to detect associations. Compared to previous studies on the impact of $PM_{2.5}$ or OP exposure on biological endpoints, this is a large-scale study of homogenous pregnant women, for which exposure was precisely assessed using personal monitors. In addition, immune blood cells were activated to assess their propensity to secrete cytokines, and urine samples were pooled to reduce intra-day variability of biomarkers. To the best of our knowledge, these are the first studies making such an effort to minimize measurement error in the assessment of air pollutant exposure through personal measurements and biomarker analysis, and employing a urinary pool methodology.

The exposure and aerosol research axis of the thesis showed an important spatial variability for OP and $PM_{2.5}$ within the cohort's study area. Redox-active species exhibited greater spatial heterogeneity

outdoors during the cold season compared to the warm season, during which non-redox species mostly influenced the magnitude of $PM_{2.5}$ concentrations. In terms of chemical tracers of PM sources in the outdoor air, a homogenous distribution of the biomass burning tracer levoglucosan was evidenced, whereas species related to road traffic exhibited some spatial contrast. Although not statistically significant, the higher levels of organic carbon, chloride and copper in the indoor *vs.* outdoor environment indicate indoor-specific sources associated to these elements, likely related to cooking, cleaning and electrical appliances. Vacuuming, by resuspending settled particles, and potentially emitting copper particles was identified as a habit that led to higher exposure to OP^{AA}. To the best of our knowledge, this was one of the first study in Europe that assessed PM constituents and two OP assays inside residences and within the city. This chapter highlights the need to take into account indoor PM exposure, particularly for OP, as well as spatially resolve exposure within the city.

Taken together, the findings of this work are complementary in showing that reduction in copper exposure would probably lead in limiting adverse health effects of $PM_{2.5}$ exposure. Indeed, higher indoor concentrations of copper compared to outdoors were found in chapter VI, and this specie was also the main indoor tracer of OP^{AA}. In the epidemiological studies of this thesis, both short-term and prenatal personal OP were found associated with biological and health endpoints, respectively. Reducing copper exposure indoors, could therefore lead to reduced exposure to the OP^{AA} of $PM_{2.5}$ and thereby limit biological and health effects. This is supported by both epidemiological and toxicological studies investigating the role of specific PM constituents in ambient air. Steenhof et al. (2011) compared $PM_{2.5}$ effects from several sites, and highlighted that $PM_{2.5}$ with the highest metal content induced the most adverse effect on cell metabolic activity compared to $PM_{2.5}$ from the other sites. A study in Canada underlined a combined effect of sulfur and transition metals, with the magnitude of association between $PM_{2.5}$ OP exposure and acute cardiovascular events being strongest when PM content of both species was elevated (Weichenthal et al., 2021). Sulfur was used as a proxy for sulfate to represent PM's acidity, that influence metals dissolution. Since there are no known indoor sources of sulfate, targeting sources of sulfate outdoors and sources of transition metals, and particularly copper both indoors and outdoors would have significant impact on personal exposure to OP. This highlights the need to raise awareness about good practices related to indoor air quality, entailing the ventilation of living spaces, including

during the moderate use of household electrical appliances such as vacuum cleaners or hair dryers, which are less recognized than smoking and cooking in terms of particle emissions. Reducing these exposures for pregnant women could result in lowered oxidative stress and inflammation levels, subsequently decreasing the risk of compromising the development of their offspring. Following the Developmental Origins of Health and Disease (DOHaD) hypothesis, this decreased exposure could also have implications for offspring's future lives, by lowering the likelihood of developing respiratory diseases.

I.2. Strength and limitations

I.2.1. Population selection

In the SEPAGES cohort, the women who volunteered for the study differed from those who did not, therefore potentially introducing a volunteer bias, which is a type of selection bias. Indeed, the participants tended to be older, had a higher education level, a lower parity, more frequently lived in a relationship and worked, compared to the women approached but not included (see Methodology section and Lyon-Caen et al., 2019). Compared to the French population, the included participants tended to smoke less before and during pregnancy. This systematic error can modify the exposure-outcome relationship in the selected population compared to the general population that would be eligible for the study (Tripepi et al., 2010). However, participants tended to be less exposed to tobacco smoke, that emit transition metals contributing to the OP of PM. They were also potentially exposed to lower levels of ambient air pollution, since they tended to live in less deprived areas. Thus, the results cannot be generalized to the whole population of Grenoble, nor to the whole population of France. However, this homogeneity of the population can limit confusion bias related to smoking or educational level, that was used as a proxy for more complex variables linked to the socioeconomical status.

