AIR POLLUTION AND PLACENTAL FUNCTION

A thesis submitted to the University of Manchester for the degree of

Doctor of Philosophy

in the Faculty of Biology, Medicine and Health

2020

NORHIDAYAH BINTI AHMAD

School of Health Sciences

Division of Population Health, Health Services Research and Primary Care

Centre for Epidemiology

Centre for Occupational and Environmental Health

LIST OF CONTENTS

Word counts: 69,183 words

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATION

LIST OF ABBREVIATIONS

8-OHdG	8-hydroxy-2'-deoxyguanosine
µg/kg	Microgram per kilogram
µg/mL	Microgram per milliliter
Ab	Antibody
APS	Aerodynamic Particle Sizer
As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
AURN	Automatic Urban and Rural Network
Ba	Barium
BC	Black carbon
BM	Basal membrane
BMI	Body mass index
BPA	Bisphenol A
BSA	Bovine serum albumin
C	Cord
Ca	Calcium
Cd	Cadmium
CDC	Centers for Disease Control and Prevention
CI	Cephalization Index
CI	Confidence Interval
CK7	Cytokeratin 7
CMRL	Connaught Medical Research Laboratories
CO	Carbon monoxide
CO_2	Carbon dioxide
COV	Coefficient of variation
Cr	Chromium
CTB	Cytotrophoblasts
Cu	Copper
DAB	Diaminobenzidine
DAQI	Daily Air Quality Index
DEFRA	Department for Environment, Food and Rural Affairs
DEP	Diesel exhaust particles
dH_2O	Distilled water

DNA	Deoxyribonucleic acid
E	Edge
EDC	Endocrine-disrupting chemicals
ELCS	Elective caesarean section
ELISA	Enzyme-linked immunosorbent assay
ESRI	Environmental Systems Research Institute
ETS	Environmental tobacco smoke
EU	European Union
FBS	Fetal bovine serum
Fe	Iron
FERA	Food and Environment Research Agency
FGR	Fetal growth restriction
FOV	Fields of view
FP	Fine particle
GC-MS	Gas chromatography with mass spectrometric
GDM	Gestational diabetes mellitus
GIS	Geographic Information System
H & E	Haematoxylin & Eosin
H_2O_2	Hydrogen peroxide
HCHO	Formaldehyde
hCG	Human chorionic gonadotrophin
HCl	Hydrochloric acid
HD	House dust
Hg	Mercury
I/O ratio	Indoor and outdoor ratio
IARC	International Agency for Research on Cancer
IBR	Individualised birth weight ratio
ICD	International Classification of Disease
ICP-MS	Inductive Coupled Mass Spectrometer
IHC	Immunohistochemistry
IL	Interleukin
IMD	Index of Multiple Deprivations
IMS	Industrial methylated spirits
IQR	Interquartile range
IUGR	Intrauterine growth restriction
K	Potassium

LBW	Low birth weight
LGA	Large gestational age
LNG	Liquefied natural gas
LOD	Limit of detection
Mg	Magnesium
MHCLG	Ministry of Housing, Communities & Local Government
MHS	Medical and Human Sciences
MP	Manchester Piccadilly
MPS	Manchester Pregnairclean Study
MSC	Microbiological safety cabinet
MT	Metallothionein
MVM	Microvillous membrane
Na	Sodium
NaOH	Sodium hydroxide
NBF	Neutral-buffered formalin
NH_3	Ammonia
NHS	National Health Service
Ni	Nickel
NIB	Non-immune block
NICE	National Institute for Health and Care Excellence
NIST	National Institute of Standards and Technology
NO_2	Nitrogen dioxide
NO_X	Nitrogen oxide
NPS	National Park Service
O_3	Ozone
OPC	Optical Particle Counter
P	Phosphate
***p*-NP**	*para*-Nonylphenol
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead
PBS	Phosphate-buffered saline
PCF	Participant consent form
PCM	Pollution Climate Mapping
PE	Pre-eclampsia
PI	Ponderal Index
PIS	Participant information sheet

PM	Particulate matter
PMC	Particle mass concentration
PNC	Particle number concentration
ppb	Part per billion
ppcf	Particle per cubic foot
PTB	Pre-term birth
RCOG	Royal College of Obstetricians and Gynaecologists
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
Se	Selenium
SE	Salford Eccles
SGA	Small gestational age
SHS	Second-hand smoker
SNA	Syncytial nuclear aggregates
SO_2	Sulphur dioxide
SOP	Standard operating procedure
SRM	Standard reference material
STB	Syncytiotrophoblast
TBS	Tris Buffered Saline
Tl	Thallium
TNF-α	Tumor Necrosis Factor Alpha
UFP	Ultrafine particles
UK	United Kingdom
UNICEF	United Nations Children's Fund
US/ USA	United States of America
USEPA	United States Environmental Protection Agency
VOC	Volatile organic compound
WHO	World Health Organization
Zn	Zinc

ABSTRACT

Scientific abstract of thesis entitled "Air Pollution and Placental Function" submitted by Norhidayah Binti Ahmad for the degree of PhD from the University of Manchester, 2020.

Maternal exposure to air pollution during pregnancy had been associated with adverse pregnancy outcomes, such as pre-eclampsia and fetal growth restriction (FGR), but the mechanism of this effect is poorly understood. Particulate matter (PM) is a ubiquitous air pollutant that is present both the indoor and outdoor environment and potentially bound with toxic materials such as heavy metals and/or polycyclic aromatic hydrocarbons (PAHs). Placental explant culture offers an *in-vitro* means to study PM induced changes in placental endocrine function and cellular turnover. This PhD explores the possible routes of air pollutant exposure and their mechanism influences on placental dysfunction that can cause adverse pregnancy outcomes.

Placentas were collected following elective caesarean section (ELCS) at-term, between October 2017 and January 2019 at St. Mary's Hospital, Manchester, and 41 villous explants were established, but 12 were excluded, as 4 were contaminated and 8 were non-responsive. Initially, placental explants were exposed to diesel exhaust particles (DEP) for 48-hour from day 5 until 7 of culture, to determine if there was a short term effect of this air pollutant on hCG secretion, and the result showed that 48-hour treatment were not give a significant changed in hCG secretion between treated and control group. Informed with the short term effect, the duration of air pollutants treatment on explants in culture was extended over 6 days. Explants were treated with DEP and house dust (HD), to mimic maternal exposure to $PM_{2.5}$ bound PAH. DEP treatment (SRM1650b; 24-hours/day; 6-days/week; mean DEP diameter ~166nm) significantly increased hCG secretion, increased 8-OHdG oxidative damage and promoted the formation of villi with an intact syncytiotrophoblast in a dose-dependent manner, whereas HD (SRM2585; 24-hours/day; 6-days/week; mean HD diameter ~166nm) significantly inhibited hCG secretion, increased the number of shed villi without regeneration and increased 8-OHdG oxidative damage. Differences in PAH levels between DEP and HD may be a reason for the difference in hCG secretion response and cellular turnover by placental explants.

Analysis of PAH and heavy metal levels in placentas and maternal blood has been conducted to indicate the functional ability of the placenta to act as a protective barrier for the fetus. Placentas and maternal blood from 53 patients were collected between June 2015 and June 2017 at St. Mary's Hospital, Manchester. Heavy metals, as detected by an inductive coupled mass spectrometer (ICP-MS), were present in the majority of samples, except for Cr and Ni which not detected in maternal blood. Cu and Pb levels were higher in maternal blood, whereas Cd and Hg levels were higher in placenta samples. PAH levels, as determined by gas chromatography with mass spectrometric (GC-MS), were low in placental samples, with only 8 out of 12 samples containing detectable levels, and most of the positive samples (n=5/8) contained only one PAH. Amongst 10 PAH compounds, fluorene, fluoranthene and pyrene detected in 8, 3 and 3 samples, respectively, whereas other compounds were detected in only one sample. Low maternal exposure on ambient PM_{10} and $PM_{2.5}$ levels throughout the gestation period, with median (IQR) levels of 17.6 (16.6 – 17.9)$\mu g/m^3$ and 10.1 (9.3 – 10.8)$\mu g/m^3$ respectively, as determined by air pollution monitoring stations, may be a reason for low heavy metal and PAH levels in the biological samples.

The investigation of maternal exposure to air pollution extended with a feasibility study of indoor air particle measurement in 20 residential homes of pregnant women across Greater Manchester (GM), between February and August 2019. The majority (n=17/20) of residential homes contained indoor $PM_{2.5}$ levels below $20\mu g/m^3$, as measured by an air particle monitor (DYLOS DC1700) for 24-hour. Simultaneously, outdoor $PM_{2.5}$ levels were estimated from air pollution monitoring stations, and the results showed that the outdoor $PM_{2.5}$ levels in most residential areas (n=16/20) were $<20\mu g/m^3$. The results suggest that pregnant women in GM were exposed to levels of outdoor PM_{10} and $PM_{2.5}$ below WHO standard limits, such that these concentrations may not have a significant effect on placental function and pregnancy outcomes.

In conclusion, interesting insights into the potential mechanism of pollution-related adverse pregnancy outcomes have been revealed by *in-vitro* experiments and the methods used in this study may be applied to future studies to investigate the effect of air pollution on placental function in other different aspects. However, this *in-vitro* investigation could not be compared with the real exposure to air pollution level, particularly in GM. Maternal exposure to air pollution in GM, at the current time, may not give a significant impact on adverse pregnancy outcomes. Further studies are needed to unravel the biological mechanism that underlies adverse pregnancy outcomes due to exposure to air pollution.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

COPYRIGHT STATEMENT

PRESENTATION AND PUBLICATION RELATING TO THIS THESIS

ORAL PRESENTATION

i. Maternal and Fetal Health Research Group, St. Mary's Hospital, Manchester, Works in progress meeting 2020

   Title: Air pollution and placental function.

ii. Maternal and Fetal Health Research Group, St. Mary's Hospital, Manchester, Works in progress meeting 2019

   Title: The modification of human chorionic gonadotrophins (hCG) secretion by cultured placental explants exposed to environmental pollutants.

iii. Maternal and Fetal Health Research Group, St. Mary's Hospital, Manchester, Works in progress meeting 2018

   Title: Indoor air quality and pregnancy outcomes.

iv. Centre for Epidemiology Seminar, University of Manchester, 2018

   Title: The modification of human chorionic gonadotrophins (hCG) secretion in cultured placental explants exposed to diesel particulate matter.

POSTER PRESENTATION

i. Centre for Trophoblast Research Annual Conference, University of Cambridge, 2019

   Title: Exposure to environmental pollutants leads to placental dysfunction *in-vitro*.

ii. Postgraduate Summer Research Showcase (PSRS), University of Manchester, 2019

   Title: Exposure to environmental pollutants leads to placental dysfunction *in-vitro*.

iii. Doctoral Academy Graduate Society (DAGS), University of Manchester 2019

   Title: Prenatal exposure to environmental pollutants leads to placental dysfunction.

iv. Institute of Federation of Placental Association (IFPA), Manchester, UK: 2017

   Title: Heavy metal levels in human placenta.

v. Division of Population Health, Health Services Research and Primary Care Away Day, University of Manchester, 2017

   Title: Heavy metal levels in human placenta.

vi. Postgraduate Summer Research Showcase (PSRS), University of Manchester, 2017

   Title: Heavy metal levels in human placenta.

CONFERENCE PROCEEDING

i. Norhidayah Binti Ahmad, Nurulshyha Md Yatim, Andrew Povey, Edward Johnstone, 2017 'Heavy metal levels in human placentas', (Published) *Placenta 57* (2017), 330.

ACKNOWLEDGEMENTS

I would like to begin by expressing my utmost gratitude and thankfulness to the 'Almighty' for giving me a beautiful path, strength and health, to carry out all the tasks and responsibilities throughout this beautiful journey.

I would like to express my deep sense of gratitude to my supervisors, Dr Andrew Povey and Prof Edward Johnstone, for guidance and encouraged me at all stages of this PhD journey. Andy, thank you very much for your invaluable help and support throughout my PhD. You shape me to be an independent student, you trust me and appreciate my work. Ed, you are always giving me a chance to be a better person, day by day. You believe in my capabilities, you show me a path to try a new thing, you guide me along the journey and you always inspired me to achieve my dream. Thank you so much for your help and guidance.

Also, I would like to extend my heartfelt and profound gratitude to a special person in my life, Dr Susan Greenwood. You are my inspiration to be a better person in everyday's life and you had shown me how I have to be as a future academician. You had guided and helped me in the laboratory for placental explants work when at that time, I did not have any knowledge in laboratory work. But you give me a chance, a big hope, a huge motivation for me to complete the project. Thank you very much for everything, Sue!

Alongside this, I would like to thanks to all women and participants who have donated placentas and blood, and completed indoor air sampling. Also, the highest appreciation to research practitioners, Christine and Jess that helped me in patient recruitment. Many thanks to Carmel Nichols, Elizabeth Cowley, Frances Beads and Dr Carolyn Jones for training me in the placental collection, immunohistochemistry and microscopes work. All members of MFHRG that welcomed me in the lab without one day I feel lost or in difficulties; thanks to Yousef, Meghan, Bo, Lewis, Daman, Zhiyong, Tatiana, and everyone in Research Floor. Also, thank you to my colleagues in Biomarkers Lab and COEH who have always shared the idea, skills and abilities during this journey.

Special thanks to Dr Michael Dickinson, from FERA, for GC-MS and ICP-MS analysis, Dr Miranda Loh, from IOM, for air pollution monitoring training, and Dr Nurulshyha for guiding me in samples collection and GIS analysis.

Support from family is a key pillar to survive and excel in this path. Special thanks to my husband, Khairi Suhaimi, my mum, Amnah Ali and my late father, Ahmad Othman, as well as other family members who always pray, trust, support me during ups and downs. Even this is not an easy path, but your love and support have brought me this far.

Last but not least, warmest appreciation to The University of Manchester for providing excellent research facilities and conducive environment for the students. Thank you to International Islamic University Malaysia and Ministry of Education for funding my study.

CHAPTER 1:

INTRODUCTION

1.1 Air pollution

Air pollution is a major risk to human health which has been estimated to cause seven million deaths every year (World Health Organization - WHO, 2019a). The WHO estimated that around 9 out of 10 people are exposed to air pollutants at levels exceeding the WHO air quality standard limits (WHO, 2019a). Air pollution is not only affecting the local or regional area but also a transboundary problem as polluted air can easily spread and circulate into other countries, without considerations of national borders (USEPA, 2019a; European Environment Agency, 2008). The air pollution such as the particulate matter of windblown dust originated from the desert areas (e.g. Africa, Mongolia, Central Asia and China) can travel for long distances and persist in the environment for up to 6 days, depending on the atmospheric conditions (WHO, 2019b). In the United Kingdom (UK), attention was drawn to air pollution as a result of the Great London Smog episodes in 1952 which resulted in ~12,000 fatalities in five days (Polivka, 2018). The pollutants released during this incident not only had an acute effect on the exposed individuals but also affected babies in utero by increasing their risk of childhood asthma (Polivka, 2018). This incident resulted in a change of perception and control measures were taken by countries to tackle the problem. In the UK, this resulted in the development of the Clean Air Act 1956 to control air pollution from residential and industrial sectors (Giussani, 2013).

Air pollution can be divided into two categories: outdoor (ambient) and indoor pollution, which is both interrelated (National Institute of Environmental Health Science – NIEHS, 2019; WHO, 2019a). Air pollutants in the outdoor environment can be emitted from both natural and anthropogenic sources, and levels of air pollution are higher in the developing countries compared to developed countries (WHO, 2019a; WHO, 2019b). Current exposure to outdoor air pollution is estimated to result in ~3,000,000 deaths every year, and the effect may be worsened when combined with indoor air pollution exposure (WHO, 2016).

The Environmental Protection Agency (EPA) defines indoor air pollution as air quality inside or around the building or structure, which relates to the health and comfort of building tenants (EPA, 2019a). Indoor air pollution may vary depending on the scope and setting, but in this context, indoor air pollution refers to air pollution in the residential home or household air pollution. According to the WHO, household air pollution refers to pollutant levels in residential homes that are generated from household fuel combustion, and at the same time contributes to the ambient air pollution level (WHO, 2014b). The effects of exposure to indoor air pollution is an issue that needs to be addressed, as people

spend about 70 – 90% of their time indoors (Dales et al., 2008; Canada, 1989). Vulnerable groups such as children, pregnant women, elderly citizens and people diagnosed with medical problems (cardiovascular and respiratory diseases) indeed may spend more time indoors and hence, may be exposed longer to indoor air pollution (Health Canada, 2013; SCHER, 2007). In recognition of this problem, the European Union (EU) Action Plan on Environmental and Health via "Action 12" has been produced (Jantunen et al., 2011; European Comission, 2007). However, indoor air pollution has still not yet been as widely investigated as outdoor air pollution; even though there is evidence linking indoor air pollution to acute and chronic human health effects (Bernstein et al., 2008).

1.1.1 Sources of air pollution

The sources of outdoor air pollution can be categorized into four groups: mobile, stationary, area and natural (National Park Service - NPS, 2018). The mobile source refers to air pollutants released from the transportation sector such as cars, heavy-duty vehicles and public transport. Vehicles may emit the pollutants from their exhaust, tyre wear, brake wear and road surface abrasion, and this is the predominant source contributing to air pollution (mainly particulate matter and polycyclic aromatic hydrocarbon (PAH)) in the ambient environment (Steiner et al., 2016; AQEG, 2012). The stationary source relates to air pollution released from industrial facilities such as power plants, sewage treatment, oil refineries or mines. Air pollutants from the industrial sector can also be emitted from waste combustion, burning of coal or natural gas and the use of chemical solvents, and these activities also contribute to the levels of particulate matter and PAH in the environment (Pruneda-Álvarez et al., 2016; Pihlava et al., 2013). The area source refers to air pollution generated from the smaller stationary sources or a specific area such as cities, agricultural and wood-burning fireplaces. The air pollutants in city areas are mainly arising due to the transportation, domestic heating and industrial sectors, whereas pollutants from agricultural areas are produced from the burning of fossil fuels, burning and clearing forests, as well as draining and degradation of peatlands (GOV.UK, 2013). Agricultural activities significantly contribute to the emission of carbon dioxide (CO_2), nitrogen oxides (NO_X), methane, ammonia and ozone-depleting substances (GOV.UK, 2013). Natural sources refer to the air pollution produced from volcanoes, wildfires or dust storms, which only tend to occur in arid regions (WHO, 2019b). The level of pollution generated from natural sources tends to be much lower compared to those arising from anthropogenic activities (WHO, 2019b).

The predominant sources of indoor air pollution in residential homes are building materials, household products/equipment and indoor activities such as cooking, smoking, heating and lighting (WHO, 2014b; WHO, 2010). Among the pollutants that can be generated from such indoor activities are particulate matter, PAH, formaldehyde (HCHO), sulphur dioxide (SO_2), carbon monoxide (CO), environmental tobacco smoke (ETS), volatile organic compounds (VOCs) and nitrogen oxides (NO_x). However, indoor air pollution levels can also be determined by outdoor air pollution, particularly from the transportation and industrial sectors (USEPA, 2019a; Balmes & Mark, 2016). Air pollution from the outdoors are able to penetrate, distribute and accumulate in the indoor environment through any opening such as windows or doors, and this can exacerbate pollution levels inside the home (Chen & Zhao, 2011). The susceptibility of individual towards air pollution exposure is varied and depends upon three important elements which are nature of pollutants, level (or dose) of exposure and time relationship of exposure (Armstrong et al., 1992). In the absence of indoor air sources, air pollution levels in the residential home are strongly correlated with outdoors levels, suggesting that the outdoor environment plays an important role in the quality of the indoor environment (Diapouli et al., 2007). Figure 1.1 illustrates the common sources of indoor air pollutants in most residential homes, whereas Figure 1.2 describes the interaction between outdoor and indoor air pollution that may result in acute and chronic human health effects.

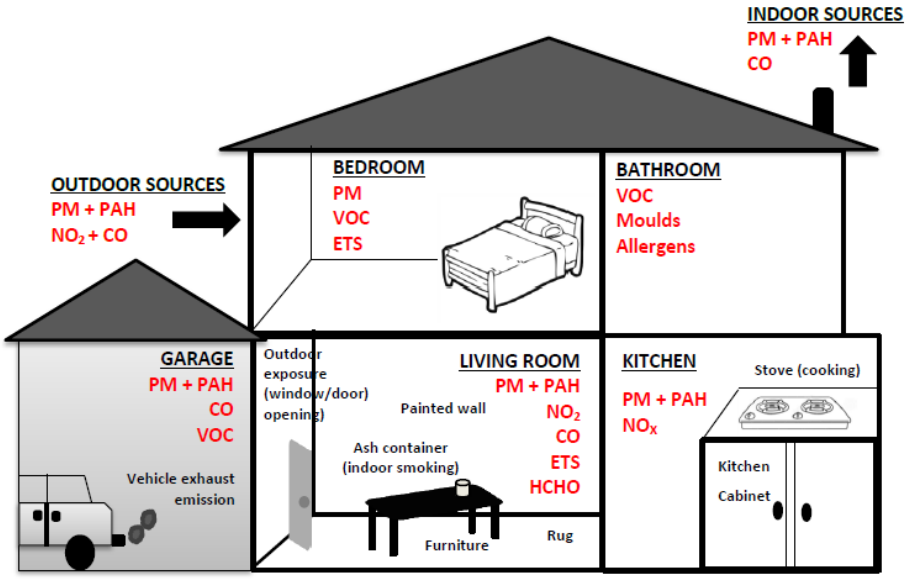


Figure 1.1: Sources of indoor air pollution (POSTnotes-UK Parliament, 2010).
Schematic diagram shows the typical sources of indoor air pollutants in the residential home. Cooking, indoor smoking and window opening are among the predominant sources that can elevate the level of indoor air pollutants in residential home, particularly particulate matter.
Emission of air pollution from indoor environment also contributes to the ambient air pollution and vice versa.

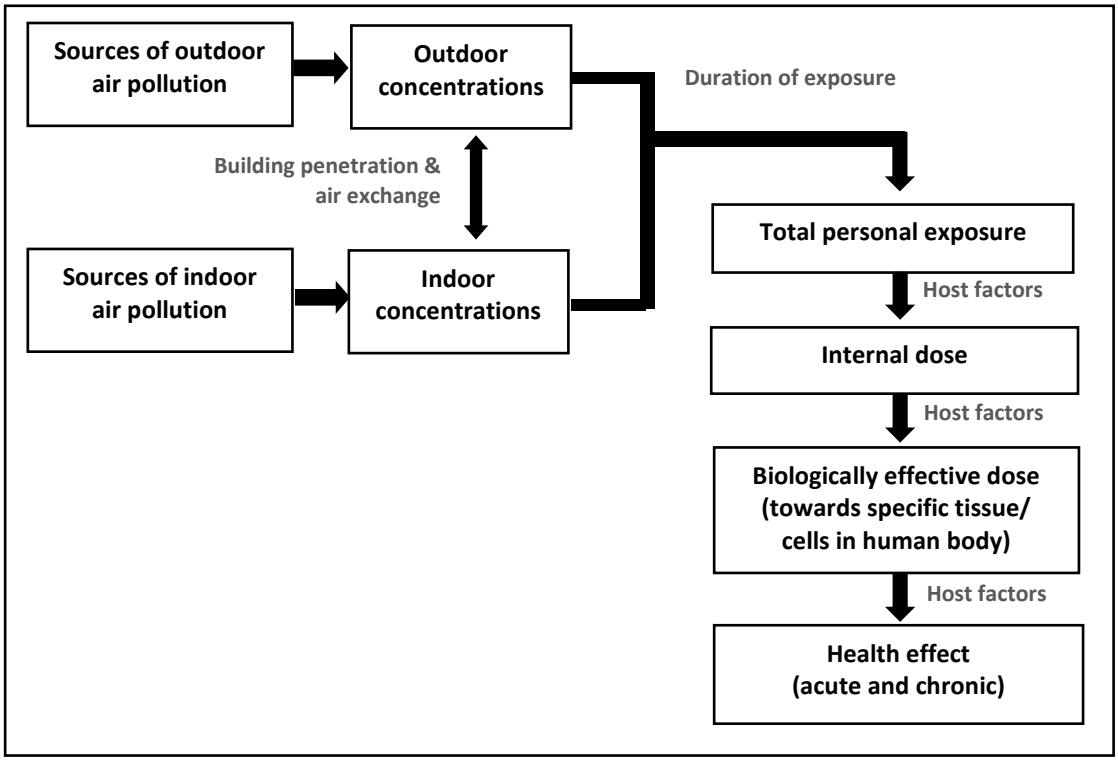


Figure 1.2: Interaction between outdoor and indoor air pollution and health effects (Adapted from Balmes & Mark, 2016).

Level of indoor air pollution in residential home is depends upon the sources of air pollution from indoor environment (as illustrated in Figure 1.1), and also strongly correlated with ambient air pollution level. The susceptibility and severity effect of individual towards air pollution is varied and depends on the nature of air pollutants, dose of exposure and time relationship of exposure (Armstrong et al., 1992).

Nature of air pollutants refer to chemical, physical and biological composition of the specific pollutants, source of pollutants, method of release and routes of exposure.

The actual amount of the pollutants coming into contact with human body is referred as administered dose (or internal dose), in which the level might be different between individuals, depends on the physiology and behaviour of the exposed individual (host factors).

Time relationship of exposure can determine the duration of exposure, total amount of exposure as well as the critical time window towards exposure to air pollutants (Armstrong et al., 1992).

1.1.2 Types of air pollutants

Air pollutants can be produced by many sources and generated from the combination of emissions and chemical reactions (Münzel et al., 2018). Air pollutants can be classified into primary and secondary pollutants and present in the environment in the form of particles or gaseous (Münzel et al., 2018; Balmes & Mark, 2016; Donohoe & Keegan, 2008). Primary pollutants are pollutants that are directly emitted from sources such as transportation or anthropogenic activities. In contrast, secondary pollutants are formed when primary pollutants undergo a chemical reaction in the atmosphere (Donohoe

& Keegan, 2008). Primary pollutants include SO_2, NO_X or NO_2, CO or CO_2, VOCs and particulate matter, whereas the commonest secondary pollutant is ozone that produced primarily by the interaction of two primary pollutants (NO_X and VOC) in the atmosphere, with the presence of heat and sunlight (Donohoe & Keegan, 2008). Particulate matter also can be categorized as the secondary pollutants as it can be formed from the particulate matter precursor gases (SO_2, NO_X, NH_3 and VOC) (Donohoe & Keegan, 2008). Table 1.1 shows the classification of primary and secondary pollutants, in which most of these pollutants resulted from the anthropogenic activities.

Table 1.1: Classification of air pollutants (Adapted from Donohoe & Keegan, 2008)

Types of air pollutants		**Primary source of pollutants**
Primary pollutants	SO_X or SO_2	Burning of coal and oil
	NO_X or NO_2	High-temperature combustion
	CO	Incomplete combustion of fuel (natural gas, coal or wood), mainly vehicle exhaust
	CO_2	Combustion
	VOCs	Hydrocarbon fuel vapours and solvents
	Particulate matter	Combustion and erosion processes
	Ammonia (NH_3)	Agricultural processes
	Lead (Pb)	Natural source, lead smelter, paints, plumbing
	Persistent organic pollutants (POPs)	Industrial processes and byproducts
Secondary pollutants	Particulate matter	Gaseous primary pollutants and compounds in photochemical smog (e.g. NO_X and VOC)
	Ozone (O_3)	Reaction between NO_X and VOCs in the presence of sunlight

1.1.3 Particulate matter

Particulate matter (PM) is defined as inhalable and respirable air particles or liquid droplets that suspended in the atmosphere (Vega et al., 2019; WHO, 2019b). PM can contain sulphates, nitrates, NH_3, sodium chloride, black carbon, VOC, mineral dust and water (Vega et al., 2019; WHO, 2019b). PM can be generated directly from the primary source of anthropogenic activities or can be formed as secondary pollutants (Donohoe & Keegan, 2008). The secondary PM can be produced after chemical or physical reactions of primary particles with atmospheric components or as a result of the chemical reaction of gaseous precursors in the atmosphere (e.g. SO_2, NO_X, NH_3) (Mangia et al., 2015; European Commission, 2008). The chemical reactions between gases can produce large numbers of

tiny particles by nucleation process or the additional size of the primary particles by condensation process (CSIRO, 2013). PM can be categorised based on particle size and so divided into total suspended, coarse, fine and ultrafine particles (CSIRO, 2013; Anderson et al., 2012). Table 1.2 provides a description of each PM category:

Table 1.2: Categories of particulate matter (Adapted from CSIRO, 2013; Donohoe & Keegan, 2008)

Category	Description	Potential sources
Total suspended particles	All particles with aerodynamic diameter up to 30µm	• All sources of coarse, fine and ultrafine particles as described above
Coarse particles	Particles with an aerodynamic diameter of 2.5 – 10µm or less (e.g. PM_{10})	• Natural sources such as dust, soil, sea salt, pollen, moulds and spores. • Crustal materials such as roads, farming, mining.
Fine particles	Particles with an aerodynamic diameter of 2.5µm or less (e.g. $PM_{2.5}$)	• Anthropogenic sources such as combustion (petrol/diesel vehicles, wood/coal burning) and industrial activities.
Ultrafine particles	Particles with an aerodynamic diameter of 0.1µm or less (typical range 1 – 100nm) (e.g. $PM_{0.1}$)	• Anthropogenic sources such as combustion from the vehicle exhaust and atmospheric chemical reactions. Usually relates to engineered material rather than described the ambient particles

PM are inhalable and able to penetrate into the human lung and are capable of being absorbed into the bloodstream (USEPA, 2018). The main route of exposure of these particles is via inhalation; hence the respiratory system is the main target organ. Exposure to PM can cause respiratory disease by affecting the upper respiratory tract and/or the lower respiratory tract, and this depends on the size of exposed particles (CSIRO, 2013). Usually, the smaller particles can affect the lower respiratory tract, while larger particles can affect the upper respiratory tract (CSIRO, 2013). Figure 1.3 illustrates the size of PM by comparing with a diameter of human hair.

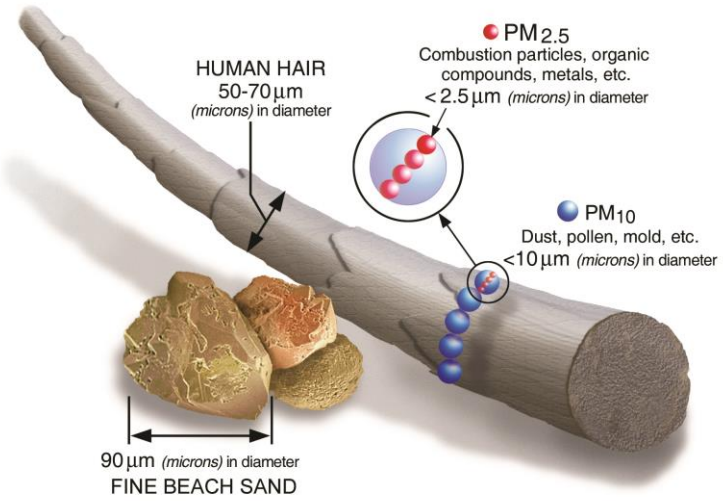


Figure 1.3: Details of PM particles size and comparison with human hair (Adapted from USEPA 2018)

The schematic diagram shows the size of PM by comparing their size with the diameter of human hair. The mean diameter of human hair is ~70µm which is 7 times larger than PM_{10} and 30 times larger than $PM_{2.5}$. Both PM_{10} and $PM_{2.5}$ are inhalable and pose a risk to human health (USEPA, 2018).

Increases in the levels of PM in the ambient and indoor environment have been associated with adverse health effects, mainly respiratory and cardiovascular disease as these particles can be inhaled and affected the systemic circulation and respiratory system (Anderson et al., 2012; WHO, 2006). The effects of PM vary for each individual as it depends on the susceptibility of the individual towards the exposure level, in which health conditions and age mainly determine sensitivity to these particles (WHO, 2006). The size of particles also determines health outcomes, with smaller $PM_{2.5}$ potentially having more adverse effects compared to PM_{10} (Air Quality Expert Group, 2012). $PM_{2.5}$ is a complex particle and can be bound with other components such as heavy metals and PAHs (Colman Lerner et al., 2018; Mostofsky et al., 2012; Pleil, Vette, & Rappaport, 2004). PAHs are known to be human carcinogens and can be a risk factor for lung cancer (WHO, 2010). Usually, PAHs are capable of binding with $PM_{2.5}$, in which $PM_{2.5}$ constituents can alter the severity effect of these particles on human health (e.g. additive, synergistic or antagonistic effects). The health outcomes of $PM_{2.5}$ exposure can vary depending on the concentrations of the constituents; hence the evaluation of $PM_{2.5}$ bound with constituents are more important rather than the physical effect of $PM_{2.5}$ (Mostofsky et al., 2012). The acute effect of $PM_{2.5}$ exposure includes increases in hospital admissions and the premature death of individuals at-risk (e.g. individuals diagnosed with the respiratory and cardiovascular diseases),

whereas the chronic effect include lung cancer, chronic obstructive pulmonary disease (COPD), tuberculosis (TB), cardiovascular disease and adverse pregnancy outcomes (Li et al., 2018; Basu et al., 2014; Air Quality Expert Group, 2012; Donohoe & Keegan, 2008).

1.1.4 Assessment of ambient air pollution level in the UK

The levels of ambient air pollution in the UK are assessed using national monitoring networks and estimations from modelling assessments, which is under the responsibility of the Department for Environment, Food and Rural Affairs (DEFRA) (DEFRA, 2019a). Modelling via Pollution Climate Mapping (PCM) was introduced to reduce the number of air pollution monitoring stations in the UK, with levels at unmeasured locations being estimated by the modelling method (DEFRA, 2019a). The UK government is responsible for reporting the air quality data on an annual basis under the following European Directives:

- Directive 2008/50/EC – The Council Directive on ambient air quality and cleaner air for Europe; responsible for setting limit values and health exposures related to $PM_{2.5}$, PM_{10}, SO_2, NO_2, lead, benzene, carbon monoxide and ozone.
- Directive 2004/107/EC – The Fourth Daughter Directive under the air quality Framework Directive (1996/62/EC) and is related to exposure to arsenic, cadmium, mercury, nickel and PAHs in ambient air.
- Directive 2015/1480/EC – the Amendment of the above Directives; concerning reference methods, data validation and sampling points of ambient air quality assessment (European Commission, 2019; DEFRA, 2019).

These Directives are responsible for setting air quality standards in the UK, which are referred to “The Air Quality Standard Regulations 2010” and they are responsible for setting “limit values”, “target values” and “long-term objectives” of ambient air pollutants (DEFRA, 2019a). Limit values are legally binding and must not be exceeded at any time. Limit values are specified for individual pollutants and consist of the concentration value, an averaging period for the concentration value, a permissible concentration value (annually), as well as a calendar date to achieve the target limit. Some limit values consisting of daily (24-hour) and annually levels are shown in Table 1.3.

Table 1.3: The Air Quality Standard Regulations 2010 (Schedule 2: Limit values and Schedule 3: Target values)*

Pollutant	Averaging period	Limit value
PM_{10}	24-hour	50μg/m^3, not to be exceeded more than 35 times per calendar year
	Calendar year	40μg/m^3
$PM_{2.5}$	Calendar year	25μg/m^3
NO_2	1- hour	200μg/m^3 not to be exceeded more than 18 times per calendar year
	Calendar year	40μg/m^3
SO_2	1- hour	350μg/m^3, not to be exceeded more than 24 times per calendar year
	24- hour	125μg/m^3, not to be exceeded more than 3 times per calendar year
O_3	8-hour mean	120μg/m^3, not to be exceeded more than 25 days per calendar year, averaged over 3 years

* Source: Environmental Protection, 2010

The level of ambient PM in the UK had been falling and improved in recent decades (DEFRA, 2019a; DEFRA, 2019b). In 2018, the annual concentration of ambient $PM_{2.5}$ for background and roadside locations had dropped to 23% and 26%, respectively, of the levels recorded in 2010 (DEFRA, 2019b). However, even though ambient $PM_{2.5}$ levels had markedly reduced over the years, current exposures are still linked to adverse human health effects, particularly for long term exposure. To resolve this issue, the UK has become the first country in the world to commit to the WHO air quality guideline, specifically for ambient $PM_{2.5}$ (DEFRA, 2019b). The WHO has set the limit for ambient $PM_{2.5}$, as exposure must not be exceed 25μg/m^3 over 24 hours, whereas annual exposure must not be exceed 10μg/m^3. The standard limit that has been set by the WHO for PM exposure is the lowest compared to other authorities of developed countries, therefore it become a challenge to the UK to commit with the target (DEFRA, 2019b). Table 1.4 compares the limit values of ambient PM ($PM_{2.5}$ and PM_{10}) that has been set by the WHO and other different authorities.

Table 1.4: Comparison between the WHO standard limit with other authorities for ambient PM levels (Adapted from Donohoe & Keegan, 2008)

Source	$PM_{2.5}$ ($\mu g/m^3$)		PM_{10} ($\mu g/m^3$)	
	24-hour	Calendar year	24-hour	Calendar year
WHO	25	10	50	20
United Kingdom	-	25	50	40
European Union	-	25	50	40
United States	65	15	150	50
California	65	15	50	20
Switzerland	-	-	50	20
France	-	-	50	40
Sweden	-	-	50	40
Japan	-	-	100	-

1.1.4.1 Daily air quality Index (DAQI)

In the UK, the daily air quality index (DAQI) was introduced to explain outdoor air pollution levels clearly and simply to the public. This system was built following a recommendation by the Committee on the Medical Effects of Air Pollutants (COMEAP), an organization that responsible for providing advice and guidance on air pollution impacts on human health to the UK government (DEFRA, 2019d). DEFRA use DAQIs to estimate the air pollution level on the next day (for up to five days ahead), and this information is publicly available from the DEFRA air quality forecasts website (https://uk-air.defra.gov.uk/forecasting/). DAQI describes exposure levels for five common air pollutants including PM_{10}, $PM_{2.5}$, SO_2, NO_2 and O_3. These pollutants are categorized into ten banding groups based on the level of exposure. These 10 banding groups are further classified into “low”, “moderate”, “high” and “very high” levels, in which each classification describes different health messages towards healthy and at-risk individuals. At-risk individuals refer to people diagnosed with respiratory and cardiovascular diseases. Table 1.5 shows the DAQI of PM_{10} and $PM_{2.5}$ exposure level, whereas Table 1.6 describes the health advice to the general population and at-risk individuals that are exposed to these air particles.

Table 1.5: Daily Air Quality Index for PM_{10} and $PM_{2.5}$ level in the UK

Index	1	2	3	4	5	6	7	8	9	10
Band	Low	Low	Low	Moderate	Moderate	Moderate	High	High	High	Very High
$PM_{2.5}$ ($\mu g/m^3$)	0 - 11	12 - 23	24 -35	36 - 41	42 - 47	48 – 53	54 - 58	59 - 64	65 - 70	> 71
PM_{10} ($\mu g/m^3$)	0 – 16	17 – 33	34 – 50	51 – 58	59 – 66	67 – 75	76 – 83	84 – 91	92 – 100	>101

Table 1.6: Health advice to healthy (general population) and at-risk individuals

Air Pollution Banding		Accompanying health messages for:	
Level	**Value**	**General population**	**At-risk individuals***
Low	1 to 3	• Enjoy your usual outdoor activities.	• Enjoy your usual outdoor activities.
Moderate	4 to 6	• Enjoy your usual outdoor activities.	• Adults and children with lung problems, and adults with heart problems, who experience symptoms, should consider reducing strenuous physical activity, particularly outdoors.
High	7 to 9	• Anyone experiencing discomforts such as sore eyes, cough or sore throat should consider reducing activity, particularly outdoors.	• Adults and children with lung problems and adults with heart problems should reduce strenuous physical exertion, particularly outdoors, and particularly if they experience symptoms. • People with asthma may find they need to use their reliever inhaler more often. Older people should also reduce physical exertion.
Very High	10	• Reduce physical exertion, particularly outdoors, especially if you experience symptoms such as cough or sore throat.	• Adults and children with lung problems, adults with heart problems, and older people, should avoid strenuous physical activity. • People with asthma may find they need to use their reliever inhaler more often.

* At-risk individuals refer to the person who diagnosed with cardiovascular and respiratory diseases.

The DAQI provides information on daily air pollutant level as estimated by automatic monitoring sites in the Automatic Urban and Rural Network (AURN) (DEFRA, 2019c). The AURN is the largest automatic monitoring network in the UK and is managed by the Environment Agency to provide information on local air quality level to the public. This organization is responsible for maintaining the 228 monitoring sites across the UK that hourly measure air pollution levels starting from 22nd February 1973 (DEFRA, 2019c). For the Greater Manchester area, 17 monitoring sites are available covering ten local authorities namely Bolton, Bury, Manchester, Oldham, Rochdale, Salford, Stockport, Tameside, Trafford and Wigan (O'Neill et al., 2017).

DEFRA categorized the monitoring stations based on their location as the following (DEFRA, 2019e):

i. Urban area;

   Monitoring station located at a continuously built-up urban area with at least two-floor buildings or large detached buildings and is not mixed with non-urbanised areas (agricultural, lakes, wood). The measurements of air quality in urban sites are within a few km^2.

ii. Suburban area;

   Monitoring stations located in largely "built-up" areas and mixed with non-urbanised areas. The measurements of air quality in suburban sites are within some tens of km^2.

iii. Rural area;

   Monitoring stations located in small areas with natural ecosystems, forests or crops, and located >20km away from agglomeration areas and >5km way from built-up areas, industrial site or motorways/major roads, with the sampling points targeted on the protection of vegetation and natural ecosystems. The measurements of air quality in rural sites are at least 1000km^2.

iv. Traffic station;

   Monitoring stations located nearby traffic sources (roads, motorways and highways). The measurements of air quality in traffic sites must be representative of air quality for a street segment with not less than 100m lengths.

v. Industrial station;

   Monitoring stations located within industrial areas that consist of single or various industrial sources emissions (e.g. power generation, incinerators and waste treatment plants). The measurements of air quality in industrial sites are at least 250m x 250m.

vi. Background station;

Monitoring stations located within an area without a specific source of air pollution or street, but based on the contribution of emission from different sources (e.g. traffic, combustion, upwind of the station in a city, industrial or rural areas). The measurements of air quality in background sites are within several km^2.

1.1.5 Measurement of indoor air pollution

In order to investigate the exposure levels to indoor air pollution, three techniques are generally applied:

i. Continuous sampling – refers to "real-time" sampling, in which temporal variation of indoor air pollutant levels can be observed over the sampling period.

ii. Integrated sampling – refers to the measurement of the air pollutants levels over a specified period (weeks, minutes or months) with the analysis of pollutants performed in the laboratory, either by gravimetric or chemical analysis.

iii. Grab sampling – refers to the single measurement of air pollutants in a very short and specific time, usually using an evacuated flask/ deflated bag (US National Research Council, 1981).

Continuous sampling has been used widely in previous studies to demonstrate the level of indoor PM (or indoor air particles) (Adesina et al., 2020; Volesky et al., 2018; Jorquera et al., 2018; Lim et al., 2018; de Gennaro et al., 2015; Rosen et al., 2015; Huang et al., 2014; Karottki et al., 2014; Jones et al., 2000). This method has numerous advantages and is simple to apply as compared to other techniques (US National Research Council, 1981). However, the limitations of this technique are that the monitor is usually expensive, requires routine calibration and maintenance, as well as potentially producing unpleasant noises and hence it may not be suitable for large-epidemiological studies (Semple et al., 2012; US National Research Council, 1981).

Due to this limitation, the "Dylos air particle monitor" (Dylos Corporation, Riverside, California, USA) was introduced to measure air particle concentrations (Dacunto et al., 2015; Semple et al., 2012). The monitor uses light scattering and photodiodes to detect the size of particles, and particles are count based on the particle number concentration in the unit of particles per cubic foot (pccf) (Brown et al., 2014; Dylos Corporation, n.d.). Dylos air particle monitors have been used in many previous studies for the measurement of air

particle levels in a various setting includes residential homes, outdoor environments and also has been used for personal monitoring (Franken et al., 2019; Gaspar et al., 2018; Lim et al., 2018; Han et al., 2017; Semple & Latif, 2014; Semple et al., 2015; Steinle et al., 2015). There are two models; Dylos DC1100 and DC1700, in which the difference between models only relates to the power supply, with the DC1100 being battery-powered and the DC1700 being either battery or mains power (Air Quality Sensor Performance Evaluation Center, n.d.).

The main advantage of Dylos monitors is its low cost, as the price is only about 10% of the price of the conventional "real-time" device, such as the Sidepak (Semple et al., 2013). The TSI Sidepak AM510 Personal Aerosol Monitor (TSI, Shoreview, MN) is a similar instrument as the Dylos, as this device operates with a real-time laser photometer and typically has been used in many air pollution studies (Jackson-Morris et al., 2016; Dacunto et al., 2015; Semple et al., 2015; Cameron et al., 2010; Edwards et al., 2006). With a smaller fan and large air inlet, the sound produced during Dylos monitoring is quieter compared to that from a Sidepak. Also, the easy application to activate this monitor makes Dylos is an ideal device to be used in a wide range of applications (Semple et al., 2012). However, conversion of particle counts to the particle mass concentration is required, as many air pollution studies and air quality guidelines present air pollution data in mass concentration ($\mu g/m^3$), instead of particle number concentration (ppcf). A comparison of air pollution data as measured by Dylos with other gravimetric methods or conventional devices is required to convert particles counts into mass concentration, as well as for calibration purpose.

Previous studies have compared the performance of Dylos counters with other air particle counters. A study conducted by Northcross et al. (2013) found that the number of the particles measured by Dylos was in good agreement with a calibrated TSI DustTrak (R^2=0.99, 0.99, 0.98 and 0.97 for four different tests, respectively), but the Dylos was able to detect finer particles down to 0.5µm as compared to the reference device. The limit of detection (LOD) of the Dylos is <1$\mu g/m^3$ and this monitor able to detect the air particles at the concentration up to 1,000$\mu g/m^3$ (Northcross et al., 2013). A study conducted by Klepeis et al. (2013) compared the Dylos performance with TSI Dustrack and found that the sensitivity, precision and baseline stability of Dylos was comparable to the reference device (R^2=0.99 for the comparison of six units of Dylos with TSI Dustrack, respectively). Also, Dylos measurement data appears not to be impacted by the temperature and other environmental conditions. The LOD of Dylos was recorded as <1$\mu g/m^3$, which is similar to that recorded by Northcross et al. (2013) (Klepeis et al., 2013).

Another previous study conducted by Semple et al. (2015) compared indoor air particles in 34 homes by using both a Dylos DC1700 and a SidePak AM510 and they found that the air pollution data generate from both devices were almost identical with only 3.2% (n=25,301) of the recorded values being outside the limits of agreement. A recent publication by Franken et al. (2019) developed a method for Dylos data conversion from particle number concentrations to $PM_{2.5}$ mass concentrations, by comparing the data generated by Dylos with the two conventional devices namely the Aerodynamic Particle Sizer (APS, TSI) and Optical Particle Counter (OPC, GRIMM). They also compared another three conversion methods from the literature and they found that the fit curve obtained from the comparison of Dylos with the OPC device was the most accurate method to convert the Dylos measurement data into mass concentration compared to another reference devices (Franken et al., 2019a). Table 1.7 summarizes the previous studies that have used Dylos and their fitted relationship, for the conversion of particle number concentration to particle mass concentration.

Table 1.7: Dylos applications in previous studies and their fitted relationship for data conversion into particle mass concentration

References	Country	Study goal	Dylos model	Concentration of $PM_{2.5}$ measured by Dylos ($\mu g/m^3$)	Reference device	Fitted relationship	Parameter
Franken et al. (2019)	Netherlands, UK and Greece	Residential home	DC1700	No information provided	Aerodynamic Particle Sizer (APS 3321)	$Y=mx^n$	$m = 9.14e^{-5}$ $n = 0.96$
					OPC 1.108 aerosol spectrometer (OPC, GRIMM)	$Y=mx^n$	$m = 7.33e^{-4}$ $n = 0.82$
Gaspar et al. (2018)	California, USA	Child care centre, home-based facility	DC1100	Median (IQR): 15 (11 – 23); n=40 child care facilities	TSI DustTrak 8520 & 8530	$Y=a_0 + a_1x$	$a_0 = 3.1$ $a_1 = 7.6e^{-5}$
Lim et al. (2018)	Mongolia	Residential homes (Gers)	DC1700	Mean (+SD) for 24 hours: 203.9 ± 195.1; n=29 traditional stoves 257.5 ± 204.4; n=31 improved stoves	MicroPEM nephelometer	$Y=a_0 + a_1x$	$a_0 = 1.354$ $a_1 = 10^{-4}$
Han et al. (2017)	Houston, USA	Outdoor environment (Residential areas)	DC1700	Mean (+SD): 13.2 ± 13.7; average concentration in the backyard of residential homes	GRIMM 11-R Mini Laser Spectrometer	$Y=a_0 + a_1x$	$a_0 = -2.8197$ $a_1 = 7.1e^{-3}$
Jackson-Morris et al. (2016)	Mexico, Chad, Bangladesh, India, Indonesia and Pakistan	Hospitality venues (bars, restaurants, cafes and hotels)	DC1700	Median (IQR) for indoor vs. outdoor: Mexico = 18 (14 – 25) vs. 19 (10 – 26); Pakistan = 69 (44 – 132) vs. 34 (30 – 52); Indonesia = 32 (12 – 148) vs. 29 (12 – 47); Chad = 51 (31 – 81) vs. 34 (13 – 39); Bangladesh = 119 (2 – 183) vs. 146 (2 – 182); India = 37 (23 – 65) vs. 49 (31 – 80)	No comparison with other conventional device or gravimetric analysis (Adapted the fitted relationship from Semple et al., 2015)		
Semple et al. (2015)	Scotland, UK	Residential homes	DC1700	Mean (range) for 24 hours: 4.4 (1 – 12); n=17 home of non-smokers 33.3 (1 – 196); n=17 home of smokers	TSI SidePak AM510	$Y=a_0 + a_1x + a_2x^2$	$a_0 = 0.65$ $a_1 = 4.16e^{-5}$ $a_2 = 1.57e^{-11}$
Dacunto et al. (2015)	California, USA	Residential homes	DC1100	Mean (range) from several sources: Cigarette = 11 (1 – 32); Stick incense = 14 (5 – 28); Overall = 23 (0 – 72)	Sidepak AM510	$Y=mx^n$	$m = 1.09e^{-7}$ $n = 2.111$
Steinle et al. (2015)	Scotland, UK	Personal monitoring (Indoor & outdoor)	DC1700	Mean (SD) from 35 participants: Home = 8.4 (±17.3); Outdoor = 6.2 (±6.9); Public building = 6.3 (±8.4); Transport = 7.0 (±6.0); Office building = 3.0 (±2.2)	TEOM-FDMS instruments (outdoor)	$Y=a_0 + a_1x$	$a_0 = 4.75$ $a_1 = 2.8e^{-5}$
Brown et al. (2014)	Connecticut, USA	Residential homes	DC1100	Mean: 37$\mu g/m^3$; n=4 residential homes near outdoor wood furnaces	Air monitoring stations	$Y=a_0 + a_1x$	$a_0 = 1.9408$ $a_1 = 0.4074$

1.2 Air pollution and placental function

The placenta allows gaseous exchange, waste removal, nutrient transport and hormone secretion, but is also an immunological barrier and physical barrier (Baker, 2015; Gude et al., 2004). Previous studies have revealed that maternal exposure to air pollution during pregnancy might increase susceptibility to abnormal placental growth and function, which may contribute to adverse pregnancy outcomes including low birth weight (LBW), intrauterine growth restriction (IUGR) and pre-term birth (PTB) (van den Hooven et al., 2012; Kannan et al., 2006). However, the evidence identifying the underlying biological mechanisms between air pollution exposure and the effect on pregnancy outcomes is still limited with several competing hypotheses including oxidative stress, systemic inflammation, coagulation, endothelial function and hemodynamic responses to air pollution (Patelarou & Kelly, 2014; Kannan et al., 2006).

1.2.1 Placenta development in normal pregnancy

The development of the placenta is a highly regulated process, which is critical for normal fetal growth and development. Disordered placental development underpins various pregnancy complication such as pre-eclampsia, fetal growth restriction (FGR), miscarriage and stillbirth (Turco & Moffett, 2019; Brosens et al.,2011).

Placental development involves three main stages; pre-lacunar, lacunar and villous stages (Huppertz, 2008). Development of the placenta starts with the implantation of the blastocyst that develops at ~5 days post fertilisation (Turco & Moffett, 2019). The blastocyst consists of two cell lineages; the trophectoderm (or trophoblast – outer layer) and inner cell mass (or embryoblast – internal layer) (Turco & Moffett, 2019; Frank, 2017). After ~6 to 7 days post-fertilisation, the polar trophectoderm (that part of the trophectoderm that is contiguous with the inner cell mass) attaches to the uterine surface epithelium and fuses to form a primary syncytium (or syncytiotrophoblast) (Turco & Moffett, 2019). This phase is referred to as the pre-lacunar stage of placental development. The implantation of blastocyst then continued with a rapid invasion of the primary syncytium into the underlying endometrium. The blastocyst is entirely embedded in the decidua and covered with the surface epithelium of endometrium at ~14 days of post-fertilisation (Turco & Moffett, 2019). The lacunae (fluid-filled spaces) then form within the primary syncytium, gradually enlarge and coalesce to form the large intervillous space (Turco & Moffett, 2019; Dunk et al., 2008). This phase is referred to as the lacunar stage of placental development,

which resulted in the formation of trabeculae (syncytiotrophoblast column) (Turco & Moffett, 2019; Dunk et al., 2008). The cytotrophoblast cell then protrudes the primary syncytium by proliferation, reaches the opposite side of the placenta until reach the maternal tissue of the decidua (Dunk et al., 2008). This process results in the formation of the extravillous trophoblast and primary villi (Turco & Moffett, 2019). At ~17-18 days post-fertilisation, extraembryonic mesenchymal cells penetrate the primary villi to form the secondary villi, following which the first blood vessels develop within the placental mesenchyme, leading to the formation of the tertiary villi (Turco & Moffett, 2019). The rapid branching of villi continues to develop a villous tree system that mainly acts in the oxygen and nutrients transport exchange between mother and fetus (Turco & Moffett, 2019; Desforges et al., 2018).

1.2.2 Placental structure at term

The structure of the human placenta is shown in Figure 1.4A. The mature placenta (at-term) is a large disk-like shape, with weight ~500g, thickness ~3cm, and a diameter from ~10 to 25cm (Vähäkangas et al., 2014; Carlson, 2014; Huppertz, 2008). However, the shape, weight, size, thickness and the location of the cord varies between placentas (Huppertz, 2008). The placenta is connected to the fetus by the umbilical cord and has a fetal and maternal side. The chorionic plate is the fetal surface of the placenta, which is covered with an amniotic membrane and can be identified by the presence of the cord (Huppertz, 2008). The cord insertion is usually located slightly eccentrically in the chorionic plate (Huppertz, 2008). The umbilical cord contains two arteries and one vein. The two umbilical arteries carry the deoxygenated blood, nutrient-depleted blood and waste products from the fetus back to the placenta, whereas the umbilical veins transfer the oxygenated blood and nutrient-rich blood from the placenta to the fetus (Vähäkangas et al., 2014; Wang & Zhao, 2010).

The basal plate represents the maternal side of the placenta and has multilobulated appearance. Typically, the human placenta consists of ~10 to 40 lobes which are separated by septae called cotyledons that contain the main stem villous trees (Carlson, 2014; Huppertz, 2008). The villous tree connects the fetal (chorionic plate) and maternal (basal plate) surface and is surrounded by the syncytiotrophoblast layer (Wang & Zhao, 2010b). Placental villi can be differentiated into three sections; stem, intermediate and terminal villi (as shown in Figure 1.4B). The exchange of oxygen and nutrients between the mother and

the fetus takes place through the surface of the villous tree, referred to as terminal villi (Vähäkangas et al., 2014). Insufficiency of oxygen and nutrient transfer to the fetus may indicate impairment of placental function, which can negatively affect fetal development.

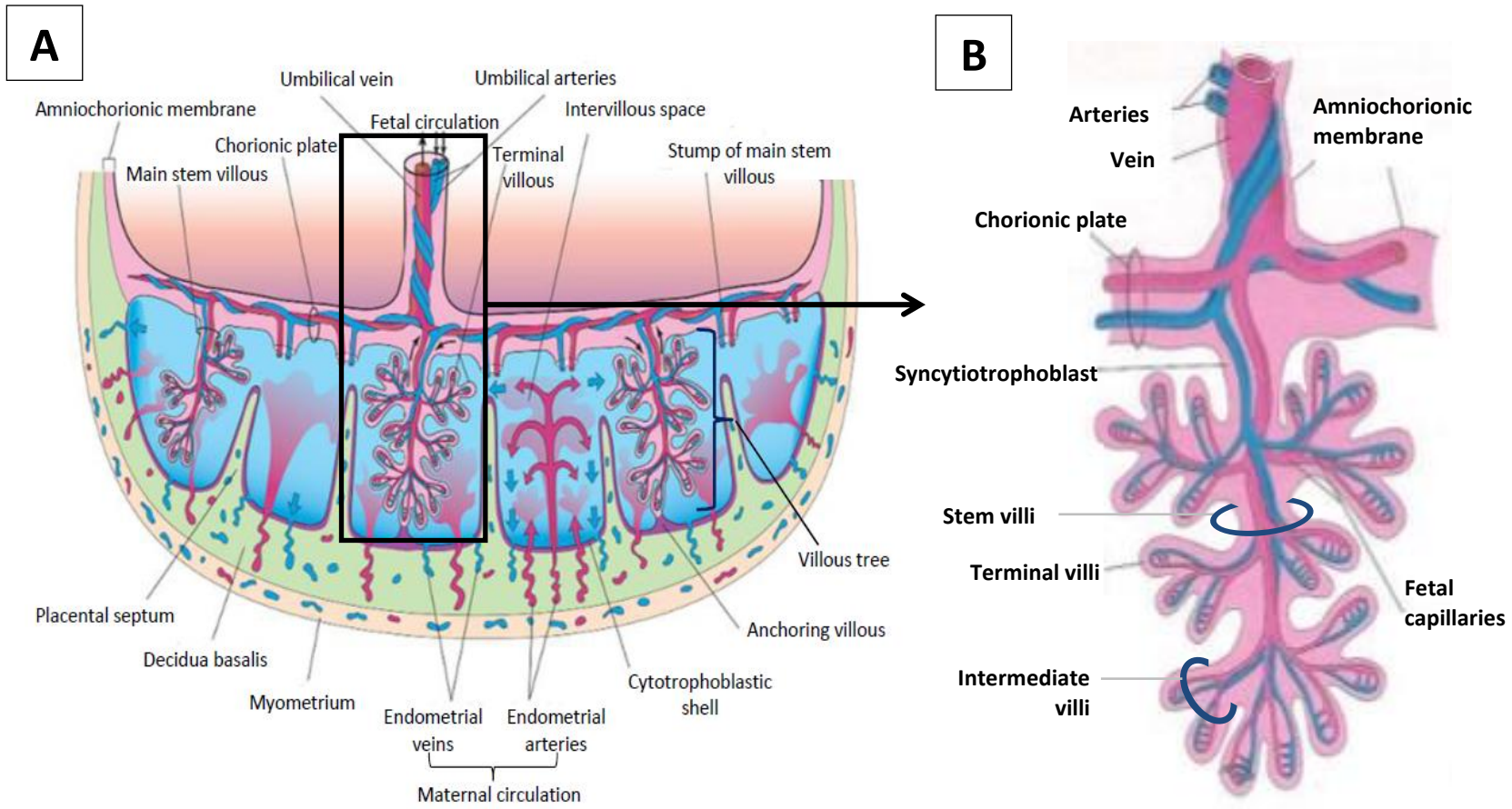


Figure 1.4: The cross-section of human placenta (Adapted from Hirst, 2015; Moore et al., 2003).

(A) The placenta is connected to the fetus by the umbilical cord and consists of a fetal and maternal side. The chorionic plate represents the fetal surface, whereas the basal plate represents the maternal side of the placenta. The umbilical cord contains two arteries (blue) and one vein (red).

(B) The villous tree connects the fetal and maternal surfaces and is surrounded by the syncytiotrophoblast layer. The exchange of the oxygen and nutrients between mother and fetus takes place through the surface of the villous tree, referred to as terminal villi.

1.2.3 Adverse pregnancy outcomes

Placental dysfunction can lead to adverse pregnancy outcomes such as pre-eclampsia, fetal growth restrictions (FGR), pre-term birth (PTB), miscarriage and stillbirth (Turco & Moffett, 2019; Brosens et al., 2011). This problem is of significant public and health concern due to the prevalence of adverse outcomes, the risk of mortality and morbidity, and long-term health effects. Pre-eclampsia is a maternal hypertensive disorder of pregnancy which affects 2 – 8% of all pregnancies; this condition can directly affect fetal health and development, as well as lead to maternal and fetal mortality and morbidity worldwide (Jeyabalan, 2014; WHO, 2011). Small gestational age (SGA) describes as infants born with a birthweight <10th centile, which sometimes used as a proxy for FGR, but 50 – 70% of the SGA babies are constitutionally small with appropriate fetal growth for maternal size and ethnicity (RCOG Guideline, 2013). FGR affects 5 to 10% of pregnancies and associated with an increased risk of neonatal mortality and morbidity (Desforges et al., 2018; Nardozza et al., 2017). Epidemiological studies often use low birth weight (LBW) when accurate data on gestation of pregnancy is missing. LBW is associated with neonatal mortality and like SGA is a risk factor of short and long term health effects including diabetes and cardiovascular disease in a later stage of life (WHO, 2014a). PTB is another factor that contributes to neonatal mortality and is a major cause of infant mortality among children below the age of 5 years (WHO, 2020). Stillbirth refers to fetal death before or during birth born dead; ~2.6 million cases occur annually, of which 98% of the cases occurred in low- and middle-income countries (Lawn et al., 2016).

1.2.3.1 Pre-eclampsia

Pre-eclampsia is defined as new-onset hypertension (diastolic blood pressure >90mmHg) and proteinuria (protein in urine >0.3g/24hours) that occurs after 20 weeks of gestation of previously normotensive women (Huppertz, 2011; WHO, 2011). Pre-eclampsia has been related to dysregulation of villous trophoblast development, poor placental perfusion, inflammation, ischemia and hypoxia (Norris et al., 2011; Huppertz, 2011). According to Huppertz (2011), pre-eclampsia can result from the failure of syncytiotrophoblast differentiation, in which the abnormal formation of necrotic or aponecrotic fragments that are released into the maternal blood and can induce an inflammatory response in the mother. The principal risk factors for pre-eclampsia include (i) antiphospholipid syndrome, (ii) a previous pre-eclampsia pregnancy, (iii) insulin-dependent

diabetes, (iv) multiple pregnancies, (v) nulliparity, (vi) a family history of preeclampsia, (vii) obesity, (viii) advanced maternal age (>40 years) and (ix) pre-existing hypertension (English et al., 2015). Preeclampsia may lead to severe maternal health complications such as seizure, liver and blood clotting disorders (HELLP syndrome), stroke, organ failure, pulmonary edema, placental abruption, and can result in maternal mortality (NHS, 2018c; English et al., 2015; Dadvand et al., 2013).

1.2.3.2 Small gestational age (SGA)

SGA refers to a baby born with a lower than the 10^{th} percentile of the standardized weight of the babies at the same gestational age and population (Barjaktarovic et al., 2017; WHO, 2010). SGA can be assessed with abnormal uterine and umbilical artery by Doppler analysis, low ponderal index, caesarean section due to fetal distress, hyperglycemia and also resulted in perinatal mortality (Gardosi, 2006). Among the risk factors of SGA are (i) demographic factors (such as maternal age >40years, BMI <20 or >25, ethnicity, parity, social-economic status), (ii) maternal dietary intake and lifestyle (such as caffeine, smoking, alcohol, nutritional supplements), (iii) maternal medical conditions (such as chronic hypertension, diabetes, renal disease, auto-immune conditions, asthma, thyroid, cardiac disease), (iv) complications during pregnancy (such as hyperemesis, bleeding in early and late gestation, placental abruption, placenta previa, hypertensive disorder) and (iv) fetal conditions (such as chromosome abnormalities, multiple pregnancies, infections) (Stanford's Children Health, 2019; McCowan & Horgan, 2009). Babies born as SGA are well documented as having a higher risk of neonatal mortality and morbidity, impaired cognitive development, non-communicable diseases and malnutrition and other health problems starting from their early life until adulthood (Muhihi et al., 2016).