I.2.2. Exposure assessment

In this work, exposure was assessed using personal monitors to limit the measurement errors related to the participant's location. Although personal PM filters could not more precisely represent the variety of environments and activities of the participant, some measurement error has probably been introduced by this estimation method, related to the chemistry of redox-active species, and to the temporal coverage of personal monitors. By using a 7-day filter, it is highly probable that reactive oxygen species with

short half-lives have already reacted when the filters were analyzed (Fuller et al., 2014; Utinger et al., 2023; Wang et al., 2023). However, the sharp decrease induced by ROS' reactivity probably happens within a few hours after collection, thereby affecting most off-line OP measurement techniques. OP levels could thereby be underestimated, and since this decrease depends on the aerosol content of ROS, which depends on the exposure sources, all filters would not be affected to the same extent. However, to this day, no other precise measurement techniques can be applied to estimate personal exposure to OP, since online measurements are only at the pilot research stage, and cannot be transported to evaluate personal exposure of participants. Regarding temporal coverage, measurement error could have been introduced in Chapter III, due to the compromise with the feasibility of personal sampling, since personal samplers cannot be worn during the entire pregnancy. Instead, two one-week $PM_{2.5}$ measurement periods were performed to estimate the prenatal exposure, and some participants of this study (N=124, 35%) only had one measurement week. Considering the heavy protocol of the cohort design, additional weeks of sampling would not have been possible, for both costs and acceptability aspects. The main concern related to the use of such temporal coverage to represent the pregnancy is to have sampled a particular week, with unusually high or low exposure. Including the season of sampling in the confounders partially accounted for the peak events that occur in winter in Grenoble, and the results excluding participants with only one measurement week tended to lead to clearer results. This measurement error related to temporal coverage of personal monitors has therefore probably diluted the associations observed, rather than inflated them.

I.2.3. Outcomes' assessment

To assess the outcomes in this thesis work, novel non-invasive techniques were used for lung function measurements. Using O_2 introduced a transient decrease of tidal volumes during N_2MBW assessment, and although FRC and LCI measurement were corrected for the degree of hypoventilation induced, there could be residual error. This demonstrates the compromise between the need to measure lung function at the earliest stage of infancy and the originality of the devices that have to be used, and how they should be adapted to comply with the French regulation regarding tracer gas.

For urinary biological marker in Chapter IV, urine pools were used for the first time among the studies investigating air pollution effects on oxidative stress biomarkers. However, the drawback from this

originality is that there is a lack of literature regarding the relevance of correcting biomarkers for specific gravity in urine pools, as the mean of concentrations corrected for specific gravity will differ from the concentration in the pool corrected specific gravity measured in the pooled sample (O'Brien et al., 2017). Indeed, mathematically, the sum of ratios generally differs from the ratio of sums. Results relying on raw biomarkers concentrations were also shown and were similar, suggesting that even if SG-correction in pooled urine samples turns out to be irrelevant, conclusions would remain unchanged. Regardless of the urine dilution, using a pooled urine sample has the advantage to reduce intra-day variability of biomarkers, which reduces measurement error.

Cytokine quantification in plasma in Chapter IV led to very few cytokine concentrations above the detection limit at baseline, and more sensitive techniques would be required to quantify very low cytokines concentration. However, a novel approach was used to activate innate blood immune cells, and a large number of cytokines secreted by monocytes, dendritic cells, granulocytes and T cells were analyzed.

I.2.4. Confounding factors

Efforts were made to collect a lot of potential confounding factors to reduce further biases. For all analyses, potential confounders were selected *a priori* and their effects were investigated to choose whether to retain them or exclude them from final models. Confounders with strong a priori knowledge such as environmental tobacco smoke or education level, were kept systematically, even when the SEPAGES data did not evidence strong effects on the associations studied. However, there could be residual confounding, as already mentioned in the corresponding chapters, and particularly chapter IV with endogenous ROS production and antioxidant supplementation that could influence the relationship between air pollution and oxidative stress levels (He et al., 2020b; Moller and Loft, 2006). The accuracy of the confounders could also introduce residual confounding. In fact, assessing socioeconomic status by using the highest level of education may not fully capture the complexity of this dimension, but this is partly compensated by the homogeneity of the cohort regarding educational level.

Overall, chapters III-VI highlighted associations between OP of PM exposure and several biological and health endpoints, with OP being more specific than $PM_{2.5}$. With the improvement of analysis techniques and the reduction of analysis costs, the amount of information per participant has largely increased in

epidemiological studies, which leads to multiple testing issues. Since results presented in this thesis work were not formally corrected for multiple testing, part of the associations observed may result from chance findings and thus should be interpreted cautiously. However, although they present some limitations, these findings add to the growing body of evidence showing that OP might be a more health-relevant metric than PM mass concentration.

II. Perspectives

The strengths and limitations of this study provide the basis for a number of recommendations and perspectives related to future research work within the SEPAGES cohort and beyond.

II.1. Further research in SEPAGES

Several aspects were investigated in this thesis work, particularly using data from the SEPAGES cohort. However, certain aspects could not be addressed or were intentionally set aside, and they will be the focus of future studies within the IAB and IGE research teams.

II.1.1. Etiological research

The follow-up of the children in the cohort is still ongoing, allowing for the replication of certain parameter measurements. Indeed, during the ongoing 8-year-old child visit, the AOS will be measured again, and the first spirometry measurements will be performed. In the study on children's lung function from Chapter III, a longitudinal analysis between 6 weeks and 3 years of age was not possible due to the use of different measurements. The measurements of AOS at 3 years and 8 years of age will enable to investigate associations between prenatal exposure to air pollutants during pregnancy and trajectories of lung function in the child.

Furthermore, personal of $PM_{2.5}$ exposure was also assessed during the week preceding the clinical visit at 3 years. These data will be validated, and the analysis of OP will be conducted on the filters, allowing for the consideration of short-term exposure in the relationship between prenatal exposure and mechanical properties of the lung in the child at 3 years. Accounting for short-term exposure in this relationship is important as it effectively distinguishes the effects of prenatal exposure from the effects

of short-term exposure. These efforts will be incorporated into a multidisciplinary thesis, once again co-supervised by IGE and IAB, that will begin in the autumn of 2023.

II.1.2. Methodological research

In this work, we aimed at getting a better overview of the suitability of the OP of PM, by evaluating its potential to predict health or biological effects compared to mass concentration, and by investigating its main tracers in the indoor and outdoor environment to propose ways to reduce exposure inside homes. However, to get a better understanding of exposure to PM and OP, a larger study focused on the assessment methods of PM available in the Grenoble basin could be performed. Currently, a one-year study relying on 3 sites with different typologies in Grenoble already investigated the sources of PM and OP, but a study comparing the results of different exposure assessments available for the SEPAGES cohort, namely personal samplers, fine-scale modelling at home address, ambient station (weighted by the distance to home) could help evaluating the suitability of using 2 weeks of personal samplers compared to other methods.

II.2. Research perspectives

In addition to the research questions that could be addressed by utilizing the research framework offered by the SEPAGES cohort, the findings of this work also raise several research perspectives.

II.2.1. Epidemiology

The results presented in this work demonstrate that the oxidative capacity of particles is a relevant indicator for considering some of the health effects of PM exposure. It would be relevant for its measurement to be more widely integrated into future epidemiological studies. Firstly, in relatively small-scale studies, including personal measurements of PM OP would be insightful in evaluating the impact of this exposure on various biological and health parameters. This would not only facilitate study comparisons but will also enhance understanding of target organs or tissues during OP of PM exposure, alongside assessing which OP tests relates best to which health or biological outcomes. This thesis employed AA and DTT assays for OP assessment, representing the main lung anti-oxidant categories, but other assays exist, some of which, might predict some specific health outcomes more effectively. Indeed, each assay's specificity toward different chemical species could also be associated with distinct

underlying biological mechanisms. A toxicological study indeed identified that $PM_{2.5}$ with the highest OP_m^{DTT}, related to high metal levels, had a strong adverse effect on cell metabolic activity, but only a moderate effect on pro-inflammatory response while a more moderate OP_m^{DTT}, mostly caused by PAH content, caused higher secretion TNF-α and IL-6, two pro-inflammatory markers (Steenhof et al., 2011). More recently, anthropogenic $PM_{2.5}$ with an important mass fraction of transition metals were found responsible for high OP levels and increased IL-6 release in cells (Daellenbach et al., 2020; Leni et al., 2020).