1.2.3.3 Low birth weight (LBW)

LBW refers to an infant weight less than 2,500g (5.5lb) at birth (Muchemi et al., 2015; WHO, 2014). LBW can be further divided into two categories, which are very LBW (less than 1500g) and extremely LBW (less than 1000g) (Muchemi et al., 2015). The birth weight of the baby is measured immediately after birth (<1 hour after delivery) before the baby has a significant postnatal weight loss (UNICEF, 2004). LBW is a major health concern being 15 – 20% of total births worldwide (WHO, 2014a). In 2003, 64% of neonatal deaths in England and Wales were associated with LBW (Bakeo & Clarke, 2006). The prevalence of

LBW is higher in developing countries rather than developed countries and is higher among pregnant women in urban areas compared to rural areas (Muchemi et al., 2015; UNICEF, 2004). Among the risk factors for LBW are (i) demographic factors (maternal BMI (<45kg), age (e.g. increased risk of LBW among mother at age between 15 – 19 and 35 – 40), ethnicity (e.g. higher occurrence of LBW among black rather than white ethnicity), socioeconomic level (e.g. low socio-economic conditions can lead to malnutrition, low educational level, stress, drug abuse and lack of access to health care), (ii) dietary intake and lifestyle factors (e.g. smoking, alcohol consumption and drug abuse) and (vi) exposure to air pollution (CDC, 2016; Muchemi et al., 2015; UNICEF, 2004; Bernabé et al., 2004).

1.2.3.4 Fetal growth restriction (FGR)

Fetal growth restriction (FGR) is a synonym of intrauterine growth restriction (IUGR) and refers to the condition of a fetus that has not achieved the growth potential expected and predominantly as a result of placental dysfunction (Nardozza et al., 2017; Berthold Huppertz, 2011). FGR babies have a higher risk of neonatal mortality and morbidity, as well as developing long term health effects include adverse neurological and cognitive development, cardiovascular and endocrine disease starting from their early life until adulthood (Nardozza et al., 2017). FGR may also induce the risk of other pregnancy complications such as PTB, stillbirth, and intrapartum asphyxia (Nardozza et al., 2017). FGR can be caused by four factors which include fetal, maternal, placental and environmental factors (Bamfo & Odibo, 2011). Fetal conditions are related to aneuploidy, fetal malformations, multifetal gestation and intrauterine infection, whereas maternal factors refer to hypertension, diabetes, renal and vascular disease, thrombophilia and hypoxia (pulmonary and cardiac disease). Also, the condition of placenta (such as inadequate placentation, reduction of uteroplacental perfusion, placenta previa and placental tumours), and intrauterine environmental factors include socioeconomic status, malnutrition, smoking habit, alcohol and drugs (Nardozza et al., 2017; Bamfo & Odibo, 2011).

1.2.3.5 Preterm birth (PTB)

PTB refers to a baby that is delivered, less than 37 weeks of a completed gestation (Arora et al., 2015; WHO, 2015). PTB can be classified into three stages that are moderate preterm (baby delivered within 32 to 37 weeks), very preterm (baby delivered within 28 to

32 weeks) and extremely preterm (baby delivered less than 28 weeks) (Howson et al., 2012). PTB can occur as a result of several factors including multiple pregnancies, infection, smoking, and maternal ill-health during pregnancy (such as obesity, diabetes, hypertension and high blood pressure) (Arora et al., 2015; Howson et al., 2012). Usually, PTB can occur spontaneously, but it also may occur due to the early induction of birth because of medical or non-medical reasons (Howson et al., 2012). Babies who are delivered as PTB are vulnerable because they may have serious health complications such as respiratory problems, higher risk of infections, feeding difficulties and unregulated body temperature (WHO, 2015). PTB is amongst the main contributors to mortality rates among infants, cardiovascular, neurological and metabolic disorder of both mother and PTB children (Arora et al., 2015). In developed countries such as the UK, eight PTB cases were recorded for every 100 births, whereas in the United States (US), there were ten PTB out of 100 deliveries (CDC, 2019; NHS, 2017). PTB occurrence is higher in Sub-Saharan Africa and Southern Asia, and is more than 60% compared to developed countries (~10%) (Howson et al., 2012).

1.2.3.6 Stillbirth

Stillbirth is where a fetus is born dead at birth in the third trimester (Lawn et al., 2016). The WHO defines stillbirth as fetus born dead as beginning from after 28 weeks of gestation with a birth weight of more than 1000g (WHO, 2019c). The exact week of gestation when stillbirth begins varies across classifications and countries (range >20 gestational weeks) (Lawn et al., 2016). The ICD defines stillbirth as:

i. Fetus weighing more than 1000g and 35cm in height, born dead at 28 weeks of gestation or more;

ii. Fetus weighing more than 500g and 25cm in height, born dead at 22 weeks of gestation or more;

iii. Pregnancy loss or miscarriage before 22 weeks of gestation (Lawn et al., 2016).

Risk factors for stillbirth include (i) demographics (age more than >35 years, BMI >30), (ii) dietary intake and lifestyle (smoking, drug use), (iii) health conditions during pregnancy (such as obesity, pre-existing diabetes, hypertensive disorder, infection), (iv) complications during pregnancy (such as haemorrhage before and during labour, placental abruption, pre-eclampsia, placental dysfunction – cord prolapse, obstetric cholestasis), (v)

non-communicable disease,(vi) exposure to indoor air pollution (especially in developing countries), (vii) sex of the baby (male), (viii) post-term pregnancy and (ix) Rhesus disease (NHS, 2019a; The Lancet, 2016; Lawn et al., 2016).

1.3 Current knowledge on the relationship between environmental pollutants with placental function and pregnancy outcomes

The environment contains various types of air pollutants that contribute to adverse effects on human health, and this can include pregnancy outcomes. Maternal exposure to air pollution during the first trimester has been associated with the disruption of placental development, can lead to IUGR, increase the risk of PTB and fetal cardiovascular problems (Ritz & Wilhelm, 2010). The effect of air pollution on pregnancy outcomes at early phases of the gestational period can inhibit the normal growth of the placenta, as the first trimester is the critical phase for placental development (Ritz & Wilhelm, 2010). A literature search was performed to identify recent studies and knowledge of air pollution exposure and pregnancy outcomes, as well as to investigate further the air pollutants that exist in the indoor and outdoor environment and their relationship with pregnancy outcomes. Among the search terms that have been used for the literature search were “air pollution” AND “indoor air pollution” AND “air particle*” AND “particulate matter*” AND “indoor air particle*” AND “polycyclic aromatic hydrocarbon*” AND “PAH” AND “PM_{10}” AND “$PM_{2.5}$” AND “pregnancy outcome*” AND “birth outcome*” AND “pregnancy complication*” AND “birth weight” AND “preterm birth” AND “gestational age” AND “intrauterine growth restriction*” AND “intrauterine growth retardation*” AND “fetal growth restriction*” AND “preeclampsia” AND “stillbirth”.

1.3.1 Maternal exposure to outdoor air pollution and pregnancy outcomes

Table 1.8 shows a summary of ten studies that investigated the relationship between maternal exposures to outdoor air pollution with pregnancy outcomes. The details of previous studies had been extracted into six primary elements, which includes (i) author, year and country of the study conducted, (ii) study design and sample size, (iii) methodology for exposure assessment and pregnancy outcome; (iv) type of outdoor pollutants, (v) type of pregnancy outcome studied and (vi) main study outcome. The selected previous studies have been published from 2013 until 2019 and conducted in various countries such as the UK, US, Norway, Canada, Denmark, Poland and China. Most of

the studies had a study design of retrospective cohort (8 out of 10), and the rest were prospective cohort study (Madsen et al., 2019; Jedrychowski et al., 2017). The study with the largest sample size was conducted in Canada with a sample size of 2,928,515 (Stieb et al., 2016) and the lowest was conducted in Poland with the sample size of 455 participants (Jedrychowski et al., 2017). The assessment of maternal exposure to outdoor air pollution level during pregnancy was performed in many ways, which include the estimation of air pollution from local monitoring stations (n=3) (Rich et al., 2015; Basu et al., 2014; Lee et al., 2013), air pollution modelling (n=3) (Madsen et al., 2019; Smith et al., 2017; Pedersen et al., 2017), a combination of the monitoring station and air pollution model (n=2) (Stieb et al., 2016; Padula et al., 2014), personal monitoring (n=1) (Jedrychowski et al., 2017) and site monitoring analysis (n=1) (Pereira et al., 2014). Most of the studies (6 out of 10) referred to the birth certificate to assess the details of pregnancy outcome and demographic characteristics (Smith et al., 2017; Stieb et al., 2016; Rich et al., 2015; Padula et al., 2014; Pereira et al., 2014; Basu et al., 2014), whereas the rest of the studies (4 out of 10) used medical health records (Madsen et al., 2019; Pedersen et al., 2017; Jedrychowski et al., 2017; Lee et al., 2013). All studies investigate the effect of "criteria pollutants" (or common air pollutants) such as NO_X, nitrogen dioxide (NO_2), PM_{10}, $PM_{2.5}$ and O_3, with one study focused on the effect of $PM_{2.5}$ with PAHs (Jedrychowski et al., 2017). The pregnancy outcomes observed included pre-eclampsia, gestational hypertension, LBW, SGA and PTB.

The effect of particulate matter (PM_{10} and $PM_{2.5}$) on pregnancy outcomes had been studied in the majority of selected studies (8 out of 10). A study conducted by Basu et al. (2014) had found that maternal exposure to $PM_{2.5}$ throughout the gestational period was significantly associated with a reduction of baby birth weight, and the most significant decrease in birth weight a by exposure of $PM_{2.5}$ constituted with heavy metals. According to studies conducted by Rich et al. (2015) and Lee et al. (2013), maternal exposure to $PM_{2.5}$ in the first trimester increased the risk of preeclampsia, gestational hypertension and PTB, whereas exposure in the third trimester was significantly associated with the LBW. A study conducted in London found that maternal exposure to $PM_{2.5}$ at a mean concentration of 14 $\mu g/m^3$ throughout the gestation period was linked to term LBW (Smith et al., 2017). For PM_{10}, only a slight risk of gestational hypertension and SGA was recorded as a result of first-trimester exposure, whereas maternal exposure to PM_{10} in the second trimester and final six weeks of the gestation increased the risk of PTB (Padula et al., 2014; Lee et al., 2013).

The effect of exposure of NO_2 during pregnancy on pregnancy outcomes has been investigated by 6 out of 10 studies. The most recent study by Madsen et al. (2019) did not

find a significant association between NO_2 exposure level during gestation with pre-eclampsia or gestational hypertension, suggesting that the exposure levels of NO_2 in Norway were low, with a mean concentration of 13.6μg/m^3 compared to the annual WHO standard limit at 40μg/m^3. However, a study conducted by Pedersen et al. (2017) revealed that an increased risk of preeclampsia and gestational hypertension was observed in every increment of 10μg/m^3 NO_2 level in the first trimester. Also, a study conducted by Stieb et al. (2016) showed that maternal exposure to NO_2 was associated with SGA and birth weight reduction, in which in every increment of NO_2 exposure level by 220ppb was associated with a reduction in baby birth weight by up to 16.2g. A study conducted by Rich et al. (2015) revealed that maternal exposure to NO_2 (IQR: 13.6ppb) at the end of gestational period, specifically the 8^{th} month of gestation was associated with a significant reduction in baby birth weight.

Table 1.8: Maternal exposure to outdoor air pollution and adverse pregnancy outcomes

Author (Year) Country	Study design Sample size (n)	Methodology	Pollutants	Pregnancy outcome	Main study outcome
Madsen et al. (2019) Norway	Retrospective cohort study (n=17,533)	• Exposure assessment: Land Use Regression (Trimester specific and averages) • Pregnancy outcome: Medical record • Lifestyle: Questionnaire	NO_2	Preeclampsia (PE) Gestational hypertension	• Maternal exposure to NO_2 level [mean ($\pm$CI) = 13.6$\pm$6.9µg/m^3] was not associated with PE/ gestational hypertension. • Increased NO_2 exposure level by 10µg/m^3 was not significantly associated with PE or hypertension.
Smith et al. (2017) London, UK	Retrospective cohort study (n=540,365)	• Exposure assessment: Dispersion model (KCLurban) (Trimester averages) • Pregnancy outcome: Birth certificate (2006 – 2010)	NO_2 NO_x PM_{10} $PM_{2.5}$ O_3	LBW SGA	• Trimester averages exposures to NO_2, NO_x, $PM_{2.5}$, PM_{10} and O_3 were at the concentrations of 41, 73, 14, 23 and 32µg/m^3, respectively. • Maternal exposure to NO_2, NO_x, $PM_{2.5}$ (overall/traffic/non-traffic source) and PM_{10} increased odds of term LBW (2 to 6%) and SGA (1 to 3%).
Pedersen et al. (2017) Denmark	Retrospective cohort study (n=72,745)	• Exposure assessment: AirGIS dispersion model (Trimester specific) • Pregnancy outcome: Medical record (1996 – 2002)	NO_2	Preeclampsia (PE) Gestational hypertension	• An increase of 10µg/m^3 NO_2 level in the first trimester had significantly increased the risk of PE by 7%.
Jedrychowski et al. (2017) Krakow, Poland	Prospective cohort study (n=455)	• Exposure assessment: Personal monitoring (PEMS) (2^{nd} trimester) • Pregnancy outcome: Medical record • Demographic and other information: Questionnaire	$PM_{2.5}$ PAH	LBW	• Maternal exposure to $PM_{2.5}$ and PAH was recorded higher in the heating season (Oct-Apr = 43.4µg/m^3 and 52.9ng/m^3) compared to non-heating season (May-Sept = 27.3 µg/m^3 and 7.6ng/m^3). • Maternal exposure to PAH during pregnancy was significantly reduced birth weight and baby length doubled compared to $PM_{2.5}$ exposure. • Effect of birthweight was 10-fold stronger in PAH exposure compared to $PM_{2.5}$.
Stieb et al. (2016) Canada	Retrospective cohort study (n=2,928,515)	• Exposure assessment: Monitoring stations & NO_2 national model (Trimester averages) • Pregnancy outcome: Birth certificate (1999 – 2008)	NO_2 $PM_{2.5}$	LBW SGA	• Trimester averages exposures to NO_2 and $PM_{2.5}$ were at the concentrations of 13.4ppb and 8.5µg/m^3, respectively. • Maternal exposure to NO_2 was significantly associated with SGA and term birth weight. • A reduction of 16.2g of baby birth weight (delivered at term) in every increment of 20ppb NO_2.

Rich et al. (2015) China	Retrospective cohort study (n=83,672)	• Exposure assessment: Monitoring stations (PM_{10}, NO_2, SO_2) Real time measurement (TEOM) (Trimester specific) • Pregnancy outcome: Birth certificate (2007 – 2010)	$PM_{2.5}$ SO_2 NO_2 CO	LBW	• Higher maternal exposure to $PM_{2.5}$ (IQR: 19.8μg/m^3), CO (IQR: 0.3ppm), SO_2 (IQR: 1.8ppb) and NO_2 (13.6ppb) at the end of gestational period (month 8^{th}) was significantly associated with a reduction in birth weight by 18g (95% CI: -32g, -3g), 17g (95% CI: -28g, -6g), 23g (95% CI: -36g, -10g) and 34g (95% CI: -70g, 3g), respectively.
Padula et al. (2014) California, USA	Retrospective cohort study (n=263,204)	• Exposure assessment: Monitoring stations + IDW (Trimester specific and averages) • Pregnancy outcome: Birth certificate (2000 – 2006)	CO NO_2 PM_{10} $PM_{2.5}$	PTB	• Maternal exposure to the high level of PM_{10} and $PM_{2.5}$ during the second trimester and last 6 weeks of pregnancy were 2-fold increased the risk of PTB.
Pereira et al. (2014) Connecticut, USA	Retrospective cohort study (n=23,123)	• Exposure assessment: Gravimetric sampling (Teflon filters – 24 hours during pregnancy) (Trimester specific and averages) • Pregnancy outcome: Birth certificate (2000 – 2006)	$PM_{2.5}$	PTB	• No significant association recorded between maternal exposure to $PM_{2.5}$ (dust, motor vehicle exhaust, oil combustion and regional sulfur) with PTB.
Basu et al. (2014) California, USA	Retrospective cohort study (n=642,296)	• Exposure assessment: Monitoring stations (20km boundary) (Trimester specific and averages) • Pregnancy outcome: Birth certificate	$PM_{2.5}$ $PM_{2.5}$ constituents	LBW	• Maternal exposure to $PM_{2.5}$ and $PM_{2.5}$ constituents were significantly associated with LBW (trimester averages). • Larger reduction of birthweight was associated with the exposure to $PM_{2.5}$ constituents (vanadium, sulfur, sulfate, iron, elemental carbon, titanium, manganese, bromine, ammonium, zinc and copper).
(Lee et al., 2013) Pittsburgh, USA	Retrospective cohort study (n=34,705)	• Exposure assessment: Monitoring stations (50km boundary) + Ordinary Kriging (First trimester) • Pregnancy outcome: Medical record	PM_{10} $PM_{2.5}$ O_3	Preeclampsia (PE) Gestational hypertension PTB SGA	• Maternal exposures to $PM_{2.5}$ and O_3 during the first trimester were associated with PE, gestational hypertension and PTB. • Slightly (5 – 8%) risk increment of gestational hypertension and SGA, by exposure to PM_{10} in the first trimester. • Maternal exposure to $PM_{2.5}$ and O_3 during the first trimester had increased the risk of PE and gestational hypertension.

1.3.2 Maternal exposure to indoor air pollution and adverse pregnancy outcomes

Table 1.9 shows the summary of eight previous studies that investigate the effect of maternal exposure to indoor air pollution and adverse pregnancy outcomes. The details of previous studies had been extracted into six primary elements, similar to those explained in Section 1.3.1. The selected previous studies were published from 2013 until 2019 and conducted in various countries such as the Poland (n=2), Nigeria (n=2), US (n=1), Australia (n=1), Bangladesh (n=1) and China (n=1). Most were prospective cohort studies (6 out of 8), and other studies were cross-sectional (n=1) or a case-control study (n=1). The numbers of participants ranged between 36 – 1,761 and the largest study was conducted in the US by Ghosh et al. (2013), whereas the smallest study was conducted in Nigeria by Dutta et al. (2018). Maternal exposure to indoor air pollution during pregnancy has been assessed in many ways including a home visit (n=1) (Dutta et al., 2018), passive sampling together with a household chemical survey (n=1) (Franklin et al., 2019), active sampling (n=1) (Zhang et al., 2016) and personal sampling (n=2) (Jedrychowski et al., 2013; Choi et al., 2012). All studies used a questionnaire to obtain information about indoor air pollution exposure or additional information about home conditions except for Dutta et al. (2018). Most of the studies (5 out of 8) referred to medical records to obtain the details of pregnancy outcomes (Franklin et al., 2019; Khan et al. 2017; Zhang et al., 2016; Jedrychowski et al., 2013; Choi et al., 2012), but one study referred to birth certificates (Ghosh et al., 2013b), one study measured the outcome (fetal size) directly after birth, together with blood samples analysis (Arinola et al., 2018), and one study assessed placental cellular turnover (Dutta et al., 2018). Among the indoor air pollutants that have been studied are $PM_{2.5}$, PAH, ETS, HCHO, NO_2 and VOC. Other than indoor air pollutants, three studies assessed indoor air pollution levels based on cooking fuel (e.g. kerosene/firewood, liquefied natural gas – LNG, ethanol, etc.) or location of cooking (Arinola et al., 2018; Dutta et al., 2018; Khan et al., 2017). The pregnancy outcomes that have been investigated include gestational age, birth weight and length, head circumference, PTB, LBW, stillbirth, children mortality (neonatal, perinatal, children <5 years), fetal growth ratio, cephalization index, ponderal index, as well as markers of placental function such as Hofbauer cells, syncytial knots, chorionic vascular density and hypoxia-inducible factor.

The effect of indoor $PM_{2.5}$ on pregnancy outcomes has been investigated in two studies. A study conducted by Zhang et al. (2016) reported that $PM_{2.5}$ levels in the residential homes of coal users for cooking fuel were ~4-fold higher compared to the electric users, and the $PM_{2.5}$ levels were significantly associated with birth weight and

gestational age. Kerosene users also had a significant reduction in birth weight compared to LNG users, and the higher heavy metal (zinc, lead, mercury) levels were detected in maternal blood of kerosene users compared to LNG (Arinola et al., 2018). A study conducted by Dutta et al. (2018) found that the placenta of kerosene and firewood users had a higher level of Hofbauer cells, syncytial knots, chorionic vascular density and hypoxia-inducible factor compared to ethanol users, in which all these parameters are the markers of placental dysfunction that associated with pregnancy complications. Other than that, the effect of pregnancy outcomes and the location of cooking (indoor vs. outdoor residential home) has been investigated by Khan et al. (2017), and they found a significant association between indoor cooking with LBW. Also, the higher risk of LBW was recorded for pregnant women who used solid fuel (e.g. charcoal, grass, agricultural waste, animal dung, etc.) for indoor cooking (Khan et al., 2017).

$PM_{2.5}$ produced by cooking activity generally contains PAHs. Two studies have investigated the association of indoor PAHs on adverse pregnancy outcomes (Jedrychowski et al., 2013; Choi et al., 2012). Both studies used personal samplers to assess maternal PAH exposure during pregnancy. The studies conducted by Jedrychowski et al. (2013) reported that mothers who consumed barbequed, smoked and grilled meat (specifically, refers to the frequency of food preparation) during pregnancy had significantly associated with LBW. Also, first-trimester exposure to PAHs significantly reduced the fetal growth ratio, birth length and birth weight (Choi et al., 2012).

Two studies investigated the effect of ETS on pregnancy outcomes, but only the study conducted by Ghosh et al. (2013) reported a significant association between maternal exposure to ETS and PTB and LBW. According to Ghosh et al. (2013), poor home ventilation and chemicals released from household products contributed to the increased risk of PTB and LBW. Formaldehyde is a by-product of cigarette smoking and can also be released from household products (e.g. furniture, fragrances and etc.), and the effect of formaldehyde of pregnancy outcomes had been investigated by Franklin et al. (2019), in which they reported that maternal exposure to formaldehyde during pregnancy was significantly associated with a reduction in baby birth weight.

1.3.3 Summary of the identified studies

Findings from the previous studies demonstrate several inconsistencies in terms of the methods used for air pollution assessment, critical windows of exposure and different

pregnancy outcomes; hence a robust association between maternal exposure to outdoor and indoor air pollution with adverse pregnancy outcomes were difficult to assess. Various air pollutants exist in the indoor and outdoor environment; however, exposure to $PM_{2.5}$ and PAH is most significantly related to pregnancy complications such as preeclampsia, gestational hypertension, LBW, SGA and PTB. $PM_{2.5}$ exist in the environment in the form of very tiny particles and inhalable to the exposed individual. These particles can penetrate deep into the lung tissues, reach the capillaries, circulates in the bloodstream and translocate to the various organs in the human body including liver, spleen, kidneys, heart, brain and the placenta (Peters et al., 2006). Exposure of $PM_{2.5}$ level, even at low concentration and below the WHO standard limits, had been proved to induce the risk of pregnancy complication (Smith et al., 2017). The level of $PM_{2.5}$ was also reported to be higher in residential homes compared to the outdoor environment, due to several indoor sources that generate these particles such as cooking, smoking, heating and household equipment. The level of these particles indoors may be exacerbated by the penetration of $PM_{2.5}$ from the outdoor environment. Also, $PM_{2.5}$ can bind with various chemicals including heavy metals and PAH, which $PM_{2.5}$ – bounded heavy metals/PAH may give a synergetic effect on pregnancy outcome. In conclusion, maternal exposure to air pollution, particularly $PM_{2.5}$ in the outdoor and indoor environment has been significantly associated with adverse pregnancy outcomes.

1.3.4 Common sources of bias in these observational studies

The inconsistencies in study design and outcome measured makes it more challenging to draw firm conclusions about the associations between air pollution and pregnancy outcomes. Due to the heterogeneity across studies, there is a potential for bias in terms of air pollution assessment, types of air pollutants measured, critical windows of exposure and outcomes of interest.

i. Air pollution assessment: Several techniques were applied to determine maternal exposure to outdoor air pollution during pregnancy. Most observational studies have applied environmental monitoring technique, by referring to the nearest air pollution monitoring stations and estimation models (e.g. dispersion model, land use regression, etc.). However, most studies that observed the effects of indoor air pollution have used personal monitoring in their assessment. The variability of techniques applied may give a significant difference in air pollution exposure level,

hence may possibly bias the association with the outcome measured. The use of air pollution personal monitors potentially can control bias by indicating a "true" exposure level throughout the sampling period, but is still limited in terms of high-cost, pregnancy burden and technical difficulties (Wang & Choi, 2014).

ii. Types of air pollutants and other confounding factors: The observational studies assessed a different kind of air pollutants, and most studies concentrated on the effects of PM ($PM_{2.5}$ and PM_{10}), NO_X, NO_2 and chemicals that can be attached to the constituent particles (PAHs and heavy metals). The mechanisms of action and effects of each pollutant are varied, depending on the nature of air pollutants, administered dose and duration of exposure towards the individual (Armstrong et al., 1992). Also, studies that observed specific air pollutants may overlook the effects of other air pollutants and/or the interaction between pollutants that exist within the same environment. In addition, other confounding factors such as maternal demographic factors (e.g. age, ethnicity, BMI, socioeconomic and employment status, etc.) and lifestyles (e.g. smoking, alcohol consumption, vitamin and mineral intake, etc.) may affect pregnancy outcomes (Woodruff et al., 2009).

iii. Critical windows of exposure: Most observational studies on outdoor air pollution and pregnancy outcomes had estimated the exposure level based on trimester averages, whereas studies relating to indoor air pollution adopted personal monitoring techniques based on trimester-specific exposures. The whole pregnancy stage starting from conception until birth is the most vulnerable life stage, in which exposure to air pollution in the first trimester was related to impaired implantation and third-trimester exposure may affect fetal growth (Shah & Balkhair, 2011: Ritz & Wilhelm, 2010). However, to measure maternal exposure level to air pollution throughout the entire pregnancy period is complicated due to technical challenges and cost burden (Wang & Choi, 2014). Hence, the use of air pollution estimation models to estimate the exposure level of air pollution during the whole gestational period seems to be the most efficient technique in this field (Wang & Choi, 2014).

iv. The outcomes of interest: The majority of observational studies have investigated the effect of maternal exposure to air pollution with standardized adverse pregnancy outcomes such as pre-eclampsia, LBW, PTB and SGA. However, several studies had also assessed other birth parameters such as gestational age, birth weight, birth length, head and chest circumference, cephalization and ponderal index, as well as markers of placental dysfunction. The used of these parameters, for example, birth

weight, may potentially cause bias as it can be related with fetal growth restriction or pre-term birth, or maybe a combination of both outcomes (Woodruff et al., 2009). The outcome of birth weight is not specific in this study, as the distribution of birth weight varies between ethnicity and baby gender, hence difficult to determine either it is caused by exposure to air pollution or resulted from other confounding factors (Woodruff et al., 2009).

Table 1.9: Maternal exposure to indoor air pollution and adverse pregnancy outcomes

Author (Year) Country	Study design Sample size (n)	Methodology	Pollutants	Pregnancy outcome	Main study outcome
Franklin et al. (2019) Australia	Prospective cohort study (n=262)	• Exposure assessment: Passive sampling for 7 days (Third trimester) Household chemical survey Questionnaire • Pregnancy outcome: Birth record	HCHO NO_2 VOC	• Gestational age • Birth weight and length • Head circumference	• All the indoor air pollutants studied were not associated with studied birth outcomes, except for HCHO. • Maternal exposure to indoor HCHO (median: 2.81µg/m^3) was significantly reduced baby birth weight.
Arinola et al. (2018) Nigeria	Cross-sectional study (n=68)	• Exposure assessment: Questionnaire • Pregnancy outcome: Direct measurement after birth Blood analysis for heavy metal levels	Indoor air pollution (kerosene & LNG)	• Gestational age • Birth weight • Chest and head circumference	• No difference in gestational age, head and chest circumference and chest to head length between kerosene and LNG users. • Significant reduction of baby birth weight of kerosene users compared to LNG.
Dutta et al. (2018) Nigeria	Prospective cohort study (n=36)	• Exposure assessment: Home visit • Sample analysis: H&E and IHC	Indoor air pollution (kerosene/firewood & ethanol)	• Hofbauer cells • Syncytial knots • Chorionic vascular density • Hypoxia-inducible factor	• The higher level of all studied placental function markers (Hofbauer cells, syncytial knots, chorionic vascular density and hypoxia-inducible factor) in placental samples of kerosene/firewood users compared to ethanol users.
Khan et al. (2017) Bangladesh	Prospective cohort study (n=22,789)	• Exposure assessment: Interview by questionnaire • Pregnancy outcome: Medical record	Indoor air pollution (cooking place – inside vs outside; *cooking fuel – clean fuel vs solid fuel; place of solid fuel use – indoor vs outdoor)	• Stillbirth • LBW • Neonatal, perinatal and children (<5 years) mortality	• Significant higher risk recorded between indoor cooking and neonatal and infant mortality. • Indoor cooking was significantly associated with LBW. • Significant higher risk of LBW for those who used solid fuel for indoor cooking.
Zhang et al. (2016) China	Prospective cohort study (n=95)	• Exposure assessment: Active sampling (20 weeks onwards) Questionnaire • Pregnancy outcome: Medical	$PM_{2.5}$ and heavy metal compositions	Gestational age Birth weight	• Higher indoor $PM_{2.5}$ levels in the residential home of coal users [median (IQR): 191.40 (51.70) µg/m^3] compared to gas [median (IQR): 71.98 (72.14)] and electric [median (IQR): 55.71 (65.94)] users. • Concentrations of heavy metals during heating (H)

		record			and non-heating (NH) season significantly associated with: - As & Cd (H) negatively associated with birth weight and gestational age. - U (H) positively associated with birth weight - Ba & Tl (NH) positive associated birth weight - Fe (NH) positive associated birth weight and gestational age
Ghosh et al. (2013) Los Angeles, California	Case-control survey (n=1,761)	• Exposure assessment: Questionnaire • Pregnancy outcome: Birth certificates and health survey by phone, mail and in person.	Indoor air pollution (SHS) Home condition (ventilation)	LBW PTB	• Poor ventilation systems at home, exposure to second-hand smoke (SHS) and pollutants released from household products increased the risk of PTB and LBW.
Jedrychowski et al. (2013) Krakow, Poland	Prospective cohort study (n=432)	• Exposure assessment: Questionnaire PAH personal sampling PEMS (48-hour) (2nd trimester) • Pregnancy outcome: Medical record Sample analysis: Cotinine level in cord blood	PAH ETS	Birth weight Birth length Head circumference	• A significant positive association between airborne PAH and cord blood adducts. • A significant reduction of birth weight was associated with maternal barbecued meat consumption during pregnancy (specifically with the frequency in preparing the food).
Choi et al. (2012) Krakow, Poland	Prospective cohort study (n=344 personal monitoring; n=76 indoor monitoring; n=70 outdoor monitoring)	• Exposure assessment: Questionnaire PAH personal sampling (48-hour) (Specific trimester) • Pregnancy outcome: Medical record	PAH $PM_{2.5}$	Fetal growth ratio (FGR, %) Cephalization Index (CI, cm/g) Ponderal Index (PI, g/cm^3)	• Maternal exposure on 8 summed PAHs during 1st trimester was associated with a significant reduction of FGR ratio, birth weight and birth length compared to other trimesters exposure.

*Cooking fuel refers to clean fuel (e.g. electricity, LNG, natural gas and biogas) and solid fuel (e.g. coal, lignite, charcoal, wood, grass, agricultural crops, animal dung and etc.) (Khan et al., 2017).

1.4 Mechanistic basis of the observed association

Maternal exposure to air pollution during pregnancy had been associated with adverse pregnancy outcome, but the biological mechanism underlying this relationship remains to be elucidated, with some emerging research suggest that this mainly occurred due to oxidative stress, placental inflammation and modification of placental endocrine function. Erickson & Arbour (2014) proposed that the repeated exposure to air particles, particularly UFP or $PM_{2.5}$ may induce oxidative stress and placental inflammation that promotes defective deep placentation. As a result, normal perfusion of oxygen and nutrient to the fetus will be reduced, and this condition may contribute to the risk of pregnancy complications. Several experimental studies have documented the evidence of air pollution and adverse pregnancy outcomes, through a mechanism of oxidative stress and placental inflammation (Familari et al., 2019; Grevendonk et al., 2016; de Melo et al., 2015; Weldy et al., 2014; Sbrana et al., 2011)**.** Also, there have been several experimental studies that investigate this association through modification of placental endocrine function (Familari et al., 2019; Kim et al., 2017; Wang et al., 2017; Mørck et al., 2010). Figure 1.5 illustrates the potential biological mechanisms that underlie the relationship between air particles exposure during pregnancy and the effect on pregnancy outcomes.

.

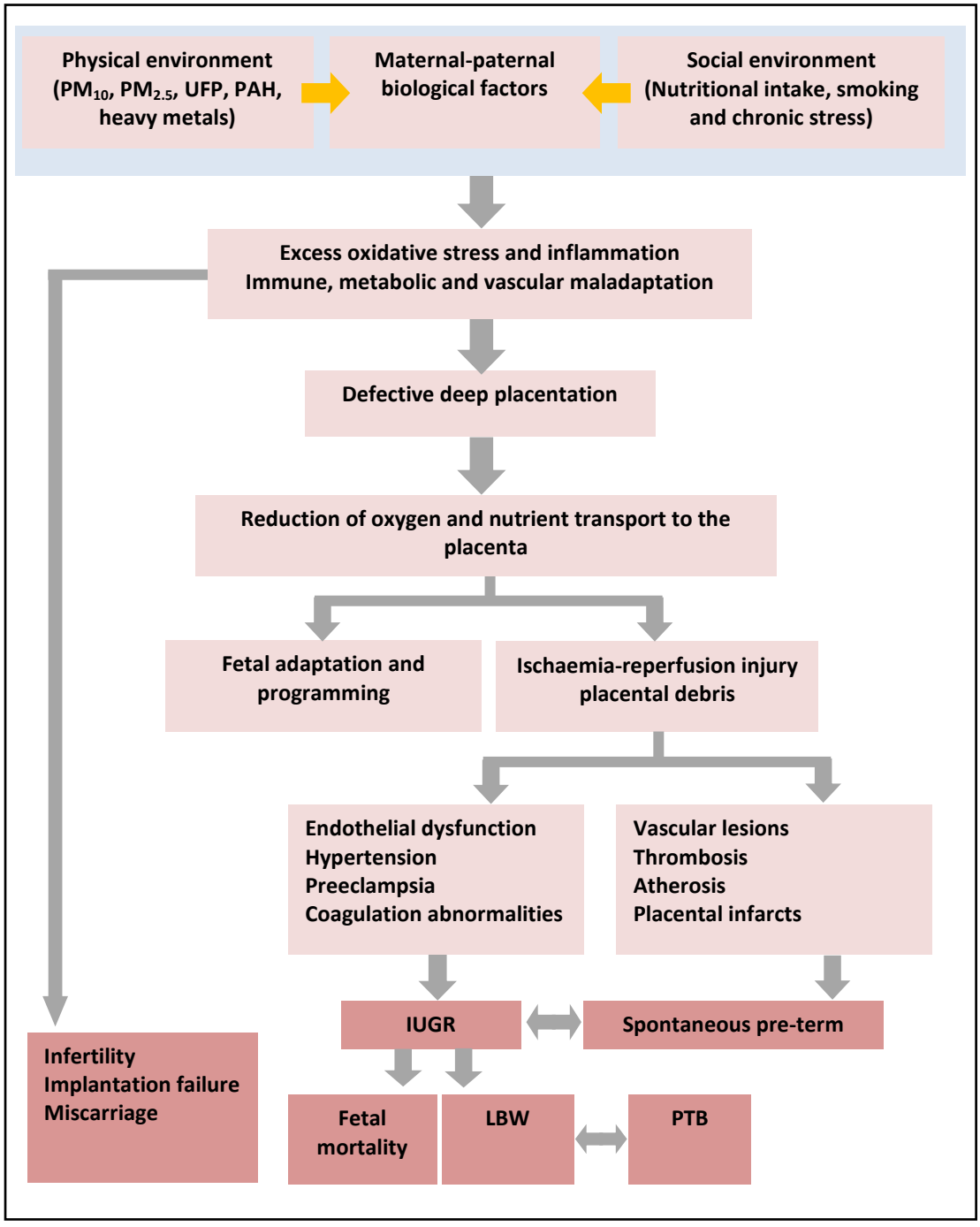


Figure 1.5: The potential biological mechanism involved in the exposure of environmental air pollutants and adverse pregnancy outcomes (Adapted from Erickson and Arbour, 2014).

Maternal exposure to air pollution during pregnancy, particularly in the early phase of gestation may induce the risk of adverse pregnancy outcomes. The risk may be exacerbated due to social environment factors such as smoking or second-hand smoker, nutritional intake and chronic stress. The cumulative exposure to air pollutants, particularly air particles bound with PAH or heavy metals may induce oxidative stress and placental inflammation. This condition leads to placental deep placentation and reduces the perfusion of oxygen and nutrient to the fetus which can result in pregnancy complications.

1.4.1 Oxidative stress

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) and the level of antioxidant species in the human body (Wu et al., 2015; Durackova, 2010; Myatt & Cui, 2004). ROS can be produced endogenously (intracellular) or exogenously (environmental factors) (Robert et al., 2015). The most common free radical is the superoxide anion radical (O2*-), which dismutates to form hydrogen peroxide (H_2O_2) and further react to form the hydroxyl radical (HO*) (Cadenas & Davies, 2000). The hydroxyl radical can react with deoxyguanosine to form 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a marker of oxidative DNA damage (Cadenas & Davies, 2000).

In pregnancy, the ROS level may vary at a certain gestation period due to the placental development (e.g. for the regulation of gene transcription, trophoblast proliferation, invasion and angiogenesis), but the overwhelming of ROS production may also be indicated with IUGR and pre-eclampsia (Wu et al., 2015; Myatt & Cui, 2004). Environmental pollutants are among the exogenous sources of free radicals that can induce cellular damage by adversely acting on proteins, lipids and DNA (Lobo et al., 2009; Myatt & Cui, 2004). Maternal exposure to environmental pollutants during pregnancy increases the level of free radicals inside the body; hence the imbalance between free radicals production and antioxidant resulted in oxidative stress. Excessive production of ROS from placental mitochondria can inhibit cellular metabolism resulting in placental endocrine dysfunction and increased trophoblast apoptosis (Wu et al., 2015).

The association between air particles exposure and markers of oxidative DNA damage had been investigated in many previous studies (Grevendonk et al., 2016; Vattanasit et al., 2014; Janssen et al., 2012; Sbrana et al., 2011). A study conducted by Grevendonk et al. (2016) found increased mitochondrial 8-OHdG levels in maternal blood following exposure to PM_{10} and $PM_{2.5}$ throughout the entire pregnancy. Increased levels of mitochondrial 8-OHdG were also recorded in cord blood following maternal exposure to PM_{10} in the first and second trimester (Grevendonk et al., 2016). $PM_{2.5}$ containing PAH can be emitted from diesel exhaust particles (DEP), and a study conducted by Vattanasit et al. (2014) found a significant positive correlation between 51 individual that were exposed to traffic particles and living within 500m from main roads in Bangkok with the level of 8-OHdG in blood. Also, an *in-vitro* study of human lymphoblasts treated with DEP confirms that 8-OHdG levels increased upon increasing the DEP concentration (Vattanasit et al., 2014). $PM_{2.5}$ containing PAHs also can be emitted from cigarette smoke, and a previous

study found that the formation of 8-OHdG was significantly higher in the placenta of smoker mothers compared to non-smokers (Sbrana et al., 2011).

1.4.2 Placental inflammation

Placental oxidative stress has been shown to mediate the placental inflammatory response, as increased placental oxidative stress can induce the production of pro-inflammatory cytokines (Tenório et al., 2019). Cytokines are small secreted protein, produced by various cells in the human body, and in particular by helper T cells and macrophages during phagocytosis (Zhang & An, 2009). The interleukin-6 (IL-6) is known as a multifunctional pro-inflammatory cytokine that can be produced by various cells, particularly at sites of the tissue inflammation (Yu et al., 2002). In the placenta, inflammation can occur at several sites such as the chorion, decidua, placental villi and fetal vessel, and this can be caused by the microorganism or by the host immune response to non-replicating antigens (Taleban et al., 2015; Redline, 2004). Increasing levels of proinflammatory cytokines such as TNF-α and IL-6 may reduce the formation of anti-inflammatory cytokines such as IL-10, and this imbalance can lead to cell damage, transplacental infection and further pregnancy complications such as pre-eclampsia (Harmon et al., 2016; Redline, 2004).

Many studies have addressed the association between air pollution exposure and the production of pro- and anti-inflammatory cytokines (Familari et al., 2019; de Melo et al., 2015; Vattanasit et al., 2014; Hougaard et al., 2008). A recent study by Familari et al. (2019) reported that exposure of trophoblast cells to urban pollution particles for 48-hours *in-vitro* significantly increased the level of IL-6 in the culture medium. In this study, trophoblast cells were exposed to particles collected from the street in Malmö to represent maternal exposure to traffic particles, as well as treatment with SRM2786 (particles collected in an urban environment), to mimic the maternal exposure to general air pollution (Familari et al., 2019). Another *in-vitro* study treated human lymphoblasts (RPMI 1788) with SRM2975 (DEP) and also demonstrated increased IL-6 and IL-8 expression in a dose-dependent manner (25 – 100µg/mL DEP) (Vattanasit et al., 2014). Furthermore, a significantly increased level of IL-6 in mice placenta following exposure to DEP has been demonstrated in an *in-vivo* study conducted by Fujimoto et al. (2005). This study exposed a group of mice to clean air in a chamber (as control), and another three groups of mice were exposed to DEP at different concentrations of 0.3, 1.0 and 3.0mg DEP/m^3, respectively, and

the highest concentrations of DEP was significantly increased IL-6 levels up to 10-fold compared to control group (Fujimoto et al., 2005). A significant increase in the anti-inflammatory cytokines IL-4 was recorded following *in-vivo* exposure of mice to particulate air pollution before and during pregnancy (de Melo et al., 2015). An increase in anti-inflammatory cytokines in the placenta of treated mice may indicate the response of placenta to overcome the inflammatory process induced by air pollution exposure, as well as proved the ability of air particles to reach the placental barrier and maternal-fetal circulation (de Melo et al., 2015).

1.4.3 Placental endocrine function

Placental endocrine function refers to the synthesis of hormones or other mediators by the placenta during gestation, which helps to maintain placental function and fetal development (Costa, 2016). Human chorionic gonadotrophin (hCG) is the most predominant pregnancy-related hormone and is mainly secreted by the differentiated syncytiotrophoblast layer of the human placenta, into the maternal circulation starting from embryo implantation until the end of pregnancy (Paulesu et al., 2018; Barjaktarovic et al., 2017; Costa, 2016). hCG is a heterodimeric glycoprotein consisting of an alpha (hCGα) and a beta subunit (hCGβ), and whilst hCGα is constitutively expressed, hCGβ is only secreted by the differentiated syncytiotrophoblast during pregnancy (Paulesu et al., 2018; Costa, 2016; Williams, 2008). hCG secretion varies throughout gestation, with peak levels recorded in the first trimester (~12 weeks gestation) and reduced levels as gestation progress (Barjaktarovic et al., 2017; Norris et al., 2011). However, changes in maternal blood hCG levels may also indicate placental dysfunction and pregnancy complications such as miscarriages, preeclampsia, FGR and gestational trophoblast disease (Paulesu et al., 2018; Costa, 2016). The production and secretion of hCG have been used as a marker of differentiation trophoblast, placental function and diagnosis of pregnancy-related disease (Costa, 2016; Williams 2008).

Maternal exposure to air pollution during pregnancy has been linked to the modification of placental endocrine function, particularly the effect of endocrine-disrupting chemicals (EDC) on hCG secretion (Paulesu et al., 2018). The WHO defines EDC as an exogenous substance or mixture that affect the function of the endocrine system, which results in adverse health outcomes to the exposed organism and population (Bergman et al., 2012). Familari et al. (2019) reported that first-trimester trophoblast cells treated with

urban air pollution (traffic particles and SRM2786) had significantly reduced hCGβ levels after 48-hour exposure *in-vitro*. This study exposed the trophoblast cell to air pollution with a concentration that mimics the mean daily exposure of $PM_{2.5}$ in Malmö, Sweden (average daily means for ambient $PM_{2.5}$: 20-30μg/m^3; 50 – 500ng $PM_{2.5}$ per day: 0.5 – 5000ng/mL stock concentration). The "realistic exposure rate" was calculated to be similar to the exposure level of $PM_{2.5}$ in the local area, as this study postulated that maternal exposure to ambient air pollution in Malmö, Sweden had contributed to PTB and LBW cases (Familari et al., 2019).

A study conducted by Kim et al. (2017) investigated the effect of ETS on the secretion of hCGβ by JEG-3 human placental cancer cells and reported that JEG-3 cells treated *in-vitro* for seven days had significantly reduced hCG expression in a time and concentration-dependent manner (Kim et al., 2017). Another *in-vitro* study of JEG-3 cells treated with environmental pollutants was conducted by Wang et al. (2017), and this study reported hCG levels increased in a time and concentration-dependent manner after exposure to $PM_{2.5}$ collected from the industrial area. The secretion of hCG by cells treated with 5μg/mL $PM_{2.5}$ for 24-hour was 2-fold higher compared to controls, whereas an incubation time of 48-hours increased hCG levels up to 3-fold, compared to the control group (Wang et al., 2017). The increase in hCG levels might possibly be due to the action of PAH compounds that are bound to air particles sampled from the industrial area; 16 PAHs (mean concentration: 119.8ng/m^3) and three nitro-PAHs (mean concentration: 0.5ng/m^3) were detected in the particles that were used in this study (Wang et al., 2017; Li et al., 2014). A summary of the previous studies that investigated the biological mechanism underlying the relationship between air pollution and adverse pregnancy outcomes is shown in Table 1.10, whereas Table 1.11 summarized previous studies that investigate the ability of air pollutants reach the human placental samples and study that observe adverse pregnancy outcomes following *in-vivo* exposure of animal models to environmental pollutants and pregnancy outcomes following exposure to air pollutants.

Table 1.10: Previous studies that explain the biological mechanism underlies the relationship between air pollution exposure and adverse pregnancy outcome

Exposure/chemical	Model	Biomarkers of exposure	Mechanism observed	Reference
Urban air pollution (SRM2786 and street particles)	*In-vitro* exposure; placental trophoblast cell	↑ IL-6 in a culture medium	Placental inflammation	Familari et al. (2019)
		↓ hCGβ secretion	Endocrine function	
ETS ($PM_{2.5}$ and PAH)	*In-vitro* exposure; JEG-3 human placental cancer cells	↓ hCG expression	Endocrine function	Kim et al. (2017)
$PM_{2.5}$ and PAH	*In-vitro* exposure; JEG-3 human placental cancer cells	↑ hCG secretion	Endocrine function	Wang et al. (2017)
PM_{10} and $PM_{2.5}$	Ambient air pollution level within residential areas; analysis of maternal and cord blood	↑ mitochondrial 8-OHdG in maternal blood/cord blood	Oxidative DNA damage	Grevendonk et al. (2016)
$PM_{2.5}$	*In-vivo* exposure; mice model; placenta	↑ anti-inflammatory cytokines of IL-4	Placental inflammation	de Melo et al. (2015)
DEP ($PM_{2.5}$ and PAH)	*In-vivo* exposure; mice model; placenta	↑ CD45 positive cells	Placental inflammation	Weldy et al. (2014)
ETS ($PM_{2.5}$ and PAH)	Smokers and non-smokers; analysis of placental tissue by immunohistochemistry	↑ 8-OHdG in placenta samples of smokers	Oxidative DNA damage	Sbrana et al. (2011)
Bisphenol A (BPA)	*In-vitro* exposure; BeWO trophoblast cell line	↑ hCGβ secretion	Endocrine function	Mørck et al. (2010)

Table 1.11: Previous studies that investigate the ability of air pollutants reach the human placental samples and study that observe adverse pregnancy outcomes following *in-vivo* exposure of animal models to environmental pollutants

Exposure/chemical	Model	Main observation/ Pregnancy outcomes	Reference
Black carbon (BC)	Ambient air pollution level within residential areas; analysis of human placental tissue	Presence of BC in human placental samples at both the maternal and fetal side. BC load in placental samples was positively associated with BC exposure level throughout pregnancy.	Bové et al. (2019)
DEP ($PM_{2.5}$ and PAH)	*In-vivo* exposure; rabbit model; placenta	↓ Head length and umbilical pulse, ↓ placental inefficiency, ↓ placental blood flow and ↓fetal vessel volume. Nanoparticles detected in maternal blood, trophoblastic cells and fetal blood.	Valentino et al. (2016)
DEP ($PM_{2.5}$ and PAH)	*In-vivo* exposure; mice model; placenta	↑ embryo resorption, ↑ placental haemorrhage, ↑ focal necrosis.	Weldy et al. (2014)
Particulate matter	Placental perfusion model; human placenta	Placental transport: Particles with diameter up to 240nm can be taken up by the placenta, able to cross the placental barrier and not affect the viability of placental explants.	Wick et al. (2010)
DEP	*In-vivo* exposure; mice model	↑ Abnormal delivery and ↓ bodyweight of the exposed group compared to control.	Tsukue & Dep (2002)

1.5 Knowledge gaps

Understanding the complexity of air pollutant's components is vitally important to determine the potential biological mechanism that leads to adverse pregnancy outcomes. A growing body of evidence has revealed that air pollution can affect oxidative stress, placental inflammation and endocrine function. Attention has been given to the effect of $PM_{2.5}$ bound PAHs, as this particle is widely generated from many sources, including the outdoor and indoor environment. Maternal exposure to particles during pregnancy can affect placental function, as these particles have been found accumulated in human placental samples (Bové et al., 2019; Wick et al., 2010). $PM_{2.5}$ bound PAH is the main components in diesel exhaust particles (DEP) and has been proved to disrupt the endocrine function (Takeda et al., 2004). For the indoor environment, activities such as smoking, cooking and residential heating are known as sources of indoor PAH, and the analysis of house dust can be used to assess indoor PAHs exposure (Whitehead et al., 2011). Level of $PM_{2.5}$ bound PAH in the indoor environment may also increase due to the penetration of particles from the outdoor environment, especially if the residence is near to busy roads. The effect of indoor $PM_{2.5}$ and PAH on pregnancy outcomes need to be further investigated, as pregnant women tend to spend more time indoors rather than outdoors, especially towards the end of pregnancy (Nethery et al., 2009).

Many previous studies have focused on *in-vivo* experiments using mice and rabbit models to investigate the effect of environmental pollutants on placental function and pregnancy outcomes, but these investigations cannot be conducted in human due to obvious ethical constraints. Direct exposure of DEP to rabbits significantly altered placental function, by a reduction in placental efficiency (fetal/placental weight), placental blood flow and fetal vessel volume (Valentino et al., 2016). Also, *in-utero* exposure of pregnant mice to DEP induced the embryo resorption, placental haemorrhage, focal necrosis, cell inflammation and oxidative stress (Weldy et al., 2014). However, the effects of air pollution on animal models may be different from effects on human placental physiology, and may not give the best indication of the effects of environmental pollutants on the human placenta or pregnancy outcomes. To our knowledge, only one recent study that has used human trophoblast cells treated with urban air pollution *in-vitro* have been conducted, to observe the effect of pregnancy outcomes through a mechanism of placental inflammation and endocrine function (Familari et al., 2019). Future research is required to extend an *in-vitro* study by using human trophoblast cells or placental villous explants treated with ubiquitous environmental pollutants, as this may give the best indication of human

placental development, function and physiological processes following exposure to environmental pollutants (Mannelli et al., 2015).

1.6 Aim and objectives of the thesis

1.6.1 Hypothesis

This study tested the hypothesis that maternal exposure to air pollution during pregnancy can cause placental dysfunction and hence adverse pregnancy outcomes.

1.6.2 Overall aim

The primary aim of this study is to explore possible routes of environmental exposure and the potential mechanism of the placental dysfunction caused adverse pregnancy outcomes.

Specifically, the objectives of this study were:

i. To determine the concentration of heavy metal and PAH in the biological samples following maternal exposure to ambient air pollution throughout the gestation period.

ii. To examine the feasibility of measuring the level of indoor air particles in the residential homes of pregnant women across Greater Manchester.

iii. To identify the primary sources that contribute to the elevation of indoor air particles in the residential homes across Greater Manchester.

iv. To adapt a well-established *in-vitro* model of placental explant culture to develop a novel model of placental air pollution exposure.

v. To investigate the effect on placental endocrine function and cellular turnover of placental villous explants treated with diesel exhaust particle and house dust *in-vitro*.

1.7 Thesis structure

This thesis is comprised of five individual chapters, of which three present the results of work with separate aims and objectives. Figure 1.6 presents a brief description of each chapter.

CHAPTER 1: **Introduction**	• This chapter describes the theory and background knowledge of the study. • The knowledge gaps were presented to indicate the relevance of this study. • Aim and objectives for the whole thesis.
CHAPTER 2: **The concentration of heavy metals and PAHs in human placenta and maternal blood**	• This chapter describes the analysis of the concentration of heavy metal and PAH in biological samples (maternal blood and placenta). • Estimation of outdoor air particles (PM_{10} and $PM_{2.5}$) exposure level throughout pregnancy.
CHAPTER 3: **Measurement of indoor air particles in the residential homes of pregnant women across Greater Manchester**	• This chapter describes the feasibility of measuring indoor air particles inside the residential homes of pregnant women across Greater Manchester. • Identification of the main sources of indoor air particles level inside home. • Collection of biological samples for future study to determine the biomarkers of indoor air exposure.
CHAPTER 4: **The modification of hCG secretion and placental cellular turnover by cultured placental explants exposed to diesel exhaust particles and house dust**	• This chapter describes an *in-vitro* model of human placental villous explants treated with environmental pollutants. • Explants treated with DEP and house dust to mimic maternal exposure to $PM_{2.5}$ and PAH from the outdoor and indoor environment. • Analysis of hCG secretion level to determine the modification of placental endocrine function following treatment. • Analysis of placental cellular turnover based on immunohistochemistry for the identification of possible markers that induced placental dysfunction.
CHAPTER 5: **Overall discussion and conclusion**	• This chapter describes the overall discussion of thesis, strengths, limitations and recommendation for future work.
References and appendices	• List of references and appendixes

Figure 1.6: Schematic of thesis structure

CHAPTER 2:

THE CONCENTRATION OF HEAVY METALS AND POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN PLACENTA AND MATERNAL BLOOD

2.1 Introduction

Air particles (or particulate matter) in the atmosphere have a strong ability to absorb hazardous pollutants such as heavy metals and PAHs which can contribute to air particles toxicity (Li et al., 2013). These particles formed in very small size and able to cross the placental barrier and accumulate in fetal tissues (Gundacker & Hengstschläger, 2012; Sawicka-Kapusta et al., 2007). Maternal exposure to air particles bound with PAH or heavy metals has globally been reported to cause adverse pregnancy outcomes such as pre-eclampsia, IUGR and PTB (Jedrychowski et al., 2017; Zhang et al., 2016; Rich et al., 2015; Padula et al., 2014; Basu et al., 2014; Lee et al., 2013; Choi et al., 2012). These particles can be exposed to individuals from many sources through environmental (outdoor and indoor sources), occupational exposures, as well as dietary intake (Amaya et al., 2013; ATSDR, 2012). According to Dermentzoglou et al. (2003), carcinogenic PAHs in the indoor environment can be emitted from smoking and incomplete combustion during food preparation. Air particles containing heavy metals such as copper (Cu) and zinc (Zn) were found at higher levels in the indoor environment compared to outdoors, whereas levels of cadmium (Cd), lead (Pb) and PAH compounds significantly higher in particles outdoors. Pregnant women were reportedly spent more time indoors, specifically at home, towards the end of the pregnancy gestation; hence the determination of the air pollutants sources is important to minimize the exposure (Nethery et al., 2009). In addition, Ritz & Wilhelm (2010) reported that maternal exposure to air particles at the end of gestation (~20-36 weeks) was associated with pregnancy complications of PTB and LBW.

The purpose of this study was to determine heavy metal and PAH concentrations in human placentas and pre-delivery maternal blood. Differences in the concentration of heavy metals and/or PAH levels between placentas and maternal blood may indicate the functional ability of the placenta to act as a protective barrier for the fetus (Sakamoto et al., 2013). The association between maternal exposure to ambient particles ($PM_{2.5}$ and PM_{10}) during pregnancy with heavy metal and PAH levels in the biological samples, may best describe the biomarkers of air pollution exposure during pregnancy, as well as can investigate the ability of these compounds transported and accumulated in the placenta and maternal blood.

2.1.1 Hypothesis

This study tested the hypothesis that maternal exposure to ambient air pollution during pregnancy leads to the presence of heavy metals and PAHs in the placenta and maternal blood, and concentrations reflect exposure to air pollution.

2.1.2 Aim and objectives

This study aimed to investigate the effect of maternal exposure to ambient air particles (PM_{10} and $PM_{2.5}$) during pregnancy on the levels of heavy metals and PAHs in placentas and maternal blood.

Specifically, the objectives were:

i. To determine the concentration of heavy metals in human placenta samples and maternal pre-delivery blood.

ii. To determine the concentration of PAHs in human placental samples.

iii. To estimate maternal exposure to ambient PM_{10} and $PM_{2.5}$ during pregnancy by using the automatic air pollution monitoring stations.

iv. To determine the correlation between heavy metal and PAH levels in placenta and maternal blood with maternal exposure to ambient PM_{10} and $PM_{2.5}$ throughout pregnancy.

2.2 Methodology

2.2.1 Study design, participants and recruitment

This study had a cross-sectional design. Biological samples were collected before (maternal blood) and after delivery (placenta and cord blood) from mothers of term pregnancy and had given birth via elective caesarean section (ELCS) in St. Mary's Hospital, from June 2015 until June 2017. Ethical approval was obtained from the North West-Haydock Research Ethics Committee, reference number 08/ H1010/ 55 (+5).

Demographic information, maternal new-born characteristics were collected before and after delivery by a research practitioner. The demographic and maternal information had obtained before the delivery included residential postcode, maternal age, height, weight, gestational age, parity, gravidity, ethnicity and smoking status. The information on

the new-born was acquired after delivery and this included information on birth weight, gender and individualised birth weight ratio (IBR). The IBR percentile was calculated by using the GROW Centile Calculator (https://icc.growservice.org/754731/), taking into account information on maternal height and weight, ethnic origin, parity, gestational age, sex and birth-weight of the new-born. After delivery, their residential postcode was confirmed to make-sure only those living in Greater Manchester for the entire pregnancy period were included in this study.

2.2.2 Collection of biological samples

The collection of biological samples was started by an online sample request in the Medical and Human Sciences (MHS) Biobank diary. This is an online shared calendar among researchers on the Research Floor, St. Mary's Hospital Manchester and allows them to request the biological sample that they need for their subsequent study. The availability of biological samples was updated routinely by research practitioners to the researchers who requested the samples. One of the researchers acted as a bleep holder and received a notification to collect the samples from the labour operating theatre and brought them to the Tissue Handling Laboratory on the Research Floor, St. Mary's Hospital. The researchers who requested the samples were notified, and samples collected immediately. The patient's identification was anonymised by replacing identifiable material with a code number.

In total, 42 placentas and 30 pre-delivery maternal bloods were collected from 53 pregnant women in term pregnancy (37 to 42 weeks). Out of these samples, 19 of placentas and maternal blood samples were received from the same mother before (maternal blood) and after (placenta) delivery, which referred to paired samples.

2.2.2.1 Collection of blood samples

Approximately, 10mL of each blood sample was collected by the research practitioner before and after delivery and stored at -80^{0}C until further analysis. However, the amount of cord blood collected in this study was small (<10mL) and not enough for the subsequent analysis. Only placenta and pre-delivery maternal blood were analysed for heavy metal and PAH concentration.

2.2.2.2 Collection of placental samples

Placentas were sampled following standard operating procedures (SOP) applied for tissue bank collection, and either collected intact (whole-placenta) or aliquoted. Placentas were initially collected as aliquots (~6g) of the whole placenta, but pilot studies indicated that this amount was not sufficient to detect PAHs. Due to this, whole placental samples (~20g) were collected. For intact samples, placentas were stored immediately in the freezer (-80^{0}C) after delivery with no cleaning or other process involved. For aliquoted samples, the placental region was first identified, and the villus tissues were taken from placenta regions identified as a cord (C), middle (M) and edge (E) as shown in Figure 2.1. The villous tissues were excised from the maternal side, as identified through its physical appearance and the lack of umbilical cord. The villus tissue was excised in the form of small chunks each approximately 2g in weight. Samples were washed by using phosphate-buffered saline, PBS (Thermo Fisher Scientific Inc.) at pH 7.4 to remove the blood, kept in Eppendorf tubes, labelled and stored at -80^{0}C.

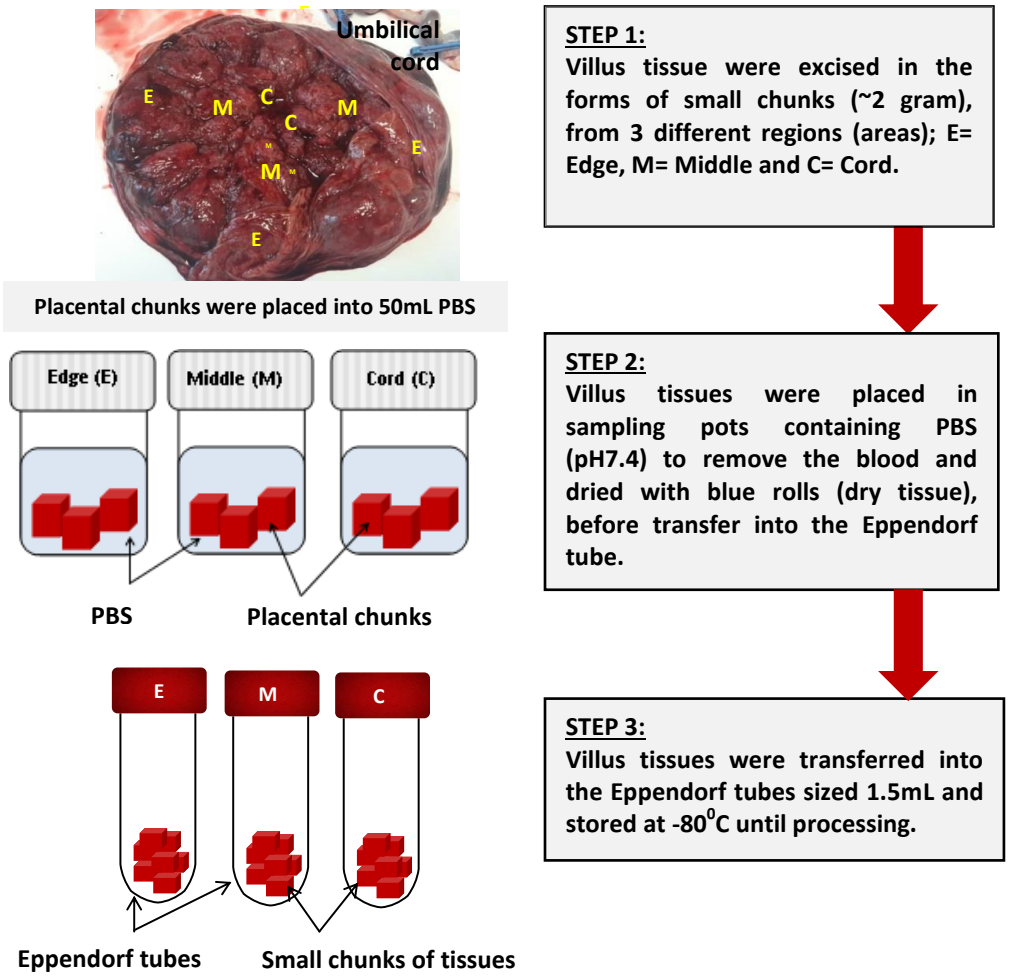


Figure 2.1 Procedure for placenta collection and storage

2.2.3 Biomarker analysis

Analysis of heavy metals and PAHs in the biological samples was conducted by the Food and Environment Research Agency (FERA) Science Limited, York, United Kingdom by Dr Michael Dickinson.

2.2.3.1 Heavy metal levels determined by Inductive Coupled Mass Spectrometer (ICP-MS) analysis

The analysis of heavy metals in placental samples (n=30) was conducted in January 2017 whereas, for pre-delivery maternal blood (n=30), the samples were analysed in August 2017. Both samples underwent a similar analytical process for heavy metal analysis.

Deionised water (18.2 MΩ cm), metal analysis grade reagents and acid-cleaned plastic ware were used throughout the analysis process. Approximately, 1g of placenta aliquots and 0.25g of maternal blood aliquots were weighed and placed into digestion vessels. A mixture of nitric acid and hydrochloric acid (4:1) was then added, the digestion vessels capped and the contents were digested under high temperature and pressure by using a single reaction chamber microwave digester system. Blank reagents and certified reference materials (NIST, Maryland, USA) were measured through the analysis. The resulting solutions were then transferred to labelled acid-cleaned plastic test tubes and diluted to 10mL with deionized water. The digest solutions and a set of standards covering the expected heavy metal concentration range were then internally standardised with rhodium in dilute nitric acid (1% v/v). The multi-element measurements were made using an Agilent 7700x ICP-MS (Santa Clara, CA, USA) with a collision cell. This analysis method was ISO17025 accredited. In total, 15 elements were measured, including heavy metals and nutritional elements, with the limits of detection (LOD) between 0.001 to 500mg/kg. There were seven heavy metals including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni) and lead (Pb) and the rest were nutritional elements which included iron (Fe), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphate (P), selenium (Se) and zinc (Zn).