Since personal measurements are feasible only in relatively small cohorts, the development of ambient OP measurements through both routine analysis at air quality monitoring stations, and modelling would be necessary. European regulations are moving in this direction, as OP has been proposed as a new parameter to be measured across European super-sites (one site per 10 000 000 inhabitant) that conduct extensive air pollution characterization. Simultaneously, an ongoing European project, RI-Urbans, aims to assess the relevance of new air pollution indicators, including OP, by creating a network that will investigate the sources of these parameters and their effects on mortality. This will be evaluated through time series analyses based on long-term ambient OP measurements and mortality data. Given the large scale of this project (across 5 European cities), more generalizable conclusions will be made on the impacts of PM OP on mortality, with regards to other air pollutants measured simultaneously.

II.2.2. Mechanistical and methodological considerations

II.2.2.1. Mechanistic research

An interesting approach to gain a better understanding of the findings of this thesis would be to study the associations between exposure to PM constituents and biomarkers of oxidative stress. OP integrates PM constituents, by accounting for their ROS-generation capacities and synergetic and antagonistic effects. However, it is necessary to disentangle which of the constituents affecting OP are affecting oxidative stress levels, while taking into account the effects of PM mass concentration. Liu et al. (2018) investigated the role of both OP_m and metal constituents in PM in relation with urinary MDA and 8-OHdG in a controlled exposure study. They reported increased urinary levels of 8-OHdG post-exposure to copper and increased urinary levels of both MDA and 8-OHdG post-exposure to OP_m (AA- and

glutathione-related). However, the role of metals in the OP-OSB relationship was not further investigated, and since they assessed exposure to concentrated ambient particles, PM mass concentrations were high compared to the average levels in cities (238.4 ± 62.0 µg/m^3). The higher mass-normalized OP for below median levels of $PM_{2.5}$ mass concentrations denotes a higher PM reactivity at these levels, which was also found in other studies in ambient air (Campbell et al., 2021), but the underlying atmospheric mechanisms remain insufficiently understood. Considering that stronger OP_m effects were observed on oxidative DNA damage at lower $PM_{2.5}$ concentrations, important questions are raised, related to the physicochemical properties of PM constituents at these low concentrations. This is of particular interest, since the low PM concentrations were also found to be critical in terms of effects on mortality and asthma incidence (S. Liu et al., 2021; Stafoggia et al., 2022). To address these research gaps, a multidisciplinary approach combining atmospheric sciences and toxicological studies could be implemented. In toxicology, cells are usually exposed to concentrations higher than the average levels to which the population is exposed. However, conducting analyses stratified by concentrations of PM, on which an extensive chemical and OP characterization would be performed, could provide insights into the cellular processes at play. In atmospheric sciences, low PM concentrations could be investigated by utilizing measurement sites where long-term chemical characterization of PM and OP were already performed. This would be achieved by focusing on days with low PM concentrations, and determining whether OP was significantly higher than on other days. Common sources contributing to this high OP could potentially be identified.

Once fully characterized, these high OP – low PM events could be simulated using atmospheric simulation platforms, such as the Pollu-Risk research platform (LISA - UMR CNRS 7583, IMRB - INSERM 955), which enables the simulation of realistic atmospheres under controlled conditions for exposing various types of cells and mice. Characterizing the physical properties of particles could also shed light on the increased reactivity of these particles at low concentrations, as well as their health effects. Parameters like particle morphology and size play crucial roles in understanding these mechanisms. These various experiments could confirm or refute the hypothesis made in Chapter IV, suggesting that certain PM constituents might have a physical effect on epithelial cells, exacerbating oxidative damage to DNA.

II.2.2.2. OP measurement methods

Some of the inconsistencies observed in the results between OP exposure and health outcomes could be attributed to the lack of standardization in measuring this parameter, which makes studies hard to compare. Indeed, protocols for OP analysis are not yet standardized, and for each OP assay, analysis protocol can vary between laboratory. Some of these differences lie in the methods for PM extraction (aqueous, organic, or in a simulated lung fluid), the concentrations of reactants and the devices used to perform the measurement (kinetic or endpoint). As part of the aforementioned RI-Urbans project, a comprehensive intercomparison of OP^{DTT} measurement protocols has been undertaken. The results of this intercomparison will help identify critical technical variables contributing to differences in OP measurements and assess variations across different protocols employed by collaborating laboratories, as compared to a reference protocol. This intercomparison pertains to OP^{DTT}, the most widely used test, that was suggested as one of the most relevant for health effects, because of its consistent associations with cardiorespiratory outcomes. Nevertheless, extending the intercomparison to other existing tests and, ultimately, developing standardized protocols could facilitate the use of a comparable exposure metric across various studies.

Although acellular tests on filters are robust and allow for the analysis of oxidative potential (OP) using multiple assays, across numerous filters simultaneously, and at a relatively low cost, there can be a rather long time between sample collection and analysis, that could potentially lead to an underestimation of OP levels. Reactive oxygen species (ROS) are highly reactive, and oxidation processes persist after PM is collected on filters, thereby decreasing their concentration (Fuller et al., 2014; Y. Wang et al., 2011). Several prototypes have been developed or are currently under development to enable continuous online measurement of OP, particularly with DCFH (King and Weber, 2013), AA (Campbell et al., 2019; Uzu, n.d.), and DTT tests (Puthussery et al., 2018; Uzu, n.d.). Presently, the use of these prototypes is limited due to challenges in employing them in moderately or low-polluted environments. However, in the long run, equipping air quality monitoring stations with automated systems could enable the assessment of short-term OP effects on a larger scale.

II.2.3. Perspectives for public health

Some tools related to OP assessment could be developed to facilitate future epidemiological studies, assist decision-making, and refine specific recommendations related to air pollution. In particular, the development of Land Use Regression (LUR) models and Chemical Transport Models (CTM) could greatly beneficiate to the epidemiology community, because such models would enable large-scale studies on OP without implementing heavy measurement campaigns, thereby increasing the weight of evidence for OP relevance and wider use. Models would also provide tools for future assessment and management of the health risks associated with exposure to the oxidative potential of PM. Currently, a jointly supervised thesis between LISA (University Paris Cité) and IGE (University Grenoble Alpes) aims to model the oxidative potential of PM using the CHIMERE model for France. LUR and CTM would be complementary since LUR models would present a fine spatial resolution that would take into account the complex topography of Grenoble and enable the mapping of vulnerable areas to inform the population and guide urban planning decisions. Scenarios related to urban planning policies (low-emission zones, creation of wooded areas) could also be evaluated. CTM would allow to study the impacts of different sources, on which reduction actions can be implemented, as well as to model scenarios for policies related to these emissions.

Another action that could be considered in regards of the findings of this thesis, along with previous findings, is to conduct awareness campaigns regarding exposure to air pollutants. These campaigns should emphasize on the gestational period's crucial window for both mother's and child's health and simple recommendations for reducing exposure in households should be provided. A challenge in implementing these awareness campaigns is related to the acceptability of new recommendations, since the pregnancy period is already characterized by many changes and recommendations. This could create a sense of guilt among pregnant women and the couples more generally. Interventional studies and citizen science could help evaluate the acceptability and effectiveness of the recommendations. Additionally, it would be beneficial for these best practices to become part of daily life, as this would also promote the health of occupants in their homes.

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Appendix A

Publications and communications

A.1 Accepted articles

Anouk Marsal, Jean-Jacques Sauvain, Aurélien Thomas, Sarah Lyon-Caen, Lucille Joanna S. Borlaza, Claire Philippat, Jean-Luc Jaffrezo, Anne Boudier, Sophie Darfeuil, Rhabira Elazzouzi, Johanna Lepeule, Ryan Chartier, Sam Bayat, Rémy Slama, Valérie Siroux*, Gaëlle Uzu*. Effects of personal exposure to the oxidative potential of $PM_{2.5}$ on oxidative stress biomarkers in pregnant women. Science of the Total Environment, 911 (2024) Available on: https://doi-org/10.1016/j.scitotenv.2023.168475 *Co-last authorship

Anouk Marsal, Rémy Slama, Sarah Lyon-Caen, Lucille Joanna S. Borlaza, Jean-Luc Jaffrezo, Anne Boudier, Sophie Darfeuil, Rhabira Elazzouzi, Yoann Gioria, Johanna Lepeule, Ryan Chartier, Isabelle Pin, Joane Quentin, Sam Bayat, Gaëlle Uzu*, Valérie Siroux*, and the SEPAGES cohort study group. Prenatal Exposure to $PM_{2.5}$ Oxidative Potential and Lung Function in Infants and Preschool-Age Children: A Prospective Study. Environmental Health Perspectives, 131:1 (2023). Available on: https://ehp.niehs.nih.gov/doi/10.1289/EHP11155. *Co-last authorship