2.2.3.2 PAH levels determined by gas chromatography with mass spectrometric (GC-MS) analysis

The analysis of PAHs in human placenta samples was conducted into two stages; that used either placental aliquots (n=10) or whole placenta samples (n=12). These groups differed by the amount of tissue used for analysis, which was 6g from the placental aliquots and 15 to 20g from the whole placental samples.

Samples were spiked with ^{13}C labelled internal standards and saponified with 200mL methanolic potassium hydroxide and extracted with 100mL cyclohexane. The crude extracts were concentrated down to 50mL and purified by partitioning into 50mL dimethylformamide. Then, the analysis was continued by their application to silica gel columns in which the silica gel was adjusted to 10% water. The extracts were then eluted with 150mL cyclohexane before being concentrated to 25µL. GC-MS (Thermo Trace) was used to determine the concentration of PAHs in these extracts with reference to the ^{13}C labelled internal standards. In total, 28 PAHs were measured with LOD being between 0.01 to 2.00µg/kg. The LODs were based on the availability of samples for analysis and interfering matrix peaks in the chromatograms. The LOD for whole placentas were lower than placental aliquots for specific analytes. This analysis method is ISO17025 accredited.

2.2.4 Estimation of ambient PM_{10} and $PM_{2.5}$ level during the pregnancy

PM_{10} and $PM_{2.5}$ levels during pregnancy were estimated based on automatic air pollution monitoring station, which the air pollutants data is publicly available from the DEFRA website (https://uk-air.defra.gov.uk/data/data_selector). The nearest monitoring station was selected by using the maternal residential postcode. The specific date for each trimester window was estimated by using the delivery date, gestational days and the expected delivery date (assume the pregnancy lasts for 280 days) for each participant. The first trimester was defined as the pregnancy in 1^{st} – 13^{th} gestational weeks, the second trimester the 14^{th} – 27^{th} gestational weeks and third trimester started from 28^{th} gestational week until birth. The level of PM_{10} and $PM_{2.5}$ were calculated on a daily mean basis (24-hour running mean), based on the specific date for each trimester period.

2.2.4.1 Selection of air pollution monitoring stations

The AURN was selected as a monitoring network, as this is the largest automatic monitoring network in the UK, which hourly measured the ambient air pollutants such as air particle (PM_{10} and $PM_{2.5}$), NO_X, SO_2 and O_3 (NICVA, 2015). The monitoring stations were selected from urban and sub-urban monitoring sites, in which the distance between monitoring stations and the residential home of the study population ranged between 1.32 to 25.7 kilometres (km) (DEFRA, 2019). The other monitoring sites such as rural area, traffic, industrial and background station were excluded in this study due to the measurement of different kinds of air pollutants and areas.

Due to the unavailability of PM_{10} levels from the Manchester Piccadilly monitoring site at certain times, the second nearest monitoring site was chosen if necessary. The only available PM_{10} data from Manchester Piccadilly was from 18th December 1995 to 15th January 2009 (DEFRA, 2019). Thus, PM_{10} estimation levels were referred to the Salford Eccles monitoring station, as a second nearest monitoring station (see Figure 2.2). The data for $PM_{2.5}$ concentration was available from all selected monitoring stations.

2.2.4.2 Interpolation map of PM_{10} and $PM_{2.5}$

The interpolation map of PM_{10} and $PM_{2.5}$ exposure level in Greater Manchester areas was created by using Geographical Information System (GIS) software. To begin this process, the postcode of participant's residential home and monitoring station was geocoded to the postcode easting (x) and northing (y) of British National Grid by using Geoconvert tools (UK Data Service Census Support, 2019). Then, the background map (Greater Manchester) was acquired from the EDINA Digimap website and used as an image base layer on the GIS software ArcMap 10.4.1 software (ESRI, Redlands, CA, USA). The geocoded postcode (x and y) of the participant's home and monitoring station were then added to the base layer and followed with the estimated level of PM_{10} and $PM_{2.5}$ of each participant's home. The interpolation technique of Ordinary Kriging applied at the end of the process, to predict the exposure level in unmeasured locations, by taking into account the distance and degree of variation from the measured area. Figure 2.2 shows the flowchart in obtaining the estimation level of PM_{10} and $PM_{2.5}$ by using air pollution monitoring stations and the process involved in creating the exposure map by using GIS software and interpolation technique.

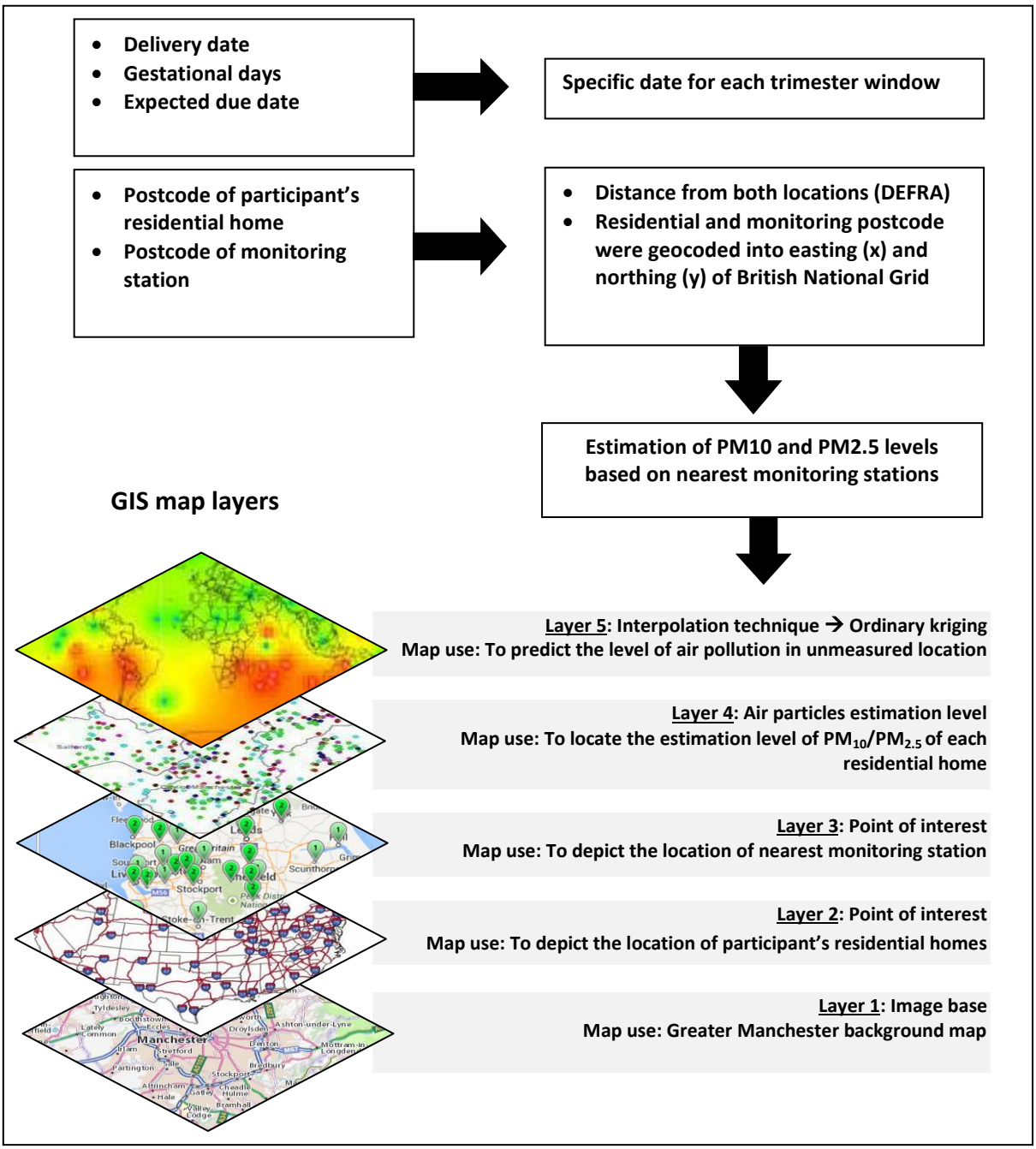


Figure 2.2 Estimation of ambient PM_{10} and $PM_{2.5}$ levels based on nearest monitoring stations and application with GIS.

The schematic diagram shows the steps in estimating maternal exposure to ambient PM_{10} and $PM_{2.5}$ levels throughout pregnancy.

The specific date for each trimester window was calculated by using the information of delivery date, gestational days and expected due date. The distance from maternal residential home and monitoring stations was acquired from DEFRA and then both postcodes (location) were geocoded. Levels of PM_{10} and $PM_{2.5}$ were obtained from DEFRA, based on nearest monitoring stations and specific trimester period corresponding to each participant.

Exposure map was created by using GIS software. The background map (Greater Manchester) was used as a base layer, and location of residential homes and monitoring sites were added. Estimation of PM_{10} and $PM_{2.5}$ levels was joined into the map, and finishing with the interpolation technique by Ordinary Kriging that provides different colour on the maps based on air pollution estimation level.

2.2.5 Statistical analysis

Data management and analysis were performed using Microsoft Excel, Graphpad Prism version 8.0 (Graphpad Software, USA) and IBM SPSS® statistics software version 22.

Demographic variables of maternal (age, BMI, ethnicity, current smoking status) and new-born characteristics (gestational days, birth-weight, baby gender and IBR) were analysed by using descriptive statistics presented as median with interquartile range (IQR), minimum and maximum values. IBR percentile was categorized into three groups; normal (10 – 90% IBR), small gestational age, SGA (<10% IBR) and large gestational age, LGA (>10% IBR). The descriptive statistics of median (IQR), minimum and maximum values were also used to present the concentrations of heavy metal and PAH in placentas and maternal blood.

In order to analyse the distribution of data, the normality test was performed by using a D'Agostino & Pearson test prior to statistical analysis. Level of heavy metals in placentas and maternal blood were not normally distributed except for copper (Cu). PAH levels in placental samples were also not normally distributed, and the sample size was small (n=12). Due to this, the non-parametric tests were performed in the analysis. Biological samples that not detected elements (negative samples) were included in the study and calculated by using the equation of LOD/√2 (Verbovšek, 2011; Ogden, 2010).

Wilcoxon signed-rank test was used to compare heavy metal and nutritional element levels in placentas and maternal blood for paired samples (n=19). A Spearman rank correlation test was used to find the correlation between; (i) heavy metal and nutritional element levels in paired samples, (ii) heavy metal, nutritional element and PAH levels in biological samples with maternal and newborn characteristics, (iii) PM_{10} and $PM_{2.5}$ levels in the first trimester compared to other trimesters, (iv) heavy metal and nutritional element levels in biological samples with maternal exposure on ambient PM_{10} and $PM_{2.5}$ during pregnancy, and (v) maternal exposure on ambient PM_{10} and $PM_{2.5}$ during pregnancy with birthweight and IBR. A simple linear regression analysis was performed to find the association between PM_{10} and $PM_{2.5}$ levels in each trimester window. The Mann-Whitney U test was performed to find the association of PAH compounds with maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy. A P-value<0.05 was considered significant.

2.3 Results

2.3.1 Experimental timeline

Figure 2.3 illustrates the total number of biological samples studied. Heavy metal was analysed in placental aliquots from non-smoker mothers (n=30) and pre-delivery maternal blood from smokers (n=5) and non-smoker's mother (n=25).

PAH was analysed in 6g placental samples, referred to placental aliquots (n=10). These samples were the same samples for heavy metal analysis and collected from non-smoker mothers only. However, PAH was not detected, so a further set of analyses (n=12) using increased amounts of placental tissue (20g) was carried out, referred to placental whole samples. This second set included five smokers, in which not present in the first analyses.

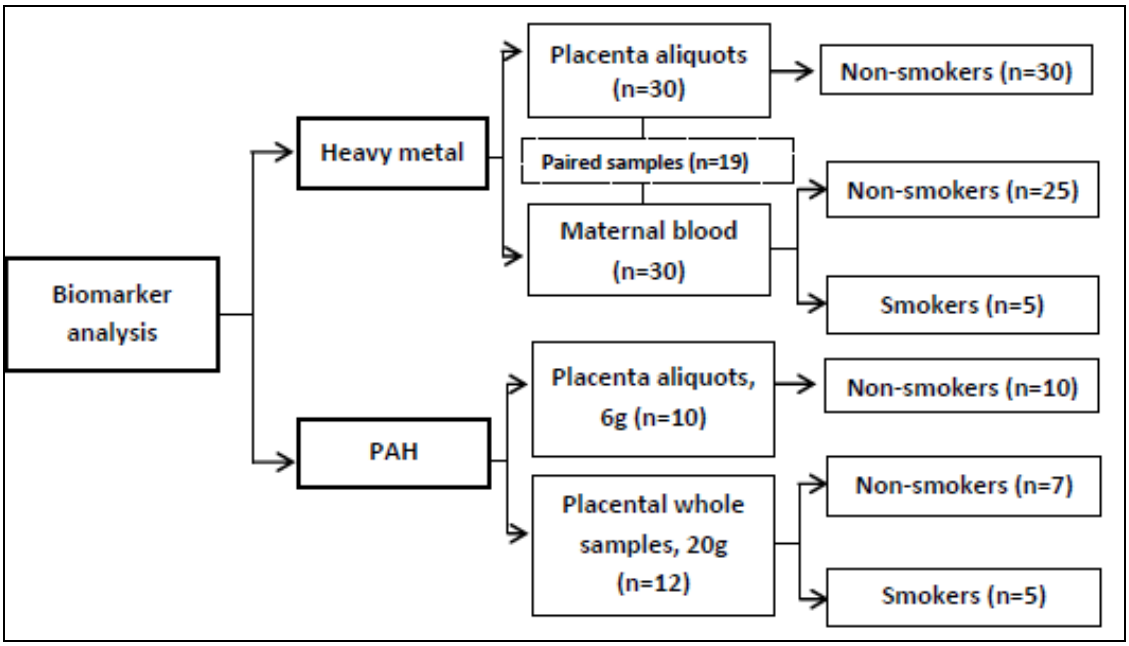


Figure 2.3: Samples collected for analysis of heavy metals and PAH levels

2.3.2 Demographic variables

Table 2.1 shows the demographic variables of the 53 participants that successfully donated their placentas and maternal blood for analysis of heavy metal and PAH levels. Of the 53 participants, the majority (46 out of 53) were Caucasian with a median age of 33 years. The median maternal BMI was 25.4kg/m^2, which indicated they were overweight. The majority (43 out of 53) of mothers were non-smokers. The median gestational age was 273 (IQR: 266 – 273) days. Over half the babies (31 out of 53) were female with a median birth weight at 3240g (IQR: 2940g – 3620g) and the median individualised birth weight ratio (IBR) was at percentile 42.6 (IQR: 19.5 – 62.0).

Table 2.1: Demographic variables of the study population

Maternal characteristics	Median (IQR)	Minimum	Maximum
Maternal age (years old)	33.0 (29.5 – 36.0)	22.0	46.0
Maternal BMI (kg/m^2)	25.4 (22.3 – 28.6)	19.0	40.8
Ethnicity			
Caucasian, n (%)	46 (86.8)		
Non-Caucasian, n (%)	7 (13.2)		
Smoking status			
Non-smokers, n (%)	43 (81.1)		
Smokers, n (%)	10 (18.9)		
Gestational age (days)	273 (266 – 273)	252	287
Baby birth weight (g)	3240 (2940 – 3620)	2328	4428
Baby gender			
Male, n (%)	22 (41.5)		
Female, n (%)	31 (58.5)		
*IBR	42.6 (19.5 – 62.0)	2.4	92.1
10 – 90 (Normal), n (%)	43 (81.1)		
< 10 (SGA), n (%)	8 (15.1)		
> 10 (LGA), n (%)	2 (3.8)		

*IBR= Individualised birth weight ratio; SGA= small gestational age and LGA= large gestational age

2.3.3 Heavy metal and nutritional element levels in biological samples

Table 2.2 shows the concentration of heavy metals (As, Cd, Cr, Cu, Hg, Ni and Pb) and nutritional elements (Ca, Fe, K, Mg, Na, P, Se and Zn) in 30 placentas and maternal blood. All placental samples contained detectable levels of heavy metals, whereas Cr and Ni were not detected in any maternal blood samples. Only Cd and Cu were detected in all 30 placental samples. The nutritional elements were detected in all placental and maternal blood samples (100% positive samples for n=30), respectively.

2.3.3.1 Comparison of heavy metal and nutritional element levels in placentas and maternal blood for paired samples

Figure 2.4 and 2.5 show the comparison of heavy metal and nutritional element levels in placentas and maternal blood for 19 paired samples. Cu and Pb levels were significantly higher in maternal blood compared to the placenta (P-value<0.0001), whereas Cd and Hg were significantly higher in placentas compared to maternal blood (P-value<0.0001 and 0.05, respectively). All nutritional element levels in placentas differed significantly from those in maternal blood (P-value<0.0001). 6 out of 8 nutritional elements were detected higher in placentas compared to maternal blood and those elements were Ca, Mg, Na, P, Se and Zn, whereas Fe and K levels were higher in maternal blood compared to placentas.

Table 2.2: Concentration of heavy metals and nutritional elements in placentas and maternal blood

Elements (mg/kg)	Placenta (n=30)				Maternal blood (n=30)			
	Median (IQR)	Min	Max	No. of positive samples (out of 30)	Median (IQR)	Min	Max	No. of positive samples (out of 30)
Heavy metals								
Arsenic (As)	0.0007 (0.0007 - 0.0008)	0.0007	0.0030	7	0.0007 (0.0007 – 0.0007)	0.0007	0.0040	3
Cadmium (Cd)	0.0030 (0.0020 – 0.0050)	0.0010	0.0120	30	0.0007 (0.0007 – 0.0007)	0.0007	0.0020	2
Chromium (Cr)	0.0250 (0.0100 – 0.0530)	0.0071	0.4000	25				*Not detected*
Copper (Cu)	0.6000 (0.6000 – 0.7000)	0.5000	0.8000	30	1.6750 (1.4880 – 1.8130)	1.2600	2.3400	30
Mercury (Hg)	0.0015 (0.0007 – 0.0020)	0.0007	0.0060	19	0.0007 (0.0007 – 0.0010)	0.0007	0.0030	9
Nickel (Ni)	0.0071 (0.0071 – 0.0100)	0.0071	0.0500	11				*Not detected*
Lead (Pb)	0.0010 (0.0007 – 0.0020)	0.0007	0.0060	20	0.0060 (0.0080 – 0.0103)	0.0030	0.0210	30
Nutritional elements								
Calcium (Ca)	130.00 (106.00 – 225.00)	94.00	1380.00	30	59.40 (56.00 – 61.43)	48.00	65.50	30
Iron (Fe)	50.20 (39.85 – 68.53)	24.10	107.00	30	418.50 (396.30 – 461.30)	286.00	574.00	30
Potassium (K)	1305.00 (1180 – 1426)	1010.00	1620.00	30	2050.00 (1910 – 2183)	1760.00	3180.00	30
Magnesium (Mg)	66.05 (61.35 – 69.43)	47.90	80.20	30	31.50 (30.50 – 33.40)	27.80	36.70	30
Sodium (Na)	2510.00 (2340 – 2743)	2190.00	2830.00	30	1880.00 (1758 – 1975)	1620.00	2100.00	30
Phosphorus (P)	1395.00 (1360 – 1493)	1180.00	1940.00	30	355.50 (336.80 – 379.50)	310.00	427.00	30
Selenium (Se)	0.13 (0.12 – 0.15)	0.12	0.24	30	0.10 (0.08 – 0.11)	0.05	0.22	30
Zinc (Zn)	8.60 (7.88 – 8.93)	6.50	10.20	30	6.47 (5.93 – 6.83)	4.59	8.71	30

LOD for As, Cr, Hg and Pb were 0.001mg/kg, respectively, whereas LOD for Ni was 0.01mg/kg.

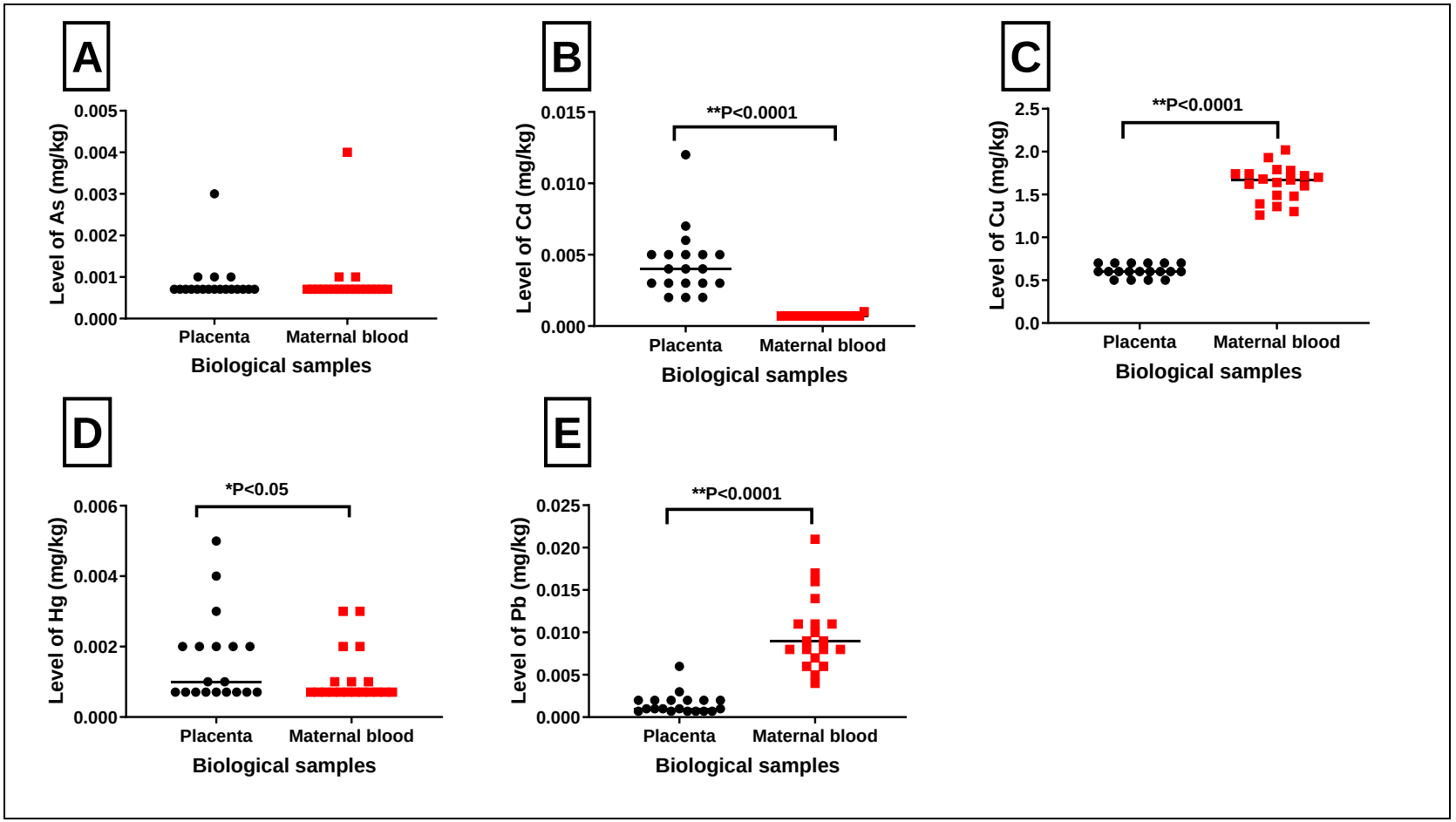


Figure 2.4: Comparison of heavy metal levels in placenta and maternal blood for paired samples.

Heavy metal levels in placenta and maternal blood for (A) arsenic, As (B) cadmium, Cd (C) copper, Cu (D) mercury, Hg and (E) lead, Pb.

Line represented median. There was no difference in As levels, but Cu and Pb levels were significantly higher in maternal blood compared to placenta (P-value<0.0001), whereas Cd and Hg were significantly higher in placenta compared to maternal blood (P-value<0.0001 and 0.05, respectively) (Wilcoxon signed rank test, n=19).

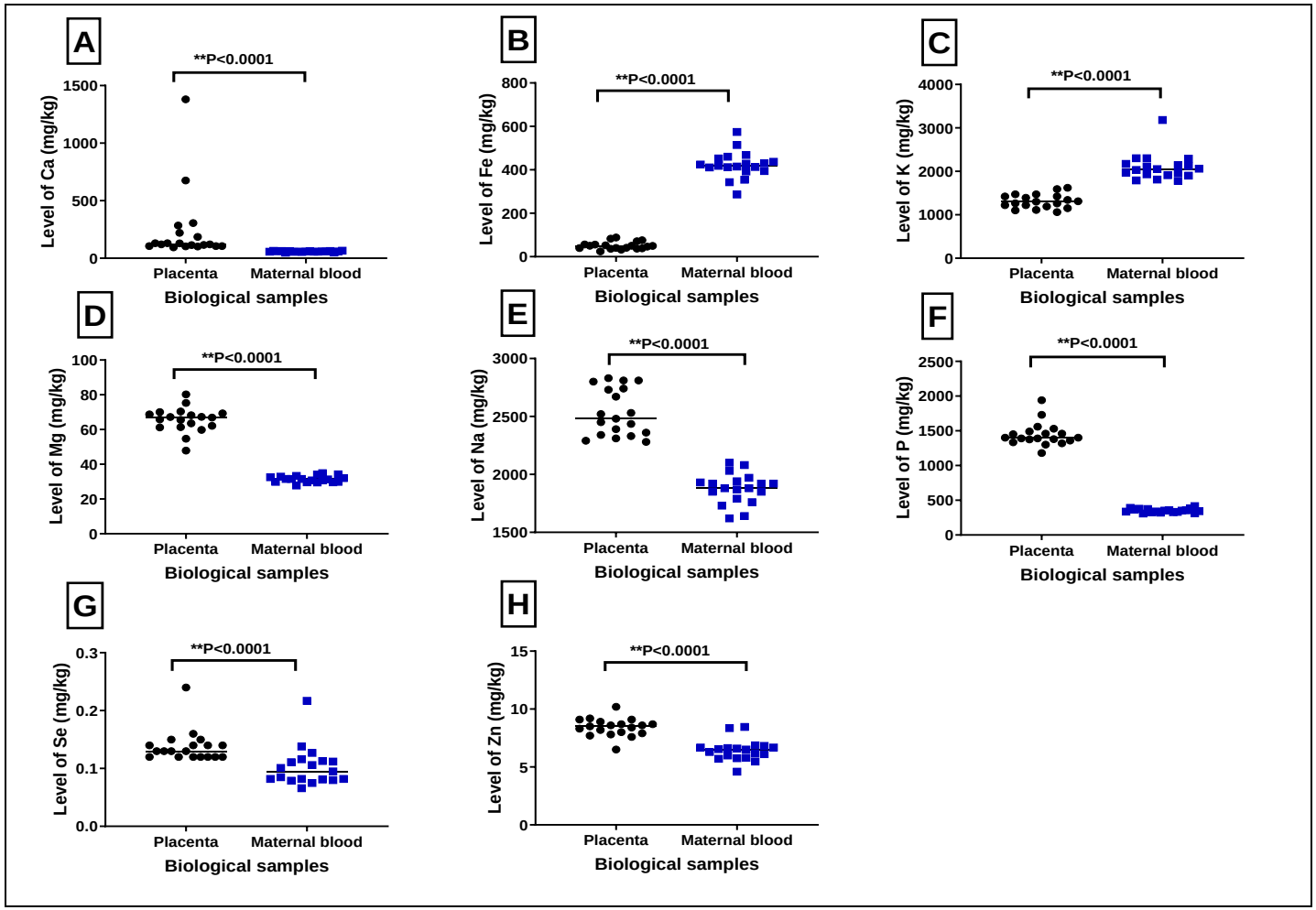


Figure 2.5: Comparison of nutritional element levels in placenta and maternal blood for paired samples.

Nutritional element levels in placenta and maternal blood for (A) calcium, Ca (B) iron, Fe (C) potassium, K (D) magnesium, Mg, (E) sodium, Na (F) phosphorus, P (G) selenium, Se and (H) zinc, Zn. Line represented median. Level of nutritional elements in both samples were significantly different; Ca, Mg, Na, P, Se and Zn were significantly higher in placentas compared to maternal blood, whereas Fe and K were significantly higher in maternal blood compared to placentas (P-value<0.0001; Wilcoxon signed rank, n=19).

2.3.3.2 Correlation between heavy metal and nutritional element levels in placentas and maternal blood

Table 2.3 shows the correlation between heavy metal and nutritional element levels in placentas and maternal blood for paired samples. There were significant positive correlations of Hg and Pb levels in placentas and maternal blood at P-value<0.0001 (r=0.88) and 0.05 (r=0.46), respectively. For nutritional elements, only Se shows a significant positive correlation in both samples at P-value<0.05 (r=0.01).

Table 2.3: Correlation of heavy metal and nutritional element levels in placentas and maternal blood for paired samples

Heavy metals	r (P-value)[a]	Nutritional elements	r (P-value)[a]
Arsenic (As)	0.23 (0.35)	Calcium (Ca)	0.15 (0.55)
Cadmium (Cd)	0.18 (0.47)	Iron (Fe)	-0.25 (0.31)
Copper (Cu)	0.41 (0.08)	Potassium (K)	0.11 (0.64)
Mercury (Hg)	**0.88 (<0.0001)****	Magnesium (Mg)	0.10 (0.67)
Lead (Pb)	**0.46 (0.05)***	Sodium (Na)	0.02 (0.93)
		Phosphorus (P)	0.07 (0.78)
		Selenium (Se)	**0.55 (0.01)***
		Zinc (Zn)	-0.25 (0.31)

[a]Spearman rank correlation test; n=19
*Significant different at P-value<0.05 or **P-value<0.0001

2.3.3.3 Association of heavy metal and nutritional element levels with maternal and newborn characteristics

Table 2.4 shows the correlation of heavy metal levels in placentas and maternal blood with maternal and newborn characteristics. There was a positive correlation between maternal age with Ni levels in placental samples at P-value=0.02 (r=0.42). The birth weight of new-borns was significantly correlated with Cr and Cu in placentas, and As, Cd and Hg in maternal blood at P-value<0.05. There was a trend towards significant between birth weight with Ni levels in placentas at P-value=0.06 (r=0.34). Cr, Cu and Ni levels in placentas and Ni in maternal blood were correlated with IBR at P-value<0.05.

Table 2.5 shows the correlation of nutritional element levels in placentas and maternal blood with maternal and newborn characteristics. Maternal age was inversely correlated with Ca levels in maternal blood at P-value=0.03 (r=-0.40), and positively correlated with Fe levels at P-value=0.04 (r=0.39). Maternal BMI was positively correlated with P levels in the placenta at P-value=0.02 (r=0.41).

Table 2.4: Correlation of heavy metal levels in biological samples with maternal and newborn characteristics

Biological samples	Heavy metals	Maternal characteristics		New-born characteristics	
		Age r (P-value)[a]	BMI r (P-value)[a]	Birth-weight r (P-value)[a]	IBR r (P-value)[a]
Placenta	Arsenic (As)	0.19 (0.32)	0.03 (0.86)	0.01 (0.96)	-0.15 (0.43)
	Cadmium (Cd)	0.16 (0.40)	-0.04 (0.82)	-0.20 (0.29)	-0.20 (0.28)
	Chromium (Cr)	0.34 (0.07)	0.09 (0.62)	**0.44 (0.01)***	**0.54 (0.002)***
	Copper (Cu)	0.27 (0.15)	-0.04 (0.86)	**0.54 (0.002)***	**0.42 (0.02)***
	Mercury (Hg)	0.08 (0.66)	0.01 (0.97)	0.28 (0.14)	0.14 (0.46)
	Nickel (Ni)	**0.42 (0.02)***	-0.13 (0.50)	0.34 (0.06)#	**0.46 (0.01)***
	Lead (Pb)	0.14 (0.48)	-0.18 (0.33)	-0.14 (0.46)	-0.23 (0.22)
Maternal blood	Arsenic (As)	0.15 (0.44)	-0.29 (0.12)	**0.38 (0.04)***	0.24 (0.20)
	Cadmium (Cd)	-0.33 (0.08)	0.04 (0.83)	**-0.40 (0.03)***	**-0.37 (0.05)***
	Copper (Cu)	-0.18 (0.33)	-0.03 (0.90)	0.15 (0.43)	0.19 (0.31)
	Mercury (Hg)	0.10 (0.61)	-0.17 (0.36)	**0.37 (0.04)***	0.19 (0.33)
	Lead (Pb)	0.31 (0.10)	-0.07 (0.73)	-0.21 (0.27)	-0.28 (0.14)

[a]Spearman Rank Correlation Test (n=30)

*Significant different at P-value<0.05; #Trend towards significant at P-value=0.06

Table 2.5: Correlation of nutritional element levels in biological samples with maternal and newborn characteristics

Biological samples	Nutritional elements	Maternal characteristics		New-born characteristics	
		Age r (P-value)[a]	BMI r (P-value)[a]	Birth-weight r (p-value)[a]	IBR r (p-value)[a]
Placenta	Calcium (Ca)	0.09 (0.62)	0.26 (0.17)	-0.26 (0.16)	-0.34 (0.07)
	Iron (Fe)	-0.28 (0.13)	0.08 (0.68)	0.01 (0.96)	-0.11 (0.58)
	Potassium (K)	0.17 (0.38)	-0.01 (0.95)	0.21 (0.28)	0.30 (0.11)
	Magnesium (Mg)	0.16 (0.40)	0.22 (0.24)	0.17 (0.37)	0.09 (0.621)
	Sodium (Na)	-0.19 (0.33)	-0.10 (0.60)	-0.17 (0.37)	-0.25 (0.18)
	Phosphorus (P)	0.17 (0.37)	**0.41 (0.02)***	0.12 (0.55)	-0.05 (0.78)
	Selenium (Se)	0.08 (0.66)	0.06 (0.74)	0.09 (0.64)	0.04 (0.85)
	Zinc (Zn)	0.10 (0.60)	0.31 (0.09)	0.17 (0.36)	0.08 (0.69)
Maternal blood	Calcium (Ca)	**-0.40 (0.03)***	-0.06 (0.77)	0.03 (0.88)	-0.04 (0.83)
	Iron (Fe)	**0.39 (0.04)***	-0.08 (0.69)	-0.15 (0.43)	-0.04 (0.85)
	Potassium (K)	0.30 (0.11)	-0.18 (0.34)	0.03 (0.88)	0.18 (0.35)
	Magnesium (Mg)	0.20 (0.28)	0.01 (0.96)	0.06 (0.77)	0.14 (0.48)
	Sodium (Na)	-0.15 (0.44)	-0.02 (0.91)	0.26 (0.17)	0.20 (0.29)
	Phosphorus (P)	0.07 (0.72)	-0.15 (0.42)	0.12 (0.54)	0.16 (0.40)
	Selenium (Se)	0.09 (0.63)	-0.19 (0.32)	-0.28 (0.13)	-0.20 (0.28)
	Zinc (Zn)	0.17 (0.38)	0.17 (0.37)	-0.20 (0.30)	-0.19 (0.31)

[a]Spearman Rank Correlation Test (n=30)

*Significant different at P-value<0.05

2.3.4 PAH in placental samples

All placental aliquot samples (n=10) were recorded as being below the LOD (data not shown), and only 10 PAH compounds were detected in placental-whole samples (Table 2.6: n=12). Of these whole placental samples, 8 contained detectable levels of PAHs. Fluorene detected in 8 placental samples, fluoranthene and pyrene detected in 3 samples, whereas other compounds were detected in only one sample.

Table 2.6: Concentration of PAHs in placental samples

PAHs (µg/kg)	Placental whole samples (n=12)			
	Median (IQR)	Min	Max	No. of positive samples (out of 12)
Anthracene	0.01 (0.01 – 0.01)	0.01	0.06	1
Benz[a]anthracene*	0.01 (0.01 – 0.01)	0.01	0.02	1
Benzo[b]fluoranthene*	0.01 (0.01 – 0.01)	0.01	0.02	1
Benzo[b]naphtho[2,1-d]thiophene	0.01 (0.01 – 0.01)	0.01	0.03	1
Benzo[e]pyrene	0.01 (0.01 – 0.01)	0.01	0.01	1
Benzo[g,h,i]fluoranthene	0.01 (0.01 – 0.01)	0.01	0.01	1
Fluoranthene	0.02 (0.02 – 0.03)	0.02	1.21	3
Fluorene	0.30 (0.25 – 0.44)	0.17	0.89	8
Phenanthrene	0.18 (0.18 – 0.23)	0.18	4.37	1
Pyrene	0.03 (0.03 – 0.04)	0.03	0.89	3

*Potentially carcinogenic PAHs as classified by EPA

LOD for anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[b]naphtho[2,1-d]thiophene, benzo[e]pyrene, benzo[g,h,i]fluoranthene were 0.01µg/kg, whereas LOD for fluoranthene, fluorene, phenanthrene, pyrene were 0.03, 0.17, 0.25 and 0.04, respectively.

2.3.4.1 Association between PAH levels in placental samples with ethnicity and smoking status

No significant association was recorded between PAH levels in placental samples with maternal (age and BMI) and newborn characteristics (birthweight and IBR) (data not shown). Also, PAH level in placental samples was not significantly associated with ethnicity groups or maternal smoking status.

2.3.5 Location of participant's residential homes and nearest monitoring stations

Figure 2.6 presents the location of the participant's residential homes and the nearest monitoring stations that were selected in this study. The participants lived in four different postcode areas ranged from Manchester (M), Oldham (OL), Stockport (SK) and Warrington (WA). Two monitoring stations were assigned to the participant's residential homes namely Manchester Piccadilly (MP) and Salford Eccles (SE).

Over half of the study population (58.5%, n=31) were assigned to MP monitoring station, whereas the rest (41.5%, n=22) were assigned to SE monitoring station. The distance of the MP station to the participant's home ranged from 2.74 to 25.70km, whereas for the SE station, the distance ranged from 1.32 to 20.78km. The details of selected monitoring stations are summarized in Table 2.7.

Figure 2.6: Location of participant's residential homes and selected air pollution monitoring stations.

The map shows the location of participant's residential homes (green dots) and monitoring stations (red stars) selected in this study. The levels of PM_{10} and $PM_{2.5}$ were estimated based on two monitoring stations; MP and SE, with the distance from monitoring stations to the residential homes within 2.7 – 25.7km and 1.3 – 20.8km, respectively.

Table 2.7: Details of selected monitoring stations

Monitoring stations	*N (%)	Distance from participants home, (Min-Max)	Pollutants measured
MP	31 (58.5)	2.74 – 25.7km	$PM_{2.5}$, **PM_{10}
SE	22 (41.5)	1.32 – 20.78km	$PM_{2.5}$, PM_{10}

*N referred to the number of residential homes of participants that assigned to the specific monitoring station

**MP monitoring station was no longer measured ambient PM_{10}; hence data were referred from SE (as the second nearest station).

2.3.5.1 Estimation of ambient PM_{10} and $PM_{2.5}$ based on trimester windows

Table 2.8 shows the estimation of ambient PM_{10} and $PM_{2.5}$ levels in each trimester window for the 53 participants. The level of PM_{10} was generally higher compared to $PM_{2.5}$ in every trimester windows. The significant positive association was recorded between PM_{10} and $PM_{2.5}$ levels in every trimester window at P-value<0.0001 (Linear regression analysis; n=53; data not shown).

By comparing the exposure level between trimester, the PM_{10} level in the first trimester was correlated with the second trimester at P-value<0.001. For $PM_{2.5}$, exposure level in the first trimester was correlated with the second trimester and third trimester at P-value<0.001, respectively (Spearman Rank Correlation Test; n=53; data not shown).

Table 2.8: PM_{10} and $PM_{2.5}$ levels for different trimester window

Ambient air particles ($\mu g/m^3$)	Median (IQR) in every trimester window		
	1st trimester	2nd trimester	3rd trimester
PM_{10}	15.85 (14.05 – 17.74)	18.06 (16.68 – 19.29)	17.84 (16.91 – 18.63)
$PM_{2.5}$	8.96 (7.00 – 11.66)	10.72 (9.08 – 11.96)	11.05 (8.53 – 11.97)

2.3.5.2 Temporal variation of ambient PM_{10} and $PM_{2.5}$ based on trimester windows

The temporal variation of ambient PM_{10} and $PM_{2.5}$ levels at Manchester Piccadilly (Figure 2.7) and Salford Eccles (Figure 2.8) were shown similar patterns across the gestation period, with a marked increment of both particles in November 2014.

$PM_{2.5}$ levels in November 2014 as determined at Manchester Piccadilly and Salford Eccles stations were the highest in a study time frame, with the peak levels at ~75 and 90µg/m^3, respectively. For PM_{10}, a peak level in November 2014 at Salford Eccles was ~90µg/m^3. The gestation period for study participants was varied, which the first trimester for the majority of participants falls between Junes and August 2015 and pregnancy ends between March and April 2016.

2.3.5.3 Spatial variation of ambient PM_{10} and $PM_{2.5}$ in Greater Manchester

The interpolation maps in Figure 2.9(A) and (B) illustrate the spatial variation of PM_{10} and $PM_{2.5}$ in Greater Manchester areas. Level of PM_{10} and $PM_{2.5}$ were categorized into quartiles.

PM_{10} levels ranged between 15.95 to 19.47µg/m^3, whereas $PM_{2.5}$ levels ranged between 8.60 to 12.55µg/m^3. Most areas in Greater Manchester were recorded similar ambient PM_{10} and $PM_{2.5}$ levels; in which the level of PM_{10} ranged between 17.01 to 17.99µg/m^3 (Figure 2.9A), whereas $PM_{2.5}$ levels ranged between 9.89 to 10.76µg/m^3 (Figure 2.9B).

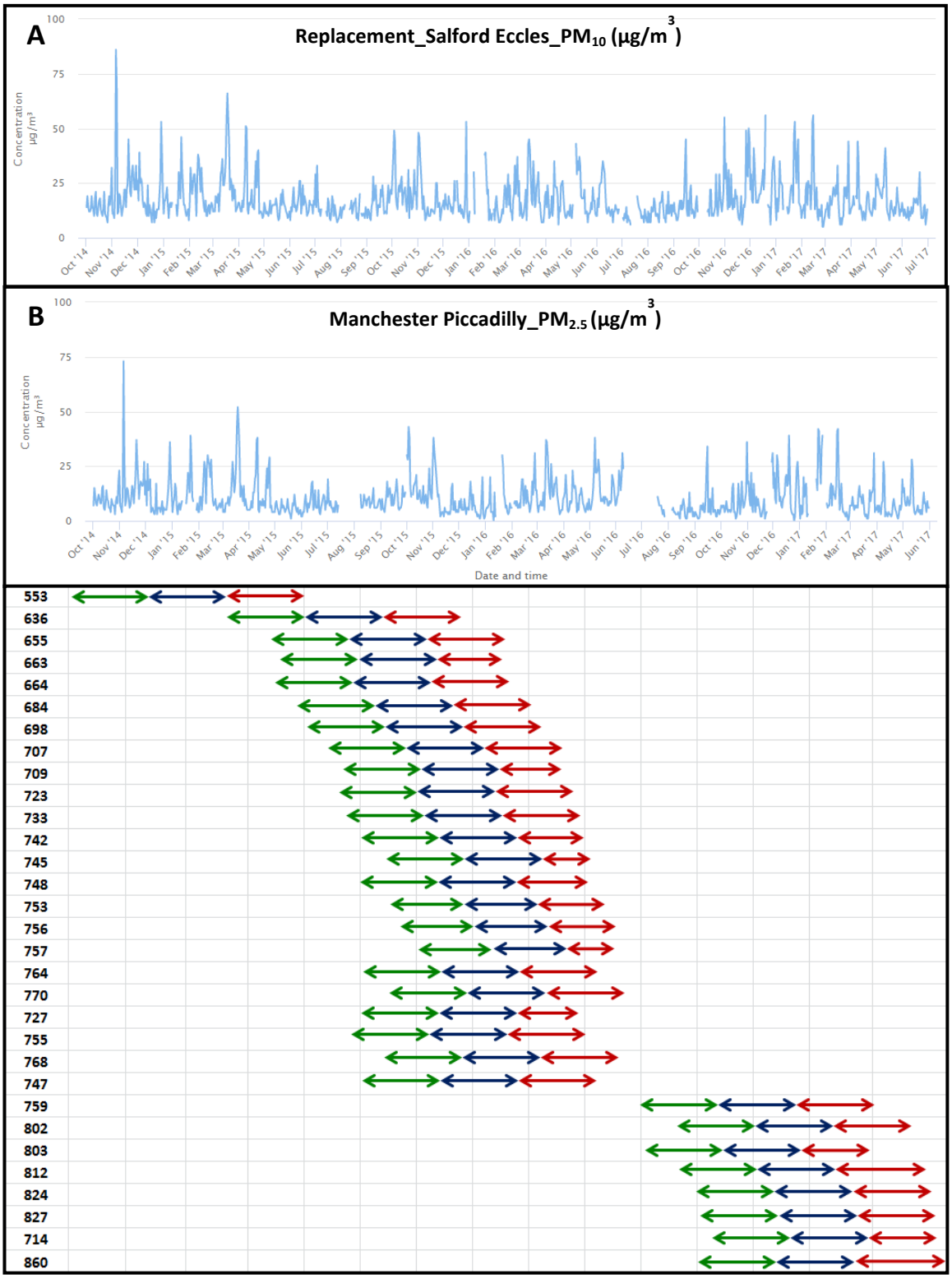


Figure 2.7: PM_{10} and $PM_{2.5}$ levels estimated from Manchester Piccadilly/Salford Eccles monitoring station during pregnancy (Adapted from DEFRA, 2019).

Figure shows the trimester window period for 31 participants assigned to the Manchester Piccadilly monitoring station, from October 2014 – June 2017. Arrows indicate trimester windows; first trimester (green arrow), second trimester (blue arrow) and third trimester (red arrow).

Data for PM_{10} (Panel A) was obtained from Salford Eccles station, as second nearest monitoring station).

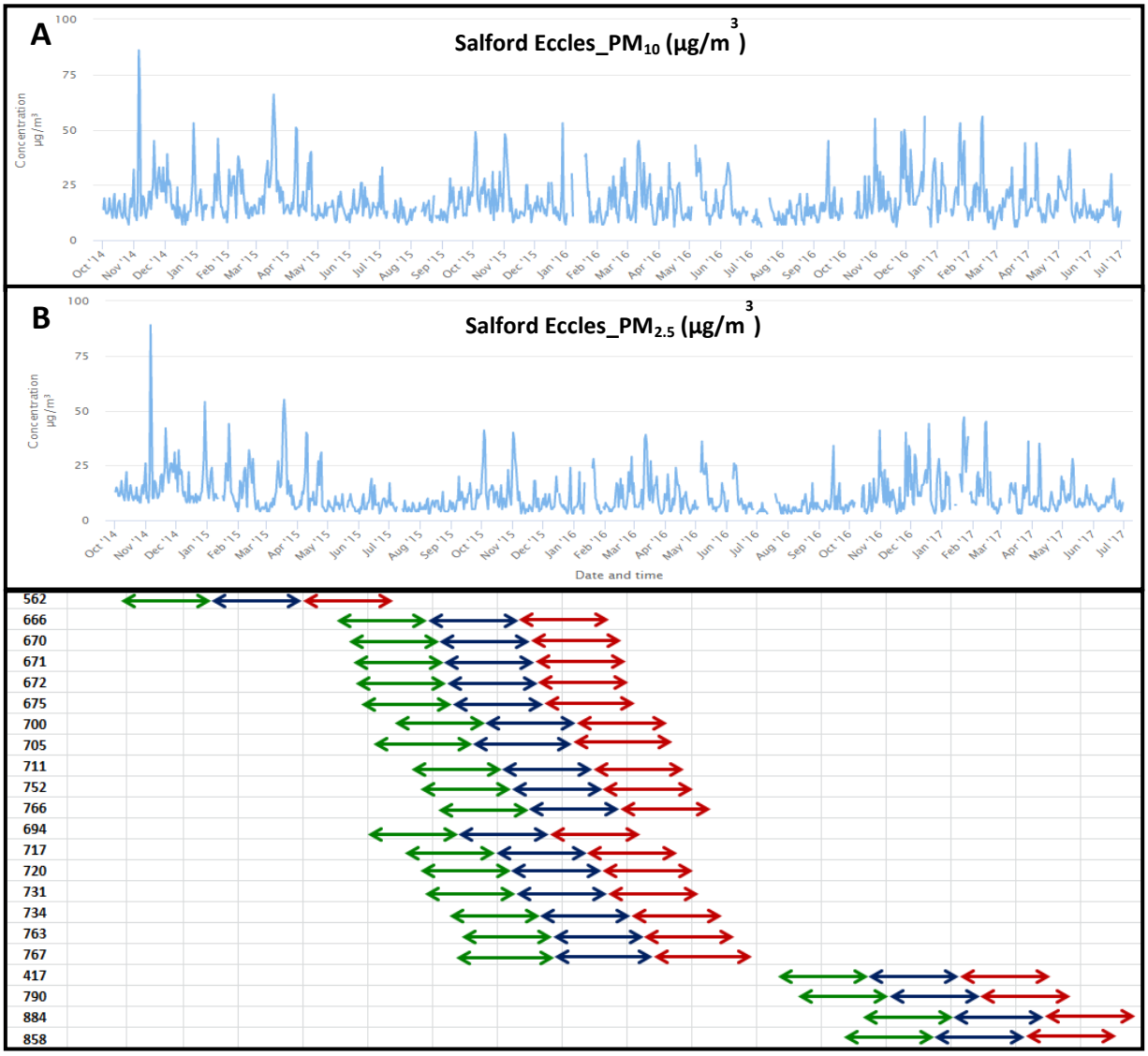


Figure 2.8: PM_{10} and $PM_{2.5}$ levels estimated from Salford Eccles monitoring station during pregnancy (Adapted from DEFRA, 2019).

Figure shows the trimester window period, for 22 participants assigned to Salford Eccles monitoring station, from October 2014 – June 2017. Arrows indicate trimester windows; first trimester (green arrow), second trimester (blue arrow) and third trimester (red arrow).

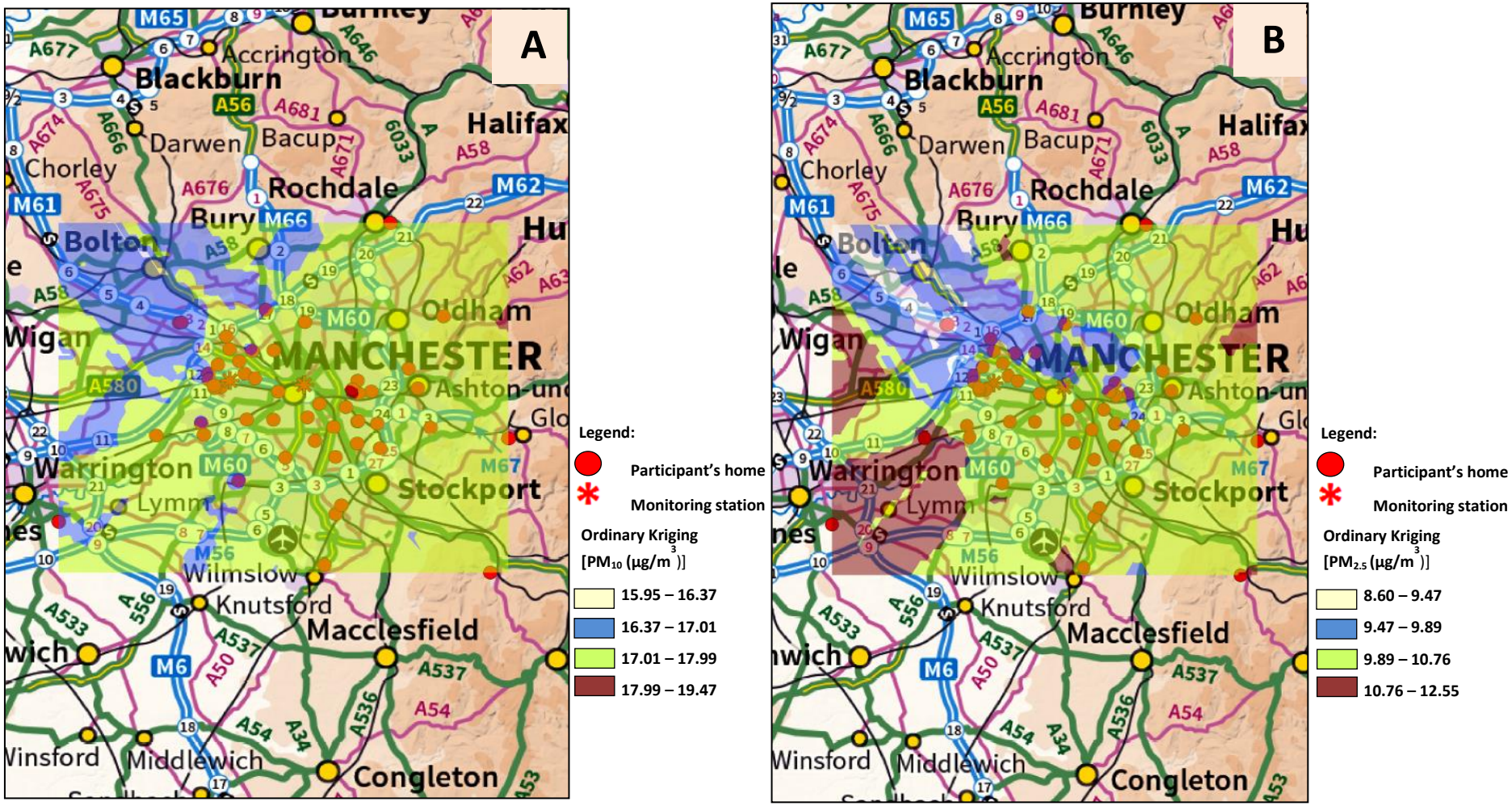


Figure 2.9: Interpolated maps showing ambient PM_{10} and $PM_{2.5}$ levels in Greater Manchester

Figures show the spatial variation of ambient PM_{10} (Panel A) and $PM_{2.5}$ (Panel B) levels in Greater Manchester areas, derived from air pollution monitoring station closest to the maternal residential homes. Red dots indicate location of maternal residential homes and red stars indicate location of monitoring stations. PM_{10} and $PM_{2.5}$ levels were calculated based on the average exposure of these particles during pregnancy (first until third trimester).

2.3.5.4 Correlation between maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy with heavy metal and nutritional element levels in placentas and maternal blood.

Table 2.9 presents the correlation between the average maternal exposure on PM_{10} and $PM_{2.5}$ throughout gestation period with heavy metal levels detected in placentas and maternal blood. No significant correlation was observed between maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy with the concentrations of heavy metals in placentas and maternal blood. Maternal exposure on ambient PM_{10} and $PM_{2.5}$ during pregnancy was significantly negatively correlated with Na level in placental samples (Spearman rank correlation; n=30; data not shown).

Table 2.9: Correlation between PM_{10} and $PM_{2.5}$ levels with heavy metal concentrations in placentas and maternal blood

Biological samples	Compounds	PM_{10} (µg/m^3) r (P-value)	$PM_{2.5}$ (µg/m^3) r (P-value)
Placentas	Arsenic (As)	0.10 (0.61)	0.02 (0.92)
	Cadmium (Cd)	0.08 (0.69)	0.03 (0.86)
	Chromium (Cr)	0.23 (0.23)	0.22 (0.24)
	Copper (Cu)	0.13 (0.49)	0.12 (0.54)
	Mercury (Hg)	0.04 (0.84)	0.04 (0.84)
	Nickel (Ni)	0.28 (0.13)	0.34 (0.07)
	Lead (Pb)	-0.08 (0.66)	-0.09 (0.64)
Maternal blood	Arsenic (As)	-0.01 (0.94)	-0.02 (0.92)
	Cadmium (Cd)	0.07 (0.70)	0.07 (0.71)
	Copper (Cu)	0.10 (0.60)	0.10 (0.62)
	Mercury (Hg)	-0.14 (0.46)	-0.18 (0.35)
	Lead (Pb)	-0.19 (0.33)	-0.13 (0.49)

PM_{10} and $PM_{2.5}$ levels calculated based on the average exposure throughout the gestation period (Spearman Rank Correlation Test; n=30)

2.3.5.5 Association between maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy with PAH level in placentas.

Table 2.10 presents the association between the average maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy with the detection of PAHs in placental samples. Maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy was not associated with the presence of PAH compounds in the placental samples.

Table 2.10: Association of maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy with the detection of PAH compounds in placental samples

Compounds	Detection in placental samples	PM_{10} (µg/m^3) (P-value)	$PM_{2.5}$ (µg/m^3) (P-value)
Anthracene	Present (n=1) Not present (n=7)	0.51	0.83
Benz[a]anthracene	Present (n=1) Not present (n=7)	0.51	0.83
Benzo[b]fluoranthene	Present (n=1) Not present (n=7)	0.51	0.83
Benzo[b]naphtho[2,1-d]thiophene	Present (n=1) Not present (n=7)	0.51	0.83
Benzo[e]pyrene	Present (n=1) Not present (n=7)	0.51	0.83
Benzo[g,h,i]fluoranthene	Present (n=1) Not present (n=7)	0.51	0.83
Fluoranthene	Present (n=3) Not present (n=5)	0.46	0.66
Fluorene	Present (n=8) Not present (n=0)	-	-
Phenanthrene	Present (n=1) Not present (n=7)	0.51	0.83
Pyrene	Present (n=3) Not present (n=5)	0.46	0.66

PM_{10} and $PM_{2.5}$ levels calculated based on the average exposure throughout the gestation period (Mann-Whitney Test; n=8).

2.3.5.6 Correlation between maternal exposure to PM_{10} and $PM_{2.5}$ with birthweight and IBR

Maternal exposure on PM_{10} and $PM_{2.5}$ levels during pregnancy were not correlated with birth-weight of babies and IBR percentile (P-value>0.05; Spearman-rank correlation test; n=53; data not shown).

2.4 Discussion

2.4.1 Principal findings

This study was designed to determine the concentration of heavy metals and PAHs in placentas and maternal blood, and their association with maternal exposure to ambient PM_{10} and $PM_{2.5}$ during pregnancy in Greater Manchester. Heavy metal levels in placentas and maternal blood, measured with ICP-MS, were detected in the majority of samples, except for Cr and Ni which not detected in maternal blood. The concentrations of Cu and Pb were detected higher in maternal blood, whereas Cd and Hg were found higher in placenta samples. The level of Cr, Cu and Ni levels in placentas and Cd level in maternal blood were correlated with birthweight and IBR percentile, whereas As and Hg in maternal blood were correlated with birthweight. The level of nutritional elements presence in placentas and maternal blood were not correlated with birth weight or IBR, but P level in placentas was positively correlated with maternal BMI, whereas Ca and Fe levels in maternal blood were correlated with maternal age.

PAHs, as determined by GC-MS, were not detected in initial small placental samples but were detected in whole placental samples, suggesting that a large amount of placental, at least 20g of villous tissue is required for PAH analysis in a future study. Of 12 whole placental samples, only 8 contained detectable levels of PAHs. Fluorene was detected in 8 samples, fluoranthene and pyrene detected in 3 samples, whereas other compounds were detected in only one sample. None of the PAH compounds was correlated with maternal or new-born characteristics. Low maternal exposure on ambient PM_{10} and $PM_{2.5}$ levels during pregnancy, which determined by air pollution monitoring stations, may be an indicator of low heavy metal and PAH concentrations in biological samples. As overall, ambient PM_{10} and $PM_{2.5}$ levels in Greater Manchester were below the WHO air quality standard limits ($<25\mu g/m^3$), in which this exposure level leads to lower detection of heavy metal and PAH in the biological samples and may not give a significant effect on placental function.

2.4.2 Heavy metal concentrations in maternal blood and placentas

It was anticipated that particles could adsorb heavy metals or toxic elements from the surrounding environment and affect the human body following inhalation (Erickson & Arbour, 2014; Li et al., 2013). There is growing evidence associating prenatal exposure to air particles bound with heavy metals and/or PAHs with adverse pregnancy outcomes by the mechanism of oxidative stress, inflammation and modification of placental endocrine

function (Kim et al., 2017; Wang et al., 2017; Grevendonk et al., 2016; Erickson & Arbour, 2014; Sbrana et al., 2011). Previous studies also compared the concentration of heavy metals in maternal blood, cord blood and placentas to investigate the ability of these particles to transfer, accumulate and affect cells and tissue in human body (Sakamoto et al., 2013). However, the present study was not able to analyse the concentrations of heavy metal and PAH in the cord blood, due to the limited amount of cord blood during sample collection. Hence, the present study only compares heavy metals concentrations in placentas and maternal blood samples.

The present study found that Cd and Hg were significantly higher in placental tissue compared to maternal blood. The level of Cd and Hg was more elevated in placentas which may due to the ability of these elements to bind to the cysteine-rich protein metallothionein (MT) which is present in the placenta (Iyengar & Rapp, 2001). MT is present in the syncytiotrophoblast layer and villous interstitial cells and plays a role in heavy metal detoxification, as well as metal and nutrient transport (Gundacker & Hengstschläger, 2012; Iyengar & Rapp, 2001; Waalkes et al., 1984). The concentration of Cd and Hg in placentas was positively associated with MT protein expression in placenta, suggesting that these heavy metals were bound to MT, hence minimize the transport of these metals to the fetus (Kippler et al., 2010; Shimada et al., 2004). Higher Hg levels in placentas compared to maternal blood was in line with the finding from the previous study (Hsu et al., 2007). The higher accumulation of Hg levels in placental samples compared to fetal tissue, suggesting that placenta successfully acts as a barrier in protecting the fetus from environmental pollutants and harmful substances (Yang et al., 1996).

Cu presence in maternal blood with several species and most of Cu (65 – 90%) bounds with a protein known as caeruloplasmin and 7-15% loosely binds with albumin and transcuprein (Catalani et al., 2018). Only ~5% of the free fraction Cu is present in blood and able to be accumulated in the cells (Catalani et al., 2018). Free fraction Cu refers to Cu bounds with amino acid or without bind with any element (Catalani et al., 2018). Only free fraction Cu able to cross the placenta from maternal blood to the fetus via facilitative diffusion process, in which this resulted to limited Cu levels accumulated in the placentas compared to maternal blood (Raghunath et al., 2000). Other than that, Pb was found higher in maternal blood compared to the placenta, in which similar with the finding from the previous study (Al-Saleh et al., 2011; Osman et al., 2000). Higher Pb level in maternal blood may indicate maternal exposure to cigarettes smoke and wine consumption during pregnancy (Sponder et al., 2014).

Individuals may be exposed to heavy metals from a variety of sources such as environmental, occupational, lifestyle (e.g. smoking) or dietary sources (ATSDR, 2012). Previous studies have reported that heavy metals such as Cd, Hg, Ni and Pb can be transported to the fetus via placenta by many mechanisms, and able to impair placental function resulting in adverse pregnancy outcomes (Zheng et al., 2014; Gundacker & Hengstschläger, 2012; Sawicka-Kapusta et al., 2007). The present study shows that Cr, Cu and Ni in placentas, and As, Cd and Hg levels in maternal blood were significantly correlated with birthweight and IBR. However, the level of As, Cd and Hg in maternal blood were low and only detected in 3, 2 and 9 maternal blood samples (out of 30), respectively. The significant correlation between these variables possibility getting by "chance" and the small sample size were not sufficient to provide robust statistical analysis; hence suggested that previous study is required to further investigate this association by increasing the number of sample size.

Cr levels in placental samples of the present study were comparable with a previous study conducted in Spain with median concentrations of 0.03 and 0.06mg/kg, respectively (Amaya et al., 2013). However, a study conducted in China showed higher Cr levels in placentas at a median concentration of 0.23mg/kg (Guo et al., 2010). The higher Cr level found in the placentas of China population may due to higher exposure of Cr level in the ambient environment. As reported by Cheng et al. (2014), the anthropogenic activities mainly coal combustion had induced the release of Cr level to the ambient environment of China, with the annual growth rate of 8.8%. In corresponding with this, a previous study in China revealed that the increment of Cr level leads to the increased risk of having LBW babies, especially for female babies (Xia et al., 2016). The present study also recorded a significant correlation between Cr level in placenta with birthweight and IBR percentile. The result in the present study may imply that the individuals still exposed to Cr level in the environment, even the concentration of air pollution are recorded low, which below than the WHO guideline.

The concentrations of Cu in placentas and maternal blood were the highest compared to other heavy metal elements. The significant positive correlation was observed between Cu levels in placentas with birthweight and IBR. Combustion activities and traffic pollution are among the sources of Cu production in the environment, and this exposure was correlated with LBW (Basu et al., 2014). However, with the median concentrations of 0.6 and 1.7mg/kg in placentas and maternal blood, Cu detected in the present study was lower compared to another study and did not show the correlation with an ambient

exposure level of PM_{10} or $PM_{2.5}$ during pregnancy. A previous study conducted in Poland shows a higher Cu level in placentas at the concentrations between 2.7 to 6.7mg/kg for the city area in Krakow and 3.5 to 6.7mg/kg for the control area in Bieszczady Mountains (Sawicka-Kapusta et al., 2007). Even this previous study showed higher Cu levels in placentas, but they have not investigated the effect on pregnancy outcomes, suggested that a higher number of samples are needed in future studies to confirm a "true" significant correlation between Cu levels in Greater Manchester areas with pregnancy outcome.