Lucille Joanna S. Borlaza, Gaëlle Uzu, Marion Ouidir, Sarah Lyon-Caen, Anouk Marsal, Samuël Weber, Valérie Siroux, Johanna Lepeule, Anne Boudier, Jean-Luc Jaffrezo, Rémy Slama and the SEPAGES cohort study group. Personal exposure to $PM_{2.5}$ oxidative potential and its association to birth outcomes. Journal of Exposure Science & Environmental Epidemiology, **33**, 416–426 (2023). Available on: https://doi.org/10.1038/s41370-022-00487-w [*My contribution to this article mainly involved checking the models and reviewing the manuscript.*]

Lucille Joanna S. Borlaza, Samuël Weber, Anouk Marsal, Gaëlle Uzu, Véronique Jacob, Jean-Luc Besombes, Mélodie Chatain, Sébastien Conil, and Jean-Luc Jaffrezo. Nine-year trends of PM_{10} sources and oxidative potential in a rural background site in France, Atmospheric Chemistry and Physics, **22**, 8701–8723 (2022). Available on: https://doi.org/10.5194/acp-22-8701-2022 [*I started working on the analyses and manuscript related to this article during an internship in 2019. The results and the manuscript were updated by L.J.S.B. and S.W.*]

A.2 Articles in preparation

Anouk Marsal*, Laurene Frau*, Laurence Chaperot, Ines Amine, Sarah Lyon-Caen, Anne Boudier, Claire Philippat, Karine Supernant, Johanna Lepeule, Joane Quentin, Ryan Chartier, Sam Bayat, Remy Slama, Gaelle Uzu, Valérie Siroux. Personal exposure to air pollutants and immune system biomarkers in pregnant women. *Co-first authorship. [*The manuscript related to this work is currently under review by the co-authors.*]

Anouk Marsal, Sarah Lyon-Caen, Lucille Joanna S. Borlaza, Jean-Luc Jaffrezo, Anne Boudier, Joane Quentin, Karine Supernant, Rhabira Elazzouzi, Sophie Darfeuil, Takoua Mhadhabi, Yoann Gioria, Rémy Slama, Valérie Siroux, Gaëlle Uzu. Characteristics of $PM_{2.5}$ and OP in indoor and outdoor environments. [*We plan to make some improvements to this manuscript before submitting it.*]

Lucille Joanna S. Borlaza, Valeria Mardoñez, Anouk Marsal, Ian Hough, Vy Thuy Dinh Ngoc, Marcos Andrade, Jean-Luc Jaffrezo, Andrés Alastuey, Jean-Luc Besombes, Griša Močnik, Isabel Moreno, Fernando Velarde, Jacques Gardon, Alex Cornejo, Paolo Laj, and Gaëlle Uzu. Oxidative potential of particulate matter and its association to respiratory health endpoints in high-altitude cities in Bolivia. [*This work was part of V. Mardoñez's thesis. My contributions relate to the statistical methods that are used, and result interpretation.*]

A.3 Poster communications

Anouk Marsal, Rémy Slama, Sarah Lyon-Caen, Lucille Joanna S. Borlaza, Jean-Luc Jaffrezo, Anne Boudier, Sophie Darfeuil, Rhabira Elazzouzi, Yoann Gioria, Johanna Lepeule, Ryan Chartier, Isabelle Pin, Joane Quentin, Sam Bayat, Gaëlle Uzu*, Valérie Siroux*, and the SEPAGES cohort study group. Prenatal Exposure to $PM_{2.5}$ Oxidative Potential and Lung Function in Infants and Preschool-Age Children: A Prospective Study. International Aerosol Conference, Athens, Sep. 2022.
*Co-last authorship

Anouk Marsal, Jean-Jacques Sauvain, Aurélien Thomas, Sarah Lyon-Caen, Lucille Joanna S. Borlaza, Claire Philippat, Jean-Luc Jaffrezo, Anne Boudier, Sophie Darfeuil, Rhabira Elazzouzi, Johanna Lepeule, Ryan Chartier, Sam Bayat, Rémy Slama, Valérie Siroux*, Gaëlle Uzu*. Effects of personal exposure to the oxidative potential of $PM_{2.5}$ on oxidative stress biomarkers in pregnant women. European Aerosol Conference, Málaga, Sep. 2023.