There has been substantial research undertaken to investigate the association of maternal exposure to Ni and adverse pregnancy outcomes such as SGA and LBW (Meyrueix et al., 2019; Chen et al., 2018; McDermott et al., 2015). Exposure to Ni may occur through the inhalation, skin contact and ingestion of food and water as this heavy metal element naturally exists in our environment (McDermott et al., 2015). The level of Ni in placentas in the present study was comparable with the study conducted among the South African population, with a median concentration of 0.0071mg/kg in both populations (Meyrueix et al., 2019). However, a study conducted in Turkey cohort was recorded higher Ni levels with mean concentrations of 0.13, 0.12 and 0.10mg/kg in maternal blood, placental and cord blood samples, respectively (Arica et al., 2013). The differences of Ni levels in these biological samples were in agreement by the fact that Ni potentially able to cross the placental barrier, accumulated in fetal tissue and associated with birth outcome (Meyrueix et al., 2019; Arica et al. 2013). However, in this present study, Ni was only detected in placental samples with <40% of positive samples (11 out of 30) and no detection in maternal blood. Ni levels in placentas were significantly correlated with the IBR percentile and recorded trend towards a significant correlation with new-born birthweight. With no detection of Ni in maternal blood, the present study was not able to compare the level of this element in both samples and to relate the placenta as a protective barrier towards this element. Table 2.11 summarizes the comparison of Cr, Cu and Ni levels in placental samples with other study populations, as discussed above.

Table 2.11: The comparison of Cr, Cu and Ni levels in placental samples with different study population (Data shows median or otherwise stated, mg/kg)

Heavy metals (mg/kg)	Manchester (n=30)	[a]Spain (n=680)	[b]China (n=220)	[c]Poland		[d]South African (n=40/41)	[e]Turkey (n=100)
				Krakow (n=20)	Bieszczady (n=25)		
Cr	0.03	0.06	0.23	-	-	0.01	0.22**
Cu	0.6	-	-	2.7 – 6.7*	3.5 – 6.7*	-	-
Ni	0.007	-	0.008	-	-	0.007	0.12**

[a]Amaya et al. (2013); [b]Guo et al. (2010); [c]Sawicka-Kapusta et al. (2007); [d]Meyrueix et al. (2019); [e]Arica et al. (2013)

*Minimum – maximum range; **Mean

2.4.2 Nutritional element concentrations in placentas and maternal blood

Previous studies have described the associations between maternal nutritional deficiencies and adverse pregnancy outcomes. The WHO in the publication entitled "*Born Too Soon*" relates the insufficiency of nutritional elements during pregnancy with PTB (Howson et al., 2012). In other publication, the WHO and United Nations Children's Fund (UNICEF) also revealed that nutritional deficiencies among pregnant women in poor socio-economic countries were a risk factor of having low birth weight babies (UNICEF and WHO, 2004). Inadequate maternal nutritional intake during pregnancy, together with other risk factors such as psychosocial stress may result in fetal growth restriction and preterm birth (Hobel & Culhane, 2003). In the present study, nutritional elements of Ca, Fe, K, Mg, Na, P, Se and Zn were detected in all placentas and maternal blood samples with 100% detection in both biological samples. None of the nutritional elements was correlated with pregnancy outcomes (birth-weight or IBR percentile); which only Ca and Fe levels in maternal blood were correlated with maternal age, and P levels in placentas were positively correlated with maternal BMI.

The negative correlation between Ca levels and maternal age factor in the present study may imply by the facts that, the ability of the intestine to absorb Ca in older people was reduced due to reduction of age-related hormone, and this condition may result in bone health effects (Veldurthy et al., 2016). On the other hand, the positive correlation of Fe levels in maternal blood with maternal age may imply that participants in this study may be exposed to Fe from the different sources, not necessarily from the nutritional intakes, but might be from the consumption of the supplements during pregnancy, as generally Fe levels will be degraded by increasing of age (Busti et al., 2014). The level of P levels in

placental samples was showed a positive correlation with maternal BMI, and this was contradicted with the previous study that demonstrated a negative relationship between both variables (Kalejaiye et al., 2016).

Previous studies reported that the elements of Mg, Fe, Zn, As, Cd, Pb and K can be found in the ambient environment and as part of the chemical compositions of $PM_{2.5}$ and traffic particles, which may be emitted from tyre wear, brake wear, vehicle component and motor exhaust (Sajani et al., 2016; Basu et al., 2014). However, the result of the present study shows that the nutritional element in placentas and maternal blood were not correlated with a maternal exposure level of PM_{10} and $PM_{2.5}$ during pregnancy. The exception was recorded with Na, which negative correlation was recorded between Na level in placentas with maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy. Generally, the primary source of Na is from the dietary intake and very limited studies investigated the level of Na in the ambient environment. Na levels in placenta may closer relate to maternal dietary or nutritional intake, rather than exposure to ambient air pollution, suggesting that this result may be recorded by "chance". However, there is a possibility that exposure to air pollution may reduce placental nutrient transport as a result of placental dysfunction (Gaccioli & Lager, 2016).

2.4.3 PAH concentrations in placentas

PAH levels in placental-whole samples were not correlated with any maternal or new-born characteristics. Also, the maternal exposure of PM_{10} and $PM_{2.5}$ during pregnancy was not associated with the accumulation of PAH compounds in the placental samples. The detection of PAH in placental samples was very low, indicates that this level may not give a significant impact on the placental function. Future studies are suggested to increase the number of smokers to investigate the concentration of PAH in placental samples, as cigarette smoke is among the primary source of PAH exposure (Health Canada, 1994).

2.4.4 Maternal exposure on PM_{10} and $PM_{2.5}$ and their effect on pregnancy outcomes

Previous studies revealed that for every incremental increase of 4.0μg/m^3 of $PM_{2.5}$ in the early gestational period was increased the risk of pre-eclampsia, gestational hypertension and PTB, whereas in every increment of 50μg/m^3 of PM_{10} in the final six weeks before birth was associated with an increased risk of having an SGA baby by 20% (Lee et al., 2013; Ritz et al., 2000). According to Ritz & Wilhelm (2010), the trimester-

dependant effect was recorded between maternal exposure on air particles during pregnancy with pregnancy outcomes, in which first-trimester exposure was associated with placental dysfunction, whereas third-trimester exposure may cause PTB and LBW. However, the results in the present study were not able to find any significant correlations between maternal exposure on PM_{10} and $PM_{2.5}$ during pregnancy with birth weight or IBR percentile (<20% indicates SGA).

The estimation of maternal exposure on ambient PM_{10} and $PM_{2.5}$ level was conducted by adapted the methodology from previous epidemiological studies (Shin et al., 2019; Chambers et al., 2018; Seo et al., 2016; Magsumbol et al., 2014; Williams & Bird, 2003). The daily average levels of ambient PM_{10} and $PM_{2.5}$ during pregnancy was estimated by using the automatic air quality monitoring stations in Greater Manchester areas. By referring to the average concentration of PM_{10} and $PM_{2.5}$ during pregnancy, the level of these ambient particles in the Greater Manchester areas was categorized as low level compared to the WHO standard limit; hence this exposure level may give only little impact on the studied outcome. A previous study within a larger UK population (n=203,562) had found a weak significant association between maternal exposure on PM_{10} and $PM_{2.5}$ levels with SGA in the third trimester, suggesting that higher sample size required in a future study to obtain this association within Greater Manchester population (Hannam et al., 2014).

2.4.5 Strengths, limitations and future work

The investigation of the association between maternal exposure of ambient PM_{10} and $PM_{2.5}$ during pregnancy with heavy metals and PAH concentrations is essential in order to understand to what extent the environmental pollutants affected human health and pregnancy outcome. The challenge surrounding this investigation is the percentage of positive samples for the detection of heavy metal and PAH levels in biological samples were low so that it was not possible to examine the association with pregnancy outcome. Also, ambient PM_{10} and $PM_{2.5}$ concentrations in Greater Manchester were low compared to the WHO standards limit (<25µg/m^3), in which this level may not give any significant impact on the pregnancy outcome.

The key strength of the present study was the measurement of heavy metals and PAHs in different biological samples, which this method may explain the placental function as a protective barrier of fetus towards hazardous pollutants and describe the ability of

environmental pollutants transported from the environment, to the mother and towards fetus (Sakamoto et al., 2013). However, it is unfortunate that this present study was not able to include the analysis of heavy metals and PAHs in cord blood as insufficient cord blood was collected, to allow the analysis of heavy metals and PAHs.

In order to understand the biological mechanism on how air pollution affected pregnancy outcome, future studies may further this analysis by looking at placental structure and function, as this relationship has been linked to the mechanism of oxidative stress, inflammation and modification of endocrine function (Erickson & Arbour, 2014). In future studies, it would be interesting to collect samples from complications pregnancy of pre-eclampsia, SGA and IUGR, as imbalance number of pregnancy with cases compared to normal in the present study. The comparison of heavy metals in biological samples and maternal exposure on ambient air pollution during pregnancy between these two groups (normal and cases) may best describe the association of air pollution and pregnancy outcomes.

The present study provides an understanding regarding the spatial variation of ambient PM_{10} and $PM_{2.5}$ levels in Greater Manchester areas by using GIS and an Ordinary Kriging technique. Ordinary Kriging is a geostatistical interpolation technique that is applied to an exposure map to produce an interpolated map (Figure 2.9) that is filled with different colours based on air pollution estimation level within that area (Tyagi & Singh, 2013). By using this interpolation technique, air pollution levels for any location within the mapped area can be estimated based on the measured points (monitoring stations). However, this technique is limited by not considering the heterogeneity of spatial variability and assumes that the mean exposure level within an area is constant (Kim et al., 2014). Thus, this technique may possibly cause bias as air pollution levels within an area may well vary depending upon local and regional sources.

The estimation of ambient PM_{10} and $PM_{2.5}$ in the present study was also limited to the estimation at residential homes without taking into consideration the exposure level of air pollutants at the workplace or during commuting. The occupational information was needed to better address the maternal exposure level on air pollution and to identify the sources of those pollutants. The proximity of residential homes to traffic pollution must also be accounted for the studies, as this factor increased the number of traffic particles inside the home (Sajani et al., 2016). In accord with this, the measurement of indoor air particles in residential homes would be an interesting approach in a future study. Other than that, the dietary intake information such as alcohol consumption, supplement intake,

drug use, smoking habits and exposure to second-hand smokers during pregnancy should be considered as these elements were the risk factors of placental dysfunction resulted to adverse pregnancy outcomes (Carter et al., 2016; Ghosh et al., 2013; Agarwal et al., 2012; Kaiser & Allen, 2002).

2.5 Conclusion

This study examined the concentrations of heavy metal and PAH in placentas and maternal blood, in which the existence of these compounds in studied biological samples, may occur due to the maternal exposure of air pollution during pregnancy. In comparison to other previous studies, heavy metal and PAH levels in placentas and maternal blood of the present study were low, indicates that the exposure level of air pollution in Greater Manchester appears to be low and may well have little impact on adverse pregnancy outcomes. An increased number of samples would be required to confirm this association. Further work is needed to identify the contributing factors attributed to the increment of heavy metal and PAH levels in the biological samples, and to determine the mechanism on how these compounds are transferred into the placenta, which lead to placental dysfunction and adverse pregnancy outcomes.

CHAPTER 3:

MEASUREMENT OF INDOOR AIR PARTICLES IN THE RESIDENTIAL HOMES OF PREGNANT WOMEN ACROSS GREATER MANCHESTER: A FEASIBILITY STUDY

3.1 Introduction

Air pollution can be divided into two categories: outdoor and indoor air pollution, which are both interrelated. The issues of indoor air pollution are not as widely discussed as that of outdoor air pollution, even though there is evidence linking indoor air pollution to acute and chronic human health effects such as acute upper respiratory infection, chronic obstructive pulmonary disease and lung cancer (Karottki et al., 2015; WHO, 2010). Susceptibility to air pollution varies between individuals and dependent on the nature, level and duration of pollutant exposure (Bruce et al., 2000; Armstrong et al., 1992). Exposure to indoor air pollution is higher among pregnant women, especially on the third trimester as they reportedly spent more time indoors, averagely 17 hours per days at home (Nethery et al., 2009). The association between maternal exposure to $PM_{2.5}$ during pregnancy and adverse pregnancy outcomes has been previously demonstrated (Jedrychowski et al., 2017; DeFranco et al., 2015; Harris et al., 2014; Basu et al., 2014; Jedrychowski et al., 2006). However, the clinical evidence identifying the underlying biological mechanism of this association is still limited, with several competing hypotheses suggesting this relationship may occur due to placental inflammation, oxidative stress, alteration of endothelial function and hemodynamic responses (Patelarou & Kelly, 2014).

This study was designed to assess the feasibility of studying indoor air pollution in the residential homes of pregnant women across Greater Manchester. Previous studies have used the Dylos air particle monitor to measure indoor particles concentration, due to its low-cost, quietness, simple application and also because results obtained with this monitor are in high agreement with other conventional devices and gravimetric methods (Franken et al., 2019; Jackson-Morris et al., 2016; Rosen et al., 2015; Semple et al., 2015; Brown et al., 2014; Semple et al., 2012). The estimation of ambient (or outdoor) $PM_{2.5}$ level, simultaneously with indoor air sampling may give a better understanding on the influence of the outdoor environment towards indoor air quality. The indoor and outdoor ratio (I/O ratio) of specific air pollutants may help to indicate the pollutant's sources and the relationship of the pollutants in both environments (Fonseca et al., 2014; Chen & Zhao, 2011; Qing et al., 2005; Jones et al., 2000). Finally, a comparison of outdoor and indoor $PM_{2.5}$ levels with local and global standards in air quality guideline may help to explain the level of maternal exposure to air pollution during pregnancy and the effects on their health.

3.1.1 Hypothesis

This study tested the hypothesis that the measurement of indoor air particles in the residential homes of pregnant women might help in identifying the level of maternal exposure to indoor air particles during pregnancy. Following that, the collection of biological samples from pregnant women before and after delivery, for the analysis of biomarkers of exposure, might help to better understand the relationship between maternal exposure on indoor air particles and pregnancy outcomes.

3.1.2 Aim and objectives

This study set out to examine the feasibility of measuring indoor air particles in the residential homes of pregnant women in Greater Manchester and the collection of biological samples before and after delivery. Biological samples were collected antenatally (maternal blood) and postnatally (placenta and cord blood), so that biomarkers of air pollutant exposure could be assessed in future studies.

Specifically, the objectives of this study were:

i. To measure indoor air particle levels in the residential homes of pregnant women across Greater Manchester.
ii. To collect biological samples from pregnant women before and after delivery for biomarkers of exposure analysis in future studies.
iii. To estimate maternal exposure to outdoor air particles during indoor air sampling by using the automatic air quality monitoring stations.
iv. To examine the contributing factors affecting indoor $PM_{2.5}$ level in residential homes.

3.2 Methodology

3.2.1 Study participants

This study was an observational cohort study. The participants were eligible for the study if they attended an antenatal visit in the Manchester Placenta Clinic, St. Mary's Hospital Manchester between February and August 2019, and meet the inclusion and exclusion criteria as described in Section 3.2.1.1.

Prior to the prospective study, a pilot study was conducted, which involved the measurement of indoor air particles in 13 residential homes of the general population across Greater Manchester. The pilot study aimed to understand the pattern of indoor air particles level in most residential homes across Greater Manchester and as a feasibility study to determine indoor air particle levels by using Dylos air particle monitor (DYLOS DC1700). There were no specific inclusion and exclusion criteria for the pilot study, and the participation was completely voluntary.

Following the pilot study, the measurement of indoor air particles was conducted in residential homes of pregnant women across Greater Manchester. The participants were approached following informed consent from East Midlands – Leicester South Research Ethics Committee, reference number 18/EM/0317 (Appendix 1). A research practitioner in St. Mary's Hospital Manchester reviewed and screened patient's records for the identification of potential participants based on the inclusion and exclusion criteria that were set-up for this study.

3.2.1.1 Inclusion and exclusion criteria

The inclusion criteria for the study participants were as follows:

i. Pregnant women between 20 to 40 weeks' gestation.
ii. Participants attending antenatal visits at St. Mary's Hospital, Manchester.
iii. Participants living in Greater Manchester.

The exclusion criteria for the study participants were as follows:

i. Participants with a known fetal abnormality.
ii. Participants with actual or potential imminent difficulties in their pregnancy.
iii. Participants with any health issues that may affect pregnancy (other than criteria for referral to the Manchester Placenta Clinic).
iv. Participants who were unable to speak or read English adequately to be able to provide informed consent and complete the questionnaire.

3.2.1.2 Sample size

This study was an exploratory study, and the data obtained in this study was used to determine the feasibility of an investigative study. The sample size of 50 participants was decided based on the practical considerations of the project time available.

3.2.2 Recruitment process

Figure 3.1 describes the recruitment process and sample collection involved in the prospective study. Firstly, the participants were recruited based on their attendance at an antenatal visit at the Manchester Placenta Clinic, St. Mary's Hospital, Manchester. A research practitioner reviewed and screened patient records to check the criteria of the patients before the clinic appointment session. The potential participants were then approached by the research practitioner at the end of the antenatal visit and given the participant information sheet (PIS) (Appendix 2). The participants were given enough time (more than 24 hours) to understand the PIS and consider their participation in this study. The research practitioner was contacted the participants to ask their willingness to participate in the study. Potential participants who showed their willingness to join in this study were approached at the second antenatal appointment, which scheduled around five weeks after the first appointment. Meanwhile, the potential participants who were not interested in participating were thanked for their time, and no further action was taken.

Full consent was taken at the second antenatal appointment by a research practitioner in the Manchester Placental Clinic. After signing the consent form (Appendix 3), the participants were given an indoor air sampling device, a “DYLOS air particle monitor” by the research team. They were asked to take home the air particles counter and given instructions regarding how to set up the device in their living room and then run it for 24 hours. The standard operating procedure (SOP) (Appendix 4) and a sampling diary (Appendix 5) were provided and clearly explained. They were also given a 60 millilitres (mL) sampling pot and asked to fill it with dust from their household hoover. A questionnaire (Appendix 6) regarding their demographic information, smoking habit and home condition were given, and they were asked to complete it at their home. Next, 15mL of maternal blood was also collected at the end of the clinic visit, by the research practitioner. Lastly, a date for collection of research materials (the DYLOS, sampling diary, house dust and questionnaire) was arranged and at a time agreed with the participant, a member of the research team visited the participant’s home to collect the research materials.

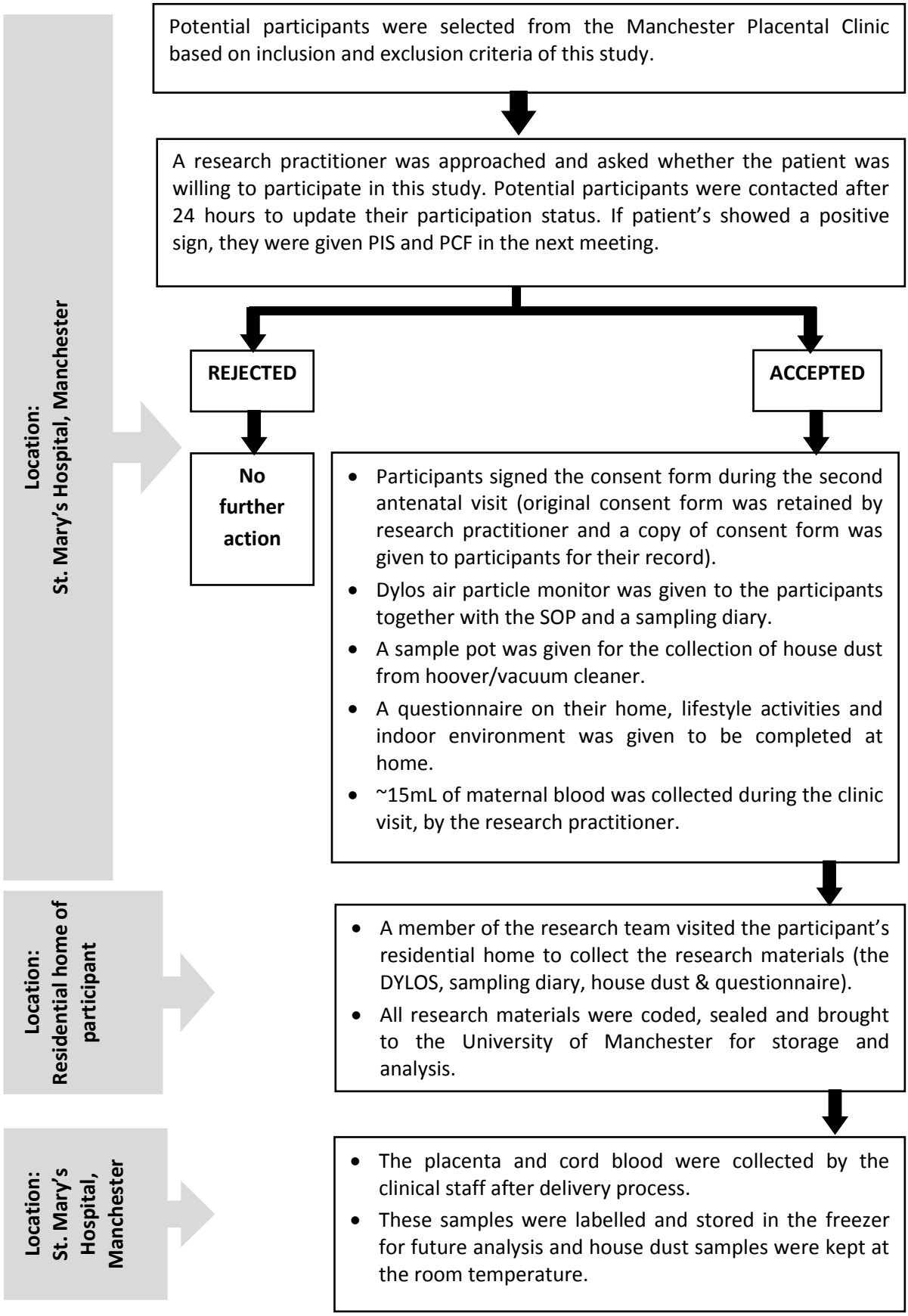


Figure 3.1: Flow diagram of participant's recruitment process and research materials collection

3.2.3 Data collection

Data collection involved the measurement of indoor air particles in the participant's residential homes by using a Dylos DC1700, the collection of research materials such as house dust, questionnaire, sampling diary and the collection of biological samples of participants including the placenta, maternal blood and cord blood.

3.2.3.1 Measurement of indoor air particles

The measurement of indoor air particles was conducted using a Dylos DC1700, as shown in Figure 3.2. The air particle monitor system was lent by the Institute of Occupational Medicine, Edinburgh, United Kingdom for the duration of the study. During the sampling process, the Dylos was to be placed in the main living room, located on a stable surface at the height between 75 and 120 centimetre (cm) from the floor and out of reach from any children. It was to be placed at least two metres from any doors or windows and not exposed to sunlight, fans, heaters or any air supply (Semple et al., 2015). The sampling duration of indoor air particles for each residential home was 24 hours.

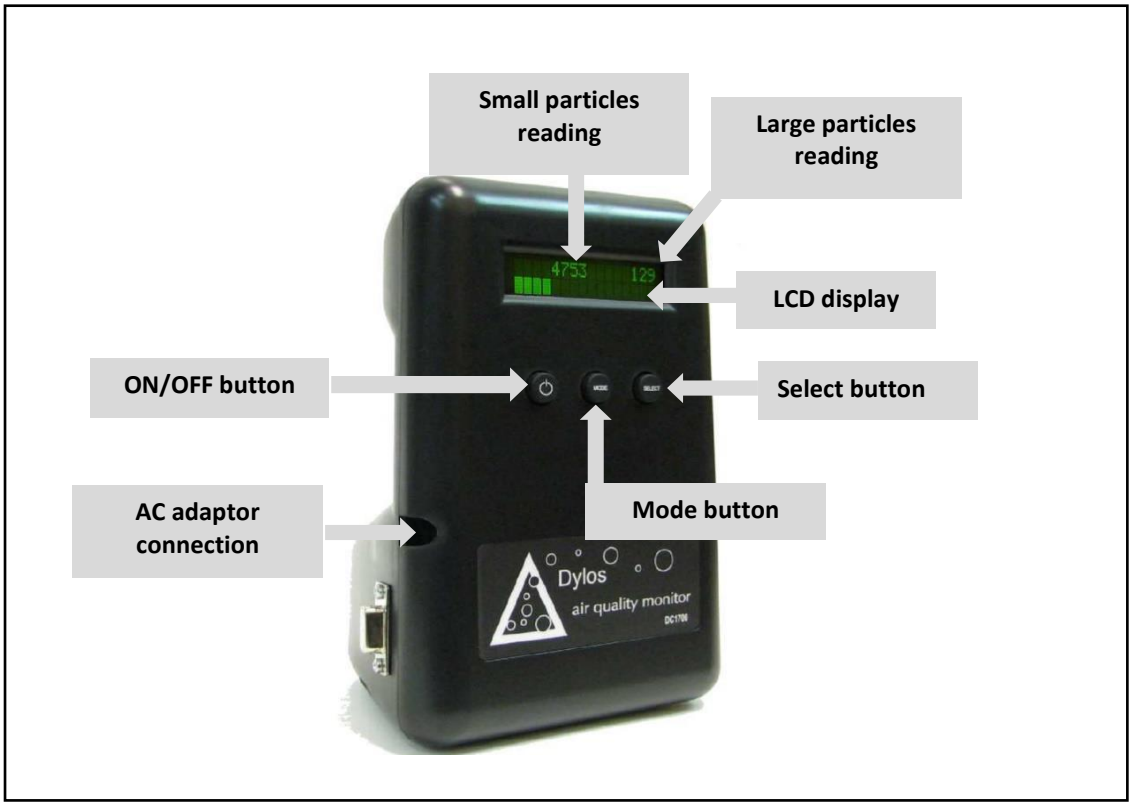


Figure 3.2: Dylos DC1700 air particles counter (Adapted from Dylos Corporation, n.d.)

The measurement of indoor air particles was started by connecting the Dylos to an AC adaptor. The monitor was plugged into the mains power at the beginning of the sampling period, and the ON/OFF button was pressed to start the sampling process. The monitor's fan starting was noted, and the word "sampling" was flashed on the LCD screen. The unit automatically configured itself and continuously counted the particle number concentration (PNC) at a one-minute resolution.

During the sampling process, the monitor counted both small and large particle sizes by using a laser light-scattering technique (Semple et al., 2015). The small particles referred to particles detected down to the detection limit of 0.5µm (reading displayed on the left side of the LCD screen), whereas large particles referred to particles detected above threshold 2.5µm (reading displayed on the right side of the LCD screen). Manual interaction was not required during the sampling process. Time for indoor activities such as smoking, cooking and windows opening was recorded in a Dylos sampling diary (Appendix 5) throughout the sampling period. After 24 hours, The ON/OFF button of the monitor was pressed to stop the sampling process.

The Dylos Logger software was used to download the sampling data from the monitor. The monitor provided air particle data as PNC of small and large particles. The PNC data was converted to particle mass concentration (PMC) by using Equation 3.1. The PNC of large particles were subtracted from the PNC of small particles to obtain the PNC for $PM_{2.5}$. Then, PNC of $PM_{2.5}$ (in unit particle per cubic foot – ppcf) was converted to PMC of $PM_{2.5}$ (in unit µg/m^3), by using Equation 3.1.

$$Y = mx^n$$

Where,

Y = $PM_{2.5}$ mass concentration

x = $PM_{2.5}$ number concentration

m = $7.33e^{-4}$

n = 0.82

Equation 3.1: $PM_{2.5}$ conversion equation (Franken et al., 2019)

Figure 3.3 illustrates the steps in the sampling technique and the conversion of PNC to PMC. For the calibration, the monitor was calibrated by the manufacturer upon purchasing and does not requires any calibration from the users (Williams et al., 2015). However, to maintain the performance and reliability of the data, a side-by-side comparison of Dylos units was conducted before and after the sampling process, to determine the agreement of PNC data between monitors.

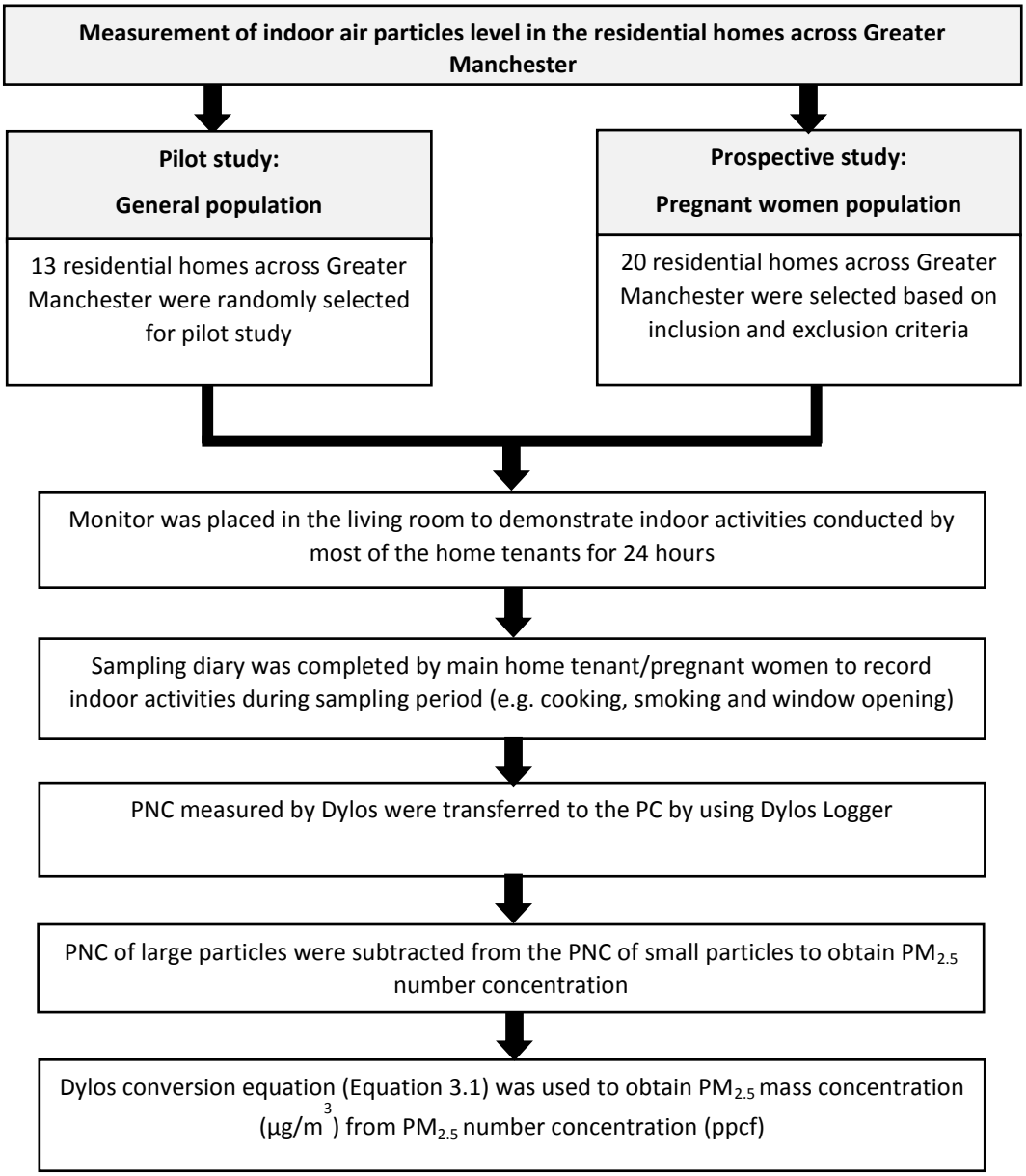


Figure 3.3: Flowchart of sampling technique and conversion to $PM_{2.5}$ mass concentration

3.2.3.2 Collection of research materials

Research materials consisted of the sampling pot containing house dust, the questionnaire, the sampling diary and the DYLOS air particle monitor were collected by the research team during a home visit. They were then coded, sealed and brought back to the University of Manchester for further processing. Sample of house dust stored at room temperature for further use to determine any trace elements or biomarkers of indoor air pollution exposure of the study population.

A short questionnaire (Appendix 6) was designed to provide background information on the home condition and to assess the relationship between indoor $PM_{2.5}$ level with demographic information and home conditions. Among the question were participant's employment status and hours at work, smoking history, exposure to second-hand smoke (SHS), location of smoking, age of residential home, years of living at this address, potential sources of indoor air pollution at home and cleaning activity. The participants completed the questionnaire at home.

3.2.3.3 Collection of biological samples

The biological samples were collected before and after the delivery. A research practitioner collected pre-delivery maternal blood at the end of the second clinic visit. Placenta and cord blood were collected after the delivery, following a similar procedure as described in Chapter 2.2.2. These biological samples were then stored at -80^0C for future study use, to look at the biomarkers related to indoor air pollution exposure.

3.2.3.4 Collection of demographic and pregnancy information

Further demographic and pregnancy information was obtained from the medical records by a research practitioner, before and after the delivery. This included information on residential postcode, maternal age, height, weight, ethnicity and smoking status that were obtained before the delivery, whereas gestational age, parity, baby weight and gender, and the percentile of individualised birth weight ratio (IBR) was obtained after the delivery.

3.2.4 Index of Multiple Deprivations (IMD)

The Index of Multiple Deprivations (IMD) data was obtained from The English Indices of Deprivation 2019 (MHCLG, 2019). The IMD is the official indicator of deprivation area of small areas in England, ranked between 1st (most deprived area) until 32,844th (least deprived area). The IMD then is categorized into ten groups of deprivation deciles, as shown in Figure 3.4. The IMD was derived from the combination of 7 deprivation domains and appropriately weighted into income (22.5%), employment (22.5%), education (13.5%), health (13.5%), crime (9.3%), barriers to housing and services (9.3%) and living environment (9.3%) (MHCLG, 2019).

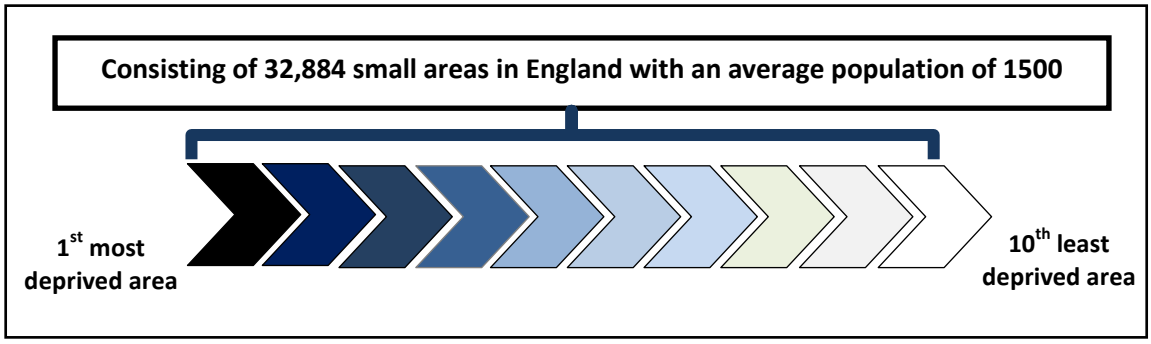


Figure 3.4: The UK Index of Multiple Deprivation Deciles (Adapted from MHCLG, 2019)

3.2.5 Estimation of outdoor $PM_{2.5}$ during the sampling period

The outdoor $PM_{2.5}$ during the sampling period was estimated based on automatic air quality monitoring stations in the Greater Manchester areas, following a similar procedure as described in Section 2.2.4.1. In brief, the monitoring stations were selected based on the closest proximity between the residential homes of the participants and air pollution monitoring stations. Two monitoring stations were assigned, which were Manchester Piccadilly (MP) and Salford Eccles (SE). The outdoor $PM_{2.5}$ level was estimated for 24 hours, simultaneously with the measurement of indoor air particles in the residential home. Maternal exposure to outdoor $PM_{2.5}$ level in every trimester window was also estimated, following a similar procedure as described in Section 2.2.4.

3.2.6 Data management and confidentiality

Access to all participants' data was limited to the research team only. All the research data were encrypted and identified using a code number. All other documents in non-digital format, such as written consent form and completed questionnaire were stored in stand-alone, locked cabinets in secure facilities located in the Centre for Occupational and Environmental Health, Ellen Wilkinson Building, The University of Manchester. Any identifying data was stored separately from anonymised data and only retained if necessary. All personal information in digital format was stored in a password-protected file on university servers with access restricted to the research team only.

3.2.7 Statistical analysis

Data management and analysis were performed by using Microsoft Excel, Graphpad Prism version 8.0 (Graphpad Software, USA) and IBM SPSS® statistics software version 22.

Demographic variables of maternal (age, BMI, ethnicity, smoking status, IMD decile, parity) and new-born characteristics (gestational days, birth-weight, baby gender and IBR) were analysed using descriptive statistics and presented as median with interquartile range (IQR), minimum and maximum values. IBR percentile was categorized into three groups; normal (10 – 90% IBR), small gestational age, SGA (<10% IBR) and large gestational age, LGA (>10% IBR). The descriptive statistics of median (IQR), minimum and maximum values were also used to describe the indoor and outdoor $PM_{2.5}$ level and the indoor and outdoor pollutant ratio.

The normality test was performed prior to statistical analysis by using a Shapiro-Wilk test. The indoor and outdoor $PM_{2.5}$ level of each residential home was not normally distributed; hence non-parametric tests were applied in the study analysis. The association between indoor and outdoor $PM_{2.5}$ with demographic factors and home condition were assessed using Spearman rank correlation test, Mann-Whitney U test and Kruskal Wallis test. A simple linear regression analysis was performed to find the association of PNC between monitors. A P-value<0.05 was considered significant.

3.3 Results

3.3.1 Participant invitation and flowchart

Figure 3.5 shows the number of participants and collected research materials in this study. Fifty potential participants were invited to participate in this study from the Manchester Placental Clinic, St. Mary's Hospital Manchester. A research practitioner screened the eligibility of potential participants based on her medical record and recruited the participants following the antenatal visit. Before recruitment, participants received an explanation of the project and their role in this study. A demonstration to use the DYLOS air particle monitor was conducted, and the SOP of the monitor was given to the participants.

Out of 50 invited participants, only 20 agreed to take part in the study (Figure 3.5). All successfully recruited participants agreed to conduct indoor air sampling in their residential homes and donated pre-delivery maternal blood. 17 out of 20 participants completed the sampling diary and collected house dust from their hoover, and 18 participants completed the questionnaire. Placentas were successfully obtained from 6 participants only, whereas no cord blood was successfully collected in this study.

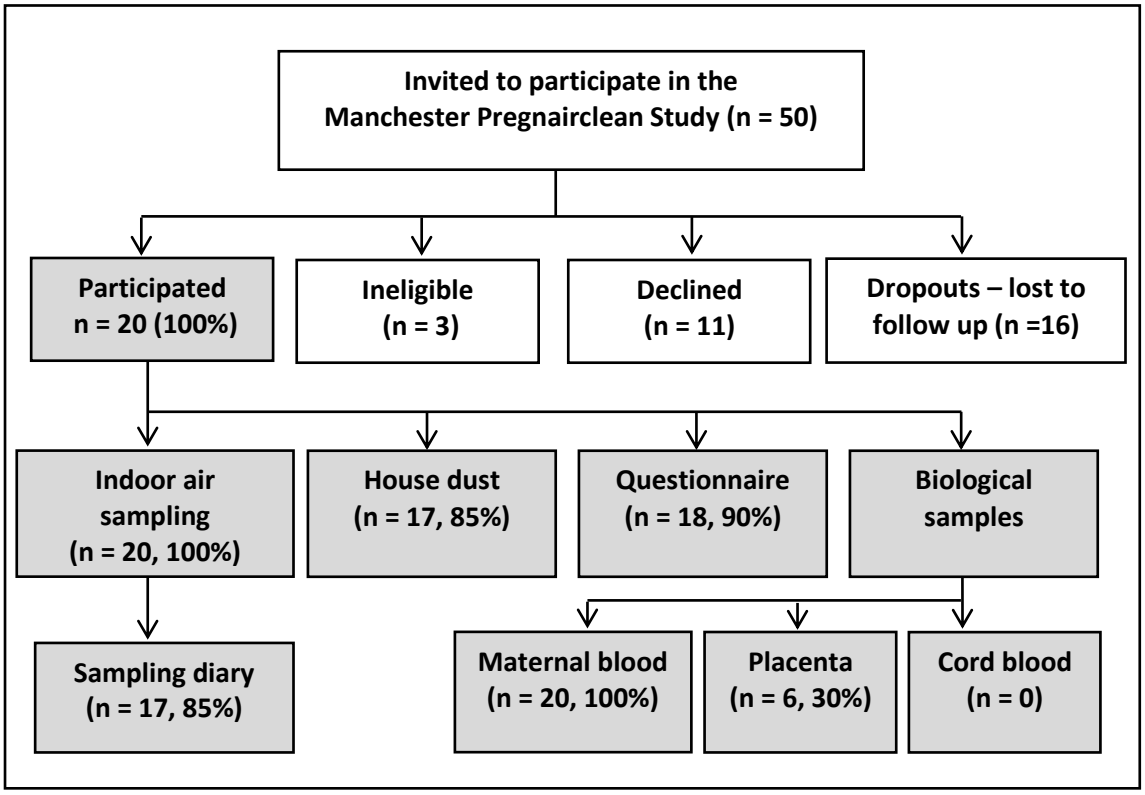


Figure 3.5: Number of participants and collected research materials in this study

3.3.2 Demographic variables

Table 3.1 shows the demographic variables of the 20 participants that were successfully recruited in this study. The majority (14 out of 20) of participants were Caucasian at a median age of 30 years. The median maternal BMI was 24.5kg/m^2 indicating normal BMI. The majority (18 out of 20) of participants were non-smokers, and 9 out of 20 were living in IMD decile 1 – 2, which indicates most deprived areas. The majority (16 out 20) of mothers were multiparous with median gestations of 270 (IQR: 266.0 – 273.8) days. Over half the babies (11 out of 20) were female with a median birth weight at 3045 (IQR: 2524 – 3288) gram and the median IBR ratio was at percentile 21.88 (IQR: 5.5 – 46.2).

Table 3.1: Demographic variables of the study population

Maternal characteristics	Median (IQR)	Minimum	Maximum
Maternal age (years old)	30.0 (28.0 – 35.8)	18.0	40.0
Maternal BMI (kg/m^2)	24.5 (21.3 – 28.8)	18.0	38.0
Ethnicity			
Caucasian, n (%)	14 (70)		
Non-Caucasian, n (%)	6 (30)		
Smoking status			
Non-smokers, n (%)	18 (90)		
Smokers, n (%)	2 (10)		
IMD decile, n (%)	3.5 (1.3 – 6.8)	1	10
1 – 2 (Most deprived)	9 (45)		
3 - 4	2 (10)		
5 - 6	4 (20)		
7 - 8	3 (15)		
9 – 10 (Least deprived)	2 (10)		
Gestational age (days)	270.0 (266.0 – 273.8)	247.0	289.0
Parity			
Primiparous, n (%)	8 (40)		
Multiparous, n (%)	16 (60)		
Baby birth weight (g)	3045 (2524 – 3288)	2228	3715
Baby gender			
Male	9 (45)		
Female	11 (55)		
*IBR	21.8 (5.5 – 46.2)	0.5	91.7
10 – 90 (Normal), n (%)	14 (70)		
< 10 (SGA), n (%)	5 (25)		
> 10 (LGA), n (%)	1 (5)		

*IBR= Individualised birth weight ratio; SGA= small gestational age and LGA= large gestational age

3.3.3 Agreement of PNC between monitors

Figure 3.6 shows the agreement of PNC between Dylos monitors, as determined by a side-by-side sampling for three Dylos monitors. A side-by-side comparison of Dylos units was carried out in two residential homes and office space in the University of Manchester, between May 2017 and February 2019 for 5 and 24 hours consecutively.

Before the main study began, side-by-side sampling between monitors was conducted in two residential homes. The PNC of Dylos monitors A and B, measured in the residential homes, was strongly positively associated with P-value<0.0001 (Figure 3.6A and B). After completion of the study, the monitors were re-tested within an office building, and the results show that Dylos A was strongly correlated with Dylos B (Figure 3.6C) and C (Figure 3.6D) with P-value<0.0001, respectively. In overall, all monitors had shown a good agreement in terms of PNC measurement, before and after the study commenced.

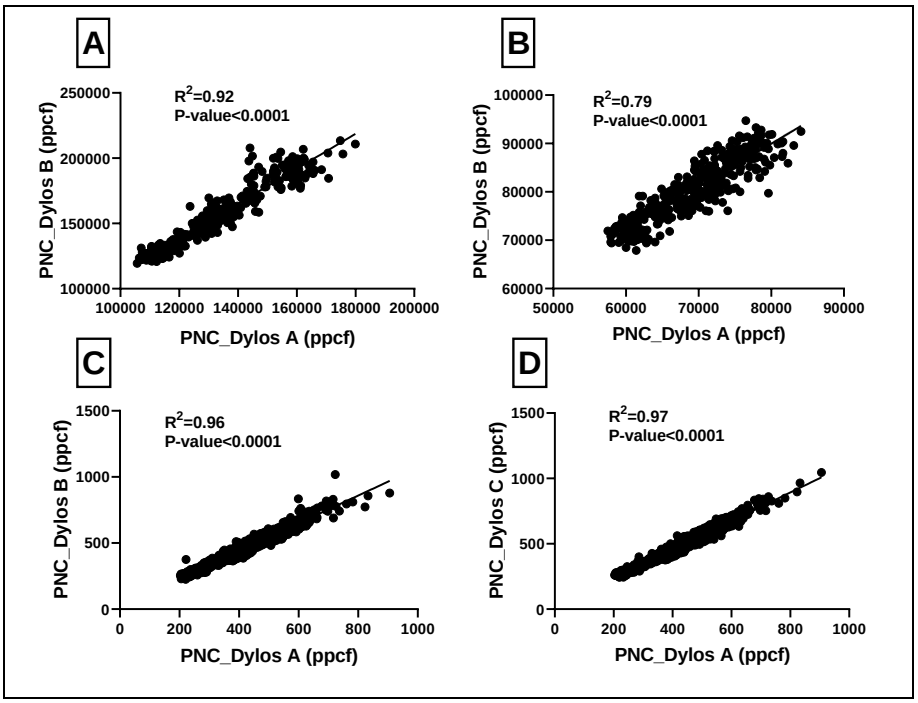


Figure 3.6: The agreement of PNC between different Dylos monitors.

Graphs show the association of PNC measured by the different monitors. The monitors were located in the same location, side-by-side, and the results were compared.

(Graph A and B) Dylos A and B were tested before study commenced in two residential homes. The PNC between Dylos A and B was strongly positive associated at both locations, with P-value<0.0001 (R^2=0.92 and 0.79, respectively) (Linear regression analysis, n=356mins and 343mins).

(Graph C and D) Dylos A, B and C were re-tested after study completed in the office building. The PNC between monitors (Dylos A & B) and (Dylos A & C) were strongly positive associated at P-value<0.001 (R^2=0.96 and 0.97, respectively) (Linear regression analysis, n=1440mins).

3.3.4 Calculated $PM_{2.5}$ mass concentration in the residential homes and comparison with the WHO air quality guideline

Table 3.2 shows the $PM_{2.5}$ level in the residential homes of general (pilot study) and pregnant women (prospective study) population, whereas Figure 3.7 compares indoor $PM_{2.5}$ level with the WHO standard limits for $PM_{2.5}$ exposure within 24-hour.

For the residential homes of a pilot study, the majority (10 out of 13) of homes recorded a median $PM_{2.5}$ level below than 20μg/m^3. PS02 recorded the lowest indoor $PM_{2.5}$ level at a median concentration of 3.74μg/m^3, with 3.68% of the sampling period exceeding 25μg/m^3. PS03 recorded the highest $PM_{2.5}$ level at a median concentration of 71.36μg/m^3, with 79.43% of the sampling period exceeding 25μg/m^3. PS10 and PS13 also recorded higher $PM_{2.5}$ levels with median concentrations of 45.70 and 29.39μg/m^3, respectively. PS10 recorded the second-highest $PM_{2.5}$ level, in which $PM_{2.5}$ level in this home exceeded 25μg/m^3 throughout the sampling period (100% >25μg/m^3, 24 hours). $PM_{2.5}$ levels in PS01 and PS06 did not exceed 25μg/m^3 at any time during the sampling (0% >25μg/m^3, 24 hours), with median concentrations of 8.64 and 8.04μg/m^3, respectively.

For the residential homes of the prospective study, the majority (17 out of 20) of homes recorded median $PM_{2.5}$ levels below 20μg/m^3. Eight homes recorded $PM_{2.5}$ levels below 25μg/m^3 throughout the sampling period (0% >25μg/m^3, 24 hours). MPS10 recorded the lowest $PM_{2.5}$ level with a median concentration of 3.77μg/m^3 and did not exceed 25μg/m^3 throughout sampling duration. MPS03 recorded the highest $PM_{2.5}$ level with a median concentration of 43.47μg/m^3 and had exceeded 25μg/m^3 for 92.99% of the sampling duration.

Table 3.2: Indoor $PM_{2.5}$ mass concentration in the residential homes across Greater Manchester

Study population	Home profile	Indoor $PM_{2.5}$ ($\mu g/m^3$)			
		N (minutes)	Median (IQR)	Min - Max	% Time >25$\mu g/m^3$
General population (Pilot study)	PS01	1441	8.64 (7.57 – 10.91)	3.98 - 18.59	0
	PS02	1441	3.74 (3.02 – 5.13)	2.18 - 54.56	3.68
	PS03	1434	71.36 (28.18 – 145.0)	10.70-256.70	79.43
	PS04	1441	8.75 (7.62 – 15.28)	6.76 - 93.58	14.23
	PS05	1441	10.74 (9.47 – 13.31)	6.35 - 52.51	7.36
	PS06	1441	8.04 (7.12 – 8.93)	4.88 - 22.09	0
	PS07	1438	7.36 (5.97 – 12.00)	3.65 - 135.40	9.46
	PS08	1441	9.95 (5.74 – 15.54)	3.44 - 116.10	8.40
	PS09	1254	12.23 (8.96 – 17.78)	3.95 - 29.52	2.39
	PS10	1441	45.70 (40.03 – 50.04)	27.88 - 143.20	100
	PS11	1441	9.20 (7.14 – 19.30)	3.07 - 100.20	17.90
	PS12	1441	14.72 (4.82 – 22.46)	2.36 - 49.70	7.84
	PS13	1441	29.39 (22.53 – 33.77)	6.69 - 54.60	69.74
Pregnant women (Prospective study)	MPS01	1414	17.63 (15.65 – 20.51)	12.87-35.92	5.94
	MPS02	1436	6.01 (5.52 – 8.58)	3.92-54.42	11.91
	MPS03	1441	43.47 (31.78 – 67.10)	8.96-120.1	92.99
	MPS04	1325	4.65 (3.19 – 5.46)	1.84-15.06	0
	MPS05	1285	6.59 (3.62 – 24.40)	2.09-246.5	24.67
	MPS06	1439	9.13 (3.64 – 9.61)	2.15-11.35	0
	MPS07	1441	10.22 (8.63 – 13.53)	5.08-67.86	6.80
	MPS08	374	11.35 (6.39 – 16.77)	5.30-89.32	11.76
	MPS09	1440	11.71 (5.92 – 23.26)	2.99-121.5	21.32
	MPS10	1441	3.77 (3.29 – 4.83)	2.15-16.31	0
	MPS11	1441	4.39 (3.60 – 4.79)	2.12-12.86	0
	MPS12	1441	4.91 (4.32 – 8.38)	3.28-11.49	0
	MPS13	1441	25.61 (14.09 – 30.39)	5.78-45.67	53.09
	MPS14	1362	25.24 (14.69 – 28.03)	6.81-53.01	51.10
	MPS15	1403	5.50 (5.03 – 6.53)	2.26-14.72	0
	MPS16	958	5.20 (3.75 – 5.95)	2.32-9.844	0
	MPS17	1441	4.72 (3.92 – 7.41)	2.70-13.83	0
	MPS18	1441	4.50 (2.51 – 6.12)	0.93-26.66	0.07
	MPS19	1441	9.29 (5.98 – 14.88)	2.76-76.61	16.86
	MPS20	1441	12.51 (3.50 – 23.24)	2.19-94.74	23.59

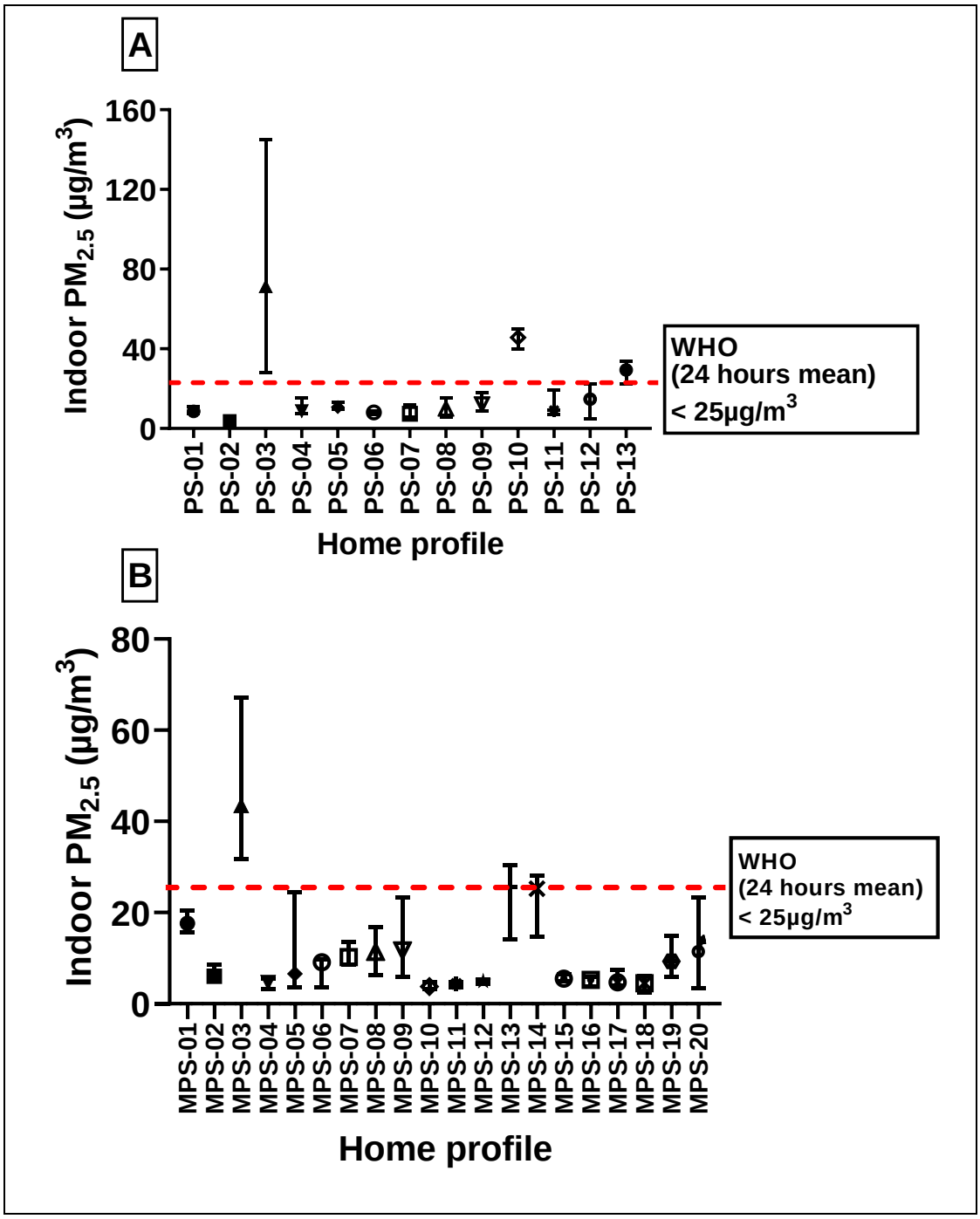


Figure 3.7: Calculated indoor $PM_{2.5}$ (µg/m^3) in the residential homes across Greater Manchester.

Graphs show $PM_{2.5}$ level in the residential homes of (A) general population (n=13) and (B) pregnant women population (n=20). Measurement of indoor air particles in 13 residential homes of general population was conducted between Junes – September 2017, whereas measurement in 20 pregnant women residential homes was conducted between Februarys – September 2019.

The WHO standard limit of $PM_{2.5}$ for 24 hours exposure is <25µg/m^3. The median concentrations of $PM_{2.5}$ in 3 residential homes of general population were exceeded the WHO standard limit, whereas only 1 residential home of pregnant women population were above the limit. Measurement of $PM_{2.5}$ levels were conducted for 24 hours. Data represented as median with IQR.

3.3.5 The temporal variation of indoor $PM_{2.5}$ level for 24 hours

Figure 3.8 shows the temporal variation of $PM_{2.5}$ level in a residential home at one-minute resolution for a 24-hour sampling period. MPS18 was selected to provide an example of the temporal variation of $PM_{2.5}$ level in the residential home of pregnant women across Greater Manchester. This home profile was selected due to complete information on indoor activities recorded throughout the sampling period. The temporal variation of all home profiles for pregnant women population is shown in Appendix 7.

The indoor $PM_{2.5}$ level was generally low in this home profile (MPS18) with a median concentration of 4.50μg/m^3, and $PM_{2.5}$ levels exceeded 25μg/m^3 for only 0.07% of the sampling duration. The measurement of $PM_{2.5}$ in this residential home was conducted for 24 hours (n=1441), started from 17:00 and ended at 17:00 on the next day. Based on Figure 3.8A, $PM_{2.5}$ levels during the day and night were markedly different, with $PM_{2.5}$ levels being higher in day time. The $PM_{2.5}$ level was low (<5μg/m^3) at the first hour of the sampling duration and dramatically increased from 18.00 to 20:00, with a peak level of 26.66μg/m^3. During this period, no information on cooking, smoking or window opening were recorded by the participant. In the following hours (~01:00), the $PM_{2.5}$ level was reduced to between 5 to 7μg/m^3 and was then stable during the night time. The lowest $PM_{2.5}$ level during the night was 0.93μg/m^3.

Figure 3.8B shows the temporal variation of $PM_{2.5}$, of the same home profile at a specific time between 00:00 until 17:00. Based on the graph, the $PM_{2.5}$ levels increased gradually at 06:00 and keep rising over time, until the end of the sampling period. The first cooking time between 08:00 to 08:15 resulted in a steep increase in $PM_{2.5}$ level, with a maximum concentration of 11.67μg/m^3. The $PM_{2.5}$ level was then quickly stabilised within several minutes at a concentration between 4 to 6μg/m^3. In the second cooking time, between 13:00 to 15:00, $PM_{2.5}$ levels increased with a maximum concentration of 8.21μg/m^3. The $PM_{2.5}$ level then slowly decreased until the end of the sampling period, and no marked increase was recorded at the third cooking time between 16.30 until 17:00.

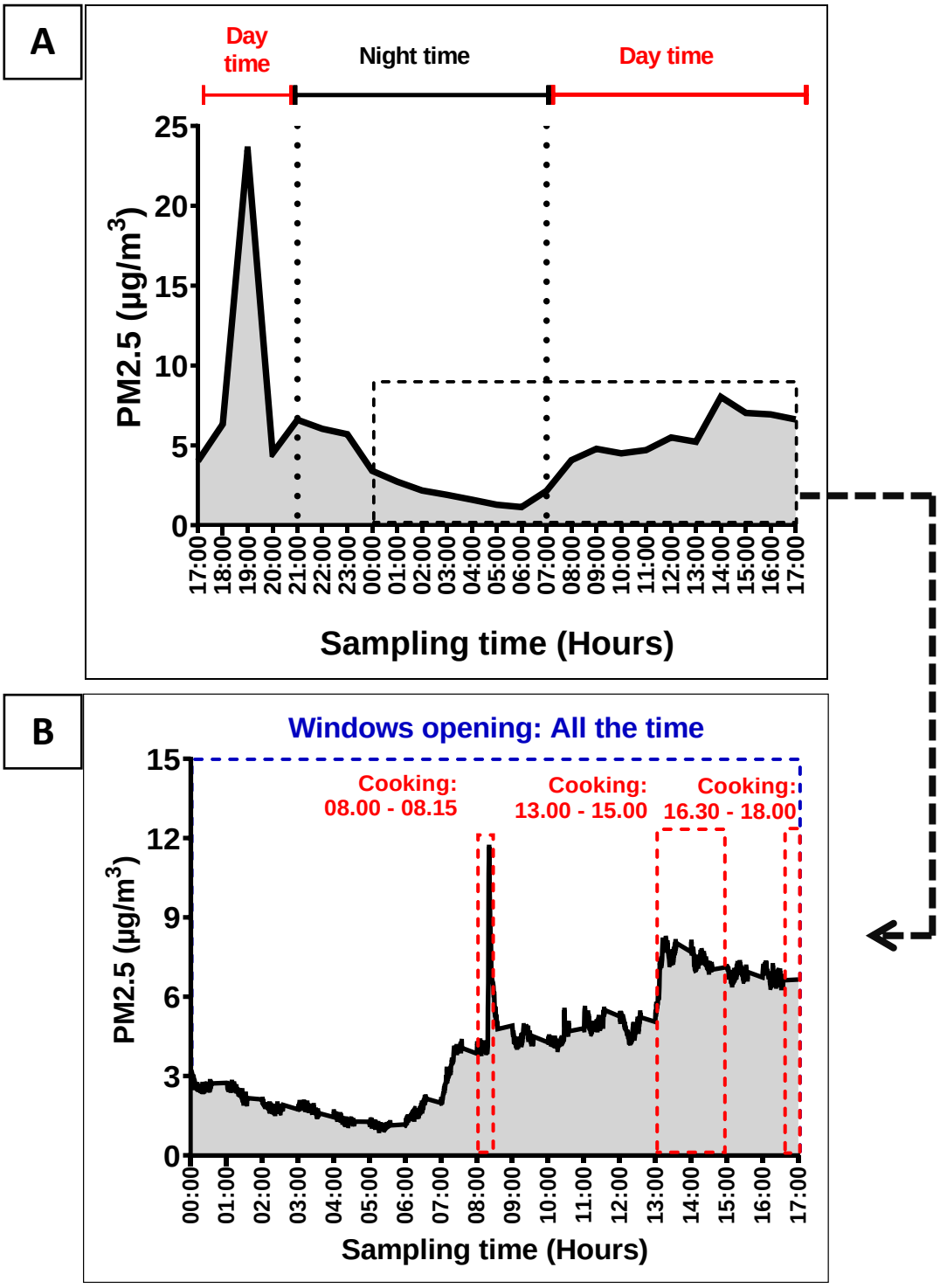


Figure 3.8: The temporal variation of $PM_{2.5}$ levels in the residential home of selected home profile (MPS18).

Panel A illustrates the temporal variation of $PM_{2.5}$ levels at one minute resolution for a 24-hour sampling period (17:00 day 1 to 17:00 day 2). $PM_{2.5}$ levels were higher in the first 3 hours of sampling (17:00 – 20.00), decreased in following hours and stable along the night time. Then, $PM_{2.5}$ levels demonstrated increment started from 06:00 until end of sampling period.

Panel B (expanded graph from Panel A) illustrates the temporal variation of $PM_{2.5}$ levels in a specific time period, from 00:00 until 17:00, for the same home profile. A marked increment in $PM_{2.5}$ level was recorded during cooking times. Windows in the living room were opened for the whole of sampling period and participant/home tenant did not smoke during sampling activity.

3.3.6 Association between indoor $PM_{2.5}$ level with maternal demographic factor and pregnancy outcomes

Table 3.3 shows the association between indoor $PM_{2.5}$ level with maternal demographic and pregnancy outcomes in the prospective study. The information on maternal demographics and pregnancy outcomes was taken either from the medical records (n=20) or the questionnaire (n=18). The level of indoor $PM_{2.5}$ was associated with the ethnicity group at P-value=0.02. The majority of participants were Caucasian (14 out of 20), and their median indoor $PM_{2.5}$ level was 9.76 (IQR: 3.78 – 19.53) $\mu g/m^3$, whereas the indoor $PM_{2.5}$ level in the non-Caucasian ethnic group (n=6) was lower with a median of 4.93 (4.47 – 7.05) $\mu g/m^3$.

3.3.7 Association between indoor $PM_{2.5}$ levels with home conditions

Table 3.4 shows the association between indoor $PM_{2.5}$ levels with home conditions, assessed by the questionnaire (n=18). The majority (13 out of 18) of participants had stayed at their current residential homes between one to five years, and most of them lived in semi-detached homes (7 out of 18). Most of the homes (8 out of 18) were built more than ten years ago, two homes were built less than ten years ago, and the rest of the participants did not give details on home ages. When asked about home renovation, two-thirds of the participants (12 out of 18) had renovated their home including wall painting (61.1%), ceiling repair (22.2%), floor repair (33.3%), window and door repair (38.9%), insulation repair (27.8%), wall construction (38.9%), heating and cooling system repair (38.9%) and also home extension (16.7%).

The main floor types of participant's home were wood (8 out of 18) or carpet (5 out of 18). The majority of participants (12 out of 18) had painted the walls of their homes, and three participants had either decorated the wall with wallpaper or used both paint and wallpaper. All participants used central heaters with radiators, except for one participant who used a wood-burning stove or fireplace. The majority of participants (16 out of 18) naturally ventilated their homes with doors and windows (double glazing). The majority of participants (10 out of 18) open windows when the weather permitted it only, and used curtains and blinds (16 out of 18) to cover the windows in the living room.

Out of 18 participants, 17 and 16 of them did not face any problems with water leakage and damp problems at their home, respectively. Fifteen participants did not report visible moulds at their home. The majority of participants (13 out of 18) cleaned their living

room once a week, by using a vacuum or hoover (18 out of 18), wet washing with a mop (10 out of 18), dry sweeping with a cloth (2 out of 18) or broom (3 out of 18). The majority of participants (15 out of 18) were took their shoes off inside homes.

Over half of the participants (10 out of 18) had a kitchen without a partition (open floor plan), used an electric stove (10 out of 18) and regularly used a kitchen hood when cooking (9 out of 18). Also, the majority of participants (13 out of 18) did not have pets inside the home, and the rest had reported having dogs, cat or rodent as their pets.

Only the usage of curtains and blinds in the living room was associated with indoor $PM_{2.5}$ level at P-value=0.03. The $PM_{2.5}$ level inside the homes that used curtains and blinds (n=16) was higher than that in homes without curtains and blinds in the living room (n=2), with median concentrations of 11.91 and 4.44 $\mu g/m^3$, respectively. Another factor of wearing shoes at home was borderline significant (P-value=0.06). The participants who took their shoes off inside the home (n=15) had higher indoor $PM_{2.5}$ levels compared to the participants who took their shoes off outside their home (n=2), with median indoor $PM_{2.5}$ levels of 12.46 and 4.69$\mu g/m^3$, respectively.

Table 3.3: Association between indoor $PM_{2.5}$ level with demographic and pregnancy outcomes

Variables	N (%)	Indoor $PM_{2.5}$	
		Median	P-value
Data taken from medical records (N=20,100%)			
Pregnancy outcomes[a]	20 (100)		0.69
Normal	14 (70)	9.35	
SGA	5 (25)	12.46	
LGA	1 (5)	17.01	
Ethnicity[b]	20 (100)		0.02*
Caucasian	14 (70)	14.70	
Non-Caucasian	6 (30)	4.86	
IMD[a]	20 (100)		0.55
1 (1 – 2)	9 (45)	11.35	
2 (3 – 4)	2 (10)	11.15	
3 (5 – 6)	4 (20)	16.59	
4 (7 – 8)	3 (15)	5.05	
5 (9 – 10)	2 (10)	5.71	
Smoking status[b]	20 (100)		0.08
Non-smoker	18 (90)	9.35	
Smoker	2 (10)	22.63	
Sampling season[b]	20 (100)		0.34
Spring	15 (75)	12.46	
Summer	5 (25)	5.53	
Data taken from questionnaire (N=18, 100%)			
Previously smoking[b]	18 (100)		0.73
No	13 (72.2)	5.89	
Yes	5 (27.8)	11.35	
Working status[a]	18 (100)		0.13
Unemployed	1 (5.6)	23.36	
Employed	16 (88.9)	9.35	
Prefer not to answer	1 (5.6)	4.37	
Hours at work[a]	18 (100)		0.21
< 20 hours	1 (5.6)	51.17	
21 – 30 hours	5 (27.8)	11.35	
31 – 40 hours	7 (38.9)	5.53	
> 41 hours	3 (16.7)	14.67	
Not working	1 (5.6)	23.36	
Prefer not to answer	1 (5.6)	4.37	

Pregnancy outcomes determined by IBR percentile; normal (10-90%), SGA (<10%) and LGA (>90%)

[a]Kruskal Wallis Test; [b]Mann-Whitney U Test (*Significant different at P-value<0.05)

Table 3.4: Association between indoor $PM_{2.5}$ levels with home conditions

Variables	N=18 (100%)	Indoor $PM_{2.5}$	
		Median	P-value
Duration living at current home[a]	18 (100)		0.23
< 1 year	2 (11.1)	32.95	
1 – 5 years	13 (72.2)	5.89	
6 – 10 years	3 (16.7)	7.34	
Home type[a]	18 (100)		0.61
Flat/apartment	5 (27.8)	5.00	
Terraced	5 (27.8)	12.46	
Semi-detached	7 (38.9)	5.89	
Detached	1 (5.6)	18.44	
Home age[a]	17 (94.4)		0.74
< 10 years	2 (11.1)	15.13	
> 10 years	8 (44.4)	13.01	
Do not know	7 (38.9)	5.05	
Home renovation[b]	17 (94.4)		0.96
No	5 (27.8)	12.46	
Yes	12 (66.7)	9.35	
Type of renovation undertaken			
Wall painting/new wallpaper	11 (61.1)		
Ceiling repair	4 (22.2)		
Floor repair	6 (33.3)		
Water or sewage system repair	0 (0)		
Window or door repair	7 (38.9)		
Insulation repair	5 (27.8)		
Wall construction	7 (38.9)		
Heating and cooling system	7 (38.9)		
Home extension	3 (16.7)		
Floor type[a]	18 (100)		0.58
Wood	8 (44.4)	6.62	
Concrete	1 (5.6)	11.35	
Stone and tile	3 (16.7)	14.67	
Linoleum	1 (5.6)	51.17	
Carpet	5 (27.8)	5.53	
Wall type[a]	18 (100)		0.46
Paint	12 (66.7)	13.57	
Wallpaper	3 (16.7)	5.53	
Paint and wallpaper	3 (16.7)	5.89	
Heating system[b]	17 (94.4)		0.41
Central heaters with radiators	16 (88.9)	6.62	
Wood burning stoves/fireplaces	1 (5.6)	17.01	
Home ventilation[a]	18 (100)		0.16
Natural ventilation	16 (88.9)	11.91	
Mechanical ventilation	0 (0)	0	
Both	1 (5.6)	5.00	
Do not know	1 (5.6)	4.37	

Frequency of opening windows[a]	18 (100)		0.34
Almost never	2 (11.1)	11.27	
Yes, occasionally	1 (5.6)	14.73	
Yes, when the weather was good	10 (55.6)	11.91	
Yes, always open	5 (27.8)	4.72	
Glazing type[b]	17 (94.4)		
Single	0 (0)		
Double	17 (94.4)		
Curtain and blind[b]	18 (100)		0.03*
No	2 (11.1)	4.44	
Yes	16 (88.9)	11.91	
Water leakage at home[b]	18 (100)		0.10
No	17 (94.4)	11.35	
Do not know	1 (5.6)	4.37	
Damp problem at home[b]	18 (100)		0.78
No	16 (88.9)	9.35	
Yes	2 (11.1)	13.87	
Moulds at home[b]	17 (94.4)		0.18
No	15 (83.3)	7.34	
Yes	2 (11.1)	19.05	
Home cleaning frequency[a]	18 (100)		0.82
Once a week	13 (72.2)	7.34	
Once a month	4 (22.2)	9.73	
Once every few months	1 (5.6)	14.67	
Method of cleaning			
Vacuum or hoover	18 (100)		
Wet washing with a mop	10 (55.6)		
Dry sweeping with a cloth	2 (11.1)		
Dry sweeping with a broom	3 (16.7)		
Wearing shoes at home[a]	18 (100)		0.06
Taken off outside the home	2 (11.1)	4.69	
Taken off inside the home	15 (83.3)	12.46	
Not taken off	1 (5.6)	4.50	
Kitchen type[a]	18 (100)		0.97
With partition	8 (44.4)	11.91	
Without partition	10 (55.6)	6.62	
Stove type[b]	18 (100)		0.66
Gas	8 (44.4)	13.01	
Electric	10 (55.6)	6.62	
Kitchen hood[a]	18 (100)		0.89
No	4 (22.2)	11.01	
Yes, but never used/seldom	2 (11.1)	9.55	
Yes, sometimes	3 (16.7)	11.35	
Yes, regularly	9 (50.0)	5.89	
Pets[b]	18 (100)		0.77
No	13 (72.2)	7.34	
Yes	5 (27.8)	11.35	

[a]Kruskal Wallis Test; [b]Mann-Whitney U Test (*Significant different at P-value<0.05)

3.3.8 Estimation of outdoor $PM_{2.5}$ level based on air pollution monitoring station

3.3.8.1 Location of participant's residential homes and air pollution monitoring stations

Figure 3.9 presents the location of the participant's residential homes and air pollution monitoring stations selected in this study. The participants lived in five different postcode areas ranged from Manchester (M), Oldham (OL), Stockport (SK), Bolton (BL) and Warrington (WA). Two monitoring stations were assigned to the participant's residential homes named as Manchester Piccadilly (MP) and Salford Eccles (SE). All the residential homes of the general population and 65% of pregnant women population were assigned to the MP monitoring station, with a distance between 0.54 to 23.77 kilometres (km) from the monitoring station (DEFRA, 2019). The rest of the residential homes (7 out of 20) were assigned to the SE station with a distance between 1.32 to 13.78km from the monitoring station (DEFRA, 2019).

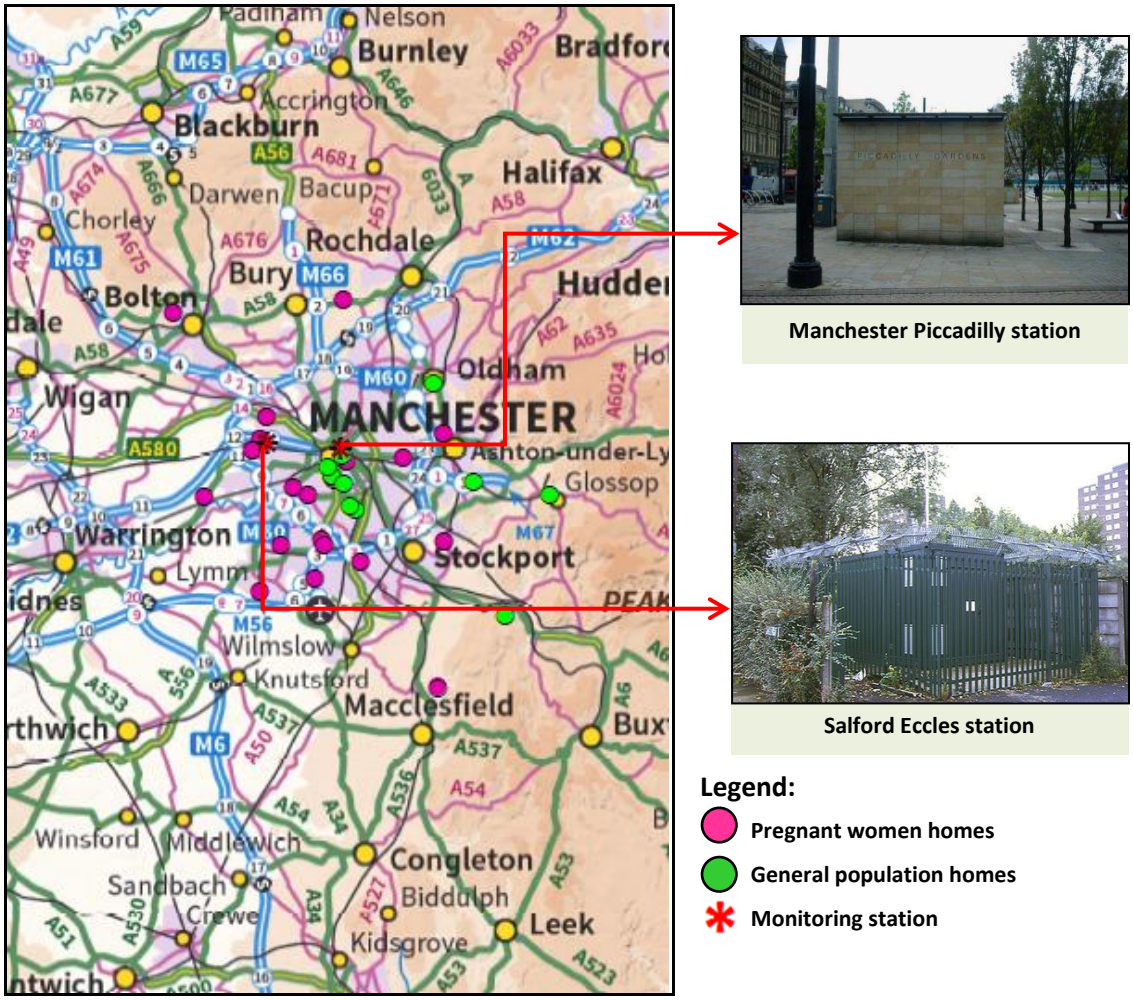


Figure 3.9: Location of participant's residential homes and selected air pollution monitoring stations.

The map shows the location of participant's residential homes of general population (green dots), pregnant women residential homes (purple dots) and monitoring stations (red stars). The outdoor $PM_{2.5}$ level was estimated based on 2 monitoring stations; MP and SE, with the distance from monitoring stations to the residential homes within 0.54 – 23.8km and 1.32 – 13.78km, respectively.

3.3.8.2 Estimation of outdoor $PM_{2.5}$ levels during indoor air sampling

Table 3.5 shows the estimation of the outdoor $PM_{2.5}$ levels at the residential areas of both populations, whereas Figure 3.10 compares outdoor $PM_{2.5}$ levels with the WHO air quality guidelines. The outdoor $PM_{2.5}$ levels were estimated for 24 hours, simultaneously with indoor air particles sampling in the residential homes.

For the pilot study, the median outdoor $PM_{2.5}$ levels in the majority of homes (12 out of 13) were below 20µg/m^3. In one house only (PS10) did the outdoor $PM_{2.5}$ level exceeded the WHO standard limits, with a median concentration of 26.00µg/m^3 and this limit was exceeded for 68% of the total sampling duration (24-hours). PS02 recorded the lowest outdoor $PM_{2.5}$ level with a median concentration of 2.00µg/m^3 and did not exceed 25µg/m^3 at any point the sampling.

For the prospective study, the outdoor $PM_{2.5}$ level at all residential home areas was below 25µg/m^3, except for MPS01. In this home, the median concentration was 46.00µg/$m^{3,}$ and the level exceeded 25µg/m^3 throughout the whole 24-hour sampling period. Homes MPS02, MPS13, MPS15 and MSP16 had low outdoor $PM_{2.5}$ levels with a median concentration of 5.00µg/m^3 for 24 hours, respectively.

3.3.8.3 Estimation of outdoor $PM_{2.5}$ in every trimester window

Table 3.6 shows the estimation of the outdoor $PM_{2.5}$ level in every trimester window for the prospective study, whereas Figure 3.11 shows the temporal variation of outdoor $PM_{2.5}$ during pregnancy, obtained from both monitoring stations. The outdoor $PM_{2.5}$ in the first trimester was at the lowest compared than other trimester levels. The median concentration of outdoor $PM_{2.5}$ in the first, second and third trimester was 9.59, 13.18 and 11.34µg/m^3, respectively. The outdoor $PM_{2.5}$ level in the first trimester was significantly correlated with second and third trimester at P-value<0.0001 and 0.0003, respectively (Spearman-rank correlation test, n=20, data not shown).

Table 3.6: Estimation of outdoor $PM_{2.5}$ in every trimester window (µg/m^3)

Trimester windows*	Median (IQR)	Min- Max
1st trimester	9.59 (8.83 - 10.81)	5.42 – 11.64
2nd trimester	13.18 (11.38 - 15.47)	9.94 – 16.22
3rd trimester	11.34 (9.81 - 14.07)	6.23 – 16.01

*1st trimester (1 – 13 weeks); 2nd trimester (14 – 27 weeks); 3rd trimester (28th weeks until birth)

Table 3.5: Estimation of outdoor $PM_{2.5}$ levels during indoor air sampling

Study population	Home profile	Outdoor $PM_{2.5}$ ($\mu g/m^3$)		
		Median (IQR)	Min - Max	% Time >25 $\mu g/m^3$
General population (Pilot study)	PS 01	17.00 (15.00 – 18.00)	13.00-19.00	0
	PS 02	2.00 (2.00 – 3.00)	1.00-3.00	0
	PS 03	5.00 (4.00 -5.00)	4.00-5.00	0
	PS 04	5.00 (5.00 -6.00)	5.00-6.00	0
	PS 05	8.00 (7.00 – 9.00)	7.00-10.00	0
	PS 06	8.00 (7.00 – 9.50)	7.00-10.00	0
	PS 07	10.00 (10.00 – 11.50)	8.00-12.00	0
	PS 08	11.00 (10.00 – 13.00)	10.00-14.00	0
	PS 09	11.00 (9.00 – 12.00)	9.00-12.00	0
	PS 10	26.00 (25.00 – 29.00)	25.00-30.00	68
	PS 11	7.00 (7.00 – 7.00)	6.00-7.00	0
	PS 12	5.00 (5.00 – 6.00)	4.00-7.00	0
	PS 13	7.00 (5.00 – 11.50)	4.00-15.00	0
Pregnant women (Prospective study)	MPS 01	46.00 (40.50 – 50.50)	37.00-53.00	100
	MPS 02	5.00 (5.00 – 6.00)	4.00-7.00	0
	MPS 03	7.00 (5.50 – 7.00)	5.00-7.00	0
	MPS 04	9.00 (9.00 – 9.00)	9.00-10.00	0
	MPS 05	9.00 (9.00 – 9.00)	8.00-10.00	0
	MPS 06	14.00 (11.50 – 14.50)	10.00-15.00	0
	MPS 07	14.00 (9.50 – 17.00)	9.00-19.00	0
	MPS 08	7.00 (5.00 – 10.00)	5.00-12.00	0
	MPS 09	10.00 (6.00 – 13.00)	5.00-13.00	0
	MPS 10	22.00 (16.00 – 28.00)	10.00-33.00	32
	MPS 11	22.00 (16.00 – 28.00)	10.00-33.00	32
	MPS 12	10.00 (9.00 – 14.50)	7.00-17.00	0
	MPS 13	5.00 (5.00 – 6.00)	4.00-8.00	0
	MPS 14	5.50 (4.00 – 8.75)	4.00-11.00	0
	MPS 15	5.00 (5.00 – 6.00)	4.00-6.00	0
	MPS 16	5.00 (4.00 – 5.00)	4.00-6.00	0
	MPS 17	7.00 (7.00 – 8.00)	7.00-10.00	0
	MPS 18	8.00 (7.00 – 17.50)	7.00-22.00	0
	MPS 19	7.00 (6.00 – 8.00)	6.00-9.00	0
	MPS 20	25.00 (22.00 – 27.50)	20.00-29.00	44

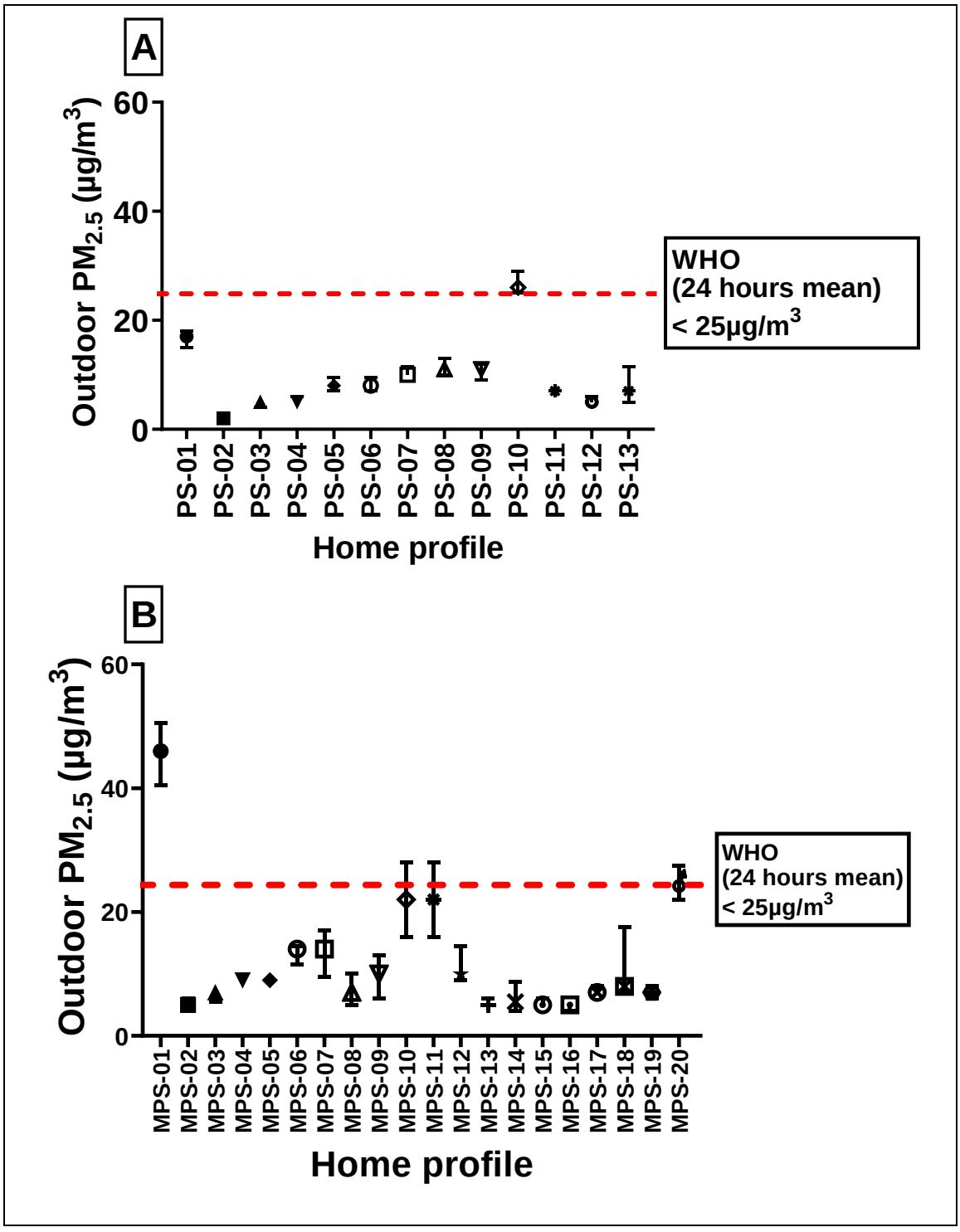
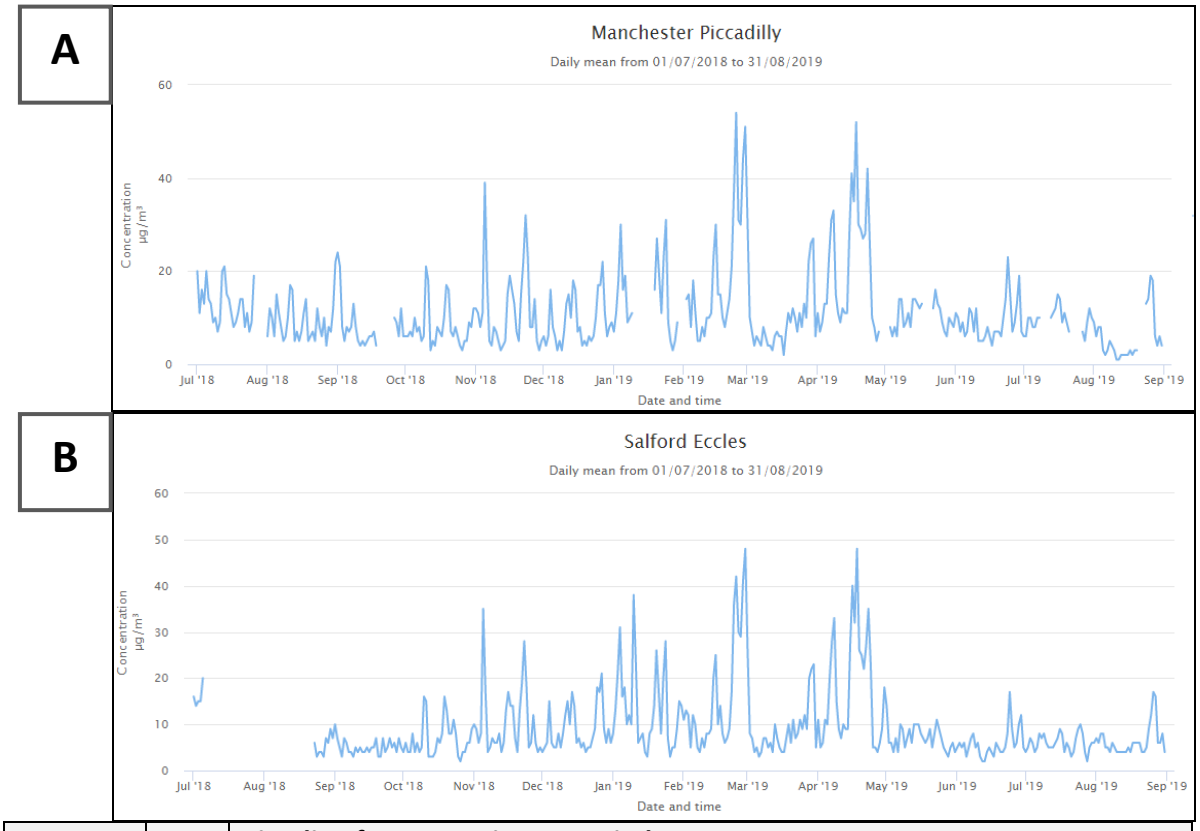


Figure 3.10: Level of outdoor $PM_{2.5}$ (µg/m^3) of the residential homes across Greater Manchester.

Graphs show outdoor $PM_{2.5}$ levels of the residential homes of (Panel A) general and (Panel B) pregnant women population. Level of outdoor $PM_{2.5}$ was referred to air pollution monitoring stations; Manchester Piccadilly and Salford Eccles. $PM_{2.5}$ level was estimated for 24-hour,simultaneously with the measurement of indoor air particles. Data represented as median with IQR.

The WHO standard limit for $PM_{2.5}$ in 24-hour exposure is <25µg/m^3. The outdoor $PM_{2.5}$ level of PS10 and MPS01 was exceeded the WHO standard limit, at median concentrations of 26.00 and 46.00µg/m^3, respectively.



ID	MS	Timeline for every trimester windows
MPS01	SE	
MPS02	MP	
MPS03	MP	
MPS04	MP	
MPS05	MP	
MPS06	MP	
MPS07	MP	
MPS08	SE	
MPS09	SE	
MPS10	MP	
MPS11	MP	
MPS12	MP	
MPS13	SE	
MPS14	SE	
MPS15	SE	
MPS16	SE	
MPS17	MP	
MPS018	MP	
MPS019	MP	
MPS20	MP	

Figure 3.11: Outdoor $PM_{2.5}$ level estimated from Manchester Piccadilly and Salford Eccles monitoring station during pregnancy (Adapted from DEFRA, 2019).

Figure shows the temporal variation of outdoor $PM_{2.5}$ level in every trimester window, started from July 2018 until August 2019. Outdoor PM2.5 levels were estimated from 2 monitoring stations (MS); Manchester Piccadilly (Panel A) and Salford Eccles (Panel B). Arrows indicate trimester windows; first trimester (green arrow), second trimester (blue arrow) and third trimester (red arrow).

3.3.9 Comparison of $PM_{2.5}$ level in the residential homes and outdoor environment

A linear regression analysis was performed to determine the relationship between indoor and outdoor $PM_{2.5}$ during the sampling period. The median concentration of indoor and outdoor $PM_{2.5}$ level was not associated either in the general population (R^2=0.09; P-value=0.33) or in the pregnant women population (R^2=0.0003; P-value=0.94) (data not shown).

3.3.9.1 Indoor and outdoor ratio (I/O ratio) of $PM_{2.5}$ level

Table 3.7 presents the I/O ratio of $PM_{2.5}$ level in both study populations. For the pilot study, only PS01 recorded an I/O ratio <1 (i.e. 0.57). PS03 recorded the highest I/O ratio at 19.13. The I/O ratio of all home profiles in the pilot study population had a median level of 1.78 (IQR: 1.15 - 2.65).

For the prospective study, the I/O ratio had a median level of 0.99 (IQR: 0.46 – 2.12). Half of the home profiles (10 out of 20) had an I/O ratio of <1. MPS11 had the lowest I/O ratio at 0.20, whereas MPS03 had the highest I/O ratio at 8.15.

Table 3.7: The I/O ratio of $PM_{2.5}$ level

Pilot study	I/O ratio	Prospective study	I/O ratio
PS 01	0.57	MPS 01	0.40
PS 02	2.55	MPS 02	2.13
PS 03	19.13	MPS 03	8.15
PS 04	2.62	MPS 04	0.52
PS 05	1.54	MPS 05	2.79
PS 06	1.02	MPS 06	0.56
PS 07	1.18	MPS 07	0.92
PS 08	1.13	MPS 08	1.94
PS 09	1.22	MPS 09	1.57
PS 10	1.78	MPS 10	0.21
PS 11	2.32	MPS 11	0.20
PS 12	2.70	MPS 12	0.44
PS 13	3.67	MPS 13	4.26
		MPS 14	3.50
		MPS 15	1.14
		MPS 16	1.07
		MPS 17	0.73
		MPS 18	0.39
		MPS 19	2.10
		MPS 20	0.69

3.4 Discussion

3.4.1 Principal findings

This study has evaluated the feasibility of measuring indoor air particles in the residential homes of pregnant women across Greater Manchester, by using a low-cost DYLOS DC1700 air particle monitor, together with the collection of biological samples from pregnant women, before and after the delivery. Out of 50 invited participants in Manchester Placental Clinic between Februarys until September 2019, 20 participants were successfully recruited in this study. These participants (n=20) were successfully involved in indoor air particle sampling in their residential homes and donated the pre-delivery maternal blood. However, only six placentas from these participants were collected after delivery, whereas no cord blood was successfully collected in this study. The most prominent finding to emerge from this study is that the majority of residential homes across Greater Manchester had indoor $PM_{2.5}$ levels below 20μg/m^3. The estimation of outdoor $PM_{2.5}$ level during the sampling period, determined by air pollution monitoring stations, showed that most areas in Greater Manchester had low outdoor $PM_{2.5}$ levels (<20μg/m^3). Maternal ethnicity was associated with indoor $PM_{2.5}$ levels, in which the residential homes of Caucasian had higher $PM_{2.5}$ levels compared to non-Caucasian. Having curtains or blinds was significantly associated with $PM_{2.5}$ levels inside the home. Also, cooking activity markedly increased indoor $PM_{2.5}$ levels in several residential homes.

3.4.2 Indoor $PM_{2.5}$ level in the residential areas across Greater Manchester

The air particle levels in residential homes in the present study were measured by using a Dylos DC1700 which is an upgraded version of the previous DC1100 model. The difference between both models is only in terms of the power supply, for which the Dylos DC1100 was battery operated, whereas Dylos DC1700 can be operated by using battery or power cable (Air Quality Sensor Performance Evaluation Center, n.d.). Previous studies (Table 3.8) demonstrated that Dylos could be used to measure air particles in various settings included residential homes, hospitality venues, child care centres, outdoor environments as well as having been used as personal monitors. The instrument provides data in terms of particle number concentration (PNC) of both small and large particles. The small particles referred to particles detected down to the detection limit of 0.5μm, whereas large particles referred to particles detected above the 2.5μm threshold. To compare air particle levels with local and global air quality guidelines, the PNC data has to be converted

into particle mass concentration (PMC). This conversion is also important as most air quality studies describe air particles exposures in terms of PMC (Franken et al., 2019). In corresponding with this, the present study converted the PNC data to PMC, by using Equation 3.1. This equation is the most suitable for the measurement of indoor air particles in the living room and showed the highest correlation with reference device of gravimetric method (Franken et al., 2019).

Table 3.8: Used of Dylos monitors in reported literature from different countries

Dylos model	Country	Sampling location	References
DC1100	Connecticut, USA	Residential homes	Brown et al., 2014
DC1100	California, USA	Residential homes	Dacunto et al., 2015
DC1100	California, USA	Child care centre, home-based facility	Gaspar et al., 2018
DC1700	Scotland, UK	Personal monitoring (Indoor and outdoor environment)	Steinle et al., 2015
DC1700	Scotland, UK	Residential homes	Semple et al., 2015; Semple & Latif, 2014
DC1700	Mexico, Chad, Bangladesh, India, Indonesia and Pakistan	Hospitality venues (bars, restaurants, cafes and hotels)	Jackson-Morris et al., 2016
DC1700	USA	Outdoor environment	Han et al., 2017
DC1700	Mongolia	Residential homes	Lim et al., 2018
DC1700	Netherlands, UK and Greece	Residential home	Franken et al., 2019

It was anticipated that variability of indoor air particles might be influenced by the outdoor environment, socio-demographic factors and indoor activities of home tenants. Median $PM_{2.5}$ levels were 11.35µg/m^3 and hence was approximately half that observed by Gee et al. (2002), which reported that the median level of $PM_{2.5}$ in 69 homes in Wythenshawe, Manchester was 22.6µg/m^3. This difference may be due to the higher number of smokers (n=12) in the earlier study. In terms of socio-demographic factors, Wythenshawe is near to the airport and this factor could contribute to the increase of $PM_{2.5}$ level in the outdoor environment, and hence potentially influence the $PM_{2.5}$ level in the residential home (Hudda et al., 2016). The median level of indoor $PM_{2.5}$ of the present study was comparable with the study conducted in Edinburgh, Scotland, for which the mean indoor $PM_{2.5}$ for 35 houses and private residential building, measured with Dylos DC1700, was at 8.4 and 10.2µg/m^3, respectively (Steinle et al., 2015).

In developing countries, median levels of indoor $PM_{2.5}$ are higher than those to the present study. As study conducted by Jackson-Morris et al. (2016) had measured $PM_{2.5}$ levels using Dylos DC1700 and found that median indoor $PM_{2.5}$ levels in Bangladesh, Pakistan and Chad were 119, 69 and 51μg/m^3, respectively and hence, exceeded the WHO standard limit for $PM_{2.5}$ exposure. The higher $PM_{2.5}$ level in study conducted by Jackson-Morris et al. (2016) might be due to the measurement being conducted in bars, restaurants, cafes and hotels, which may have higher levels due to several contributing factors such as smoking, cooking and ventilation. Also, particle penetration from outdoors to the indoor environment may be a reason for higher indoor $PM_{2.5}$ level in the developing countries. The outdoor $PM_{2.5}$ level in Bangladesh, Pakistan and Chad during the sampling period was 146, 34 and 34μg/m^3, respectively, and these levels are far higher than those in the present study. Hence, the internal and external factors described could be a strong reason for higher indoor $PM_{2.5}$ recorded in the previous study by Jackson-Morris et al. (2016).

Home conditions were assessed by using a questionnaire among the pregnant women population (n=18). The use of curtains and blinds in the living room was associated with significantly increased indoor $PM_{2.5}$ levels at P-value=0.03. To date, there was limited study discussed the relationship between having a curtain or blind at home with the level of indoor air pollutants, specifically $PM_{2.5}$. The use of curtains is associated with increases in microbiological contaminants inside the home, such as mould and fungi, viruses, bacteria, algae pollen and spores (WHO, 1990). However, this data is contradicted by the finding of this study, as the majority of participants in this study (16 out of 18) used curtains and blinds at home, but did not report any problem with moulds (15 out of 17) or damp (16 out of 18). The small number of sample size and imbalance number of participants between characteristics observed may reduce the validity of results; hence further research is required to investigate this relationship by increasing the number of sample size.

Another factor of “taken off shoes inside the home” was borderline significant with indoor $PM_{2.5}$ level (P-value=0.06). A previous study conducted by Harnly et al. (2009) reported that house dust containing pesticides, as analysed with GC/MS, were detected higher in a farmer who kept their shoes on inside the home. Also, a study conducted by Tagiyeva (2012) found the wheat flour allergen (WFA) and fungal α-amylase (FAA) from the workplace were found inside the shoes and home of the bakers, confirming that particles from the outdoor environment could become attached to the shoe surface, and be spread indoors. According to Sippola (2014), particles sized 5 to 10μm were easily attached to shoes, and could be deposited deep in carpet fibres and held by higher electrostatic charge.

Hence, this might be a reason for higher $PM_{2.5}$ level in the residential homes of the participants that took their shoes off inside the home. The removal of shoes before entering the home is the ideal method to reduce the levels of outdoor pollutants inside the home.

3.4.3 Outdoor $PM_{2.5}$ level at the residential areas across Greater Manchester

The infiltration of outdoor air particles into the indoor environment, through cracks of home/building structure, was hypothesized as an important factor in the elevation of indoor particle levels inside the home (Chen & Zhao, 2011; Meng et al., 2005; Jones et al., 2000; Byrne, 1998). However, the present study did not find a significant association between indoor and outdoor $PM_{2.5}$ levels. This result may imply that the concentration of outdoor $PM_{2.5}$ in Greater Manchester was low, at the level may not have a marked influence on indoor air quality, and suggested that the variation of the $PM_{2.5}$ level inside the homes may closely be related to the indoor sources rather than the outdoor environment. However, the inability to determine the association between indoor and outdoor $PM_{2.5}$ levels may due to the methodology used for the estimation of outdoor $PM_{2.5}$ levels. The present study only applied a crude analysis by obtaining the outdoor $PM_{2.5}$ levels from the nearest air pollution monitoring station, rather than the use of air pollution estimation model that provides a robust spatial and temporal air pollution prediction such as the spatio-temporal or land use regression model (Madsen et al., 2019; Smith et al., 2017; Hannam et al., 2014).

The level of outdoor and indoor $PM_{2.5}$ in the residential homes of the study participants (n=33) were summarised and shown in Figure 3.12. The concentrations of $PM_{2.5}$ in the residential homes were higher compared to the outdoor environment, with median concentrations of 11.35 and 8.40µg/m^3, respectively. The majority of areas (28 out of 33) in Greater Manchester recorded outdoor $PM_{2.5}$ levels below 20µg/m^3. This level was in agreement with Jackson-Morris et al. (2016), which reported that outdoor $PM_{2.5}$ levels in developed countries of the UK, US and Australia were usually lower than 20µg/m^3. As reported by the Air Quality Expert Group (2012), $PM_{2.5}$ levels in the UK are generally higher during the winter due to use of residential heating, especially in the morning and late evening.

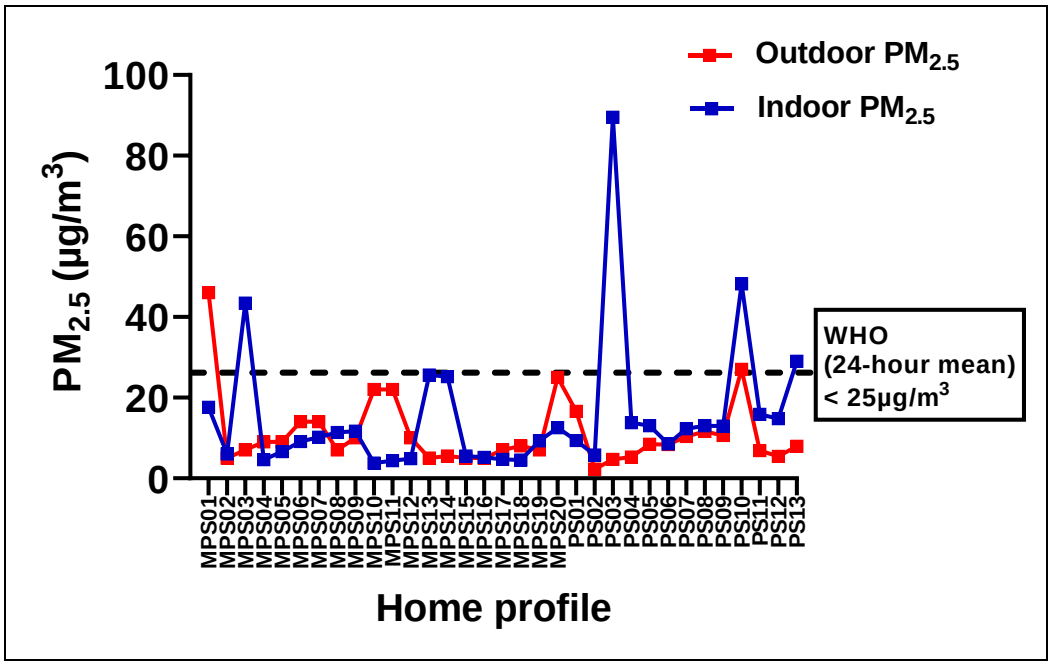


Figure 3.12: Outdoor and indoor $PM_{2.5}$ levels in Greater Manchester areas for 24-hour.

The blue line shows the median level of indoor $PM_{2.5}$ in 33 residential homes across Greater Manchester areas, whereas red line indicates the ambient (outdoor) $PM_{2.5}$ level at the residential areas. Most of the homes recorded indoor and outdoor $PM_{2.5}$ level <25µg/m^3 (WHO standard limit).

The measurement of indoor $PM_{2.5}$ level was conducted by using Dylos air particle monitor, whereas outdoor $PM_{2.5}$ levels estimated from air pollution monitoring stations.

Legend: MPS=Prospective study; PS=Pilot study

A high level of outdoor $PM_{2.5}$ level was recorded in MPS01, with the median concentration at 46µg/m^3 for 24-hour (Figure 3.12). This level was very high compared to other home profiles and exceeded the WHO standard limit. The outdoor $PM_{2.5}$ level of MPS01 was estimated by referring to the Salford Eccles monitoring station on 27th – 28th February 2019. By referring to the temporal variation of outdoor $PM_{2.5}$ level in Figure 3.11, the $PM_{2.5}$ level was generally higher in February. However, this level was spiked on the 27th of February 2019 due to a Saharan dust cloud at that time which increased the pollution level in the UK. This condition also was worsened by the pollution level and light wind condition during that period (Manchester Evening News, 2019).

The UK Daily Air Quality Index (DAQI) (as explained in Section 1.1.4.1) was referred to further investigate the level of outdoor $PM_{2.5}$ in the study population. The DAQI provides banding categories of daily air pollution level and recommends actions that need to be taken by the individuals who are exposed to a certain level of pollutants. The result in the present study shows that the outdoor $PM_{2.5}$ level at the residential areas of all study population was considered “low”, between banding 1 to 3. Only the exposure of outdoor

$PM_{2.5}$ level for MPS01 was classified as “moderate” at DAQI banding number 5. The maximum concentration of outdoor $PM_{2.5}$ in this home profile was 53μg/m^3, which is considered as a borderline to “high” banding level. The exposure to the “moderate” level of outdoor $PM_{2.5}$ may not affect healthy individuals, but attention must be given to the susceptible groups. In this context, at-risk groups refer to individuals who are diagnosed or have symptoms caused by lung or heart problems, in which they should minimize their outdoor activities and limit the exposure to the outdoor environment.

3.4.4 Indoor and outdoor (I/O) ratio and sources of indoor pollution

Many previous studies have used the I/O ratio, to determine the relationship between indoor and outdoor pollutants level (Bo et al., 2017; Radaideh & Shatnawi, 2015; Chen & Zhao, 2011; Jones et al., 2000). This ratio may indicate the sources of pollutants, either generated in the indoor environment or derived externally from the outdoor environment. An I/O ratio less than or equal 1 indicates an excellent indoor environment which minimum sources of indoor air pollutants exist (Jones et al., 2000). The median I/O ratio for the prospective study was 0.99, but was higher at a median level of 1.78 in the pilot study, with only one residential home having an I/O ratio <1. The increase in the I/O ratio in the pilot study was due to the higher indoor $PM_{2.5}$ level recorded at the home profile PS03.

The temporal variation of indoor $PM_{2.5}$ level in PS03 was further investigated and the $PM_{2.5}$ level for 17-hours sampling period (starting from 00:00 until 17:00) is shown in Figure 3.13. Based on a sampling diary, the participants did not open the windows during the sampling duration, did not smoking inside the home and cooked on two occasions. The increase in $PM_{2.5}$ levels of PS03 was due to the cooking activity (Figure 3.12). However, even without cooking activity, the indoor $PM_{2.5}$ level was high, with levels exceeding 25μg/m^3 at almost 80% of the time. In 24-hour measurement, PS03 recorded indoor $PM_{2.5}$ level at a median concentration of 71.36μg/m^3, whereas the outdoor $PM_{2.5}$ level was only at a median level of 5.00μg/m^3. The significant difference between $PM_{2.5}$ levels in both environments leads to an I/O ratio of 19.1. Based on observation, PS03 is an apartment unit located on the ground floor, closed to the main road (<100metres) and construction site (<200m) and the kitchen was designed without a partition to the living room. These demographic factors and building/home design may contribute to the level of indoor $PM_{2.5}$ in this home profile.

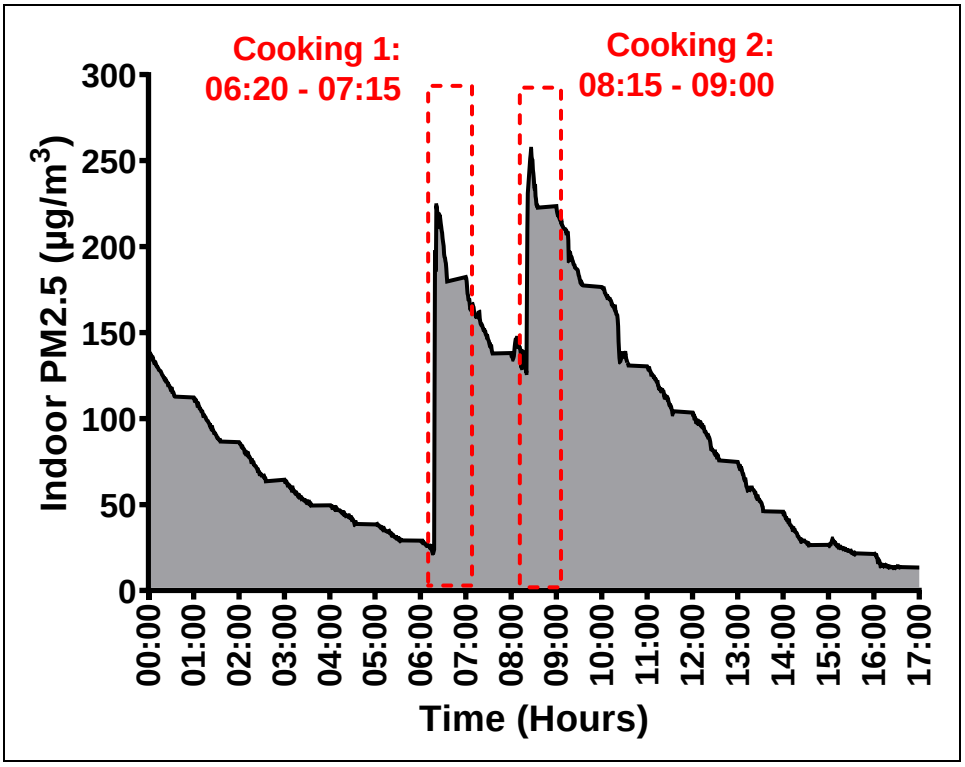


Figure 3.13: The temporal variation of $PM_{2.5}$ levels in the residential home of PS03. Graph illustrates the temporal variation of $PM_{2.5}$ levels at one-minute resolution for 17 hours sampling duration (00:00 to 17:00). The marked increase in $PM_{2.5}$ level was recorded during cooking times. Windows in the living room were not opened and no indoor smoking was recorded within the duration of indoor sampling.

For the other home profiles (MPS01, MPS04, MPS05, MPS18 and MPS19) (see Appendix 7), cooking activities also lead to increases in indoor $PM_{2.5}$ levels, but the median level of indoor $PM_{2.5}$ within 24-hours remained below 20µg/m^3. Increase of indoor $PM_{2.5}$ level by cooking activity has been demonstrated previously by Steinle et al. (2015) and Rosen et al. (2015). Also, the cooking style by frying and use of gas stoves may contribute to significant increases in particle generation in residential homes (Jones et al., 2000; Chao et al., 1998; Monn et al., 1997).

The variability in indoor $PM_{2.5}$ levels may result from other indoor activities conducted by the home tenants during the sampling duration (see Appendix 7). The majority of home profiles showed an increase in $PM_{2.5}$ levels in the early morning (06:00 – 09:00) and late afternoon (16:00 – 17:00), and this trend was consistent with other reported studies (Gaspar et al., 2018; Lim et al., 2018; Jones et al., 2017; Jones, 2015; Steinle et al., 2015; Klepeis et al., 2013). According to Gaspar et al. (2018), this pattern of

indoor $PM_{2.5}$ levels may be due to the influence of traffic density from the outdoor environment. Steinle et al. (2015) also reported that $PM_{2.5}$ levels peaked between 09:00 – 10.00 and 19:00 – 20:00 due to transportation. In the present study, indoor $PM_{2.5}$ level showed an increase by "windows opening", which was recorded by home profile MPS04 and MPS12. This result was supported by Rosen et al. (2015) which reported indoor $PM_{2.5}$ levels was increased when the window was opened and dropped when the window was closed.

Smoking is also reported as an important source of indoor $PM_{2.5}$ in residential homes (Jones et al., 2000). However, the present study was not able to demonstrate this relationship due to the low number of smokers in the study population. The interesting finding was reported by Semple & Latif (2014), which measured the time taken to reduce $PM_{2.5}$ level after smoking inside the home. According to this previous study, the median time needed to reduce $PM_{2.5}$ levels by 50% was 55 minutes, and was nearly three hours to reduce $PM_{2.5}$ levels at the concentration <25μg/m^3 (Semple & Latif, 2014).

The present study reported a significant association between ethnicity and indoor $PM_{2.5}$ level. This association may result from differences in the frequency and method of food preparation, as well as differences in residential areas for the Caucasian and Non-Caucasian population. A study of home food preparation in the large UK cohort (n=12,070) reported that Caucasian ethnic was lower (25.9%) in energy consumption of preparing food at home compared to Black ethnicity (37.8%) (Astbury et al., 2019). However, the contradicted results obtained in the present study, in which indoor $PM_{2.5}$ levels were recorded higher in the residential homes of Caucasian compared to non-Caucasians, with the median level of 14.70 (n=14) and 4.86 (n=4) μg/m^3, respectively.

Residential areas might cause differences in both internal and external exposures, and it is reported that non-Caucasian populations in the UK tend to live in more deprived areas compared to Caucasians (Office for National Statistics, 2018). However, the present study did not show a significant difference of IMD decile between the Caucasian and non-Caucasian groups. The significant association between deprivation areas in the UK and indoor $PM_{2.5}$ level have been reported previously (Edwards et al., 2006), and other air pollutants such as PM_{10} and NO_2 have been reported to be higher in the most deprived (IMD decile: 20%) neighbourhood areas in England (Fecht et al., 2015). Furthermore, residential areas in England which accommodated by >20% non-White (e.g. Birmingham and London) was recorded higher outdoor PM_{10} and NO_2 level compared to residential areas with <20% non-White populations (e.g. Bristol, Leeds, Liverpool) (Fecht et al., 2015).

3.4.5 Strengths, limitations and future work

The key strength of this study was the demonstration of the ability to measure indoor air particles inside the residential home of pregnant women, simultaneously with the estimation of outdoor $PM_{2.5}$ level by the monitoring stations in Greater Manchester. The use of sampling diary to record indoor activities at home during sampling duration was partially successful, but was limited in certain areas, particularly with a consistency of recording. When recorded, there was a strong association with cooking activity and increased $PM_{2.5}$ levels inside homes.

The collection of house dust is a potentially useful strategy to identify the types of contaminants inside the home, as house dust may accumulate heavy metals and toxic materials (Kurt-karakus, 2012). The house dust analysis may help to identify the sources of indoor particles, as the majority of participants in the present study were took their shoes off inside the home. This practice may increase the probability of toxic materials (e.g. heavy metals and PAHs from the outdoor environment) attached to the shoe surface and transferred to the indoor environment. The collection of biological samples (pre-delivery maternal blood and placenta) from pregnant women is also a good strategy to understand the relationship between maternal exposure on $PM_{2.5}$ and pregnancy outcomes. The improvement, in terms of study design in biological samples collection is needed in future studies (e.g. arrangement of samples collection out of hours), as the present study demonstrated the low number of placental samples were successfully collected.

The main weakness of the present study is relating to the estimation of outdoor $PM_{2.5}$ levels at residential areas of study population. The present study was estimated outdoor $PM_{2.5}$ levels by referring to the closest air pollution monitoring stations, where the distance from the residential home to the monitoring station was within 0.54 to 23.8km. This method may not represent the actual outdoor $PM_{2.5}$ levels in the specific area, as outdoor air pollutants levels are varies depending on the activities around the neighbourhood; hence, suggested future study may apply the air pollution estimation model (such as spatio-temporal model, land use regression and etc.) that provides deeper insight on spatial and temporal variation of air particle level in the outdoor environment (Madsen et al., 2019; Smith et al., 2017; Hannam et al., 2014).

Another weakness of the study is related to the limited nature of the measurement, as indoor air sampling was only conducted for 24-hours and the duration may not represent the actual exposure level of pregnant women to indoor air particles

throughout the entire pregnancy. The “one-shot” measurement of indoor air particles as applied in the present study assumes that the exposure levels in pregnant women homes did not change significantly across the pregnancy period. Moreover, the present study was not able to compare indoor $PM_{2.5}$ level with indoor air quality guideline for the residential home within the UK population, as the guideline is under the development and expected to be published by 20th October 2020 (National Institute for Health and Care Excellence, 2019). Due to this limitation, the indoor $PM_{2.5}$ level in the present study was compared with the WHO guideline for the outdoor environment, as this guideline has been used for explaining indoor air particles level, particularly for the developing countries (WHO, 2006).

The comparison of $PM_{2.5}$ level between Dylos monitor with the gravimetric method and/or other conventional devices also needs to be considered, for the purpose of data validation (Steinle et al., 2015; Semple et al., 2012). The data comparison with other reference devices is important to convert the PNC (ppcf) to the PMC ($\mu g/m^3$), as well as can be applied for calibration purposes. The direct data conversion by using an equation may be prone to produce an error, as previous studies have suggested different equations, based on the reference device. The present study applied the conversion equation from a recent study conducted by Franken et al. (2019), in which the equation proposed by this study was considered the most accurate to convert indoor air particles data in a residential home. The equation was developed based on the side by side measurement of indoor air particles in the living room with Dylos and conventional device of OPC 1.108 aerosol spectrometer (GRIMM Aerosol Technik GmbH & Co, Germany), in which the data obtained from both devices was highly correlated (Franken et al., 2019). Additionally, the present study conducted side by side comparison with all Dylos monitors, in which the data from all monitors were highly correlated, suggesting that the measurement data obtained in the present study was valid.

The majority of pregnant women in the present study were working, so the exposure of $PM_{2.5}$ might be from different sources, not only from the residential homes. The exposure of $PM_{2.5}$ at the workplace and during commuting needs to be considered and hence, personal monitoring may be a better method of estimating total air pollution exposure. Also, due to practical constraints, the measurement of indoor particles in the residential homes was conducted by the participants, not by a trained researcher. There remains the possibility that the Dylos was not located at the appropriate place, even though instruction and a SOP was given to the participants. Another possibility is the participants may not demonstrate their “actual” indoor activities during sampling duration,

as they may limit their indoor activities due to their intention to control particles level inside their home, as the reading of particle levels are clearly displayed on the screen. Besides, improvements to the questionnaire in future would be useful, as this study lacked of information on the cooking method, dietary intake, use of candles or any other potential activities that may contribute to the level of indoor $PM_{2.5}$.

3.5 Conclusion

The main focus of this study was the feasibility testing of the methodology in recruiting the participants and measurement of indoor air particles in pregnant women residential homes across Greater Manchester by using a low-cost air particle counter, Dylos DC1700. Overall, this study was successful in achieving recruitment within the project period. The most prominent finding to emerge from this study is that the level of indoor and outdoor $PM_{2.5}$ in this study population was low and did not exceed the air quality guidelines. The variation of $PM_{2.5}$ level in the residential homes depends on the indoor activities conducted, especially cooking, but did not appear to be related to outdoor air pollution. Future studies should seek to confirm to this finding by increasing the sample size and the potential range of outdoor air pollution exposures, by focusing on residences with proximity to busy roads. Overall, there was a low exposure level of indoor and outdoor $PM_{2.5}$ in the residential homes across Greater Manchester, suggesting that maternal exposure on $PM_{2.5}$ level during pregnancy may have a minimum impact on pregnancy outcomes.

CHAPTER 4:

THE MODIFICATION OF HUMAN CHORIONIC GONADOTROPHIN SECRETION AND PLACENTAL CELLULAR TURNOVER BY CULTURED PLACENTAL EXPLANTS EXPOSED TO DIESEL EXHAUST PARTICLES AND HOUSE DUST

4.1 Introduction

Exposure to air pollution during pregnancy raises concerns due to the increased risk of pre-eclampsia, SGA, LBW, PTB and stillbirth (DeFranco et al., 2015; Olsson et al., 2013; Shah & Balkhair, 2011). Air pollutants, specifically PM (or air particles), may exist in different sizes and are generated from a variety of sources either from the ambient (outdoor) or indoor environment (USEPA, 2018). Diesel exhaust particles (DEP) from transportation sector contributed up to 50% of PM level in the ambient environment, which is concerning as DEP are toxic and carcinogenic to human health (Krivoshto., 2008). DEP contains PAHs that are able to penetrate deep into the lungs, be absorbed in the bloodstream and be distributed around the body including placenta (Bové et al., 2019; USEPA 2018; Krivoshto et al., 2008). In indoor environments, activities such as smoking, cooking and residential heating are known as sources of indoor PAH and house dust has been reported as the best predictor of indoor PAH exposure (Whitehead at al., 2011).

Previously, animal studies revealed that prenatal exposure to DEP is associated with reduced birth weight and altered placental function in term of placental efficiency (fetal/placental weight), placental blood flow and volume of fetal vessels (Valentino et al., 2016; Hougaard et al., 2008). However, in this context, animal studies might not be appropriate to indicate the effect of air pollutants towards human placenta as there is different physiology between species (Paulesu et al., 2018; Mannelli et al., 2015). Hence, the current study was designed to conduct an *in-vitro* study of placental explant culture exposed to DEP and HD, as this method may better represent maternal exposure to air pollution during pregnancy period.

The *in-vitro* technique used for this study was adapted from a well-established placental explant *in-vitro* study by Simán et al., (2001), in which the secretion of human chorionic gonadotrophins (hCG) and cellular turnover was determined following treatment. Furthermore, the temporal change of hCG secretion by villous explants in culture is well established in many previous studies and acts as a useful biomarker of placental dysfunction that may underlie adverse pregnancy outcomes such as pre-eclampsia, IUGR and miscarriages (Paulesu et al., 2018; Díaz et al., 2016; Audette et al., 2010; Simán et al., 2001). Furthermore, placental oxidative stress, inflammation and alteration of cellular turnover are associated with placental dysfunction in complicated pregnancies of pre-eclampsia and IUGR (Cindrova-Davies et al., 2018; Donaldson & Stone, 2003).

4.1.1 Hypothesis

This study tested the hypothesis that treating human placental tissue in explant culture with air pollutants, to mimic maternal exposure during pregnancy, will cause altered placental endocrine function and cellular turnover, reminiscent of the placental dysfunction that underlies adverse pregnancy outcomes.

4.1.2 Aim and objectives

The overall aim of this study was to investigate the effects of diesel exhaust particles (DEP) and house dust (HD) on placental endocrine function and cellular turnover.

Specifically, the objectives of this study were:

i. To develop an *in-vitro* model of placental explant culture treated with DEP and HD, based on the well-established placental *in-vitro* model.
ii. To determine whether the secretion of human chorionic gonadotrophin (hCG) by placental villous explants is altered by treatment with DEP and HD.
iii. To determine whether exposure of placental villous explants to DEP and HD was associated with alteration of cellular turnover.

4.2 Materials and Methods

4.2.1 Placental collection

Placentas (n=41) were collected from mothers who gave birth by elective caesarean section (ELCS) between October 2017 and January 2019 in St. Mary's Hospital, Manchester following ethical approval for the Maternal and Fetal Health Research Centre Biobank, reference number 08/H1010/55 (+5). The process to obtain the placentas from the labour room was similar to those described in Chapter 2.2.2. The criteria for placenta selection were:

i. Placentas collected at term (37 to 42 weeks gestation)
ii. Placentas collected from non-smoking mothers.
iii. Normal pregnancy with no evidence of pre-eclampsia, FGR and diabetes mellitus.

4.2.2 Preparation of placental villous explants

Four full-thickness samples of the placenta (~2cm^3) were taken from between the chorionic plate and decidua at different sites avoiding the edge, the site of cord insertion, and areas with blood clots or fibrinoid deposits. Placental tissues were rinsed in sterile PBS with Ca^{2+} and Mg^2 (Sigma-Aldrich, UK) to remove the maternal blood and transferred to a microbiological safety cabinet (MSC) for explant processing.

In the MSC, the villous tissue samples were placed in sterile PBS in a culture dish, the chorionic plate was removed, and small fragments of villous tissue were dissected (~3mm^3). On average, 40-60 fragments (explants) were dissected from each of the four placental samples. The number of placental explants used varied according to the experimental protocol. Three explants, chosen at random, were placed onto a Netwell permeable supports (74µM mesh; Corning Lifesciences) which were housed in a 12-well culture dish and the explants were supported at the liquid/air interface (Figure 4.1).

Each well contained 1.5mL culture medium [per litre: 100mL 10x CMRL 1066 (Gibco), 100µL 10mg/mL insulin, 2.2g sodium bicarbonate ($NaHCO_3$), 100mg streptomycin sulphate, 60mg penicillin, 0.1µg/mL hydrocortisone, 0.1µg/mL retinol acetate, 100mg L-glutamine, 5% fetal bovine serum (FBS) and distilled water], pH adjusted to 7.2 and sterile filtered with 0.22µm cellulose acetate filter (CORNING®, Corning Incorporated, New York, USA). All chemicals were purchased from Sigma-Aldrich (UK) unless otherwise stated.

Care was taken to ensure that the explants remained separate and did not adhere to the walls of the Netwell. At the end of the process, the culture dishes were placed in a humidified tissue culture incubator (5% CO_2; 95% air) at 37 ^{0}C for seven days and the time was recorded. The overall process of placental villous explant preparation was illustrated in Figure 4.1.

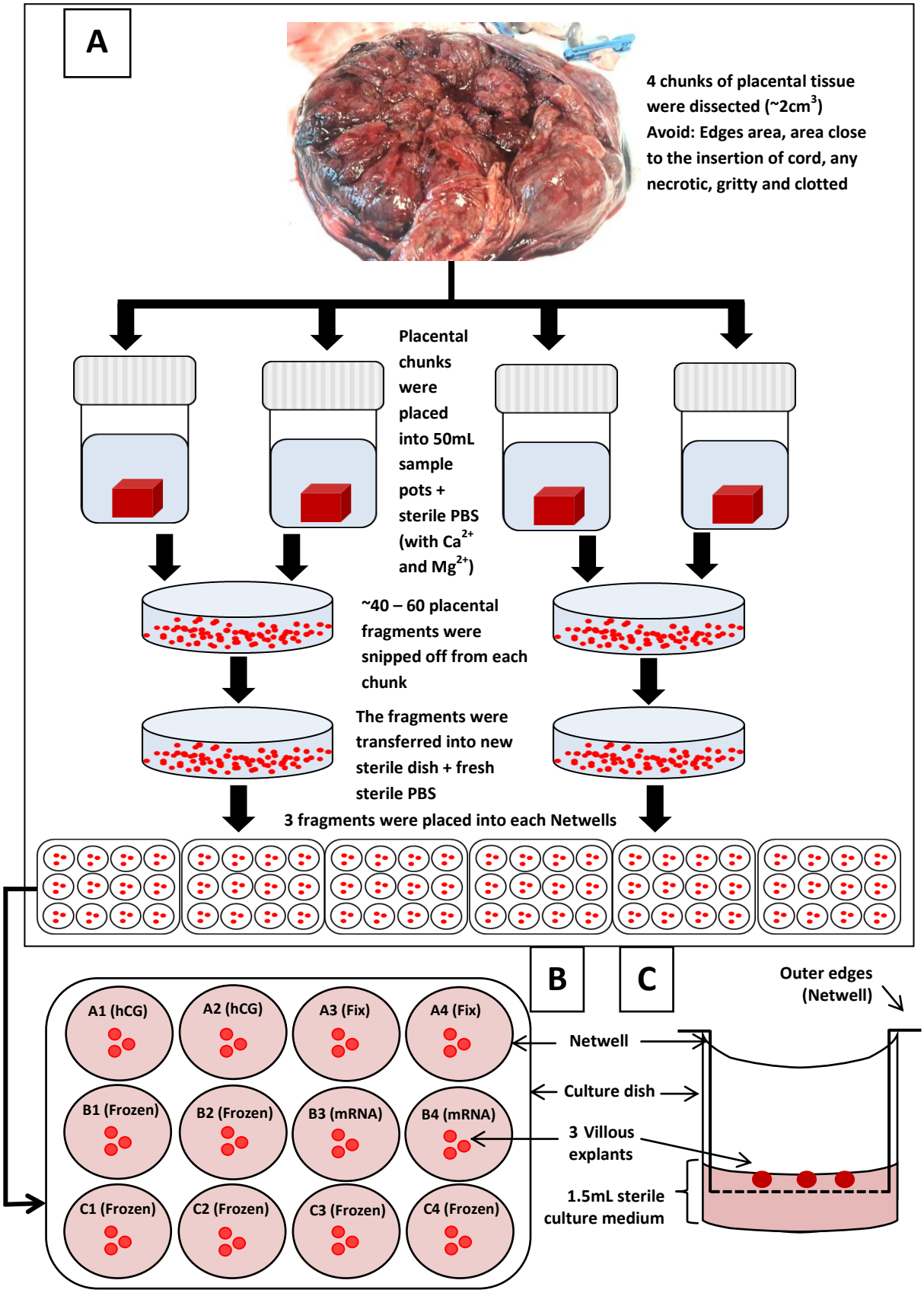


Figure 4.1: Process in preparation of placental villous explants.
Figures illustrate the (A) process of explants preparation, (B) arrangement of wells in a culture dish and (C) structure of Netwell support. The method for preparation of the explants, the experimental treatments and the outcome measures are described in Sections 4.2.3, 4.2.4 and 4.2.5, respectively

4.2.3 Culture of placental villous explants

Explants were maintained in a humidified incubator with 95% air and 5% CO_2 (21% oxygen) at 37 (^{0}C). Explants were cultured for seven consecutive days, with day 0 being the day of placental collection and day 7 the harvesting day for placental explants. The number of explants prepared depended on each experimental objective (protocol described in Section 4.2.4) and the outcome measures (described in detail in Section 4.2.5). Culture medium was replaced daily. From day 2, culture medium was collected from 2 wells (wells A1 and A2; Figure 4.1B) and aliquoted and stored at -20^{0}C for later analysis of hCG (see Section 4.2.3.1). The explants in all wells were harvested on day 7.

On day 7, placental explants in wells A1 and A2 (Figure 4.1B) were lysed in 4mL 0.3M NaOH at 37^{0}C overnight. The explant was lysates and stored at 4^{0}C for later analysis of protein content using a Biorad protein assay (see Section 4.2.3.2). Placental explants in wells A3 & A4 (Figure 4.1B) were fixed in 4% neutral buffered formalin (NBF) for analysis of cellular turnover determined by immunohistochemistry (IHC) (Section 4.2.4.1). These explants were kept at 4^{0}C overnight, then washed with PBS and stored in a cold room prior to processing for IHC. Explants in wells B3 & B4 (Figure 4.1B) were immersed in RNA later solution and placed in a fridge for three days, washed with PBS and stored in a -80 freezer for future studies. Explants in wells B1, B2, C1, C2, C3 and C4 (Figure 4.1B) were snap frozen on the harvesting day and stored in a -80 freezer. The mRNA and frozen explants were not analysed in this study and kept in the biobank for subsequent analysis.

4.2.4 Experimental treatments

4 series of experiments were performed, described in details in Sections 4.2.4.1 until 4.2.4.2:

1. Explants were treated with control medium throughout (negative control) or H_2O_2 at 0.5, 1.0 and 5.0mM on days 5 and 6 of culture (positive control).
2. Explants were treated with SRM1650b (DEP) at 2.5, 25 and 100µg/mL, or 5mM H_2O_2, on day 5 of culture.
3. Explants were treated with SRM1650b (DEP) at 2.5, 25 and 100µg/mL, or 5mM H_2O_2 on days 1 to 6 of culture.
4. Explants were treated with SRM2585 (HD) at 2.5, 25 and 100µg/mL, or 5mM H_2O_2 on days 1 to 6 of culture.

The number of sterile culture dishes, number of Netwell and number of placental explants required for each experiment were varied, depended on the treatment plan as shown in Figure 4.2.

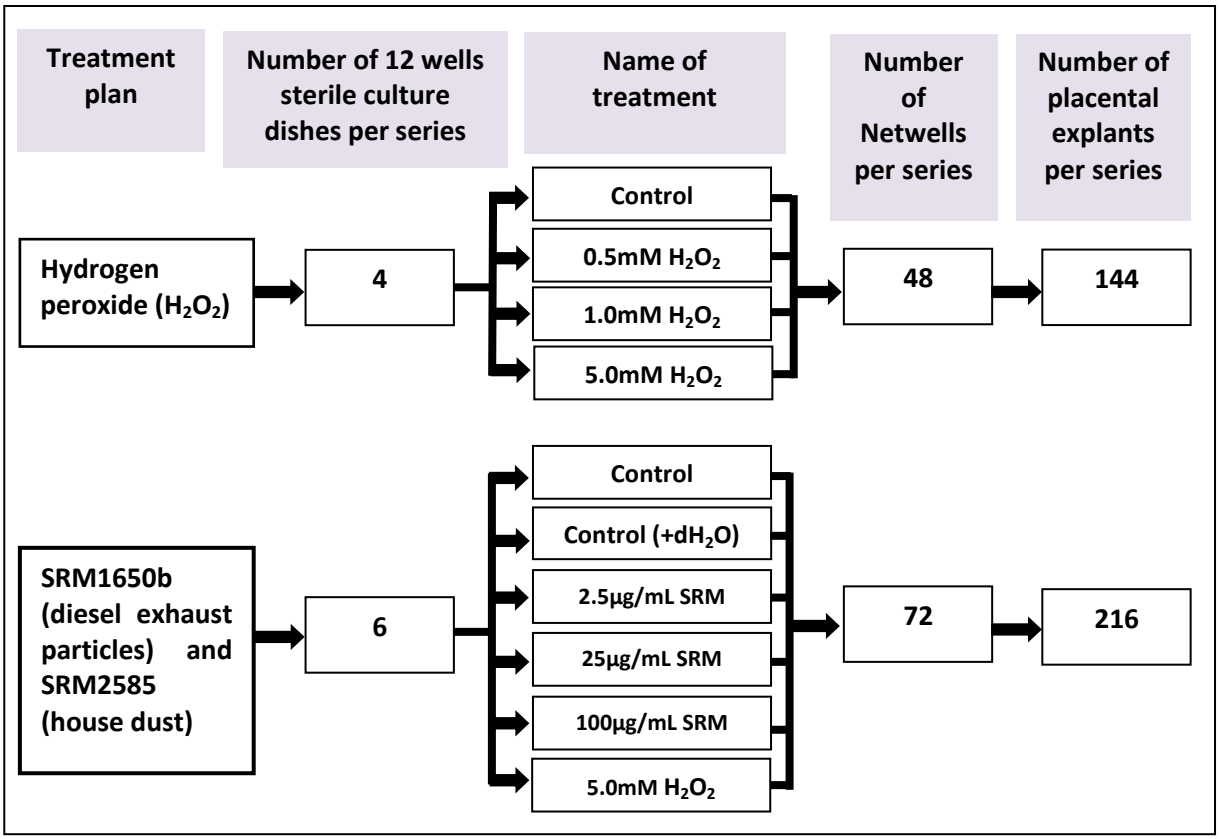


Figure 4.2: Treatment plan for placental explants culture

4.2.4.1 Placental explant culture treated with hydrogen peroxide *in-vitro*

Placental explants were treated with H_2O_2 as a positive control in order to develop an *ex-vivo* model of placental explants treated with DEP and HD. It was anticipated that DEP and HD would be detrimental to explant endocrine function and cellular turnover in part, through inducing oxidative stress. Thus, the experiment with H_2O_2 was conducted prior to the experiment with DEP and HD, as a proof of principle study.

H_2O_2 was prepared fresh at concentration of 0.5, 1.0 and 5.0mM on each day of treatment. A stock solution of 5.0mM was prepared from 30% H_2O_2 (1:2000 in culture medium) and protected from light. The stock was further diluted in culture medium at 1 in 5 and 1 in 10 to achieve a final concentration of 1.0 and 0.5mM H_2O_2, respectively. Explants were treated with H_2O_2 on days 5 and 6 of culture (series 1 experiment). Having determined that 5mM H_2O_2 reduced explants hCG secretion (see Section 4.3.5) in subsequent

experiments with DEP and HD (series 2, 3 and 4), 5mM H_2O_2 was used as a positive control and applied on day 5 (series 2) or daily over days 2 to 6 (series 3 and 4).

4.2.4.2 Placental explants treated with diesel exhaust particles and house dust *in-vitro*

Standard reference materials (SRM) 1650b and 2585 were purchased from Sigma Aldrich (Schnelldorf, Germany). SRM1650b is diesel exhaust particulate matter collected from heavy duty diesel engines with well characterized physical and chemical contents such as PAHs (NIST, 2013; Jantzen et al., 2012; Danielsen et al., 2008). SRM2585 contains organic contaminants of house dust, collected from various indoor environments such as homes, cleaning services and hotels (NIST, 2014).

To investigate the effect of DEP on placental villous explant endocrine function and cellular turnover, explants were exposed to SRM1605b at 2.5, 25 and 100µg/mL. In this present study, placental explants were treated with DEP in two series of experiments, referred to as series 2 and 3. In series 2, explants were treated with DEP on day 5 for 48hr (the medium was not changed on day 6) whereas in series 3, explants were treated with SRM1650b daily from days 1 to 6. Explants treated with 5mM H_2O_2 was used as positive controls. In series 2, the effect of short term (48 hours) treatment with DEP at 2.5, 25 and 100µg/mL was investigated on explant hCG secretion. Informed by the results of this experiment, subsequent experiments (series 3) were performed to investigate the longer term effect of DEP and explant were treated with SRM1650b daily from day 1 to 6.

Due to the insolubility of SRM1650b (DEP) in organic solvents and water, the DEP suspension was prepared by sonication, adapted from a study conducted by Jantzen et al. (2012) as described in Figure 4.3. The sonication process was started by added 50mg of SRM1650b into 50mL sterile dH_2O to get a stock concentration of 1.0mg/mL. As recommended by the supplier, the minimum amount of SRM1650b to be taken from the original supply must not be less than 50mg so as to maintain the homogeneity of PAHs in SRM1650b sub-samples. The suspension was then initially mixed by using a vortex and sonicated (Sonoplus Ultrasonic Homogenizers, HD 2070). The sonication process was performed at a frequency of 20 kHz, 10% amplitude and for a total of 8mins with 10s intervals to avoid overheating. The suspension was aliquoted, labelled and stored in a cold room (4^0C) until required. For effect comparison, the same concentration and preparation process were applied to SRM2585 (HD). Placental explant culture treated with HD (series 4) followed the longer term application protocol as described in experiment with DEP (series 3).

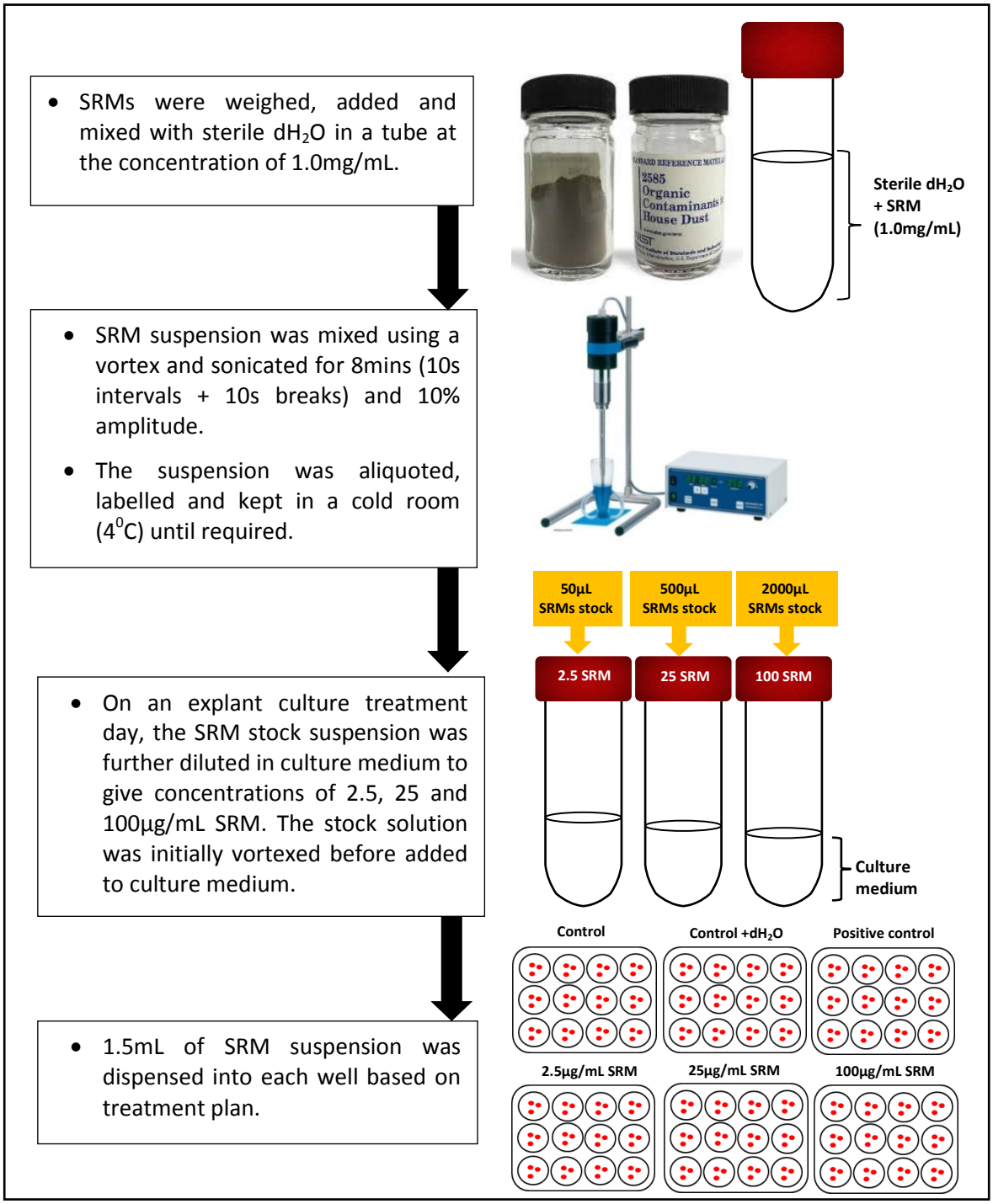


Figure 4.3: Preparation of SR1650b (DEP) and SRM2585 (HD)

Following the sonication process to achieve a stock concentration of 1mg/mL, a further dilution was performed to achieve the final concentrations of 2.5, 25 and 100µg/mL in the culture medium. Because the SRM has to be prepared in water, the culture medium was diluted by 10% with distilled water (dH_2O). To correct for any effects that a 10% dilution of culture medium might have on hCG secretion, a control experiment was performed with additional 10% dH_2O alone, referred as "Control 2".

4.2.3 Outcome measures

4.2.3.1 Analysis of hCG secreted in culture media determined by ELISA

The culture medium was collected every 24 hours (except series 2) from day 2 of culture and the levels of hCG secreted by placental explants into the medium was assayed using an enzyme-linked immunosorbent hCG kit (ELISA: EIA-1469, DRG Diagnostic, Marburg, Germany) according to standard manufacturer's protocol. Samples and standards were assayed in duplicate and the absorbance was determined by using a FLUOstar Omega microplate reader (BMG Labtech, Aylesbury, UK) at 450nm wavelength. A standard curve (double log plot) was constructed using Graphpad Prism (Figure 4.4) and the unknown hCG concentrations in the culture medium samples determined by interpolation. The duplicate samples had a coefficient of variance (COV) of 4.8% (IQR: 3.2 – 7.6). The total protein content of explants was measured using a Biorad protein assay (see Section 4.2.3.2) and hCG secretion was expressed in mIU/mL/mg protein.

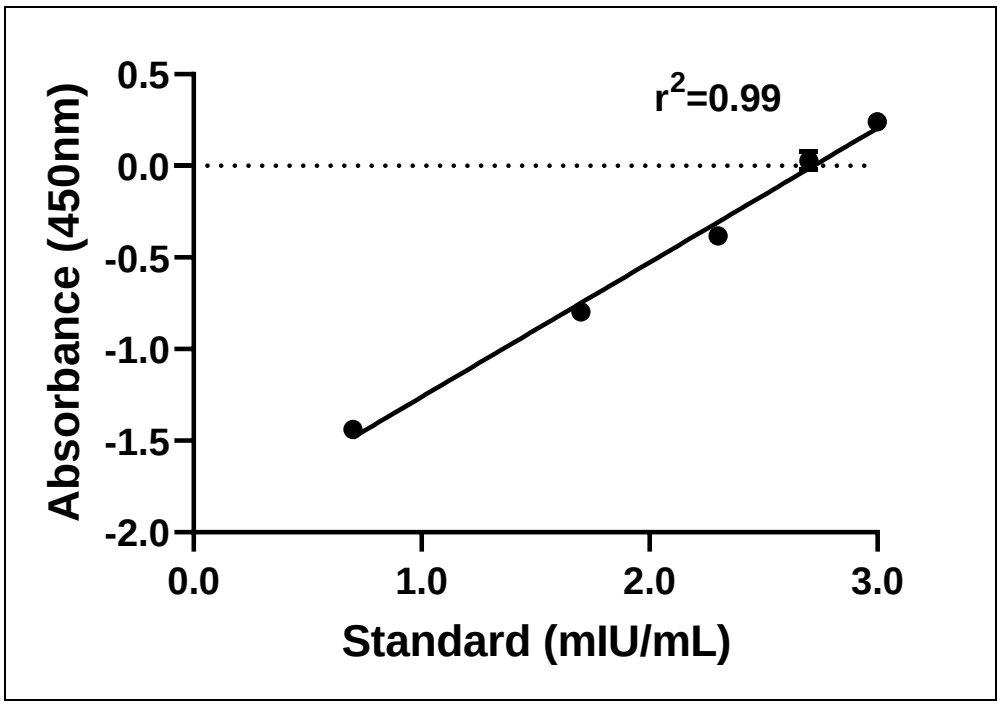


Figure 4.4: A double log plot standard curve for the hCG ELISA assay

Standards were assayed in duplicate ranging from 5 to 1000mIU/mL hCG. Absorbance (arbitrary unit) was measured at a wavelength of 450nm; Linear regression, r^2=0.99

4.2.3.2 Analysis of protein content determined by Biorad protein assay

The protein content of explants was analysed by using a Biorad protein assay. The placental explants were denatured in 4mL of 0.3M NaOH on the harvesting day. The protein assay was performed in duplicate in 96 well plates and each well contained 20µL of standards or samples. A stock solution of 0.25mg/mL bovine serum albumin (BSA) was prepared in 0.3M NaOH and serially diluted in NaOH to achieve a standard content of 0 to 5µg/20µL. Then, 180µL neutralizing solution [0.3M NaOH: 0.3M HCl (1:1.25)] was added to the standards and samples followed by 50µL Biorad Protein Assay Dye Reagent Concentrate (Biorad Laboratories Ltd, Hemel Hempstead, UK). The samples was thoroughly mixed (~10 times) for 5 minutes until the colour was even. The samples absorbance was determined by using a FLUOstar Omega microplate reader (BMG Labtech, Aylesbury, UK) at 595nm wavelength. A standard curve was generated using Graphpad Prism (Figure 4.5) and use to interpolate the protein content of the explants. The protein content of the explants was used to standardise the hCG secreted into the culture medium (Section 4.2.31.1).

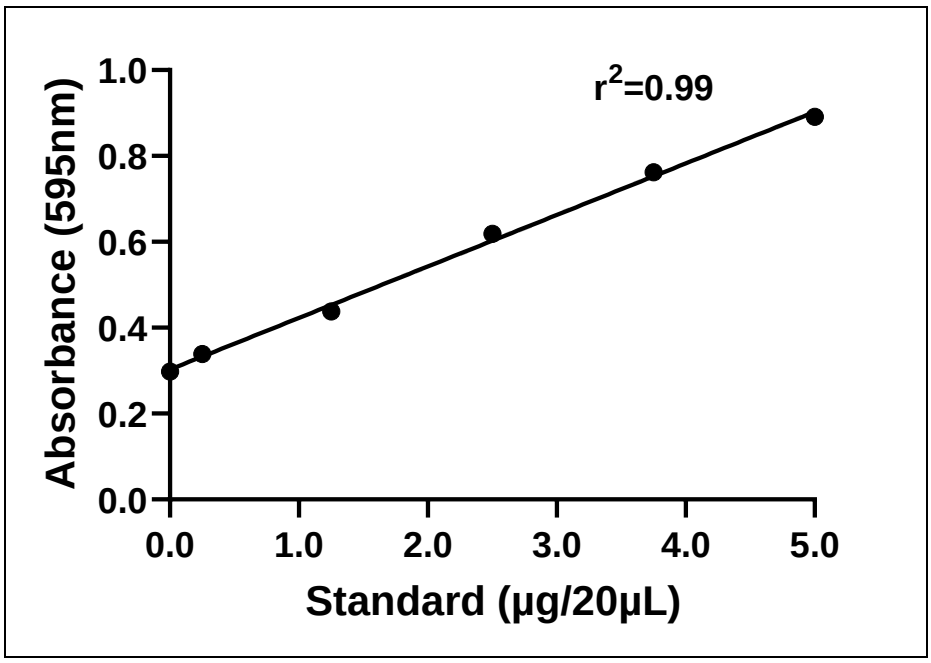


Figure 4.5: Standard curve for Biorad protein assay

Standards were assayed in duplicate ranging from 0 to 5µg of protein in 20µL of standard. Absorbance (arbitrary units) was measured at a wavelength of 595nm; Linear regression, r^2=0.99

4.2.4 Fixation and processing of villous explants

Figure 4.6 illustrates the processing of placental villous explants for IHC. Explants were dehydrated in a series of ethanol solutions before being paraffin wax embedded using Tissue Process Leica TP1020. The paraffin blocks were kept in a fridge or on ice for chilling prior to cutting with a microtome (Leica RM2245, Wetzler, Germany) to produce 5µm sections. The sections were mounted on poly-L-lysine pre-coated microscope slides and kept in the incubator at 37^0C overnight. The sections were immunostained using six primary antibodies (Table 4.1); Ki67 to determine the proliferation index, M30 for the apoptotic index, CK7 for the syncytiotrophoblast layer, hCG for syncytiotrophoblast hCG production, 8-OHdG for oxidative damage to nuclei and CD163 for macrophages.

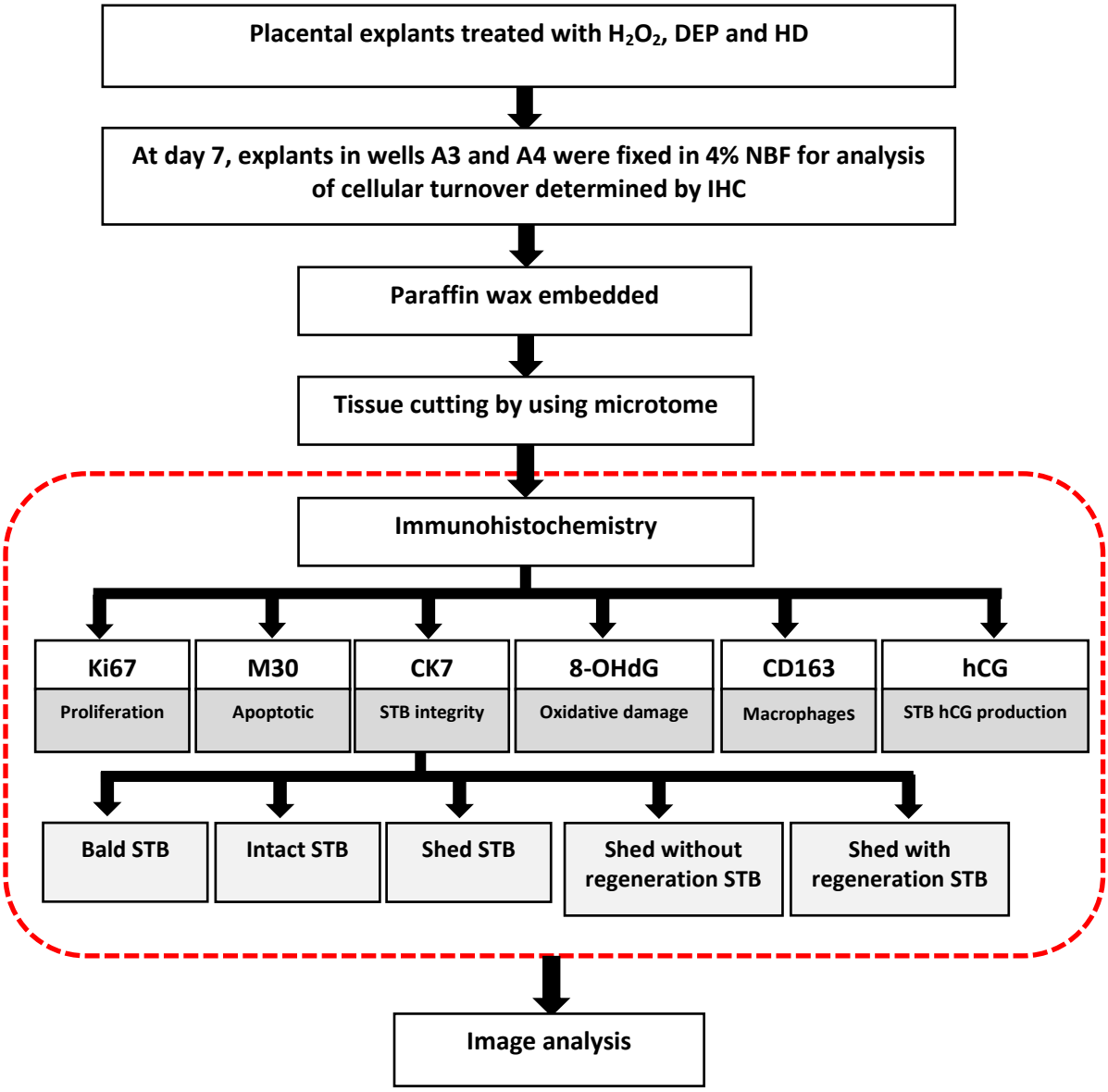


Figure 4.6: Flowchart of explants preparation for immunohistochemistry

4.2.4.1 Immunohistochemistry staining for explants

The first step of IHC process was dewaxing and dehydration of the section. The slides were heated in a warming oven for 10mins at 60°C and dewaxed in Histoclear for 3 x 5mins. Then, the section was rehydrated in a series of dilutions of industrial methylated spirits (IMS), starting from 100% IMS 2 x 2mins, 70% IMS 2 x 2mins and ending in running tap water for 5mins.

Then, antigen retrieval was carried out by immersing the slides in 400mL 0.01M sodium citrate at pH 6.0, boiling in the microwave for 10mins at 800W and cooling in a hot buffer for 20mins. Slides were then rinsed with tap water. Each section was circled with a PAP pen and covered with Tris Buffered Saline (TBS). Endogenous peroxidase was quenched for 10mins by 100µL of 3% H_2O_2 diluted with dH_2O at 1:10. Then, the section was washed with TBS for 2 x 5mins and dabbed with tissue to remove excessive liquid. 50µL non-immune block (NIB), consisting of 10% normal goat serum (or swine) and 2% human serum in TBS Tween 0.1%, was applied onto the section to block non-specific binding of antibodies. After incubating the section with NIB for 30mins, the NIB was tapped off and primary antibody was applied immediately and incubated overnight at 4^0C. The primary antibody was diluted in NIB to achieve the final concentration as shown in Table 4.1. For the negative control, non-immune mouse (or rabbit) IgG was diluted in NIB at the same working concentration as the primary antibody.

The next day, the primary antibody was washed off the section using TBS for 1 x 5mins, followed by TBS-Tween (0.6%) for 2 x 5mins and rinsed with TBS for 1 x 5mins. The section was dabbed to remove the excess liquid. 50µL secondary antibody was then applied at the appropriate concentration (Table 4.1) and incubated for 30mins at room temperature. Following incubation with secondary antibody, the section was washed again with TBS for 1 x 5mins, TBS-Tween (0.6%) 2 x 5mins, rinsed with TBS for 1 x 5mins and dabbed to remove excessive liquid. 50µL avidin peroxidase was diluted in TBS (1:200) and applied on the section for 30mins at room temperature. The section was then washed again with TBS-Tween (0.6%) 2 x 5mins, rinsed with TBS for 1 x 5mins and dabbed. The details of primary and secondary antibodies used in this study are shown in Table 4.1.

Table 4.1: Details of primary and secondary antibodies used for IHC

Target	Primary Ab	Concentration & Dilution	Source	Secondary Ab	Concentration & Dilution	Source
Ki-67	Monoclonal mouse anti-human Ki-67 antigen Clone MIB-1	4600µg/mL (1:250)	Dako	Polyclonal goat anti-mouse biotinylated	650 µg/mL (1:200)	Dako
M30	Monoclonal mouse M30 Cytodeath	6.6µg/mL (1:50)	Roche	Polyclonal goat anti-mouse biotinylated	650 µg/mL (1:200)	Dako
CK-7	Monoclonal mouse anti-cytokeratin 7	37.49µg/mL (1:200)	Invitrogen	Polyclonal goat anti-mouse biotinylated	650 µg/mL (1:200)	Dako
hCG	Polyclonal rabbit anti-hCG	7100µg/mL (1:600)	Dako	Polyclonal swine anti-rabbit biotinylated	0.93 µg/mL (1:200)	Dako
8-OHdG	Monoclonal mouse Clone 15A3	1000µg/mL (1:500)	Abcam	Polyclonal goat anti-mouse biotinylated	650 µg/mL (1:200)	Dako
CD163	Monoclonal mouse anti-human CD163	200µg/mL (1:400)	Biorad	Polyclonal goat anti-mouse biotinylated	650 µg/mL (1:200)	Dako

For the colour development, 50µL of the chromogen diaminobenzidine (DAB) (Sigma-Aldrich, UK) was applied onto the section and the colour development was observed under the microscope. The time for colour development was depended on the target, and ranged from 2 to 10mins. After that, the section was washed and incubated with dH_2O for 5mins. Then, the section was counterstained with filtered Harris's hematoxylin (Sigma-Aldrich, UK) for 1 to 2mins and washed with running tap water. In order to remove excessive hematoxylin, slides were dipped in acid alcohol for 2 to 3s and rinsed immediately with running tap water. Slides were incubated in hot tap water for 5mins for the "blueing" process. At this point, the negative sections were checked under the microscope for staining quality. The slides were transferred to the cold tap water, ready for dehydration process. For dehydration, the slides were immersed in a series of IMS starting with 70% IMS for 2 x 3mins, 95% IMS for 2 x 3mins, 100% IMS for 3 x 3mins and followed by immersion in Histoclear for 20mins. Finally, the coverslips were mounted by using DPX mounting medium (Sigma-Aldrich, UK).

4.2.4.2 Image analysis

4.2.4.2.1 Digital image analysis

Digital image analysis was performed by using an Olympus BX41 microscope (Olympus, Tokyo, Japan) with a QICAM Fast 1394 camera (QImaging, Canada). At least 6 sections were prepared for each sample and stained for proliferated nuclei (Ki67), apoptotic nuclei (M30), oxidative damage nuclei (8-OHdG) and the number of macrophages (CD163). 6 random fields of view (FOV) were captured for each section and analysed by using Histoquest Tissue Analysis software (TissueGnostics GmBH, Vienna) at x20 magnification. The positive staining nuclei and total nuclei were determined by adjusted the threshold intensity of Haematoxylin (determined by blue colour) and DAB (determined by brown colour). Haemotoxylin staining was picked as a "master" marker to define nuclei and DAB staining as a second marker to define positive staining nuclei. Ki67, M30, 8-OHdG and CD163 staining were quantified by calculating the number of positively stained nuclei (DAB) as a percent of total number of nuclei in the villous area (Haematoxylin + DAB). Representative photomicrographs of each staining using Ki67, M30, 8-OHdG and CD163 primary antibodies are illustrated in Figure 4.7.

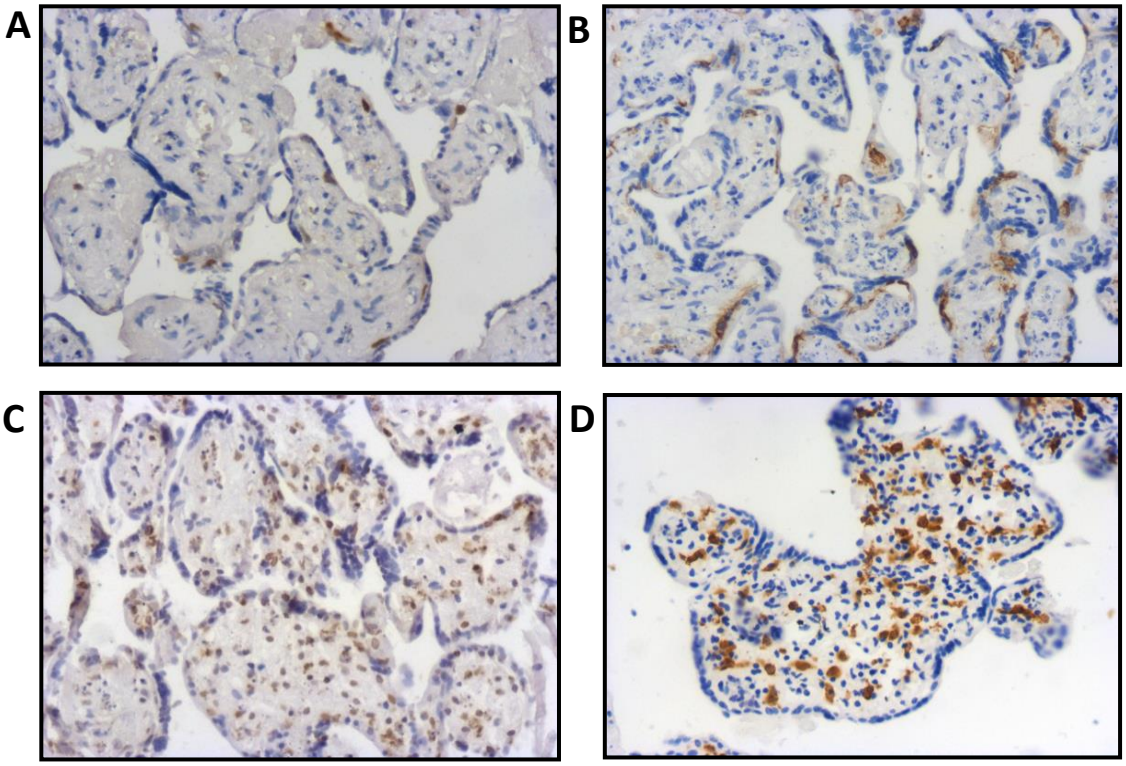


Figure 4.7: Representative photomicrograph of Ki67, M30, 8-OHdG and CD163 staining

Figure shows sections that stained with (A) Ki67, (B) M30, (C) 8-OHdG and (D) CD163. Blue indicates nuclei staining by Haematoxylin and brown indicates nuclei positively stained by DAB. The total number of nuclei in the field was calculated based on the combination of Haematoxylin and DAB staining nuclei. Image was taken at x20 magnification.

4.2.4.2.2 Observer scoring

Qualitative analysis of hCG intensity and cytokeratin 7 (CK7) staining was conducted by an observer blinded to the identity of samples. To assess the intensity of hCG staining, the image was assigned on four categories: 0 indicated negative staining, 1 slightly positive staining, 2 medium positive staining and 3 indicated highly positive staining as shown in Figure 4.8.

For CK7, only complete villi were chosen for analysis and the blinded observer assessed: i) the total number of villi, (ii) the number of bald villi (refers to villi with complete absence of STB), (iii) the number of villi with intact STB, (iv) the number of villi with shed STB and no regeneration and (v) the number of villi with shed STB but signs of STB regeneration. The data for each category ((ii) – (v)) was expressed as a percentage (%) of the total number of villi. The representative photomicrograph for each category is present in Table 4.9.

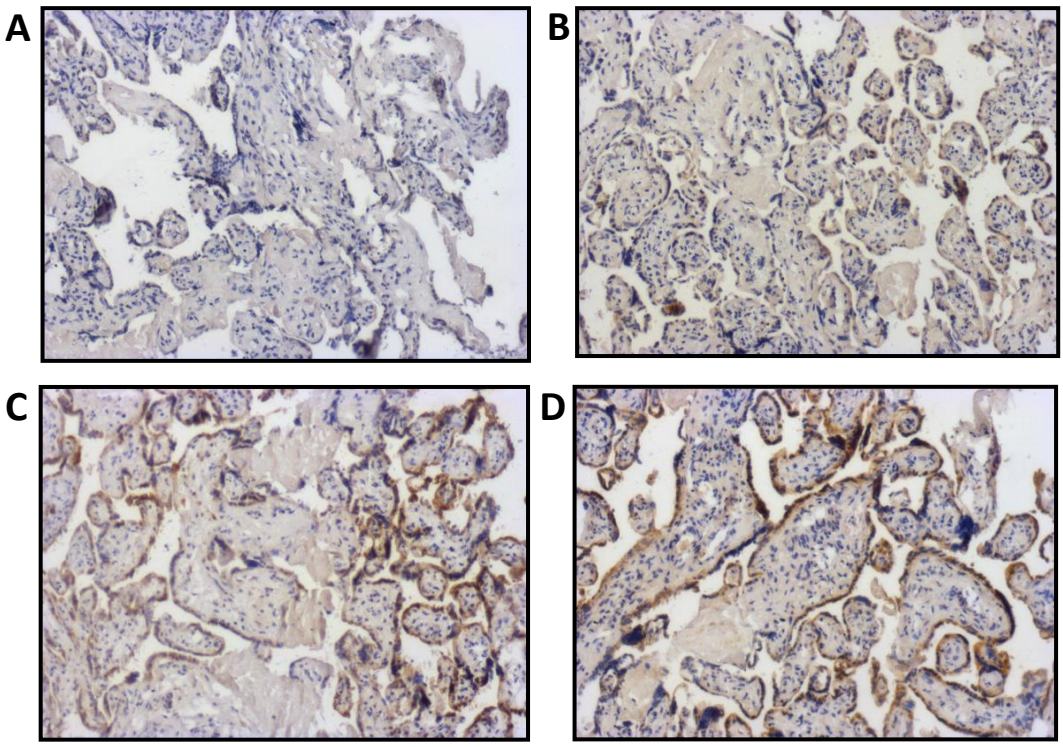


Figure 4.8: Representative photomicrograph for hCG intensity staining

Figures show section that stained with hCG and image indicate (A) negative staining, (B) slightly positive staining, (C) medium positive staining and (D) highly positive staining. Image was taken at x20 magnification.

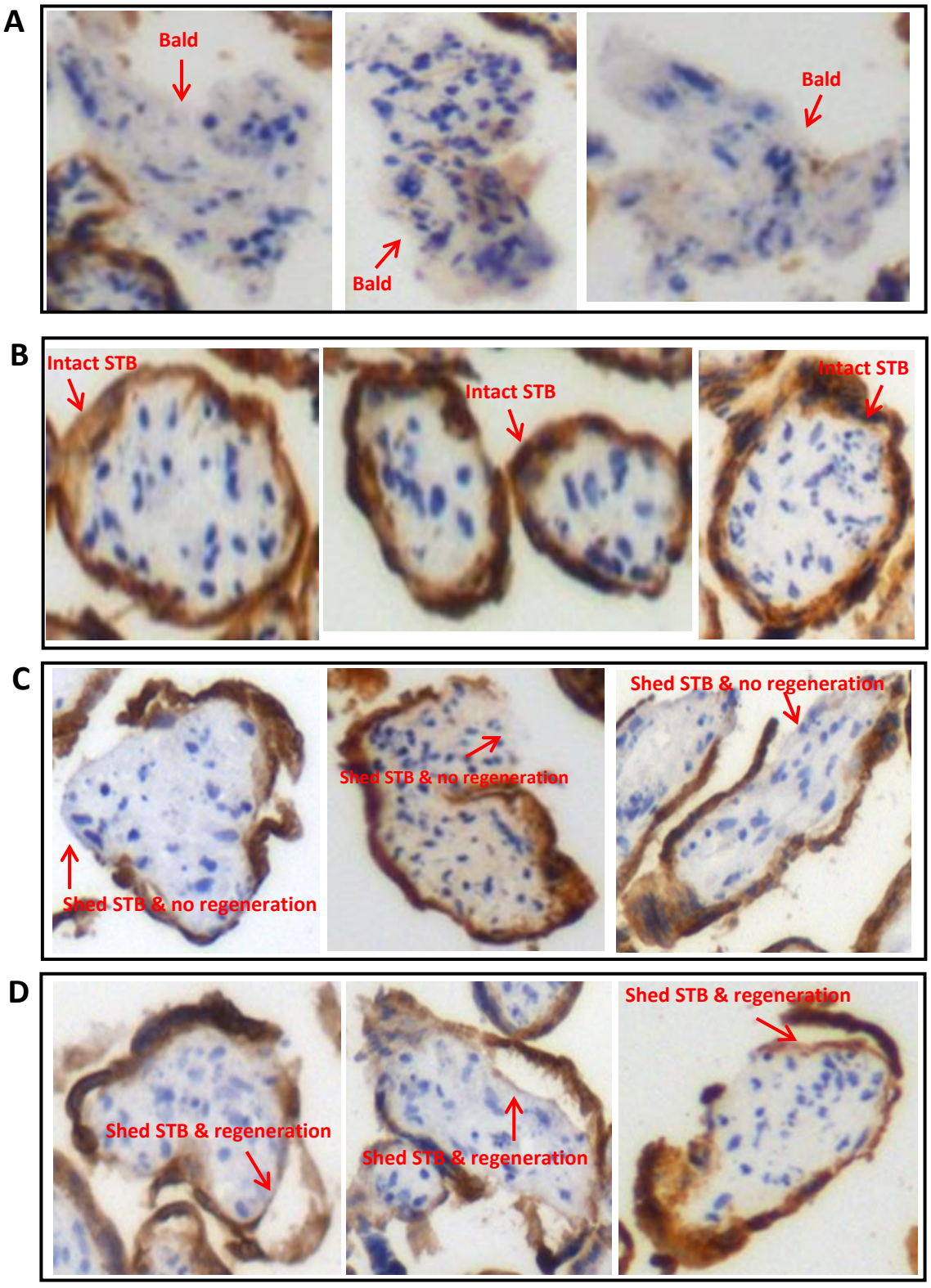


Figure 4.9: Representative photomicrograph for CK7 staining

Figures show section that stained with CK7 and the STB showing (A) bald villi, (B) villi with intact STB, C) villi with shed STB and no STB regeneration and D) villi with shed STB and STB regeneration. Original image was taken at x40 magnification.

4.2.5 Statistical analysis

Data analysis was performed by using GraphPad Prism version 8.0 (Graphpad Software, USA).

Descriptive statistics was used to describe the demographic variables of samples, presented as median (IQR), minimum and maximum. The number of experiments was conducted at least 6 in every series of treatments, which considered as appropriate to show a statistical significant difference in response to H_2O_2, DEP and HD on placental villous fragments (Audette et al., 2014; Hirst, 2015). Due to sample size (n=6) or the data being not normally distributed, the non-parametric test (Wilcoxon signed-rank, Mann-Whitney U) was performed to compare the data between groups.

The Mann-Whitney test was performed to assess the difference of hCG secretion by explants maintained in standard control medium and medium containing 10% dH_2O (Control 2) (n=16). The time course of hCG secretion by explants in control medium and treatments, over 7 days of culture was represented as mean (±SEM). hCG secretion on days 6 and 7 (series 1 and 2; see Section 4.2.4) was calculated as a percentage of secretion on day 5 and expressed as a percentage of time matched-control (100%). The area under the curve (AUC) over 7 days of culture was calculated to determine the effect of longer treatments (series 3 and 4; Section 4.2.4) on hCG secretion and expressed as a percentage (%) of time-matched control. Wilcoxon Signed rank test was used to assess the difference of hCG secretion in response to treatment with the corresponding control. Data are presented as scatter dot plots with median line shown. P-value<0.05 was considered significant.

The IHC markers (Ki67, M30, CD163, 8-OHdG, hCG production staining and CK7) was expressed as a percentage of time matched-control and the difference in response to treatment was analysed by using Wilcoxon signed rank. Data are presented as scatter dot plots with median line shown and P-value<0.05 was considered significant. To analyse the relationship between hCG secretion and IHC markers, linear regression was performed and P-value<0.05 was considered significant.

4.3 Results

4.3.1 Experimental timeline

Figure 4.10 illustrates the total number of placentas studied. 41 villous explants experiments were performed; 29 were successfully cultured and treated with H_2O_2 (series 1; n=7), DEP (series2; n=6), DEP (series 3; n=8) and HD (series 4; n=8). 12 placental explants experiments were excluded from the study due to bacterial contamination (n=4) or failure to secrete hCG (n=8). Contaminated explants usually were detected by day 4 based on four criteria which were unpleasant smell, orange/yellow culture medium, cloudy supernatant and presence of bacteria in the culture medium on microscopic examination. Non-responsive (unviable) explants were detected retrospectively, following the measurement of hCG secretion level; experiments were rejected if explant secretion (control) of hCG into the culture medium at day 4 was not at least double that at day 2 (see Figure 4.11).

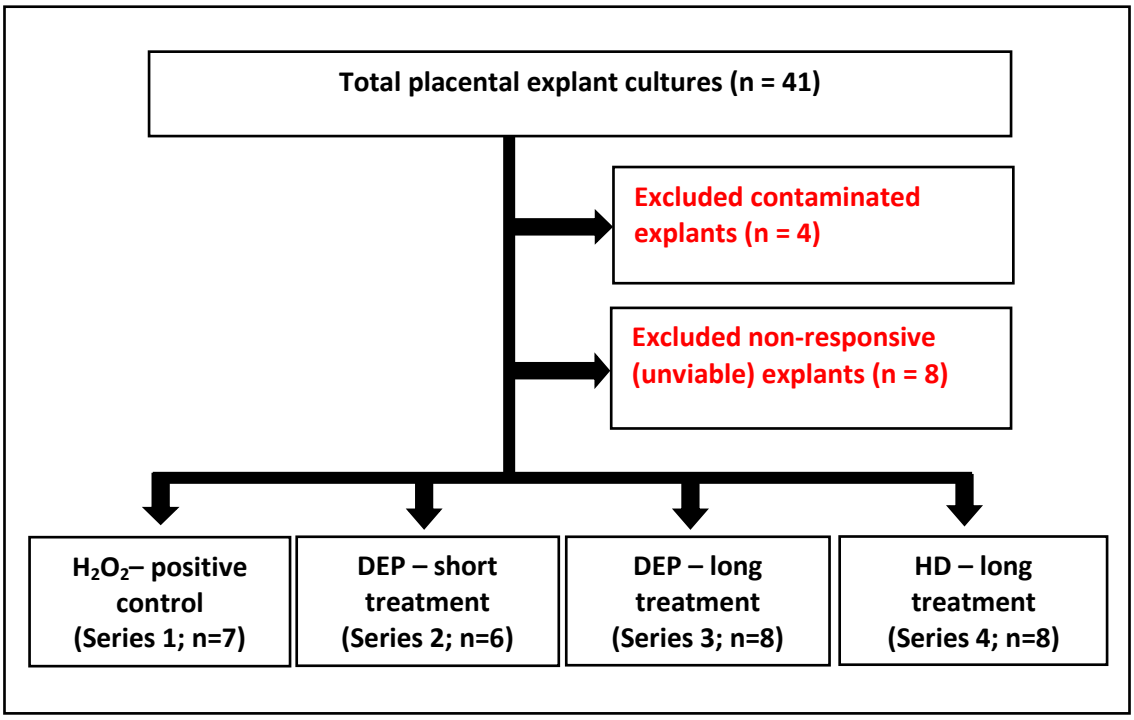


Figure 4.10: Placental samples collected for *ex-vivo* study

4.3.2 Demographic variables

Of the 29 samples that were successful cultured with H_2O_2, DEP and HD, demographic information was successfully collected from 28 samples only (Table 4.2). The majority (22 out of 28) of placental samples were from Caucasian mothers with a median age of 31 years. The median maternal BMI was 21.73kg/m^2 which indicated normal weight. The majority (21 out of 28) of mothers were multiparous with median gestations at 273 (IQR: 273.0 – 276.8) days. Over half of the babies (15 out of 28) were male with a median birthweight at 2966 (IQR: 3370 – 3668) grams and the median individualised birth weight ratio (IBR) was at percentile 54.05 (IQR: 15.05 – 75.00).

Table 4.2: Demographic variables of mothers whose placentas were successfully cultured

Maternal characteristics	Median (IQR)	Minimum	Maximum
Maternal age (years old)	35.50 (31.00 – 38.00)	23.00	42.00
Maternal BMI (kg/m^2)	23.80 (21.73 – 29.80)	18.30	35.70
Ethnicity			
Caucasian, n (%)	22 (78.6)		
Non-Caucasian, n (%)	6 (21.4)		
Gestational age (days)	273.0 (273.0 – 276.8)	264.0	287.0
Parity			
Primiparous, n (%)	7 (25)		
Multiparous, n (%)	21 (75)		
Baby birth weight (g)	3370 (2966 – 3668)	2436	4468
Baby gender			
Male, n (%)	15 (53.6)		
Female, n (%)	13 (46.4)		
*IBR (%)	54.45 (15.05 – 75.00)	4.60	97.10

*IBR= Individualised birth weight ratio

4.3.3 Time course of hCG secretion by control explants

Figure 4.11A shows the time course of hCG secretion by placental explants in control medium (n=29) for 7 days of culture, with hCG secretion low at day 2 and peaking at day 5 (hCG secretion increased ~5 fold from day 2 to day 5).

Figure 4.11B compares hCG secretion by explants in standard control culture medium and control medium 2 (n=16). Control medium 2 was prepared by adding 10% dH_2O into the culture medium (2mL dH_2O into 18mL of culture medium). This approach was applied to mimic the addition of 10% DEP and HD (in water) into the culture medium and determines whether dilution of the culture medium would affect explant function. There was no significant effect of diluted culture medium on hCG secretion when compared to standard medium (Figure 4.11B).

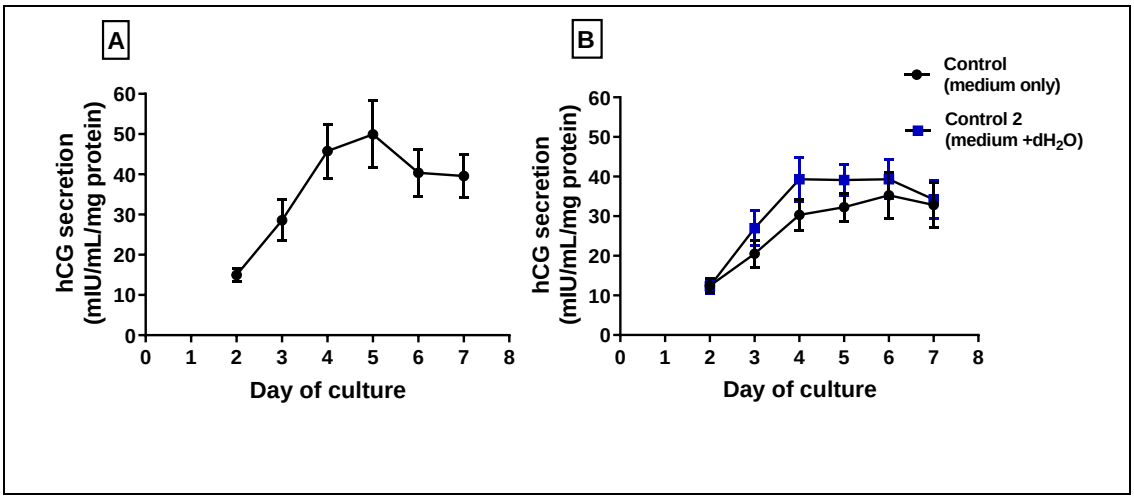


Figure 4.11: Comparison of hCG secretion between standard and diluted control culture medium

(A) The time course of hCG secretion by explants in control medium over 7 days of culture (n=29). (B) hCG secretion by placental explants in control medium and control medium 2 (added 10% dH_2O).

hCG secretion from explants maintained in normal control or diluted culture medium (control 2) was not significantly different (Mann-Whitney Test; P-value=0.31; n=16). Data represented as mean $\pm$ SEM.

4.3.4 Determination of optical density generated by DEP and HD

As described in Section 4.2.4.2, following the sonication process, DEP and HD were further diluted in culture medium to treat the villous explants. However, the culture medium containing DEP was black (Figure 4.12A), raising the possibility that it could interfere with the measurement of hCG by ELISA with spectrophotometry. To assess whether the colour interfered with the ELISA, the hCG assay was performed on a culture medium, not exposed to explants but containing DEP and HD SRM's (2.5, 25, 100 and 500µg/mL) and H_2O_2 (5mM). Culture medium with explants treated with DEP and HD on day 2, 5 and 7 were also tested, and the results are shown in Figure 4.12C. Blank wells and wells filled with dH_2O were also tested in this experiment.

By referring to Figure 4.12C, the level of absorbance of culture medium without explants was recorded far below than culture medium with explants. The absorbance of wells filled with culture medium, blank wells, wells with dH_2O and wells with H_2O_2, DEP and HD were at the same level (~6 to 7), whereas the absorbance of wells with explants was at the level ~8 to 74. This result suggested that the black colour of DEP and HD in a culture medium did not influence the reading of absorbance in hCG ELISA assay.

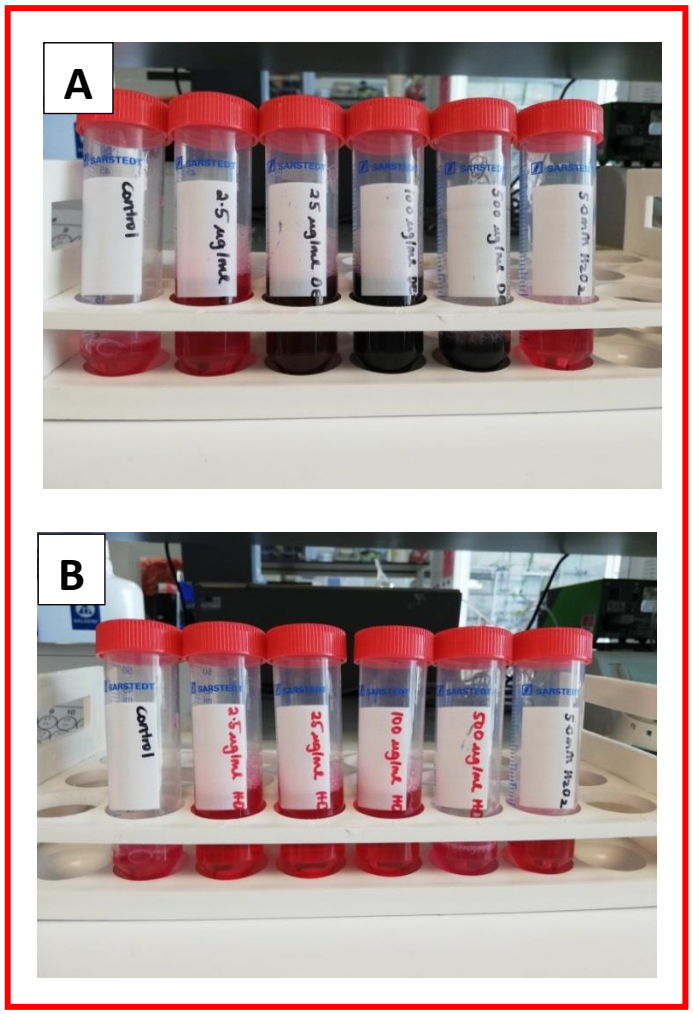


Figure 4.12: Optical density generated by DEP and HD

Figure A & B shows the fresh culture medium after diluted with (A) DEP and (B) HD, whereas graph in (C) shows the absorbance of culture medium that diluted with DEP and HD, with and without exposed to explants, and assayed with hCG ELISA at 450nm absorbance.

4.3.5 Series 1: Effect of H_2O_2 on hCG secretion by placental explants

Figure 4.13 shows the effect of treating villous explants with H_2O_2 at 0.5, 1.0 and 5.0mM on day 5 and 6 of culture on hCG secretion (n=7). The time course of hCG secretion by explants in control and treatment groups, over 7 days of culture is shown in Figure 4.13A. In control conditions, hCG secretion increased from day 2 to peak at day 5 (~5-fold greater than day 2) and then fell and stabilised at days 6 and 7. H_2O_2 at 5.0mM induced a greater drop in hCG secretion at day 7 compared to the time-matched control.

Figure 4.13B and C shows hCG secretion on day 6 and 7, expressed as a percentage of secretion on day 5. H_2O_2 did not significantly change hCG secretion on day 6, but 5.0mM H_2O_2 significantly reduced hCG secretion by 42%, on day 7 compared to control [Median (IQR) = Control: 67.05 (65.03 – 78.28) vs. 5.0mM H_2O_2: 45.08 (30.01 – 62.48)]. Due to this significant reduction of hCG secretion, 5.0mM H_2O_2 was used as a positive control in subsequent experiments where explants were treated with DEP and HD.

4.3.6 Series 2 and 3: Effect of diesel exhaust particles on hCG secretion by placental explants

4.3.6.1 48-hour treatment

Initially, villous explants from 6 placentas were cultured for 7 days and treated with DEP for 48 hours from day 5, to determine if there was a short term effect of this environmental pollutant on hCG secretion. Figure 4.14 shows the effect of DEP (SRM1650b) treatment for 48 hours on hCG secretion. Figure 4.14A shows the time course of hCG secretion by explants in control and treatment groups, over 7 days of culture. In this experiment, the control culture medium contained an additional 10% dH_2O, to match the SRM1650b media. hCG secretion in the control culture medium increased ~5-fold from day 2 to day 5 and then fell to day 7. hCG secretion by positive control explants treated with 5.0mM H_2O_2 showed a similar trend as previous experiments (Figure 4.13B).

Figure 4.14C shows the comparison of hCG secretion level by explants in day 7 as percentage of day 5. SRM1650b did not significantly alter hCG secretion at any of the concentrations used when applied to explants for 48hr. Therefore in the subsequent series of experiments (series 3), longer treatment process was introduced, when explants were treated with SRM1650b from day 1 until 6.

4.3.6.2 Daily treatment on days 1 to 6

Figure 4.15 shows the effect of DEP and H_2O_2 on hCG secretion. DEP (SRM1650b), at concentrations of 2.5, 25 and 100µg/mL, or 5.0mM H_2O_2 was added to the medium daily from day 1 until day 6 (n=8). The time course of hCG secretion by explants in control and treatment groups, over the 7 days of culture is shown in Figure 4.15A. The temporal change of hCG secretion by control explants (black line) was similar to that obtained obtained in a previous experiment (see Figure 4.14); hCG secretion increased ~4-fold from day 2 to day 4 and then plateaued for the remainder of the culture. hCG secretion by explants treated with 2.5µg/mL DEP (green line) was similar to control, whereas for 25 (blue line) and 100µg/mL DEP (red line), hCG secretion was greater than control from day 4 to 7 of culture. The hCG secretion level on day 4, by explants treated with 25 and 100µg/mL DEP was significantly higher, up to 5-fold, compared to day 2 [Median (IQR) = Day 2: 12.98 (8.16 – 16.61) vs. Day 4: 58.97 (31.76 – 84.00)]. Meanwhile, the temporal change of hCG secretion by positive control explants (see Figure 4.15B) was similar to previous experiments.

The area under the curve (AUC) was calculated to determine the effect of DEP on hCG secretion and is plotted in Figure 4.15C, expressed as a percentage (%) of time-matched control. By comparing with control group [Median (IQR) = Control: 37.64 (25.86 – 41.50)], SRM1650B (DEP) significantly elevated hCG secretion at 25 and 100µg/mL by 29% [25µg/mL DEP: 49.83 (27.43 – 58.01)] and 49% [100µg/mL DEP: 57.86 (31.66 – 61.67)], respectively. A non-significant reduction in hCG secretion was recorded in positive control explants (5.0mM H_2O_2) compared to the corresponding control (Figure 4.15D).

4.3.7 Series 4: Effect of house dust on hCG secretion by placental explants

Figure 4.16 shows the effect of HD and H_2O_2 on hCG secretion. HD (SRM2585), at concentrations of 2.5, 25 and 100µg/mL, or 5mM H_2O_2 was added to the medium daily from day 1 until day 6 (n=8). The time course of hCG secretion by explants in control and treatment groups over 7 days of culture is shown in Figure 4.16A. The temporal change of hCG secretion by control explants (black line) was increased 5-fold from day 2 until day 5 and declined for the remainder of the culture. hCG secretion by explants treated with HD shown a similar pattern as control, but the progression of hCG secretion over 7 days of culture was lower than control, and only increased 3-fold from day 2 to day 5.

The AUC of hCG secretion by control explants and treated with HD is shown in Figure 4.16C and expressed as % of time-matched control. By comparing with control

group [Control: 33.65 (20.78 – 38.14)], SRM2585 (HD) significantly reduced hCG secretion at 2.5 and 25µg/mL HD, by 32% [2.5µg/mL HD: 21.94 (17.74 – 23.51)] and 29% [25µg/mL HD: 21.21 (16.90 – 26.51)], respectively. 100µg/mL HD [100µg/mL HD: 23.99 (15.13 – 31.30)] was also reduced hCG secretion level by 31%, with trend towards significant was recorded. Positive control explants was reduced hCG secretion compared to corresponding control (Figure 4.16D).

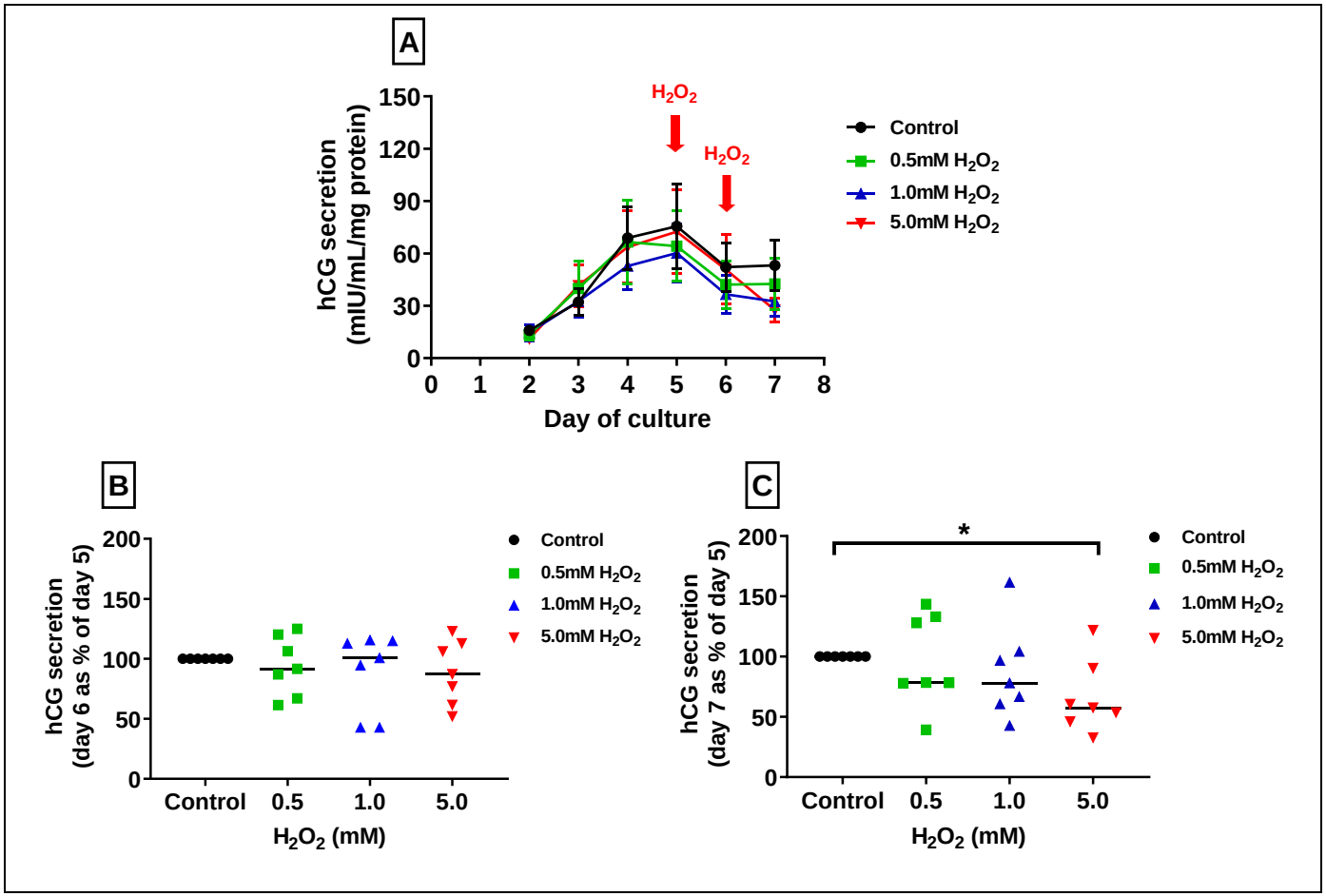


Figure 4.13: Effect of H_2O_2 on hCG secretion by placental explants (Series 1)

(A) Time course of hCG secretion by placental explants treated with H_2O_2, on days 5 and 6 of culture, data represented as mean $\pm$ SEM (n=7), (B) hCG secretion by explants treated with H_2O_2 on day 6 expressed as a percent of secretion at day 5, (C) hCG secretion by explants treated with H_2O_2 on day 7 expressed as a percent of secretion at day 5. Data in B and C expressed as % of control and the line represents the median. *P-value<0.05 vs. Control (100%) (Wilcoxon signed rank test, n=7).

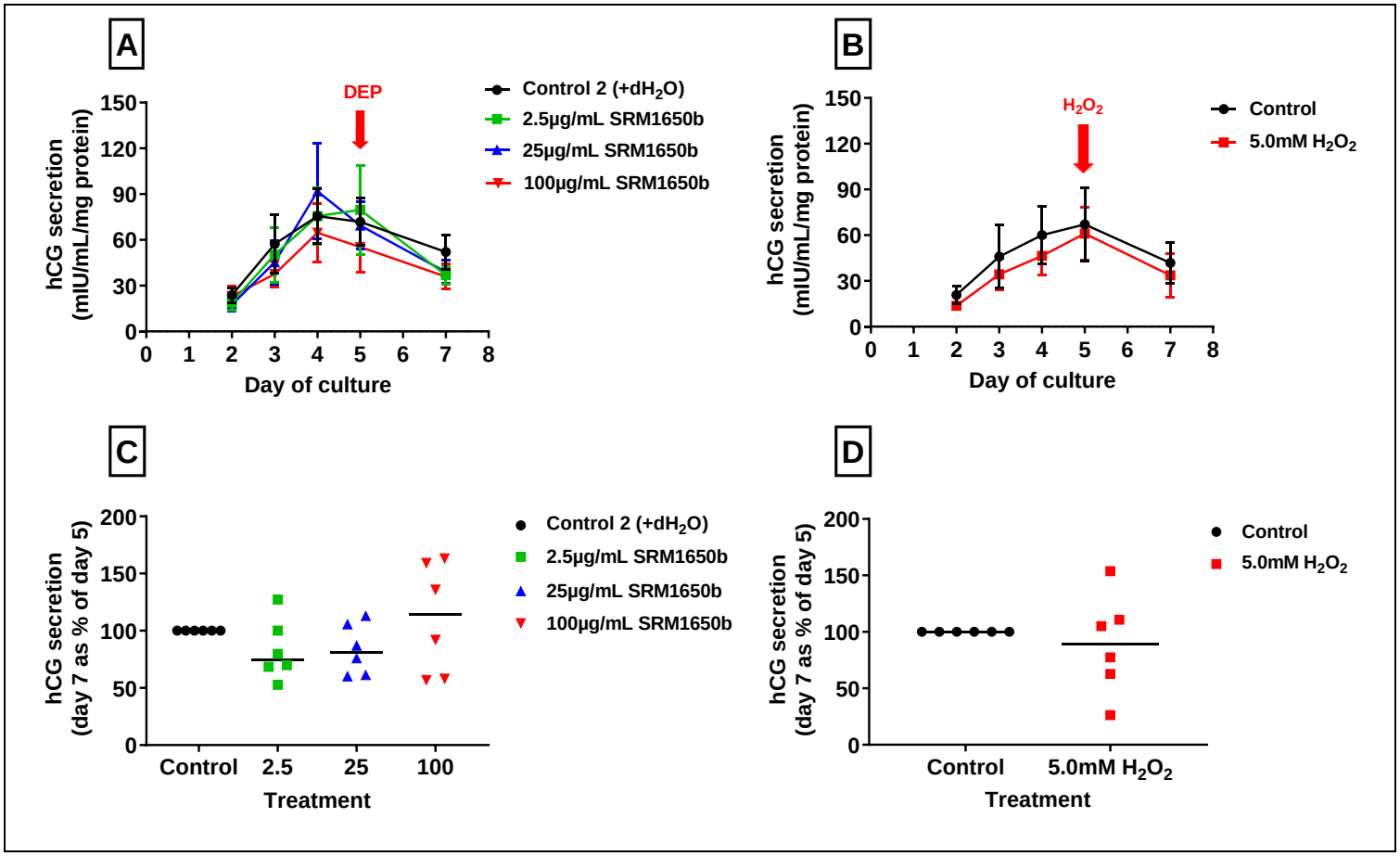


Figure 4.14: Effect of DEP applied for 48 hours on hCG secretion from placental explants (Series 2)

Time course of hCG secretion by explants treated with (A) DEP and (B) 5.0mM H_2O_2, for 48 hours (days 5 to 7). Data represented as mean ± SEM (n=6). The comparison of hCG secretion level by explants treated with (C) DEP and (D) 5.0mM H_2O_2 on day 7 as percentage of secretion at day 5. Data in C and D expressed as % of control and the line represents median.

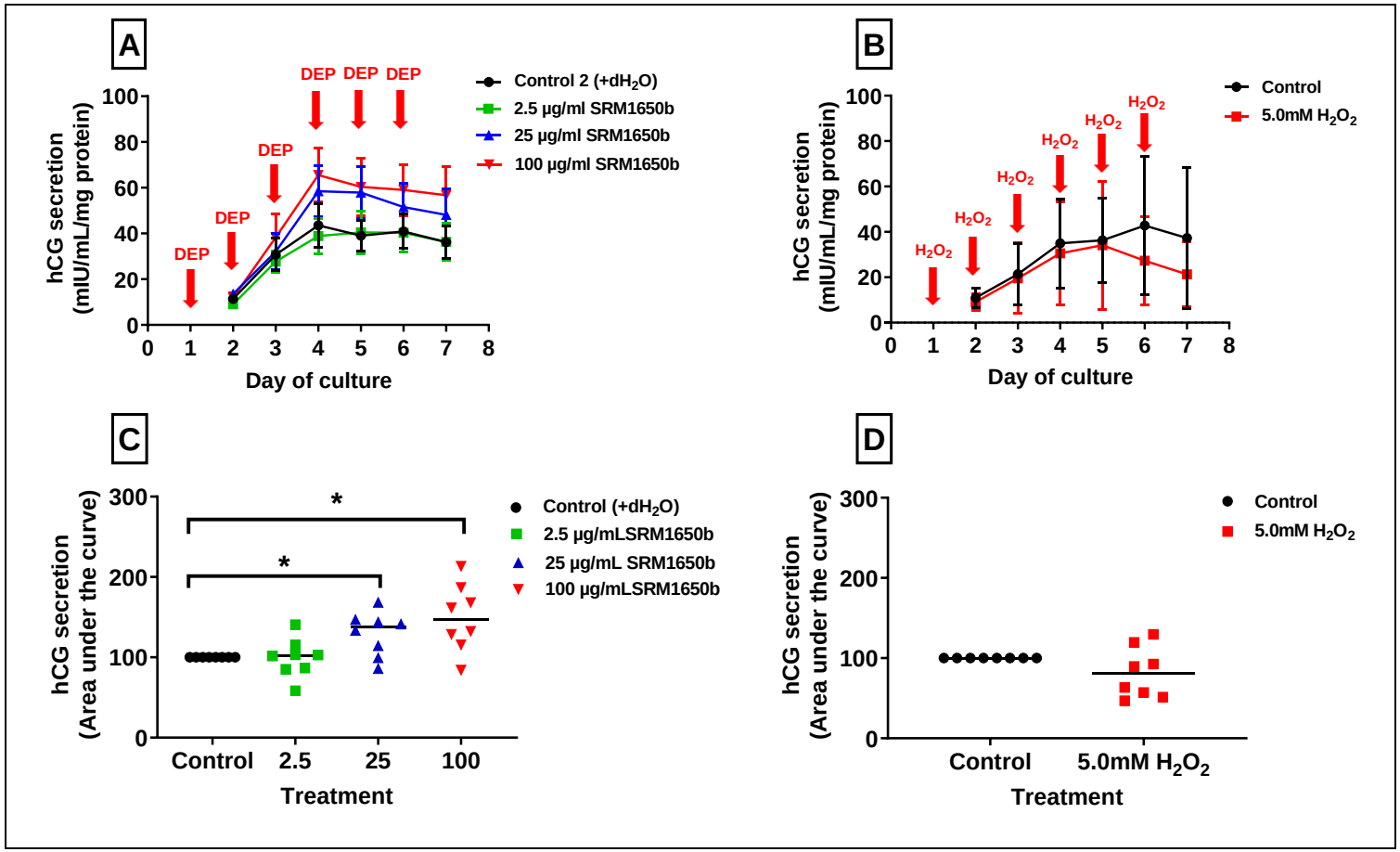


Figure 4.15: Effect of DEP applied for 6 days on hCG secretion from placental explants (Series 3)

Time course of hCG secretion by explants treated with (A) DEP and (B) 5.0mM H_2O_2, for 6 days (days 1 to 6). Data represented as mean ± SEM (n=8). The comparison of hCG secretion by explants treated with (C) DEP and (D) 5.0mM H_2O_2 for 6 days. Data calculated as AUC over 7 days of culture, expressed as % of control and the line represents median. *P-value=0.04 and 0.02 for 25 and 100µg/mL DEP, respectively (Wilcoxon signed rank test, n=8).

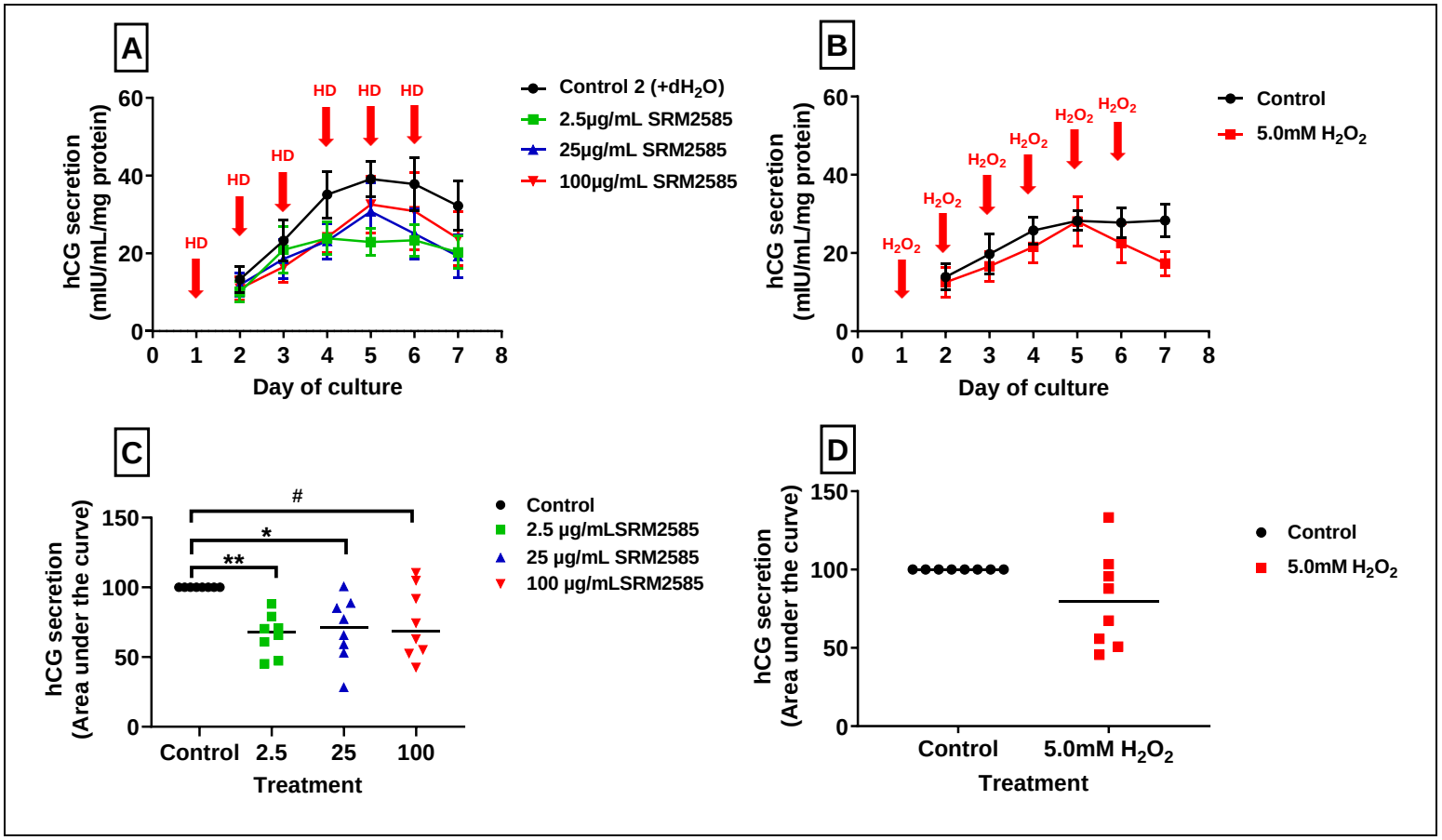


Figure 4.16: Effect of HD applied for 6 days on hCG secretion from placental explants (Series 4)

Time course of hCG secretion by explants treated with (A) HD and (B) 5.0mM H_2O_2, for 6 days (days 1 to 6). Data represented as mean ± SEM (n=8). The comparison of hCG secretion by explants treated with (C) HD and (D) 5.0mM H_2O_2 for 6 days. Data calculated as AUC over 7 days of culture, expressed as % of control and the line represents median. **P-value<0.01 for 2.5µg/mL; *P-value=0.01 for 25µg/mL; $^{\#}$P-value=0.05 for 100µg/mL HD (Wilcoxon signed rank test, n=8).

4.3.8 Assessment of cell turnover in explants treated with environmental pollutants

4.3.8.1 Proliferation: Ki67 staining

Figure 4.17 shows representative photomicrographs of Ki67 staining, to determine the proliferation index of placental explants, assessed at day 7 following treatment with H_2O_2, DEP and HD. The proliferation index was determined by calculating the positively stained with Ki67 and expressing this as a percentage of the total number of nuclei within the FOV. The mean percentage of proliferation was determined using six FOV per section, with at least four independent placental explant cultures for each experimental series (n=4/5).

First trimester placenta was used as a positive control to determine the proliferation index, as in early pregnancy there are more cytotrophoblast cells and a higher rate of proliferation. Ki67 staining was not observed in the negative control (primary antibody replaced with non-immune IgG: Figure 4.17A) but high levels of positive staining were seen in the first trimester explant positive controls (Figure 4.17B) indicating successful staining. Figure 4.17C – F shows that there were fewer Ki67 positive cells in explants, maintained in control medium and following treatment with H_2O_2, DEP and HD, indicated lower proliferation rates occurred in cytotrophoblast cell of term placenta compared with the first trimester placenta.

Figure 4.17G – I illustrates the proliferation index of explants treated with H_2O_2, DEP and HD, expressed as a % of the corresponding control, whereas Figure 4.17J compares the proliferation index between treatments, at the highest concentration plotted on the same scale for ease of comparison. There were no significant differences in proliferation between treatments and control. There was a tendency for an incremental increase in proliferation, in response to increasing concentrations of HD, but this was not significant with limited number of experiments (n=5) and the proliferation index was variable even in the control explants [Median (IQR) = Control: 2.28 (1.17 – 4.36); 25HD: 4.14 (2.34 – 4.46); 100µg/mL HD: 2.97 (2.83 – 5.76)].

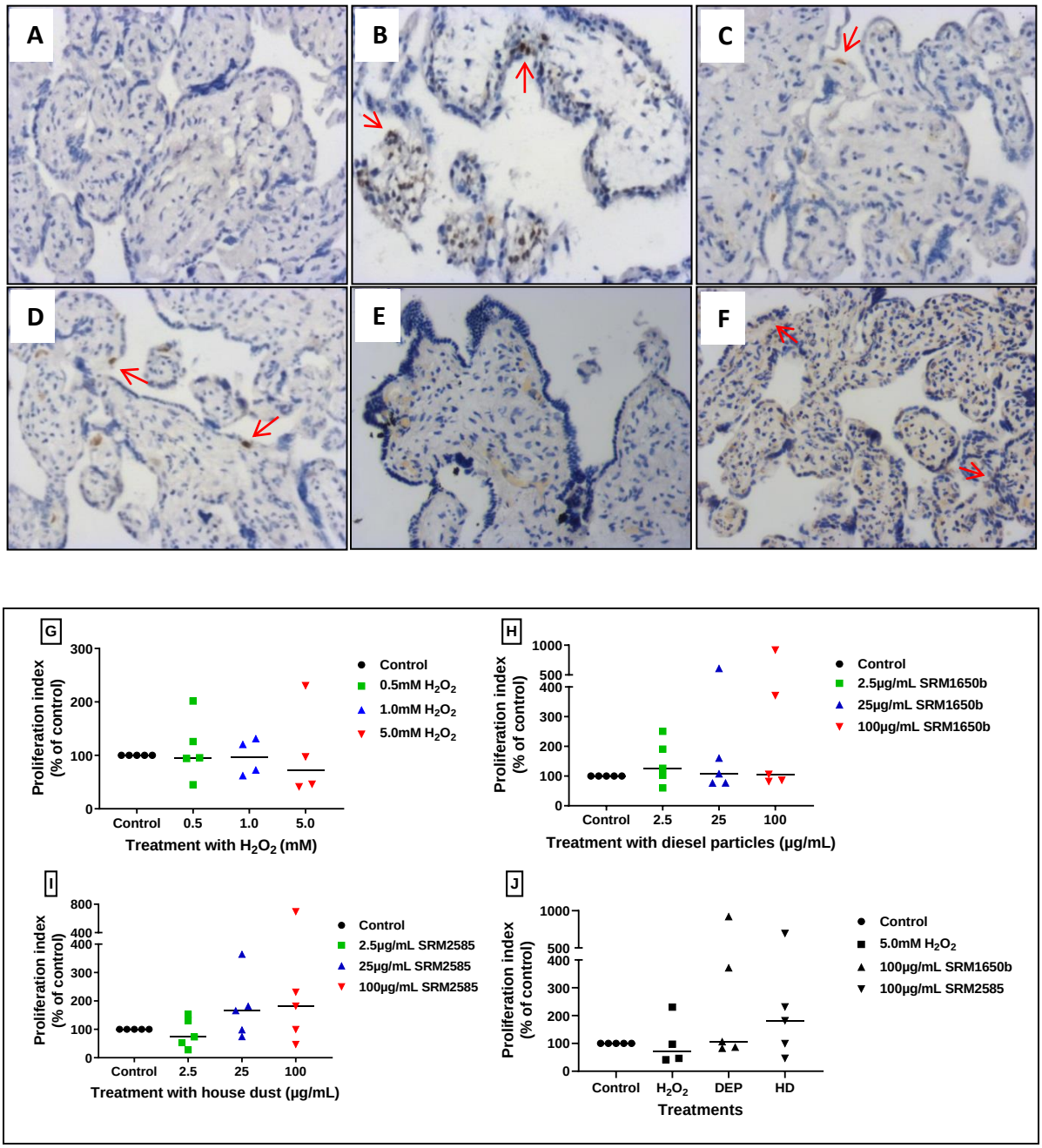


Figure 4.17: Effect of exposure to H_2O_2, SRM1650b (DEP) and SRM2585 (HD) on explant proliferation index.

Representative photomicrographs show Ki67 staining for (A) negative control explants (B) positive control explants – first trimester placenta (C) explants maintained in control medium (D) explants treated with 5.0mM H_2O_2 (E) explants treated with 100µg/mL DEP and F) explants treated with 100µg/mL HD. IHC was performed at day 7 of culture, following 2 and 6 days treatment with H_2O_2, DEP and HD, respectively. Image was taken at x20 magnification. Red arrows indicate Ki67 positive staining.

Graphs show the proliferation index of explants treated with (G) H_20_2, (H) DEP, (I) HD, expressed as a % of control, (J) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=4/5; line shows median). No significance difference of proliferation index in all treatment groups vs. control (100%) (Wilcoxon signed rank; P-value>0.05).

First trimester placenta (as showed in B) was a gift via Zhiyong Zou (MFHRG) from Professor Melissa Westwood.

4.3.8.2 Apoptosis: M30 staining

Figure 4.18 shows the representative photomicrographs of M30 staining, to determine the apoptotic index of placental explants, assessed at day 7 following treatment with H_2O_2, DEP and HD. The apoptotic index was determined by calculating the positively stained with M30 and expressing this as a percentage of the total number of nuclei within the FOV. The mean percentage of apoptotic index was determined using six FOV per section, with at least five independent placental explant cultures for each experimental series (n=5/6).

M30 staining was not observed in the negative control (primary antibody replaced with non-immune IgG: Figure 4.18A). Figure 4.18B – E shows M30 positive staining, localized in STB layer of explants maintained in control medium and treated with H_2O_2, DEP and HD.

Figure 4.18F – H illustrates the apoptotic index of explants treated with H_2O_2, DEP and HD, expressed as a % of the corresponding control, whereas Figure 4.18I compares the apoptotic index between treatments, at the highest concentration plotted on the same scale for ease of comparison. There were no significant differences in apoptotic index between treatments and control. There was an increased apoptotic index of explants treated with 0.5mM H_2O_2 compared to corresponding control. Increment of apoptotic index was also recorded between control and 100µg/mL DEP. Explants treated with HD had a lower apoptotic index compared than control. By comparing the apoptotic index between treatment groups, explants treated with 100µg/mL DEP recorded higher level of apoptosis compared to control and other treatment groups.

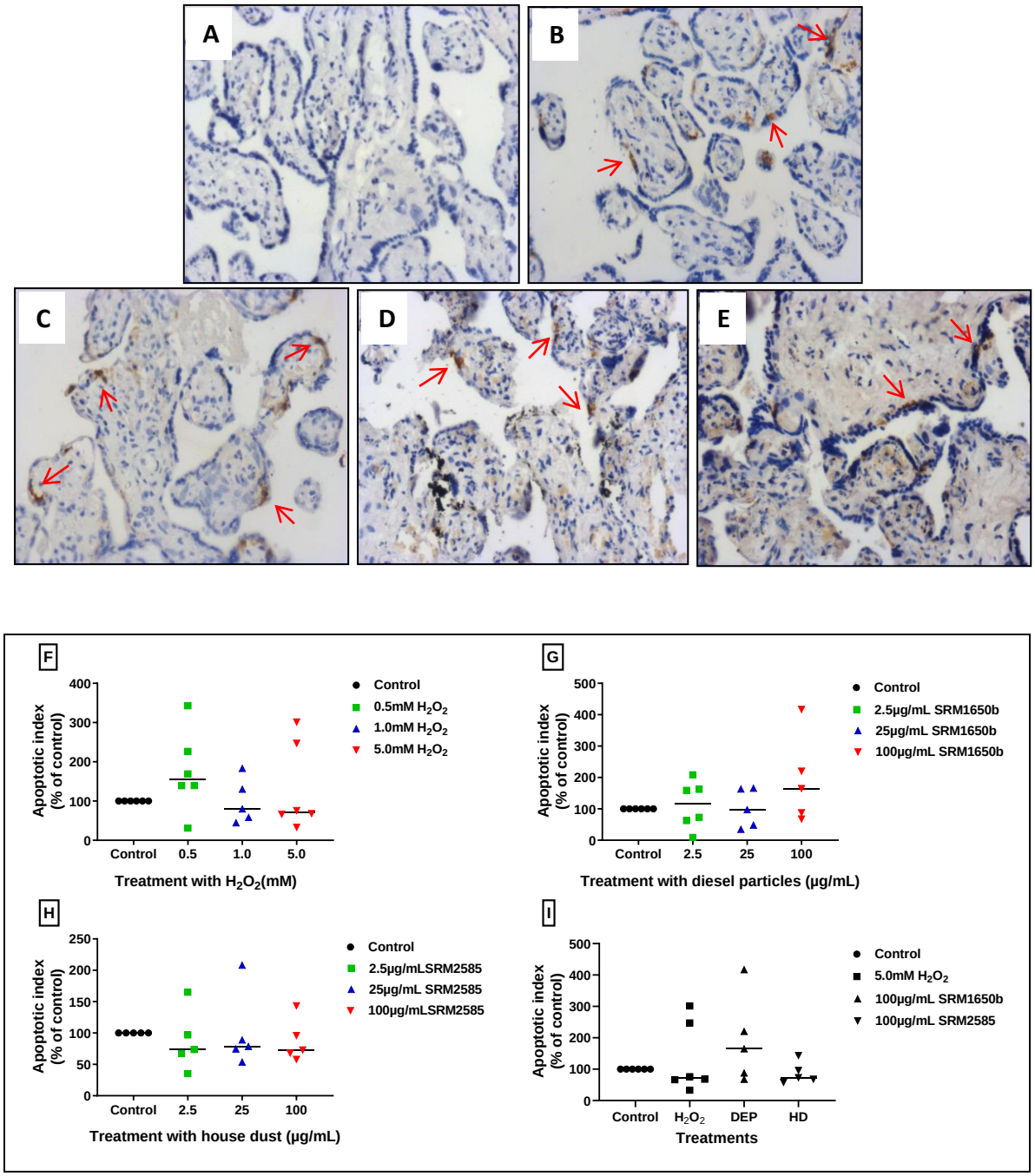


Figure 4.18: Effect of exposure to H_2O_2, SRM1650b (DEP) and SRM2585 (HD) on explant apoptotic index.

Representative photomicrographs show M30 staining for (A) negative control explants, (B) explants maintained in control medium, (C) explants treated with 5.0mM H_2O_2,(D) explants treated with 100µg/mL DEP, and (E) explants treated with 100µg/mL HD. IHC was performed at day 7 of culture, following 2 and 6 days treatment with H_2O_2 and SRMs, respectively. Image was taken at x20 magnification. Red arrows indicate M30 positive staining.

Graphs show the apoptotic index of explants treated with (F) H_20_2, (G) DEP, (H) HD, expressed as a % of control, (I) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=5/6; line shows median). No significance difference of apoptotic index in all treatment groups vs. control (100%) (Wilcoxon signed rank; P-value>0.05).

4.3.8.3 Oxidative damage to DNA: 8-OHdG staining

Figure 4.19 shows representative photomicrographs of 8-hydroxy-2'-deoxyguanosine (8-OHdG) staining, to determine oxidative DNA damage of placental explants treated with H_2O_2, DEP and HD, following 7 days of culture. The oxidative damage was determined by calculating the positively stained nuclei with 8-OHdG and expressing this as a percentage of the total number of nuclei within the FOV. The mean percentage of oxidative damage was determined using six FOV per section, with at least five independent placental explant cultures for each experimental series (n=5/6).

8-OHdG staining was not observed in the negative control (primary antibody replaced with non-immune IgG: Figure 4.19A). The proportion of 8-OHdG positive nuclei was higher in explants treated with DEP (Figure 4.19D) and HD (Figure 4.19E) compared to explants cultured in control medium (Figure 4.19B). Positive staining of 8-OHdG was observed in the villous core which could be localised in Hofbauer cells and less intense staining in the syncytiotrophoblast layer.

Figure 4.19F – H shows the oxidative damage index of placental explants treated with H_2O_2, DEP and HD which expressed as a percentage of the matched control. H_2O_2 did not have any effect on 8-OHdG staining (Figure 4.19F). Figure 4.19G shows that DEP increased oxidative damage index; 8-OHdG staining was significantly higher than control with 100µg/mL DEP and of borderline significance with 25µg/mL DEP [Median (IQR) = Control: 43.41 (41.40 – 45.09) vs. 25µg/mL DEP: 50.73 (47.36 – 54.03); 100µg/mL DEP: 52.20 (48.14 – 55.42)]. HD also increased oxidative damage at 100µg/mL (Figure 4.19I), although there was only a trend towards significance compared to control group [Median (IQR) = Control: 27.49 (22.62 – 36.55) vs. 100µg/mL HD: 44.74 (39.88 – 49.67)].

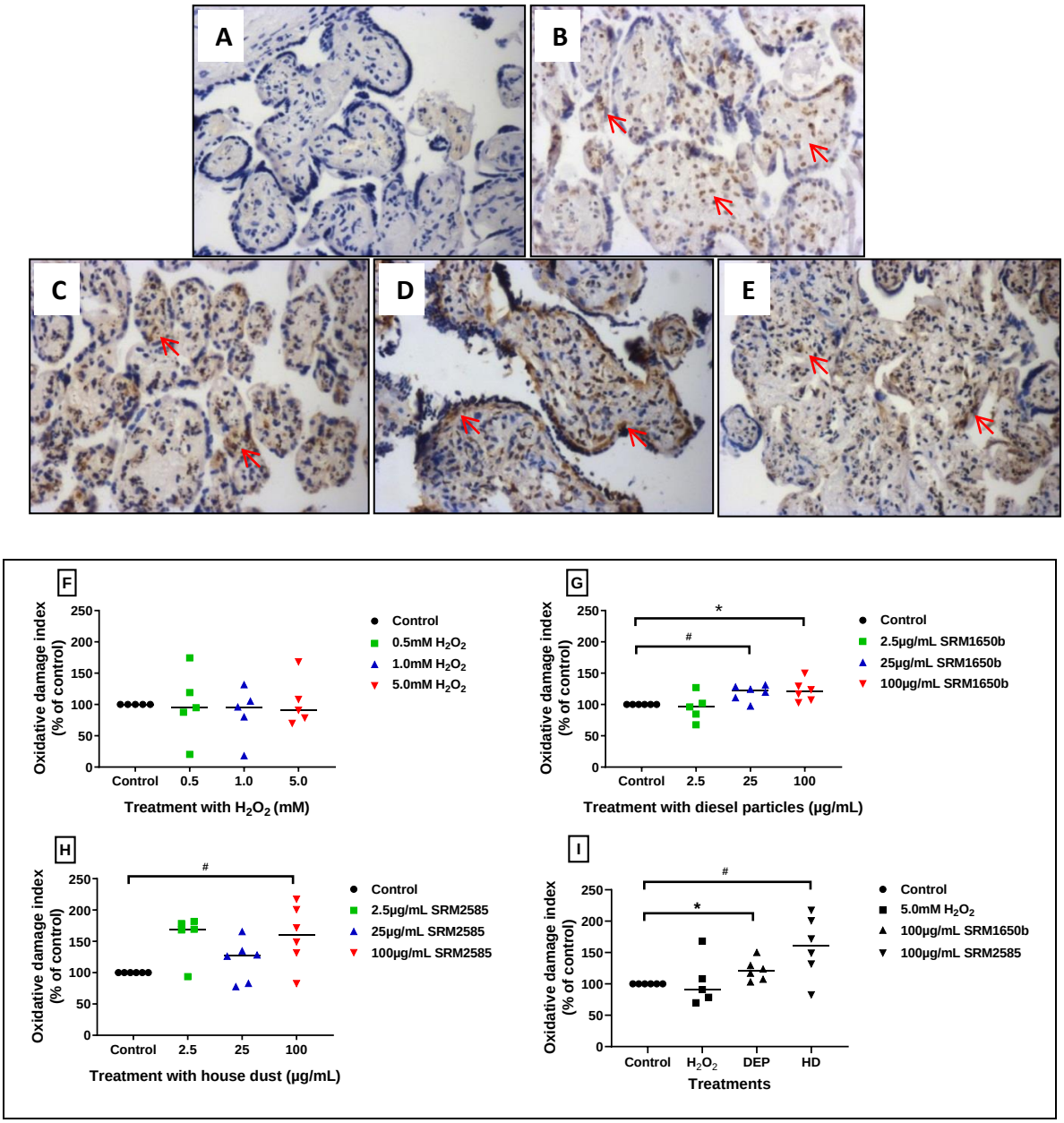


Figure 4.19: Effect of exposure to H_2O_2, SRM1650b (DEP) and SRM2585 (HD) on explant oxidative damage index.

Representative photomicrographs show 8-OHdG staining for (A) negative control explants, (B) explants maintained in control medium, (C) explants treated with 5.0mM H_2O_2, (D) explants treated with 100µg/mL DEP, and (E) explants treated with 100µg/mL HD. IHC was performed at day 7 of culture, following 2 and 6 days treatment with H_2O_2 and SRMs, respectively. Image was taken at x20 magnification. Red arrows indicate 8-OHdG positive staining.

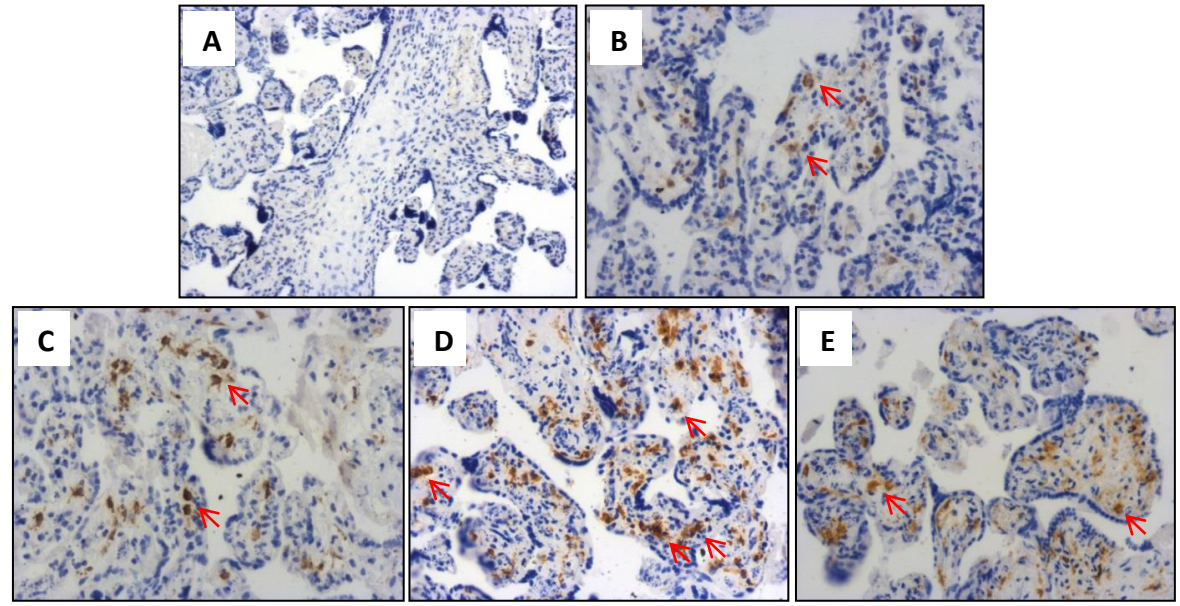
Graphs show the oxidative damage index of explants treated with (F) H_20_2, (G) DEP, (H) HD, expressed as a % of control, (I) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=5/6; line shows median). *Significance at P-value=0.03 vs. control (100%); $^{\#}$Trend towards significance at P-value=0.06 vs. control (100%) (Wilcoxon signed rank; n=6).

4.3.8.4 Number of macrophages: CD163 staining

Figure 4.20 shows representative photomicrographs of CD163 staining, to determine the number of macrophages which act as a marker of placental inflammation, assessed at day 7 following treatment with H_2O_2, DEP and HD. The number of macrophages was determined by calculating the positively stained nuclei with CD163 and expressing this as a percentage of the total number of nuclei within the FOV. The mean percentage of CD163 positive staining was determined using six FOV per section, with at least five independent placental explant cultures for each experimental series (n=5/6).

CD163 staining was not observed in the negative control (primary antibody replaced with non-immune IgG: Figure 4.20A). The positive staining of CD163 in placental explants cultured in control medium, H_2O_2, DEP and HD are shown in Figure 4.20B – E. Positive staining of CD163 was observed in the villous core which could be localised in Hofbauer cells.

Figure 4.20F – H show the CD163 positive staining of explants treated with H_2O_2, DEP and HD, expressed as a % of the corresponding control, whereas Figure 4.20I compares the CD163 positive staining between treatments, at the highest concentration plotted on the same scale for ease of comparison. There were no significant differences of CD163 positive staining between treatments and control. There was a tendency for an incremental increase in CD163 positive staining, in response to increasing concentrations of H_2O_2, but this was not significant with large variation of CD163 positive staining index in the control and treatment explants (Figure 4.20F). By comparing between treatments (Figure 4.20I), CD163 positive staining was recorded higher in explants treated with 5.0mM H_2O_2, compared to 100µg/mL DEP or HD.



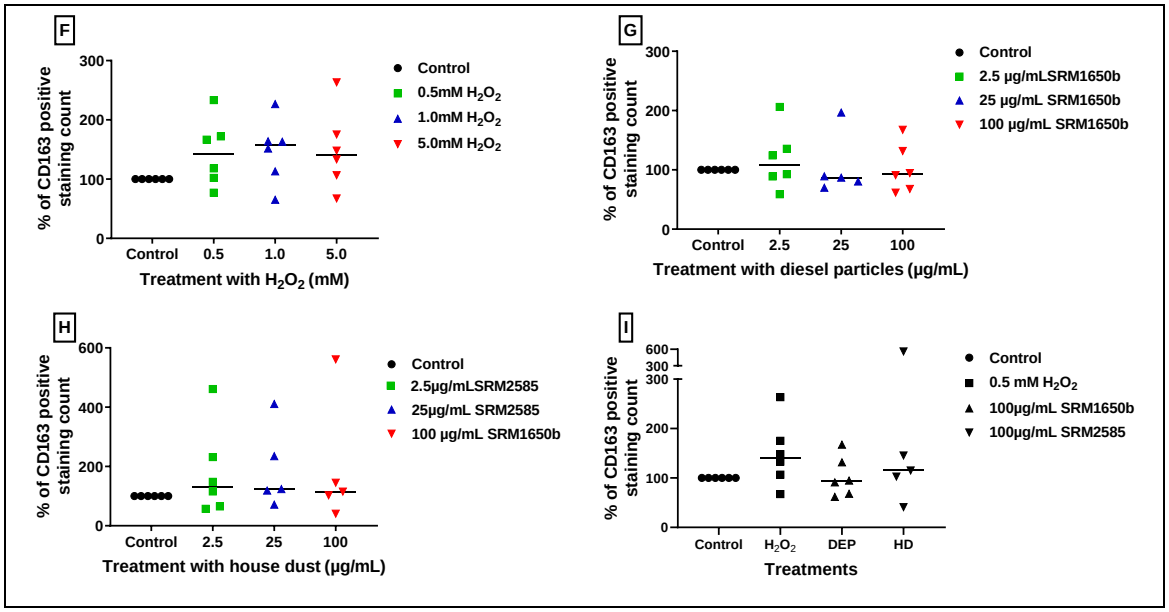


Figure 4.20: Effect of exposure to H_2O_2, SRM1650b (DEP) and SRM2585 (HD) on explant macrophages index.

Representative photomicrographs show CD163 staining for (A) negative control explants, (B) explants maintained in control medium, (C) explants treated with 5.0mM H_2O_2, (D) explants treated with 100µg/mL DEP, and (E) explants treated with 100µg/mL HD. IHC was performed at day 7 of culture, following 2 and 6 days treatment with H_2O_2 and SRMs, respectively. Image was taken at x20 magnification. Red arrows indicate CD163 positive staining.

Graphs show CD163 positive staining counts of explants treated with (F) H_20_2, (G) DEP, (H) HD, expressed as a % of control, (I) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=5/6; line shows median). No significance difference of macrophages index in all treatment groups vs. control (100%) (Wilcoxon signed rank; P-value>0.05).

4.3.8.5 hCG production staining

Figure 4.21 shows representative photomicrographs of hCG production staining of placental explants treated with H_2O_2, DEP and HD, following 7 days of culture. The hCG staining intensity was arbitrary scored by a blind observer, following method described in Section 4.2.4.2.2 and expressing this as a percentage of control. The mean percentage of hCG staining intensity of explants in treatment group was determined using six FOV per section, with at least three independent placental explant cultures for each experimental series (n=3/4/5/6).

hCG staining intensity was not observed in the negative control (primary antibody replaced with non-immune IgG: Figure 4.21A). hCG staining intensity was clearly observed in STB layer of terminal, also observed in shed and regenerated STB layer (Figure 4.21B – E).

Figure 4.21F – H show hCG staining intensity of placental explants treated with H_2O_2, DEP and HD, expressed as a percentage of the corresponding control, whereas Figure 4.21I compares the hCG staining intensity between treatments, at the highest concentration plotted on the same scale for ease of comparison. There were no significant differences of hCG production staining between treatments and control. There was a tendency for a reduction of hCG production staining, in response to increasing concentrations of H_2O_2, but this was not significant with limited number of experiments (n=4/5) and large variation of hCG staining score in control and treatment groups. A decrease in hCG production staining in explants treated with DEP was also observed, with reduction towards significant recorded in explants treated with 2.5µg/mL DEP [Median (IQR) = Control: 2.25 (1.83 – 2.67) vs. 2.5µg/mL DEP: 2.00 (1.50 – 2.17)]. By comparing between treatments (Figure 4.21I), hCG production staining was recorded lower in all treated groups compared to time matched control.

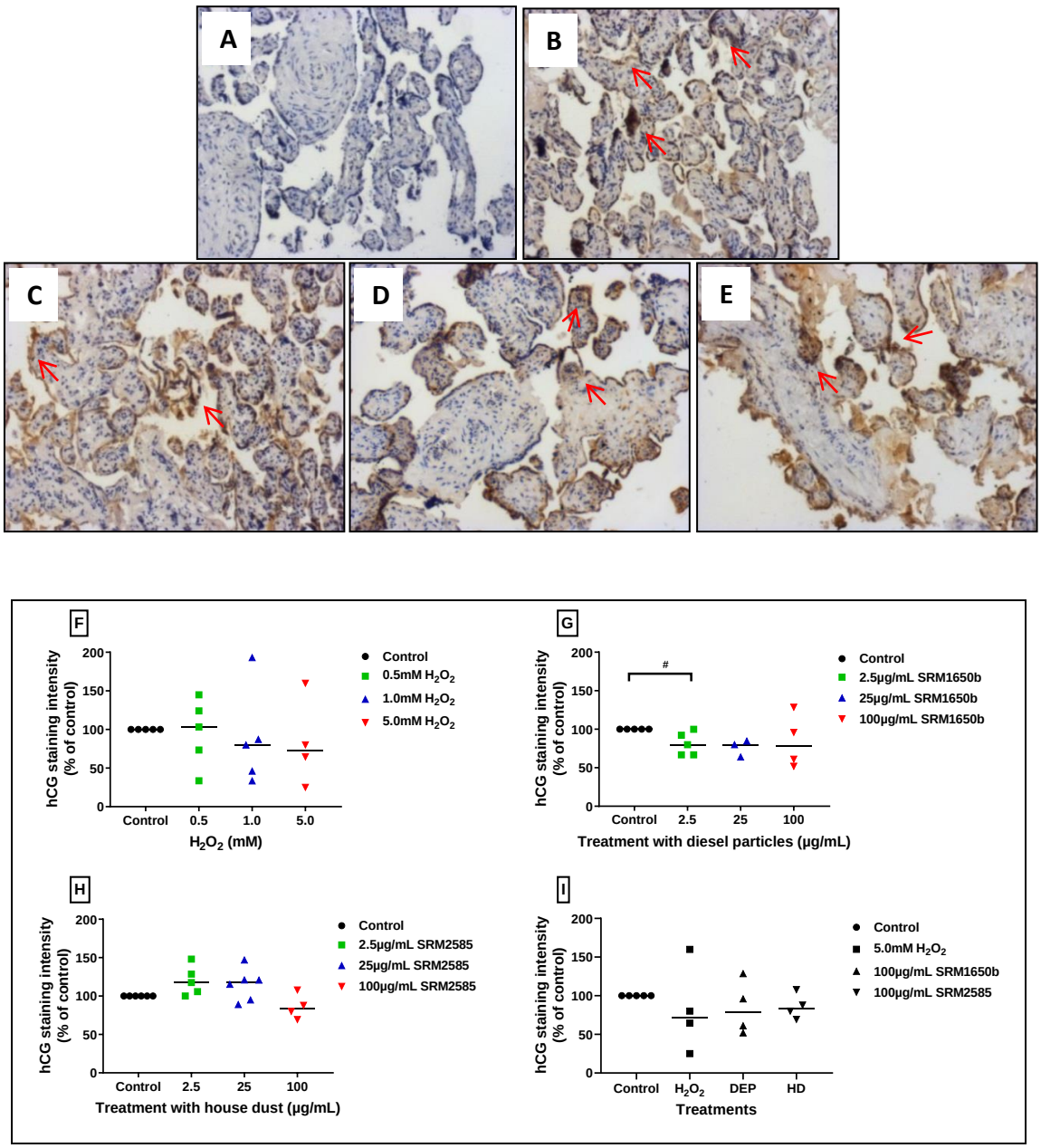


Figure 4.21: Effect of exposure to H_2O_2, SRM1650b (DEP) and SRM2585 (HD) on hCG production staining.

Representative photomicrographs show hCG staining for (A) negative control explants, (B) explants maintained in control medium, (C) explants treated with 5.0mM H_2O_2, (D) explants treated with 100µg/mL DEP, and (E) explants treated with 100µg/mL HD. IHC was performed at day 7 of culture, following 2 and 6 days treatment with H_2O_2 and SRMs, respectively. Image was taken at x40 magnification.

Graphs show hCG staining intensity of explants treated with (F) H_20_2, (G) DEP, (H) HD, expressed as a % of control, (I) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=3/4/5/6; line shows median). #Trend towards significance at P-value=0.06 vs. control (100%) (Wilcoxon signed rank; n=5).

4.3.8.6 Syncytiotrophoblast integrity: CK7 staining

Figure 4.22 (A – E) shows representative photomicrographs of explants, cultured in control medium, H_2O_2, DEP and HD and stained with cytokeratin 7 (CK7) to assess the integrity of STB layer, following 7 days of culture. CK7 was qualitatively scored by a blind observer, following the method described in Section 4.2.4.2.2. Villi were assigned to four categories: (i) bald villi with no STB surrounding the villous core, (ii) villi with intact STB, (iii) villi with shed STB and signs of STB regeneration, and (iv) villi with shed STB and no signs of STB regeneration. The mean percentage of each categories was determined using six FOV per section, with at least five independent placental explant cultures for each experimental series (n=5/6).

Figure 4.22A shows the absence of positive staining in the negative control section (CK7 antibody replaced with non-immune IgG). CK7 positive staining shows STB layer observed in all placental explants, cultured in control medium (Figure 4.22B), H_2O_2 (Figure 4.22C – E), DEP (Figure 4.22F – H) and HD (Figure 4.22I – K). The shed and regeneration of STB layer was observed in all explants and positive staining was also detected in regenerated layer.

H_2O_2 did not significantly change any of the categories identified, indicating that the STB integrity assessed at day 7 was similar to controls. In contrast, explants treated with 100µg/mL DEP had lower bald villi (Figure 4.23B), compared to control group, but the reduction was not significant. The increment towards significant of villi with intact STB was recorded in explants treated with 100µg/mL DEP (Figure 4.24B) [Median (IQR) = Control: 26.63 (22.57 – 33.33) vs. 100µg/mL DEP: 36.45 (32.08 – 40.23). No significance changes of intact villi by treatment with H_2O_2 and HD were observed (Figure 4.24A&C). There was no difference between control and treatments, in the percentage of total villi which showed shedding and the percentage of villi with shed STB that showed signs of regeneration (data not shown). The percentage of shed villi without STB regeneration was slightly higher in explants treated with H_2O_2 and HD, at all concentrations (Figure 4.25A&C). The increasing trend towards significant was shown in explants treated with 2.5µg/mL HD, compared to control [Median (IQR) = Control 52.83 (51.65 – 54.43) vs. 2.5µg/mL HD: 62.90 (56.40 – 64.90)]. In contrast, there was a tendency for a decrease in shed villi without STB regeneration, in response to increasing concentrations of DEP, with a trend towards significance was recorded in explants treated with 100µg/mL DEP (Figure 4.25B) [Median (IQR) = Control: 63.99 (56.51 – 68.23) vs. 100µg/mL DEP: 53.16 (47.57 – 59.12)].

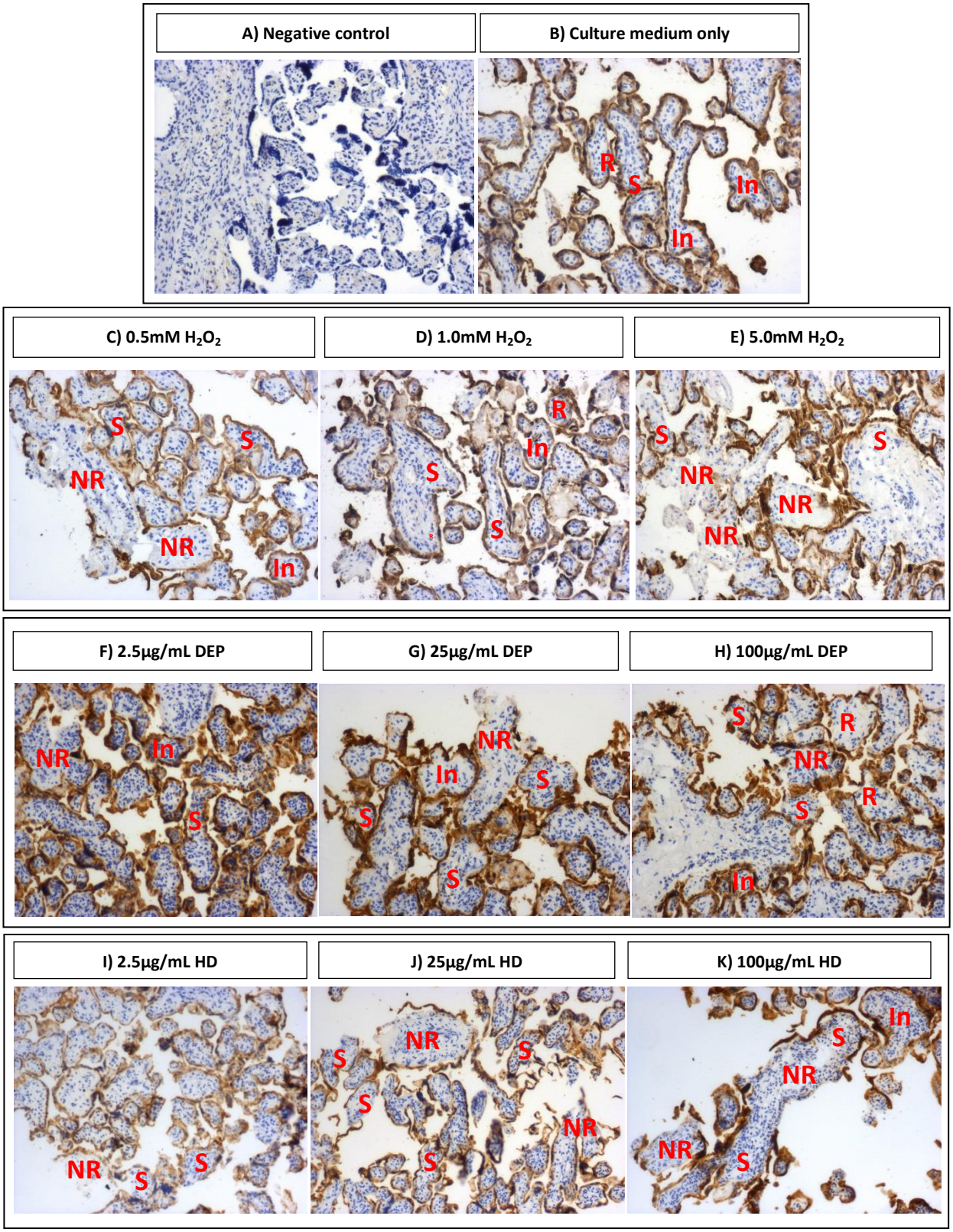


Figure 4.22: Effect of exposure to H_2O_2, SRM1650b (DEP) and SRM2585 (HD) on syncytiotrophoblast integrity staining.

Representative photomicrographs show CK7 staining for (A) negative control explants, (B) explants maintained in control medium, (C, D, E) explants treated with 0.5, 1.0, 5.0mM H_2O_2, (F, G, H) explants treated with 2.5, 25, 100µg/mL DEP, and (I, J, H) explants treated with 2.5, 25, 100µg/mL HD. IHC was performed at day 7 of culture, following 2 and 6 days treatment with H_2O_2 and SRMs, respectively. Image was taken at x40 magnification. Picture legend indicates (in) = intact; (s) = shed; (r) = regeneration; (nr) = no regeneration

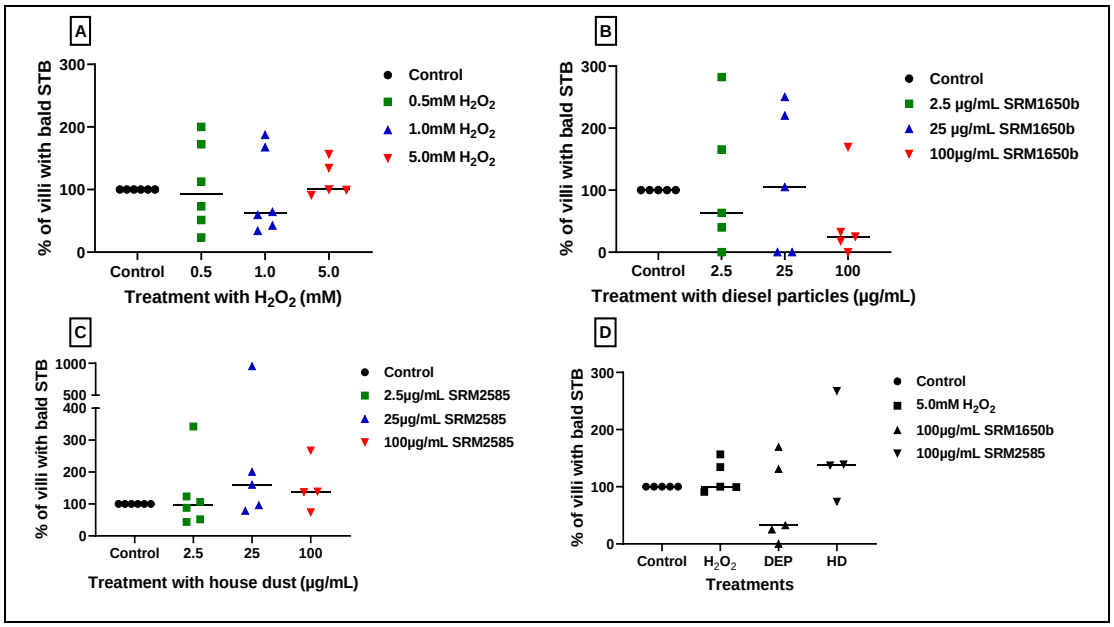


Figure 4.23: Percentage of villi with bald syncytiotrophoblast

Graphs show % of villi with bald STB of explants treated with (A) H_2O_2, (B) DEP, (C) HD, expressed as a % of corresponding control, and (D) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=4/5; line shows median).

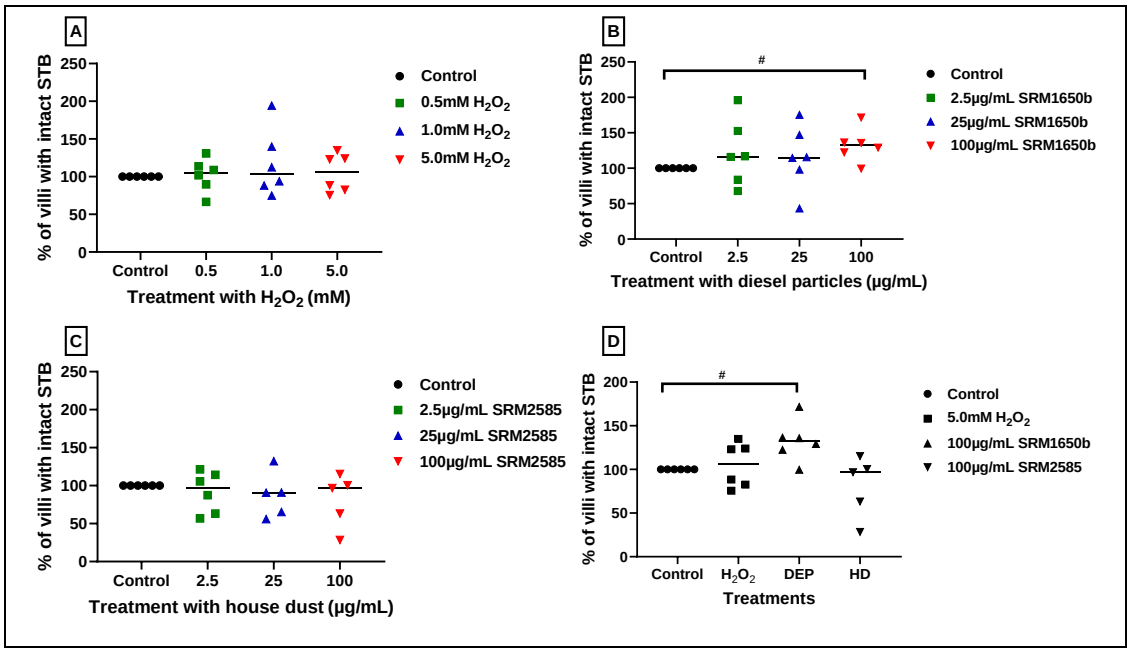


Figure 4.24: Percentage of villi with intact syncytiotrophoblast

Graphs show % of villi with intact STB of explants treated with (A) H_2O_2, (B) DEP, (C) HD, expressed as a % of control, and (D) compares the effect of the highest concentrations of treatments vs. control on the same scale (n=5/6; line shows median). #Borderline significance at P-value=0.06 vs. control (100%) (Wilcoxon signed rank; n=6).

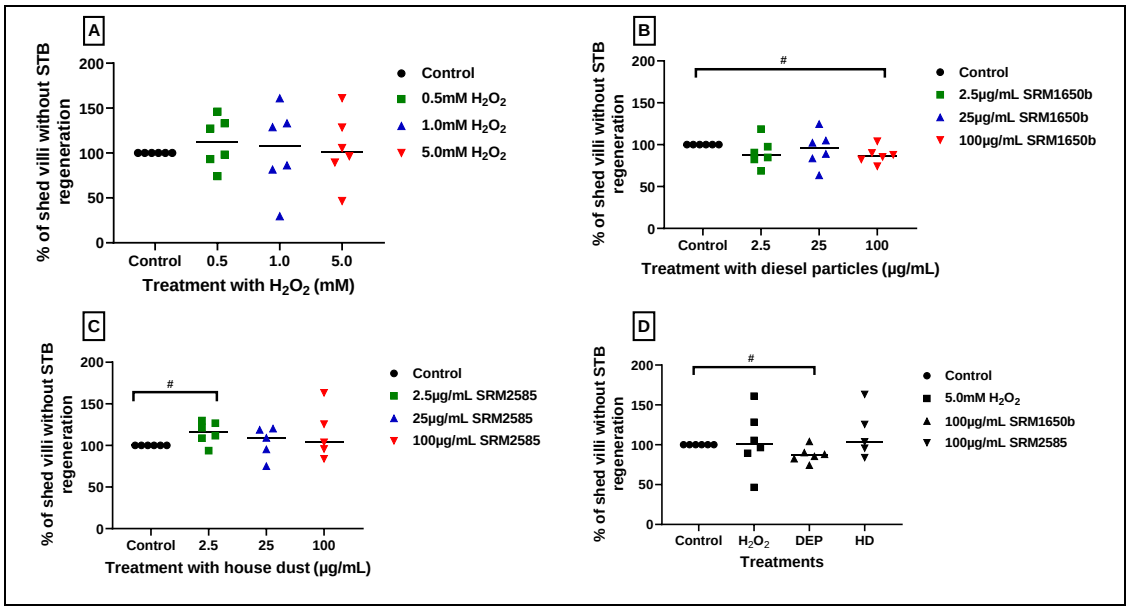


Figure 4.25: Percentage of shed villi without syncytiotrophoblast regeneration

Graphs show % of shed villi without STB regeneration of explants treated with (A) H_2O_2, (B) DEP, (C) HD, expressed as a % of control, and (D) compares the effect of the highest concentration of treatments vs. control on the same scale (n=5/6; line shows median). #Borderline significance at P-value=0.06 vs. control (100%) (Wilcoxon signed rank; n=6).

4.6 Discussion

4.6.1 Principal findings

This study set out to determine whether diesel exhaust particles (DEP) and house dust (HD), affect placental endocrine function and cellular turnover using term placental villous tissue explants *ex-vivo*. Both DEP and HD contain polycyclic aromatic hydrocarbons (PAHs), ubiquitous in the environment, and have been associated with adverse pregnancy outcome (Jedrychowski et al., 2017; Jedrychowski et al., 2013; Choi et al., 2012). The present study demonstrated that treating explants for six days in culture with SRM1650b (DEP) significantly elevated hCG secretion (49%) compared to time-matched controls. In contrast, six-day treatment with SRM2585 (HD) significantly reduced hCG secretion (32%). hCG in the syncytiotrophoblast, estimated by the intensity of immunostaining for hCG in explants at the end of the culture period, was not affected significantly by either DEP or HD. DEP-stimulated hCG secretion was associated with a trend towards a greater number of villi with intact syncytiotrophoblast compared to controls (P-value=0.06), consistent with an effect of DEP to promote the formation of differentiated syncytiotrophoblast in culture. The reduction in hCG secretion with HD was associated with a trend towards a greater number of villi with shed syncytiotrophoblast that had no signs of regeneration (P-value=0.06), suggesting that HD can inhibit the formation of syncytiotrophoblast. Both DEP (P-value<0.05) and HD (P-value=0.06) increased oxidative damage, but neither pollutant altered explant proliferation, apoptosis nor the number of macrophages compared to the untreated control. A summary of the key findings is shown in Table 4.3.

Previous studies reported that air pollutants could disrupt endocrine function, that may lead to adverse impacts on pregnancy, as well as future health and development of the fetus (Paulesu et al., 2018; Barjaktarovic et al., 2017). Paulesu et al. (2018) reviewed the effects of low and non-toxic levels of chemicals such as Atrazine, Bisphenol A (BPA), Chlorpyrifos, Diethylstilbestrol (DES), Resveratrol and *para*-Nonylphenol (*p*-NP) on hCG secretion by BeWo cells, trophoblast cells and placental explants. This review highlighted that altered hCG secretion induced by exposure to endocrine-disrupting chemicals had a dose-dependent relationship, with significant modification of hCG secretion observed by exposure to the low dose of chemicals that mimic individual daily exposure (Paulesu et al., 2018). Previous research had shown that BPA was able to be transferred into the placenta, and *in-vitro* experiments revealed that exposure of BPA on Bewo cells and villous explants increased hCG secretion and cell apoptosis (Mørck et al., 2010). This was interesting facts to

relate with the action of DEP, as the publication by Bové et al. (2019) revealed that black carbon particles from the ambient environment could be found on the fetal side of the placenta, and is consistent with the present study showing that DEP significantly increased hCG secretion level of placental explants.

Table 4.3: Summary of the key effects of H_2O_2, DEP and HD on placental villous explants

ELISA on culture medium/IHC (primary antibody)	Outcome measures	Treatment vs. Control						
		H_2O_2, n=16 (mM)	SRM1650b (DEP, n=8) (µg/mL)			SRM2585 (HD, n=8) (µg/mL)		
		5.0	2.5	25	100	2.5	25	100
hCG ELISA	hCG secretion by explants	↓ (P<0.02)		↑ (P<0.04)	↑ (P=0.02)	↓ (P<0.01)	↓ (P<0.02)	↓ (P=0.05)
hCG STB staining intensity	hCG production by STB		↓ (P=0.06)					
Cytokeratin 7 (CK7)	No STB (Bald)							
	Intact STB				↑ (P=0.06)			
	STB not regenerated				↓ (P=0.06)	↑ (P=0.06)		
	STB regenerated							
Ki67	Proliferation							
M30	Apoptosis							
8-OHdG	Oxidative damage			↑ (P=0.06)	↑ (P=0.03)			↑ (P=0.06)
CD163	Number of macrophages							

Placental villous explants were treated on days 1 to 6 of a 7 day culture with 5.0mM H_2O_2 (positive control), SRM1650b (DEP) or SRM2585 (HD).

hCG concentration in the culture medium was measured daily from day 2 until 7, and expressed as area under the curve (AUC).

IHC for hCG staining in syncytiotrophoblast, cytokeratin 7 (syncytiotrophoblast integrity), Ki67 (proliferation), M30 (apoptosis), 8-OHdG (oxidative damage) and CD163 (number of macrophages) was determined in explants harvested at day 7.

Arrow indicates significant changes (P-value<0.05 vs. control) or trend towards significance (P-value=0.06), whereas results for non-significant changes were not shown (empty cells).

Particles released from engine combustion contain PAHs that are carcinogenic to human health. PAHs levels are generally higher in the outdoor environment due to transportation sector, but PAH also can be derived from the indoor environment, especially residential homes due to smoking, cooking and domestic heating (Zhu et al., 1997). PAHs are usually bound to air particles($PM_{2.5}$) and can be transported to the human body via inhalation, capable of being penetrated deep into the lung, absorbed into the bloodstream and distributed around the body including the placenta (Ritz & Wilhelm, 2010; Dejmek et al., 2000). Maternal exposure to PAHs are associated with an increase risk of IUGR, SGA and PTB (Choi et al., 2008; Dejmek et al., 2000). By comparing with the chemical properties of both SRMs (Table 4.4), SRM1650B (DEP) contained higher concentrations of carcinogenic PAHs, which almost ~6-fold higher levels of benzo[a]anthracene and chrysene compared to HD. However, in this present study, it is still not known which of the constituents of the SRMs (DEP and HD) are the active components mediating increased and decreased hCG secretion, respectively. Disruption of hCG secretion, particularly if related to abnormal syncytiotrophoblast renewal *in-situ*, could contribute to adverse pregnancy outcome for women exposed to these pollutants.

Table 4.4: Comparison of certified PAHs concentrations in SRM1650b and SRM2585

PAHs compound name	Group[a]	Mass Fraction (mg/kg)*		Ratio
		SRM1650b (DEP)	SRM2585 (HD)	
Benzo[a]pyrene	1	1.25	1.14	1.1
Benzo[a]anthracene	2B	6.45	1.16	5.6
Benzo[b]fluoranthene	2B	6.77	2.70	2.5
Benzo[j]fluoranthene	2B	3.24	1.32	2.5
Benzo[k]fluoranthene	2B	2.30	1.33	1.7
Chrysene	2B	13.4	2.26	5.9
Dibenz[a,h]anthracene	2A	0.37	0.30	1.2
Indeno[1,2,3-cd]pyrene	2B	4.48	2.08	2.2

[a]As defined by International Agency for Research on Cancer (IARC): Group 1 = Carcinogenic to humans; Group 2A = Probably carcinogenic to humans; Group 2B = Possibly carcinogenic to humans

*Data taken from the certificate of analysis of SRM1650b (NIST, 2013) and SRM2585 (NIST, 2014).

4.6.2 Human chorionic gonadotrophins (hCG)

Human chorionic gonadotrophins (hCG) is a specific pregnancy hormone, which is produced and secreted by the differentiated multinucleated syncytiotrophoblast of the human placenta and functions to supports and regulate placental development and the maintenance of pregnancy (Paulesu et al., 2018; Barjaktarovic et al., 2017; Korevaar et al., 2015). hCG is a heterodimeric glycoprotein consisting of two subunits of alpha (hCGα) and beta (hCGβ) (Paulesu et al., 2018; Williams, 2008). The alpha unit is constitutively expressed, but the beta unit is only expressed by the differentiated syncytiotrophoblast (Williams 2008; Rull & Laan, 2006). Dimer hCG is secreted by two mechanisms; constitutive release and exocytosis, which the latter mechanism depends on intracellular calcium and is modulated by hypoxia and syncytiotrophoblast potassium (K^+) channel in the microvilli membrane (Díaz et al., 2016; Williams, 2008).

hCG secretion by the placenta depends on the gestational period, with high rates of secretion in the first trimester which fall as the pregnancy progresses (Barjaktarovic et al., 2017; Norris et al., 2011). The decrease in hCG secretion in the late first trimester is significantly associated with pregnancy complications of FGR, LBW, miscarriage and ectopic pregnancy, whereas increases in hCG levels were linked to pre-eclampsia (at term) and gestational trophoblast diseases (Paulesu et al., 2018; Barjaktarovic et al., 2017; Norris et al., 2011; Tong et al., 2006; Rull & Laan, 2006). Up to now, there is an inadequate understanding of how maternal exposure to air pollutants may affect the placental function and increase the risk of pregnancy complications. Hence, studies of syncytiotrophoblast hCG secretion using placental villous tissue *ex-vivo* in explants culture is a valuable strategy to discover the potentially damaging effect of air pollutants on pregnancy outcomes (Paulesu et al., 2018).

4.6.3 Placental explants in culture

The dissection of villous tissue from term placentas and its maintenance in explant culture was described by this laboratory ~20 years ago (Simán et al., 2001). This model system has been utilised extensively to study effects of interventions on placental endocrine function, nutrient transport, cellular turnover, production and secretion of microparticles, as well as toxicology and diseases. The viability, morphology, endocrine function and nutrient transport of villous explants in culture have been described previously (Simán et al., 2001). In the first 2 days of culture, the syncytiotrophoblast

detaches from the villous core, and this is followed by regeneration of a new syncytiotrophoblast layer, which by day 7 of culture is morphologically similar to fresh tissues as shown by electron microscopy (Simán et al., 2001). This process of syncytiotrophoblast repair and regeneration resembles the renewal of the syncytiotrophoblast that occurs throughout the gestation of normal pregnancy, as shown in Figure 4.26.

The cytotrophoblast was stimulated into syncytiotrophoblast layer via the proliferation process, followed by differentiation and fusion with the syncytiotrophoblast layer (Moll et al., 2007). This process continuously replenishes the syncytiotrophoblast layer by differentiation and apoptosis, and hCG produced and secreted from the fully differentiated syncytiotrophoblast. Nuclei in syncytiotrophoblast undergo apoptosis or necrosis (aponecrosis) and form syncytial knots, or syncytial nuclear aggregates (SNAs), which are shed into the maternal circulation (Mayhew, 2014; Huppertz, 2008; Moll et al., 2007). The balance between cytotrophoblast proliferation, differentiation and fusion, nuclear apoptosis/aponecrosis in the syncytiotrophoblast and the shedding of material into the maternal blood is essential to maintain the syncytiotrophoblast and support normal fetal growth. In pregnancy complications such as IUGR and pre-eclampsia, the rate of trophoblast cellular turnover is modified, with more apoptotic nuclei and rapid formation of syncytial knots compared to normal pregnancy (Sharma et al., 2012; Huppertz, 2008; Heazell & Crocker, 2008; Moll et al., 2007; Allaire, 2000).

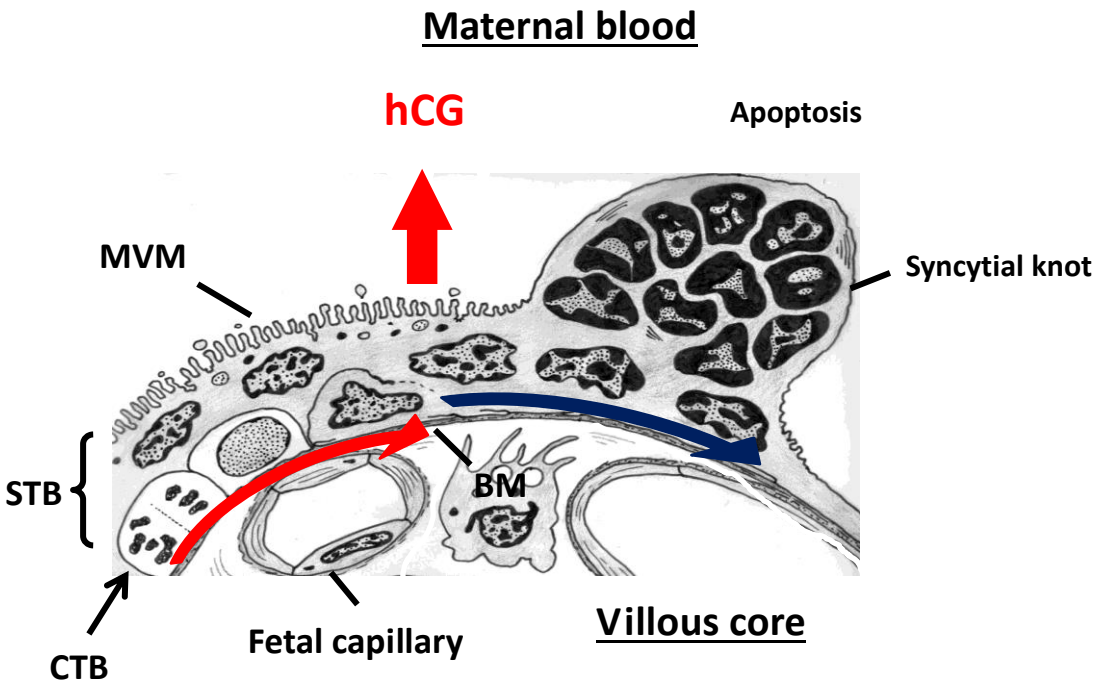


Figure 4.26: Cellular renewal of syncytiotrophoblast (Adapted from Huppertz, 2008)

This schematic diagram shows the process proposed for STB renewal, which occurs *in situ* throughout normal pregnancy, by cellular turnover. Cytotrophoblasts (CTB) which underlie the syncytiotrophoblast (STB) undergo proliferation and differentiation and then fuse with the STB, donating their nuclei to this cell layer (red arrow). Nuclei in the STB do not divide, but gather in syncytial knots or syncytial nuclear aggregates undergo apoptosis/aponecrosis and are shed into the maternal blood (blue arrow). The differentiated STB produces human chorionic gonadotrophin (hCG) which is secreted into the maternal circulation.

(BM) = Basal membrane; (MVM) = Microvillous membrane

The timecourse of hCG secretion from villous explants in culture shows a distinctive pattern, whereby secretion is low on day 2, in accord with syncytiotrophoblast shedding, and then increases by ~5 fold to reach a peak at days 4 or 5, when regeneration of the syncytiotrophoblast is occurring, and thereafter, the hCG secretion falls until day 7 (Díaz et al., 2016; Williams, 2008; Simán et al., 2001). hCG secretion on the first day of culture was not determined due to explant stabilisation in the culture medium. Previous studies recorded higher hCG secretion in the first day of culture compared than the second day, suggested that hCG measured on the first day was not solely secreted by explants, but was influenced from the hCG in maternal blood, and also as a result of the damage syncytiotrophoblast encountered during explant processing (Williams, 2008; Turner et al., 2006). However, it is well documented that there is considerable intra- and inter-placental variability in explant hCG secretion (mg explant protein) and that consistency in explant size (estimated by protein content) is important (Turner et al., 2006; Miller et al., 2005). In the

present study, hCG secretion by control, untreated explants followed the temporal profile previously described, increasing from day 2 to peak at day 4 or 5 and falling off after that. If hCG secretion did not increase by at least 2 fold between days 2 and 4, the explants were judged to be non-responsive, and the experiment was discarded.

4.6.4 Effects of H_2O_2, DEP and HD on hCG secretion and cellular turnover of placental explants *in-vitro*

4.6.4.1 Hydrogen peroxide (H_2O_2)

It was anticipated that DEP and HD would have damaging effects on placental function and cellular turnover, and this could be through increasing of oxidative damage. Therefore, to confirm that these changes could be detected in explants, a pilot series of experiments were performed using H_2O_2 as a positive control treatment. H_2O_2 has been used widely in placental *ex-vivo* studies and has been shown to induce oxidative stress in cultured placental explants (Cindrova-Davies et al., 2018). In this present study, H_2O_2 was applied to placental explants on day 5 and 6 of culture, for 24 hours, and the culture medium was assayed for hCG secretion. A significant reduction in hCG secretion was observed when explants were treated with 5mM H_2O_2, compared to the time-matched control. This reduction of hCG secretion by explants treated with H_2O_2 was observed on day 7, expressed as a percentage of day 5, implying that H_2O_2 was able to inhibit the regeneration of the syncytiotrophoblast layer. hCG secretion was similar between days 2 until 5, indicating that the syncytiotrophoblast regeneration of explants was at the same stage before H_2O_2 treatment, and the reduction after day 5 was due to the action of H_2O_2.

Informed by this pilot experiment, 5mM H_2O_2 was used as a positive control in the subsequent experiments to investigate the effects of DEP and HD, applied over a longer period (days 1 until 6 of culture). H_2O_2 significantly inhibited hCG secretion, expressed as area under the curve (AUC), over the treatment period (n=16). However, in this present study, 5mM H_2O_2 did not alter syncytiotrophoblast hCG content or any of the measures of syncytiotrophoblast integrity, as assessed by CK7 staining. In addition, in contrast to the studies by Cindrova-Davies et al. (2018), 5.0mM H_2O_2 did not induce oxidative damage or affect proliferation or apoptosis. The effect of oxidative damage by explants treated with H_2O_2 was unable to be proved in the present study, as lower H_2O_2 concentrations (0 – 5mM) was used compared than previously reported study (Cindrova-Davies et al., 2018).

The effects of H_2O_2 in the present study imply that hCG secretion can be altered in the absence of altered oxidative stress or syncytiotrophoblast turnover.

4.6.4.2 SRM1650b (DEP) and SRM2585 (HD)

The effect of DEP (SRM1650b) and HD (SRM2585) was tested on 16 placentas at concentrations of 2.5, 25 and 100µg/mL. A previous study by Jantzen et al. (2012) revealed that treatment of lung epithelial cells with DEP (SRM1650b) at concentrations of 25 and 100µg/mL significantly increased the level of DNA strand breaks, and these concentrations of DEP may give significant effects on other human cells. The present study is the first to use SRMs of DEP and HD, to investigate the effects of these ubiquitous air pollutants on placental endocrine function and cellular turnover using villous tissue explants. Thus, the same concentration of SRM1650b that was used by Jantzen et al. (2012) was applied to villous explants in this study. The method of Jantzen et al. (2012) to sonicate SRM1650b was used in the present study and these authors reported that the mean diameter of DEP (SRM1650b) after sonication was ~166nm, which this size replicates the daily exposure of DEP in the environment (Kittelson, 1998). To be consistent with DEP, the same sonication method was applied to prepare HD (SRM2585). Due to the dilution and sonication process of SRMs in water, a control contained 10% dH_2O was performed and shown not to alter hCG secretion (see Figure 4.11).

DEP and HD were applied onto culture medium from day 1 until day 6, and the culture medium was collected every 24 hours and assayed for hCG by ELISA. AUC was calculated to determine the modification of hCG secretion following treatment and expressed as a percentage of time-matched control. In this study, an unexpected finding was obtained, in that hCG secretion by placental explants treated with DEP was significantly increased, in a concentration-dependent manner, whereas treatment with HD significantly reduced hCG secretion, compared to the time-matched control.

4.6.4.3 Effect of DEP on hCG secretion, STB integrity and cellular turnover

hCG secretion by explants exposed to the lowest concentration of DEP (2.5µg/mL) was similar to control, but the exposure to 25 and 100µg/mL DEP significantly increased the hCG secretion level by 29 (P-value=0.04) and 49% (P-value=0.02), respectively, suggesting that rapid regeneration of the syncytiotrophoblast had occurred in explants treated with DEP. Based on CK7 staining (determined at day 7 of culture), the increase in intact villi and reduction of shed villi without syncytiotrophoblast regeneration in explants treated with 100µg/mL DEP were of borderline significance (P-value=0.06). In order to confirm the significance of this result, more experiments are required in future to determine whether this trend indicates a truly significant difference.

DEP did not alter the hCG staining in the syncytiotrophoblast, implying that hCG production was not elevated, and it is probable that the elevated hCG secretion with DEP was a consequence of the treatment promoting syncytiotrophoblast regeneration. DEP also did not alter proliferation or apoptosis, but these measures were taken at the end of the culture period and it is difficult to relate this "snapshot" to the dynamic change in syncytiotrophoblast layer that is reported to occur over the culture period (Simán et al., 2001). Some of the proliferating cells appeared to be in the villous stroma and not cytotrophoblast that would contribute to syncytiotrophoblast regeneration. Although DEP did not alter apoptosis compared to control, there was a significant positive correlation between hCG secretion and apoptosis (both expressed as % of control; Figure 4.27A), which is consistent with the proposal that higher rates of syncytiotrophoblast renewal are associated with increased hCG secretion. However, the lack of effect of DEP on proliferation and apoptosis suggests that DEP does not increase the risk of adverse pregnancy outcome by reducing trophoblast viability.

4.6.4.4 Effect of HD on hCG secretion, STB integrity and cellular turnover

hCG secretion by explants treated with HD was reduced, with 2.5 and 25µg/mL HD significantly reducing hCG secretion by 32% (P-value<0.01) and 29% (P-value=0.01), respectively, whereas 100µg/mL HD reduced hCG secretion level by 31% (P-value=0.05). A previous study postulated that the significant decrease of hCG secretion level by placental explants was associated with the modification of secretory mechanism of the trophoblast cells, by which hCG inhibited to secrete from the syncytiotrophoblast layer, or possibly this condition due to the degradation of hCG production by the cells (Williams, 2008).

There was no effect on hCG staining intensity in the syncytiotrophoblast, suggesting that hCG production was not inhibited by HD. There was a trend towards an increased number of villi that had not regenerated with 2.5μg/mL HD (P-value=0.06), which suggests that a reduced ability to repair syncytiotrophoblast could contribute to lower hCG secretion into the culture medium. Interestingly, hCG secretion was significantly positively related to proliferation in explants treated with HD (both expressed as % of control; Figure 4.28B). However, it is not possible to know whether the proliferating cells were cytotrophoblasts without conducted dual staining to prove the identity of these cells, but most were probably cytotrophoblasts as the positive Ki67 staining was close to the periphery. It is plausible that cell proliferation was promoted by HD, but that these cells failed to undergo differentiation or fusion with syncytiotrophoblast.

There was no significant association observed between hCG staining and secretion in the present study by explants treated with HD. hCG secretion was measured as AUC during days 2 until 7 of the culture period, and staining was measured at day 7. hCG secretion by exocytosis is regulated by intracellular calcium and potassium (K^+) channel activity in the microvillus membrane, which in turn regulates intracellular calcium (Díaz et al., 2016; Williams 2008). There is a possibility that a component of HD (SRM2585) blocks K^+ channels to inhibit hCG secretion directly, but this remains to be investigated. This also could be the explanation for H_2O_2 inhibition of hCG secretion. In common with DEP, HD did not alter villous explant proliferation or apoptosis indicating that HD did not have toxic effects on cellular integrity.

4.6.5 Effect of DEP and HD on oxidative damage

In the present study, DEP significantly increased oxidative damage to DNA as shown by elevated 8-OHdG staining (25μg/mL, P-value=0.06; 100μg/mL, P-value<0.05) and HD showed a trend towards increasing oxidative damage (100μg/mL, P-value=0.06). These results reflect those of Jantzen et al. (2012) who found that DEP (SRM1650b) significantly induced DNA strand breaks and oxidative damage in lung epithelial A549 cells in a concentration-dependent manner (0 – 100μg/mL DEP). As neither DEP nor HD significantly altered syncytiotrophoblast integrity, proliferation, apoptosis or macrophage number, the data suggest that these outcome measures were not affected by oxidative damage. However, there was a significant positive association between apoptosis and oxidative damage in explants treated with DEP, suggesting that oxidative damage induced by DEP

could increase the susceptibility of trophoblast to apoptosis (both expressed as % of control; Figure 4.29A). As DEP increased, but HD decreased the hCG secretion, it is unlikely that elevated oxidative damage accounted solely for these disparate changes in hCG secretion.

Oxidative stress is associated with inflammation; however this was not assessed in the current study, but in future, the production and secretion of inflammatory cytokines in response to DEP or HD could be evaluated (Dybdahl et al., 2004; van Eeden et al., 2001). Oxidative damage could result in other detrimental effects on placental function not assessed in the current study; for example mitochondrial dysfunction and reduced nutrient transport (Miller et al., 2005; Li et al., 2003; Kowaltowski & Vercesi, 1999). Other markers of oxidative damage, such as protein carbonyl and malondialdehyde (MDA) could also be assessed in a future study (Goncalves et al., 2016; Zusterzeel et al., 2001). If the concentration of DEP or HD in the actual environment cause elevation of placental oxidative stress; hence, it could compromise placental function and increase the risk of pregnancy complications.

4.6.6 Effect of DEP and HD on the number of macrophages

DEP and HD did not change the number of macrophages, as indicated by CD163 positive staining, compared to the controls. It is not known if macrophages proliferate in culture, but in the current study, there was little positive Ki67 staining in the villous core that could have been associated with macrophages. It is possible that the pollutants altered macrophage activation, but this was not assessed in the current study.

The elevation of airway inflammation by individuals exposed to DEP, which observed by the elevation of sputum neutrophils and concentrations of interleukin-6 (IL-6) has been reported previously (Nordenhäll et al., 2000). Also, the elevation of the inflammatory response by exposure of DEP to human lung epithelial cells *in-vitro* and inhalation of DEP by mice *in-vivo* has been reported by Dybdahl et al. (2004). Exposure of DEP on human alveolar macrophages significantly impaired macrophage phagocytosis, resulting in an increased risk of lung infection (Lundborg et al., 2006). However, in this present study, the CD163 expression of treated placental explants recorded similar to that of control group; thus the effect of inflammation between treatment groups was not able to be compared. It would be useful to measure the production of cytokines by the explants

in response to DEP and HD in the future, particularly as both induce oxidative damage which can lead to inflammation.

As a marker of inflammation, CD163 expression was reported higher in the placenta of mothers who had gestational diabetes mellitus (GDM) compared to normal pregnancy (Barke et al., 2018). The maternal hyperglycemia occurring as a result of GDM, and this condition can modify the placental morphology, affected syncytiotrophoblast integrity and placental inflammation (Barke et al., 2018). In this present study, CD163 expression showed a significant negative association with the number of shed villi without regeneration of syncytiotrophoblast layer (both expressed as % of control; Figure 4.30), suggested lower CD163 expressions were associated with an increase of the syncytiotrophoblast layer without regeneration.

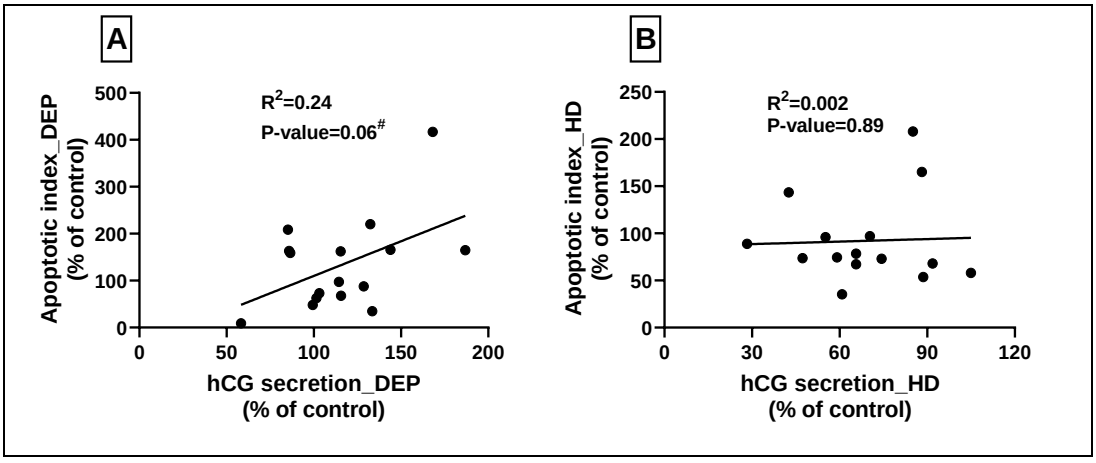


Figure 4.27: Relationship of hCG secretion (ELISA) with apoptotic index (M30)

Graphs show the relationship between hCG secretion with apoptotic index on explants treated with 2.5, 25 and 100µg/mL (A) DEP and (B) HD on day 1 until 6 of culture. Data in both axes represented as a percentage of control ($^{\#}$P-value=0.06 indicates trend towards significant; Linear regression analysis).

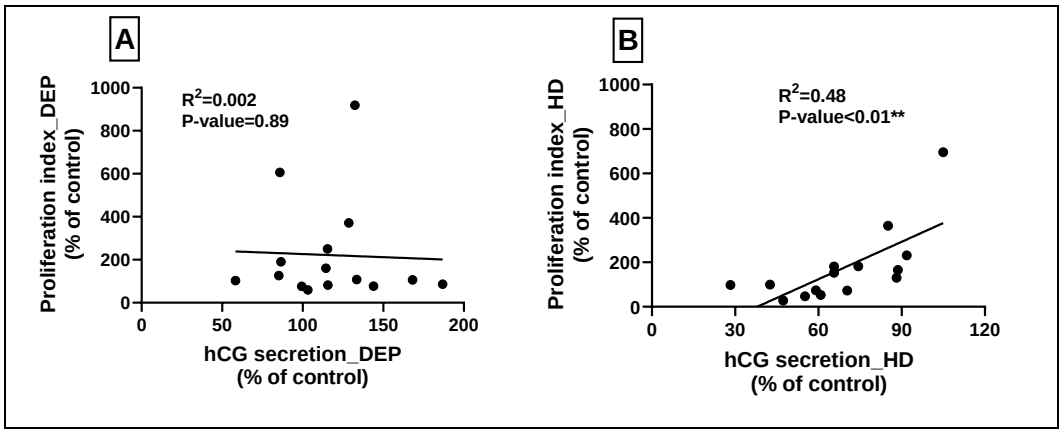


Figure 4.28: Relationship of hCG secretion (ELISA) with proliferation index (Ki67)

Graphs show the relationship between hCG secretion with proliferation index on explants treated with 2.5, 25 and 100µg/mL (A) DEP and (B) HD on day 1 until 6 of culture. Data in both axes represented as a percentage of control (**P-value<0.01 indicates significant relationship; Linear regression analysis).

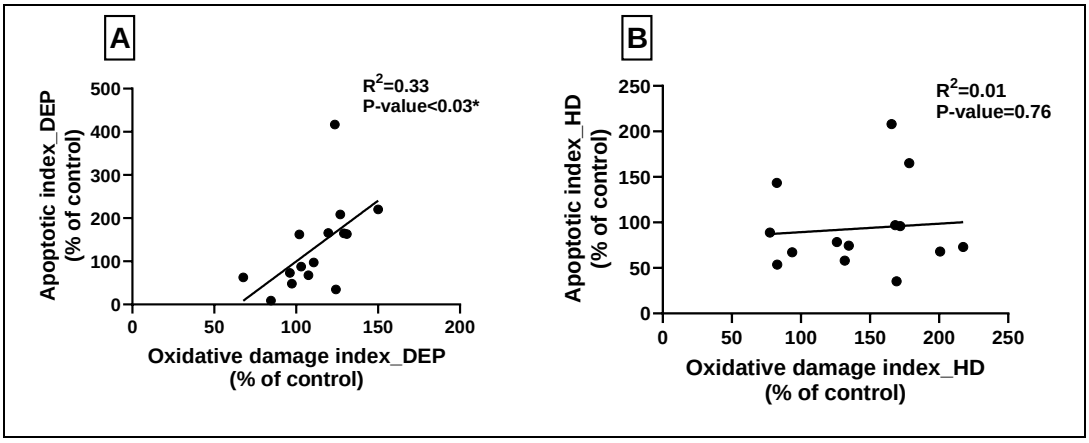


Figure 4.29: Relationship of oxidative damage index (8-OHdG) with apoptotic index (M30)

Graphs show the relationship between oxidative damage index with apoptotic index on explants treated with 2.5, 25 and 100µg/mL (A) DEP and (B) HD on day 1 until 6 of culture. Data in both axes represented as a percentage of control (*P-value<0.03 indicates significant relationship; Linear regression analysis).

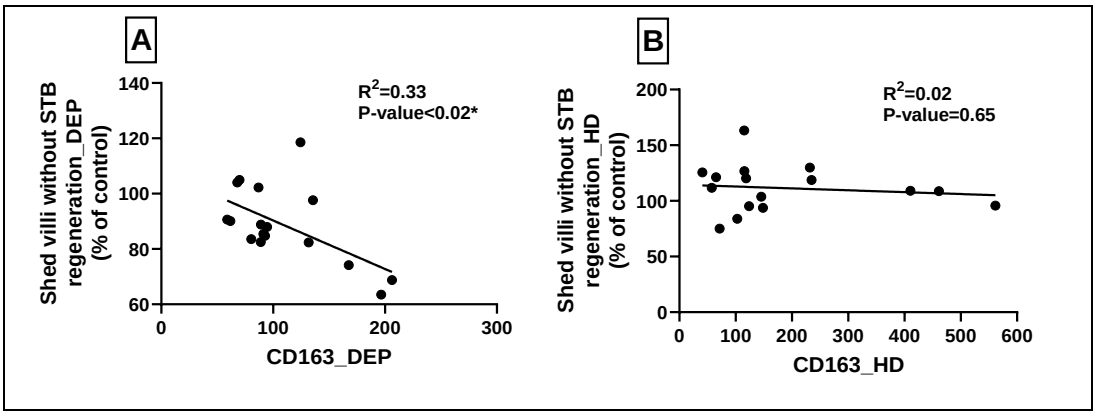


Figure 4.30: Relationship of CD163 expression with the number of shed villi without syncytiotrophoblast regeneration (CK7)
Graphs show the relationship of CD163 expression with the number of shed villi without syncytiotrophoblast regeneration on explants treated with 2.5, 25 and 100µg/mL (A) DEP and (B) HD on day 1 until 6 of culture. Data in both axes represented as a percentage of control (*P-value<0.02 indicates significant relationship; Linear regression analysis).

4.6.7 Strengths, limitations and future work

The villous tissue explant model is a well characterised *ex-vivo* preparation with which to study the effects of treatments in the medium term (days). It is a multicellular preparation which undergoes syncytiotrophoblast regeneration similar to renewal *in-situ*, and which retains endocrine and nutrient transport function. It is well documented that, there is intra- and inter-placental variability in hCG secretion from placental explants and in the present study, experiments were rejected if hCG secretion at day 4 or 5 was not progressed at least double that at day 2 (Turner et al., 2006).

The main key strength of the present study is the adaptation of well-established placental *in-vitro* technique to assess the effects of exposure to DEP and HD on placental explant culture, by using standard reference materials (SRM). To our knowledge, this is the first study that used SRM of ubiquitous air pollutants DEP and HD on placental explant culture, and our results showed a significant alteration of hCG secretion by explants treated with DEP and HD. The modification of hCG secretion into culture medium may not mimic actual pathophysiological conditions, but this can be a key determinant of placental dysfunction, as dysregulation of hCG is a signal of adverse pregnancy outcomes (Paulesu et al., 2018; Norris et al., 2011).

A limitation of the study design was that treatments were applied over days 1 until 6 of culture, but the tissue immunostaining carried out on samples at the end of culture. The present study shows an increment of hCG secretion by explants treated with DEP

between day 2 until 4, suggested that the aberrant cellular turnover of explants treated with DEP occurred during this period and was inhibited at the end of culture. Thus, future studies should sample tissues daily to relate endocrine function with tissue turnover at the same time point.

For any future study, it is recommended to compare the oxidative damage of explants treated with DEP and HD, with pathological placental of pre-eclampsia and IUGR pregnancy, as oxidative damage index was reported higher in both complicated pregnancies (Cindrova-Davies et al., 2018). Also, a future study investigating the formation of syncytial nuclei aggregates (SNA) in placental explants treated with DEP and HD would be interesting, as SNAs are more prevalent in pregnancy complications and could act as an indicator of a deficient uteroplacental circulation (Vähäkangas et al., 2014).

4.7 Conclusion

This study examined the effect of medium-term application (6 days) of DEP and HD on placental explants from normal pregnancy *ex-vivo*, to stimulate maternal exposure to these air pollutants during pregnancy. The effect of DEP to elevate hCG secretion and HD to lower secretion, suggests that components of SRM1650b and SRM2585 differentially alter placental endocrine function, putatively through promoting or inhibiting syncytiotrophoblast renewal, respectively. Both pollutants elevated oxidative damage to DNA which, if true *in utero*, could compromise placental function that may lead to adverse pregnancy outcomes. As a conclusion, DEP and HD modified the placental endocrine function and cellular turnover, by elevated oxidative stress which could underlie adverse pregnancy outcomes in mothers exposed to these environmental pollutants. Further work is required to identify the active components of the SRMs for DEP and HD, particularly PAHs, which attribute to the outcomes and determine the mechanisms of action.

CHAPTER 5:

OVERALL DISCUSSION

5.1 Summary

Previous studies have reported that environmental pollutants can disrupt placental development, increase the risk of an adverse pregnancy outcome, and negatively affecting the future health and development of the fetus (Paulesu et al., 2018; Erickson & Arbour, 2014). The work presented in this thesis addressed the hypothesis that maternal exposure to air pollution during pregnancy can increase the risk of placental dysfunction and hence adverse pregnancy outcomes. The thesis has been conducted based on the following aims:

i. To determine the concentration of heavy metal and PAH in biological samples following maternal exposure to ambient air pollution throughout the gestation period.

ii. To examine the feasibility of measuring the level of indoor air particles in the residential homes of pregnant women across Greater Manchester.

iii. To identify the primary sources that contribute to the elevation of indoor air particles in the residential homes across Greater Manchester.

iv. To adapt a well-established *in-vitro* model of placental explant culture to develop a novel model of placental air pollution exposure.

v. To investigate the effect on placental endocrine function and cellular turnover of placental villous explants treated with diesel exhaust particle and house dust *in-vitro*.

The main focus of this study was the air pollutant $PM_{2.5}$ that potentially is bound with PAHs and heavy metals. These particles are ubiquitous in the indoor and outdoor environment, and have been reported to induce oxidative DNA damage, placental inflammation and modify placental endocrine function (Familari et al., 2019; Kim et al., 2017; Wang et al., 2017; Grevendonk et al., 2016; Weldy et al., 2014; Sbrana et al., 2011). The first aim of this study is described in Chapter 2, in which I investigated the association between maternal exposure to ambient air particles (PM_{10} and $PM_{2.5}$) during pregnancy with the concentration of heavy metals and PAHs in biological samples. The second and third aims are addressed in Chapter 3, in which I examined the level of indoor air particles inside the residential homes of pregnant women across Greater Manchester and attempted to identify sources that contribute to $PM_{2.5}$ levels inside the home. Finally, in Chapter 4, I investigated the modification of placental endocrine function and cellular turnover of placental explants treated with SRM1650B (diesel exhaust particles) and SRM2585 (house dust) *in-vitro*. The summary of key findings is illustrated in Figure 5.1.

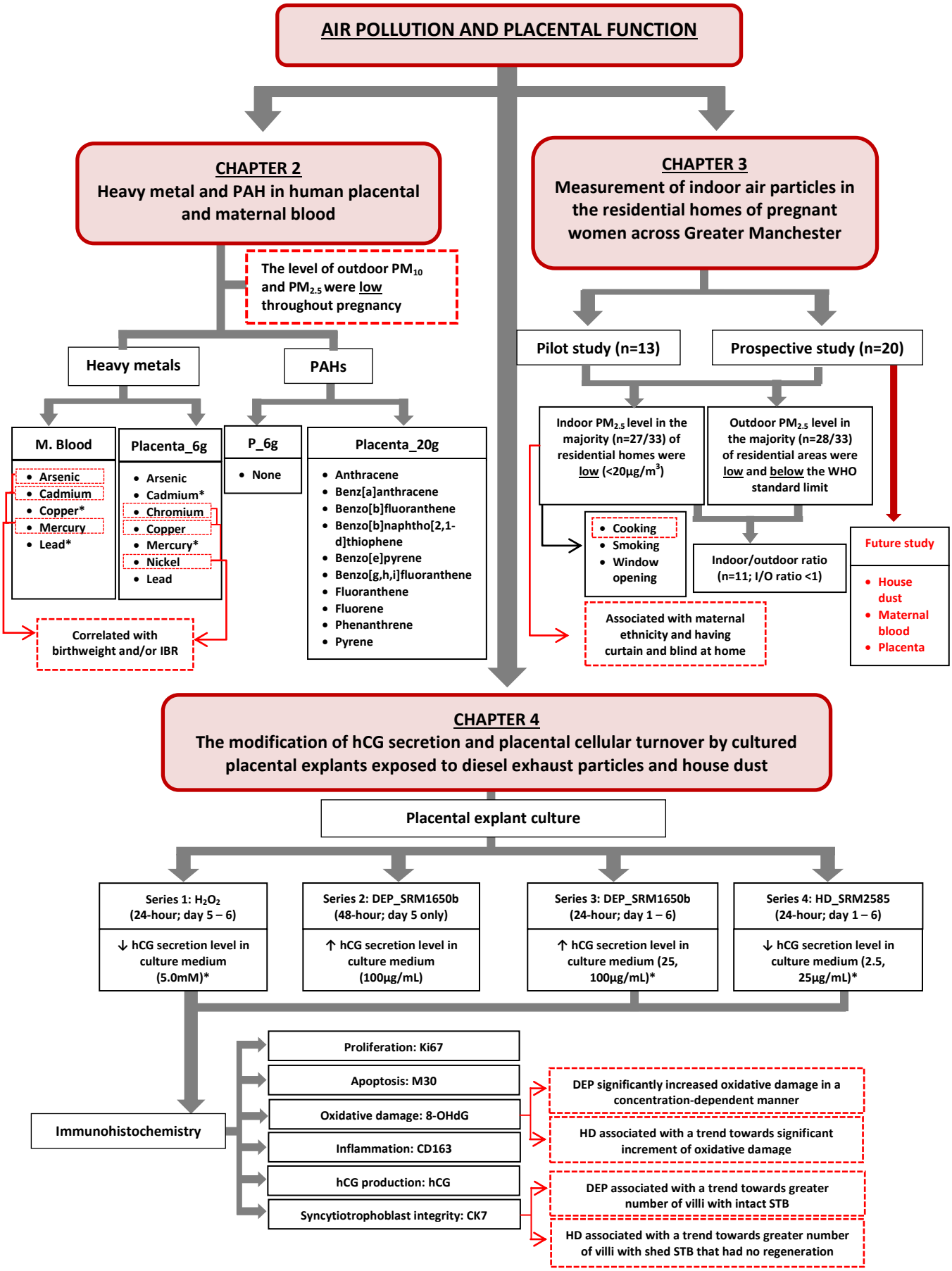


Figure 5.1: Schematic summary of the key findings in the investigation between the effect of air pollution and placental function

The effect of air pollution on placental function and/or pregnancy outcomes has been investigated in many previous studies, mainly in *in-vivo* experiments using animal models (e.g. mouse and rabbit), but limited investigations using human placental tissue have been conducted (Valentino et al., 2016; de Melo et al., 2015; Weldy et al., 2014; Tsukue & Dep, 2002). Animal studies may not adequately represent the effects of air pollution on human placental function or pregnancy outcomes due to different physiology between species (Paulesu et al., 2018; Mannelli et al., 2015). Previous studies investigated the effect of placental endocrine function, placental inflammation and oxidative damage on human placental cells (JEG-3, BeWo and trophoblast cells) treated with air pollutants *in-vitro* (Familari et al., 2019; Kim et al., 2017; Wang et al., 2017; Mørck et al., 2010), but only two studies have used placental villous explants to investigate this relationship (Mannelli et al., 2014; Bechi et al., 2006). A study conducted by Mannelli et al. (2014) examined the effect of BPA treatment on first-trimester placental explants, and they found a significant increase in β-hCG secretion levels by explants in culture medium compared to control group for 48-hour culture. Bechi et al. (2006) investigated the effect of *p*-NP treatment on first-trimester placental explants, and found that β-hCG secretion by explants into the culture medium was increased over time.

The use of human term placental villous explants is potentially advantageous, compared to the first-trimester placental explants as the latter can only be collected following a pregnancy termination. Both early and term placenta can be used to study the effect of placental endocrine function *in-vitro*; however, the culture techniques and methods are slightly different, particularly in terms of oxygen concentration during incubation (Miller et al., 2005). The present study extends the knowledge by investigating the effects of diesel exhaust particles (DEP, SRM150b) and house dust (HD, SRM2585) on term placental explants *in-vitro*. The well established *in-vitro* model of term placental explants has been adapted to develop a novel *in-vitro* technique to enable the relationship between air pollutants and placental function to be investigated (Simán et al., 2001).

The modification of placental endocrine function and cellular turnover of term villous tissue of normal pregnancy has been examined following a 6-day treatment with DEP and HD, to mimic maternal exposure to these environmental pollutants during pregnancy. The present study provided evidence that DEP treatment of placental explants (25 and 100µg/mL SRM1650b; 24-hours/day; 6-days/week; mean DEP diameter ~166nm) significantly increased hCG secretion, increased 8-OHdG oxidative damage and promoted the formation of villi with an intact syncytiotrophoblast in a dose-dependent manner. In

contrast, treatment of placental explants with HD (2.5, 25 and 100µg/mL SRM2585; 24-hours/day; 6-days/week; mean HD diameter ~166nm) significantly inhibited hCG secretion, increased the number of shed villi without regeneration (lowest dose only), and increased 8-OHdG oxidative damage (highest dose only). Neither DEP nor HD altered explant proliferation, apoptosis or the number of macrophages compared to the untreated control. By comparison, both SRMs contained PAHs, with DEP containing higher concentrations of carcinogenic PAHs, with almost a 6-fold higher levels of benzo[a]anthracene and chrysene compared to HD. Differences in PAH levels between DEP and HD may be a reason for the difference in hCG secretion response and cellular turnover by placental explants. However, it is not known which of the constituents of the SRMs (DEP and HD) are the active components mediating the modification of hCG secretion, oxidative damage and syncytiotrophoblast turnover.

DEP and HD were sonicated in prior use as in placental explant culture following the procedure reported by Jantzen et al. (2012); this results in particle sizes that correspond to the actual size of $PM_{2.5}$ existing in the environment. The sonication procedure by Jantzen et al. (2012) has been shown to produce DEP (SRM1650b) with a diameter of ~166nm. This small size of particles may facilitate passage across placental barriers and accumulation in cells as it has been demonstrated that particles with a diameter up to ~240nm were reported to cross the placental barrier (Wick et al., 2010).

According to Valentino et al. (2016), the *in-vivo* exposure of mice models to DEP at these concentrations (1mg/m^3 DEP; 2-hour/day; 5-day/week for 20 days over 31-days of gestation; mean DEP diameter=69nm) corresponds to exposure of $PM_{2.5}$ at a mean concentration of 80µg/m^3 over 24 hours. Another *in-vivo* exposure study of DEP to mice reported that exposure of DEP at these parameters (300µg/m^3 DEP; 6-hour/day; 5-day/week) corresponds to exposure of $PM_{2.5}$ at a mean concentration of 53µg/m^3 over one hour, and this concentration significantly induced placental injury observed by haemorrhage, vascular compromise, focal necrosis, embryo resorption, inflammation and oxidative stress (Weldy et al., 2014). The concentration of DEP and HD used in the present study (2.5, 25 and 100 µg/mL DEP; 24-hour/day; 6-day/week) was higher than concentration used presented in previous *in-vivo* studies and was also higher than air particle levels in Greater Manchester.

In the environment, $PM_{2.5}$ has a high ability to bind with heavy metals and PAHs, and exposure to these particles during pregnancy has been associated with adverse pregnancy outcomes (Smith et al., 2017; Jedrychowski et al., 2017; Zhang et al., 2016; Basu

et al., 2014; Choi et al., 2012). Due to their small size, these particles easily penetrate the placental barrier and accumulate in the mitochondria (Erickson & Arbour, 2014a). As a result, PAHs bound with $PM_{2.5}$ can induce the formation of pro-inflammatory cytokines and increase ROS levels in the cells, and these events may lead to cell damage (Vattanasit et al., 2014). Also, exposure to PAHs can result in increased levels of endogenous ROS produced by mitochondria, due to interference with mitochondria electron transfer and altered permeability of transition pores (Xia et al., 2007). Toxic ROS in the placenta may cause oxidative damage to DNA, proteins, lipids and other biological molecules, and this may lead to placental dysfunction by affecting the trophoblast turnover and vascular reactivity (Myatt & Cui, 2004).

Markers of air pollution exposure can be detected in the placenta, maternal blood and cord blood, in which the detected level may represent the level of air pollution that pregnant women are exposed to during the gestation period (van den Hooven et al., 2012). An analysis of PAH and heavy metal concentrations in placentas and maternal blood has been presented in Chapter 2. The results showed that pregnant women in Greater Manchester were exposed to a low level of outdoor PM_{10} and $PM_{2.5}$ during pregnancy, with median levels of 17.56 and 10.14μg/m^3, respectively. Air pollution levels in the Greater Manchester have shown an improvement year by year, with air particles (PM_{10} and $PM_{2.5}$) since 2012 being recorded at low levels and not exceeding the annual permissible limit (O'Neill et al., 2017). Due to the low concentration of these particles in the ambient environment, it was unsurprising that the concentration of PAH and heavy metals in the placenta and maternal blood samples were also low, such that these concentrations do not have a significant effect on placental function and pregnancy outcomes.

The result of the pilot study determining the analysis of indoor air particles in the residential homes of pregnant women during pregnancy was reported in Chapter 3. According to Nethery et al. (2009), pregnant women have been reported to spend the majority of their time at home, particularly at the end of gestation. Hence, the focus of this investigation was to better understand maternal exposure level to indoor air particles during the gestation period and to identify the primary sources of indoor air pollution in residential homes across Greater Manchester. The most prominent finding to emerge from this study is that the level of indoor $PM_{2.5}$ for 33 houses in Greater Manchester areas was low, with the median level of 11.35μg/m^3. Simultaneously, outdoor $PM_{2.5}$ levels during the sampling period were estimated based on air pollution monitoring stations, and showed that most participants (n=28/33) in Greater Manchester had low ambient $PM_{2.5}$ levels

(<20μg/m^3) that were below the WHO air quality standard limit for outdoor $PM_{2.5}$ (<25μg/m^3 for 24-hour exposure). The median level of outdoor $PM_{2.5}$ for 33 houses was lower than indoor levels, at the concentration of 8.40μg/m^3. These results indicate that the low outdoor $PM_{2.5}$ levels in Greater Manchester do not influence indoor air quality. Variations in $PM_{2.5}$ levels inside residential homes are, therefore, dependent on the indoor generation of these particles. The low exposure level to indoor and outdoor $PM_{2.5}$ in the residential homes of pregnant women across Greater Manchester may have impacted the low levels of heavy metals and PAHs in placental samples as presented in Chapter 2.

5.2 Strengths of the study

The main strength of this study is the adaptation of the well-established placental *in-vitro* technique to enable the investigation of placental explants treated with air pollutants *in-vitro*. This *in-vitro* study produced solid foundations of novel experimental work to enable further investigations regarding the effects of air pollution on placental function and pregnancy outcomes. To our knowledge, this is the first study that used standard reference materials (SRM) of DEP and HD in placental explant culture, in which the usage of both SRMs mimic to some degree maternal exposure to the ubiquitous air pollutants, $PM_{2.5}$ and PAHs that exist within the outdoor and indoor environments. Also, this study has provided a deeper insight of maternal exposure to air pollution during pregnancy, which not only focused on the ambient air pollution level but simultaneously investigated the indoor air pollution level in the residential homes of pregnant women across Greater Manchester. Future studies may potentially use the methodology applied in the measurement of indoor air particles in pregnant women homes to investigate a robust relationship between maternal exposure to air pollution and pregnancy outcomes.

5.3 Limitations and recommendations for future research

Some of the findings of this study are limited by the use of sample sizes that may result in bias which can lead to Type 1 (false positive) and Type 2 (false negative) errors. For example, significant associations between indoor $PM_{2.5}$ levels and ethnicity, wearing shoes at home and use of curtain and blinds were observed, but the small sample size may not adequately reflect all pregnancies in Greater Manchester at the current time. Also, a typical unaccounted issue with studies relating to health outcomes is the lack of adjustment for the multiple comparisons that can be corrected, for example, by using Bonferroni

correction. The present study lacks adjustment for multiple comparisons, and therefore, it is possible that the results obtained are overly reassuring with regard to the correlation of traces elements in biological samples (heavy metals and nutritional elements) with maternal and new-born characteristics (Table 2.4 and 2.5). Furthermore, the estimation of ambient air pollution based on monitoring stations may be limited due to the lack of monitoring stations available in the study area. The present study found that the distance range monitoring stations and the residential homes of the study population were within 0.54 to 25.7 kilometres and hence, the actual level of ambient air particles at each residence may differ significantly from the level estimated by the monitoring stations. Future studies are therefore recommended to apply air pollution estimation models such as the spatio-temporal model, land use regression or dispersion model that provide a robust spatial and temporal resolution of air pollution level in the monitoring areas (Madsen et al., 2019; Smith et al., 2017; Hannam et al., 2014).

Another limitation is due to the practical constraints, in which the monitoring of indoor air pollution in the residential homes across Greater Manchester was conducted by the participants, not by a trained researcher. There might be a possibility that the air particle monitor was not operated as per instructed in the SOP, and it is suggested that future studies should improve the study design to overcome this limitation. In addition, the majority of pregnant women in the present study were working, so that exposure to $PM_{2.5}$ might be from several different sources, and not limited to the residential home only. The exposure to $PM_{2.5}$ at the workplace and during commuting needs to be considered, and hence personal monitoring may be an ideal method of obtaining "true" overall air pollutants exposure. The proximity of the residential home to the main road or traffic pollution must also be taken into account in any future study, as this factor has been reported to increase the number of traffic particles inside the home. Also, the level of indoor $PM_{2.5}$ obtained in the present study was not able to be compared with indoor air quality guideline for the residential home within the UK population, as the guideline is under development and expected to be published by 20th October 2020 (National Institute for Health and Care Excellence, 2019).

A potential limitation in the *in-vitro* experiment of placental explant culture with air pollutants is the study design, in which the concentration of DEP (SRM1650b) and HD (SRM2585) used to expose placental explants *in-vitro* was far higher than the actual exposure to ambient $PM_{2.5}$ in the environment. In order to obtain outcomes that are realistic and comparable to the real exposure of air pollution in the actual environment,

particularly in Greater Manchester, the concentration of the chemicals (air pollutants) should be similar to the levels found in the human body (Mannelli et al., 2015). In addition, the identification and the analysis of active components of the SRMs for DEP and HD, particularly PAHs, is essential to understand the outcomes and mechanisms of action.

The analysis of placental cellular turnover was only conducted at day 7 of explant culture, and it is likely that aberrant cell turnover (e.g. proliferation, apoptosis, etc.) of explants treated with DEP or HD occurred throughout the culture period and was inhibited at the end of culture. It would be interesting in future studies to collect explants earlier in the culture period, to examine the time course of placental cellular turnover *in-vitro* throughout the culture process, not only at the end of the culture. The investigation of syncytial nuclei aggregates (SNA) in placental explants treated with DEP and HD would be interesting, as SNAs are more prevalent in pregnancy complications (Vähäkangas et al., 2014). Future studies would also be recommended to compare the effect of oxidative damage of explants treated with DEP and HD, with pathological placental conditions such as pre-eclampsia and IUGR pregnancy, as a marker of oxidative damage (8-OHdG) was reported to be higher in such pregnancies compared to normal pregnancy (Cindrova-Davies et al., 2018). To extend the analysis, a future study could compare placental oxidative stress, apoptosis, etc. of mothers who were exposed, or resided, in low and high air pollution areas during pregnancy, to discover the effect of real air pollution exposure on placental function and pregnancy outcomes.

5.4 Conclusion

This study was undertaken to determine the effect of air pollution on placental dysfunction that may exacerbate adverse pregnancy outcomes. Interesting insights into the potential mechanism of pollution-related adverse pregnancy outcomes have been revealed by *in-vitro* exposure experiments and the methods used in this study may be applied to other future studies to investigate the effect of air pollution and placental function in other different aspects. In Greater Manchester at the current time, maternal exposure to indoor and outdoor air pollution during pregnancy does not suggest high exposure to $PM_{2.5}$ bound PAH and heavy metals. Further studies are needed to unravel the biological mechanism that underlies adverse pregnancy outcomes due to exposure to air pollution.

LIST OF REFERENCES

Adesina, J. A., Piketh, S. J., Qhekwana, M., Burger, R., Language, B., & Mkhatshwa, G. (2020). Contrasting indoor and ambient particulate matter concentrations and thermal comfort in coal and non-coal burning households at South Africa Highveld. *Sci Total Environ*, *699*, 134403.

Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A., & Gupta, S. (2012). The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol*, *10*, 1.

European Environment Agency. (2008). Air *pollutants and global effects*.

Air Quality Expert Group. (2012). Fine Particulate Matter in the United Kingdom. Available at:h*ttp://ukair.defra.gov.uk/assets/documents/reports/cat11/1212141150_AQEG_Fine_Particulate_Matter_in_the_UK.pdf*, 1–191.

Air Quality Sensor Performance Evaluation Center. (n.d.). *Dylos - DC1700 PM Sensor Background*.

Armstrong, B. K., White, E. & Saracci, R. (1992). *Principle of exposure measurement in epidemiology*; *Monographs in Epidemiology and Biostatistics* (21st ed.). United States, Oxford University Press Inc., New York.

Allaire, A. D., Ballenger, K. A., Wells, S. R., McMahon, M. J., & Lessey, B. A. (2000). Placental apoptosis in preeclampsia. *Obstet Gynecol*, *96*(2), 271–276.

Al-Saleh, I., Shinwari, N., Mashhour, A., Mohamed, G. E. D., & Rabah, A. (2011). Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women. *Int Hyg Environ Health*, *214*(2), 79–101.

Amaya, E., Gil, F., Freire, C., Olmedo, P., Fernández-Rodríguez, M., Fernández, M. F., & Olea, N. (2013). Placental concentrations of heavy metals in a mother-child cohort. *Environ Res*, *120*, 63–70.

Anderson, J. O., Thundiyil, J. G., & Stolbach, A. (2012). Clearing the Air: A Review of the Effects of Particulate Matter Air Pollution on Human Health. *J Med Toxicol*, *8*(2), 166–175.

AQEG. (2012). Fine particulate matter (PM2.5) in the UK, 1–203. Available at: https://www.gov.uk/government/publications/fine-particulate-matter-pm2-5-in-the-uk

Arica, E., Kayaalti, Z., Kaya Akyuzlu, D., & Soylemezoglu, T. (2013). Assessment of chromium and nickel levels in maternal blood, placenta and cord blood by graphite furnace atomic absorption spectrometry. *Toxicol Lett*, *221*(2013), S248.

Arinola, G. O., Dutta, A., Oluwole, O., & Olopade, C. O. (2018). Household air pollution, levels of micronutrients and heavy metals in cord and maternal blood, and pregnancy outcomes. *Int J Environ Res Public Health*, *15*(12).

Arora, C. P., Kacerovsky, M., Zinner, B., Ertl, T., Ceausu, I., Rusnak, I., Shurpyak, S., Sandhu, M., Hobel, C. J., Dumesic, D. A., & Vari, S. G. (2015). Disparities and relative risk ratio of preterm birth in six Central and Eastern European centers. *Croat Med J*, *56*(2), 119–127.

ATSDR. (2012). Toxicological Profile for 2-Hexanone. *ATSDR's Toxicological Profiles*, (September).

Audette, M. C., Challis, J. R. G., Jones, R. L., Sibley, C. P., & Matthews, S. G. (2014). Synthetic glucocorticoid reduces human placental system a transport in women treated with antenatal therapy. *J Clin Endocrinol Metab*, *99*(11), E2226–E2233.

Audette, M. C., Greenwood, S. L., Sibley, C. P., Jones, C. J. P., Challis, J. R. G., Matthews, S. G., & Jones, R. L. (2010). Dexamethasone stimulates placental system A transport and trophoblast differentiation in term villous explants. *Placenta*, *31*(2), 97–105.

Baker, B. C. (2015). The Effect of Folate Deficiency on Placental Function. PhD. The University of Manchester.

Balmes, J. R., & Eisner, M. D., (2016). Indoor and Outdoor Air Pollution. In *Murray and Nadel's Textbook of Respiratory Medicine (Sixth Edition)* (Sixth Edit, pp. 1331–1342). Elsevier Inc.

Bamfo, J. E. A. K., & Odibo, A. O. (2011). Diagnosis and management of fetal growth restriction. *J Pregnancy*, *2011*, 640715.

Barjaktarovic, M., Korevaar, T. I. M., Jaddoe, V. W. V., de Rijke, Y. B., Visser, T. J., Peeters, R. P., & Steegers, E. A. P. (2017). Human chorionic gonadotropin (hCG) concentrations during the late first trimester are associated with fetal growth in a fetal sex-specific manner. *Eur J Epidemiol*, *32*(2), 135–144.

Barke, T. L., Goldstein, J. A., Sundermann, A. C., Reddy, A. P., Linder, J. E., Correa, H., Velez-Edwards D. R., & Aronoff, D. M. (2018). Gestational diabetes mellitus is associated with increased CD163 expression and iron storage in the placenta. *Am J Reprod Immunol*, *80*(4).

Basu, R., Harris, M., Sie, L., Malig, B., Broadwin, R., & Green, R. (2014). Effects of fine particulate matter and its constituents on low birth weight among full-term infants in California. *Environ Res*, *128*(2), 42–51.

Bechi, N., Ietta, F., Romagnoli, R., Focardi, S., Corsi, I., Buffi, C., & Paulesu, L. (2006). Estrogen-like response to *P*-nonylphenol in human first trimester placenta and BeWo choriocarcinoma cells. *Toxicol Sci*, *93*(1), 75–81.

Bergman, Å., Heindel, J., Jobling, S., Kidd, K., & Zoeller, R. T. (2012). *State-of-the-science of endocrine disrupting chemicals, 2012*. *Toxicol Lett* (Vol. 211).

Bernstein, J. A., Alexis, N., Bacchus, H., Bernstein, I. L., Fritz, P., Horner, E., Li, N., Mason, S., Nel, A., Oullette, J., Reijula, K., Reponen, T., Seltzer, J., Smith, A., & Tarlo, S. M. (2008). The health effects of non-industrial indoor air pollution. *J Allergy Clin Immun*, *121*(3), 585–91.

Bo, M., Salizzoni, P., Clerico, M., & Buccolieri, R. (2017). Assessment of indoor-outdoor particulate matter air pollution: A review. *Atmosphere*, *8*(8).

Bové, H., Bongaerts, E., Slenders, E., Bijnens, E. M., Saenen, N. D., Gyselaers, W., Van Eyken, P., Plusquin, M., Roeffaers, M. B. J., Ameloot, M., & Nawrot, T. S. (2019). Ambient black carbon particles reach the fetal side of human placenta. *Nat Commun*, *10*(1),1-7.

Brosens, I., Pijnenborg, R., Vercruysse, L., & Romero, R. (2011). The "great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol 204*(3), 193–201.

Brown, D. R., Alderman, N., Weinberger, B., Lewis, C., Bradley, J., & Curtis, L. (2014). Outdoor wood furnaces create significant indoor particulate pollution in neighboring homes. *Inhal Toxicol 26*(10), 628–635.

Bruce, N., Perez-Padilla, R., & Albalak, R. (2000). Indoor air pollution in developing countries: a major environmental and public health challenge. *Bull. World Health Organ*, *78*(9), 1078–1092.

Busti, F., Campostrini, N., Martinelli, N., & Girelli, D. (2014). Iron deficiency in the elderly population, revisited in the hepcidin era. *Front Pharmacol*, *5 APR*(April), 1–9.

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med*, *29*(3–4), 222–230.

Health Canada. (1989). *Exposure Guidelines for Residential Indoor Air Quality*. *Safe Environments Programme*. Available at: http://www.hc-sc.gc.ca/ewh-semt/pubs/air/exposure-exposition/index-eng.php

Carlson, B. M. (2014). Formation of Chorionic Villi. In *Placenta* (pp. 1–8).

Carter, R. C., Wainwright, H., Molteno, C. D., Georgieff, M. K., Dodge, N. C., Warton, F., Meintjes, E. M., Jacobson, J. L., & Jacobson, S. W. (2016). Alcohol, Methamphetamine, and Marijuana Exposure Have Distinct Effects on the Human Placenta. *Alcohol. Clin. Exp. Res.*, *40*(4), 753–764.

Catalani, S., Paganelli, M., Gilberti, M. E., Rozzini, L., Lanfranchi, F., Padovani, A., & Apostoli, P. (2018). Free copper in serum: An analytical challenge and its possible applications. *J Trace Elem Med Biol*, *45*(November 2017), 176–180.

CDC. (2016). Reproductive and Birth Outcome. Available at: https://ephtracking.cdc.gov/showRbLBWGrowthRetardationEnv.action (Last accessed 20/12/2019).

CDC. (2019). Premature birth. Available at: https://www.cdc.gov/reproductivehealth/features/premature-birth/index.html (Last accessed 20/12/2019).

Chambers, L., Finch, J., Edwards, K., Jeanjean, A., Leigh, R., & Gonem, S. (2018). Effects of personal air pollution exposure on asthma symptoms, lung function and airway inflammation. *Clin Exp Allergy*, *48*(7), 798–805.

Chao, C. Y. H., Tung, T. C. W., & Burnett, J. (1998). Influence of different indoor activities on the indoor particulate levels in residential buildings. *Indoor Built Environ*, *7*(2), 110–121.

Chen, C., & Zhao, B. (2011). Review of relationship between indoor and outdoor particles: I/O ratio, infiltration factor and penetration factor. *Atmos Environ*, *45*(2), 275–288.

Chen, X., Li, Y., Zhang, B., Zhou, A., Zheng, T., Huang, Z., Pan. X., Liu. W., Liu. H., Jiang. Y., Sun. X., Hu. C., Xing. Y., Xia. W., & Xu, S. (2018). Maternal exposure to nickel in relation to preterm delivery. *Chemosphere*, *193*, 1157–1163.

Cheng, H., Zhou, T., Li, Q., Lu, L., & Lin, C. (2014). Anthropogenic chromium emissions in China from 1990 to 2009. *PLoS ONE*, *9*(2).

Choi, H., Rauh, V., Garfinkel, R., Tu, Y., & Perera, F. P. (2008). Prenatal exposure to airborne polycyclic aromatic hydrocarbons and risk of intrauterine growth restriction. *Environ Health Perspect*, *116*(5), 658–665.

Choi, H., Wang, L., Lin, X., Spengler, J. D., & Perera, F. P. (2012). Fetal window of vulnerability to airborne polycyclic aromatic hydrocarbons on proportional intrauterine growth restriction. *PLoS ONE*, *7*(4).

Cindrova-Davies, T., Fogarty, N. M. E., Jones, C. J. P., Kingdom, J., & Burton, G. J. (2018). Evidence of oxidative stress-induced senescence in mature, post-mature and pathological human placentas. *Placenta*, *68*(January), 15–22.

Clifford Astbury, C., Penney, T. L., & Adams, J. (2019). Home-prepared food, dietary quality and socio-demographic factors: a cross-sectional analysis of the UK National Diet and nutrition survey 2008–16. *Int J Behav Nutr Phys Act*, *16*(1), 1–11.

Collingwood Bakeo, A., & Clarke, L. (2006). Risk factors for low birthweight based on birth registration and census information, England and Wales, 1981-2000. *Health Stat Q*, *30*(2), 15–21.

Colman Lerner, J. E., Elordi, M. L., Orte, M. A., Giuliani, D., de los Angeles Gutierrez, M., Sanchez, E. Y., Sambeth, J. E., & Porta, A. A. (2018). Exposure and risk analysis to particulate matter, metals, and polycyclic aromatic hydrocarbon at different workplaces in Argentina. *Environ Sci Pollut Res*, *25*(9), 8487–8496.

Costa, M. A. (2016). The endocrine function of human placenta: An overview. *Reprod BioMed Online*, *32*(1), 14–43.

CSIRO. (2013). Community Affairs Inquiry into the impacts on health of air quality in Australia, (March).

Dacunto, P. J., Klepeis, N. E., Cheng, K. C., Acevedo-Bolton, V., Jiang, R. T., Repace, J. L., Ott, W. R., & Hildemann, L. M. (2015). Determining $PM_{2.5}$ calibration curves for a low-cost particle monitor: Common indoor residential aerosols. *Environ Sci Proces Impacts*, *17*(11), 1959–1966.

Dadvand, P., Figueras, F., Basagaña, X., Beelen, R., Martinez, D., Cirach, M., Schembari, A., Hoek, G., Brunekreef, B., & Nieuwenhuijsen, M. J. (2013). Ambient air pollution and preeclampsia: A spatiotemporal analysis. *Environ Health Perspect*, *121*(11), 1365–1371.

Dales, R., Liu, L., Wheeler, A. J., & Gilbert, N. L. (2008). Quality of indoor residential air and health. *Can Med Assoc J*, *179*(2), 147–152.

Danielsen, P. H., Loft, S., & Møller, P. (2008). DNA damage and cytotoxicity in type II lung epithelial (A549) cell cultures after exposure to diesel exhaust and urban street particles. *Part Fibre Toxicol*, *5*, 1–12.

de Melo, J. O., Soto, Sô. F., Katayama, I. A., Wenceslau, C. F., Pires, A. G., Veras, M. M., Furukawa, L. N. S., de Castro, I., & Saldiva, P. H. N., & Heimann, J. C. (2015). Inhalation of fine particulate matter during pregnancy increased IL-4 cytokine levels in the fetal portion of the placenta. *Toxicol Lett, 232*(2), 475–480.

DEFRA. (2019a). Air pollution in the U.K. 2018 (Vol. 1).

DEFRA. (2019b). Assessing progress towards WHO guideline levels of PM2.5 in the UK. Available at: www.gov.uk/government/publications.

DEFRA. (2019c). Automatic Urban and Rural Network (AURN). Available at: https://uk-air.defra.gov.uk/networks/network-info?view=aurn (Last accessed 18/12/2019)

DEFRA. (2019d). What is the Daily Air Quality Index. Available at: https://uk-air.defra.gov.uk/air-pollution/daqi?view=more-info (Last accessed 18/12/2019)

DEFRA. (2019e). Site environment types. Available at: https://uk-air.defra.gov.uk/networks/site-types (Last accessed 03/01/2020)

DeFranco, E., Hall, E., Hossain, M., Chen, A., Haynes, E. N., Jones, D., Ren, S., Lu, L., & Muglia, L. (2015). Air pollution and stillbirth risk: exposure to airborne particulate matter during pregnancy is associated with fetal death. *PloS One*, *10*(3), e0120594.

Dejmek, J., Solanský, I., Benes, I., Lenícek, J., & Srám, R. J. (2000). The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. *Environ Health Perspect*, *108*(12), 1159–64.

Dermentzoglou, M., Manoli, E., Voutsa, D., & Samara, C. (2003). Sources and patterns of polycyclic aromatic hydrocarbons and heavy metals in fine indoor particulate matter of Greek houses. *Fresenius Environ*, *12*(12), 1511–1519.

Desforges, M., Rogue, A., Pearson, N., Rossi, C., Olearo, E., Forster, R., Lees, M., Sebire, N. J., Greenwood, S. L., Sibley, C. P., David, A. L., & Brownbill, P. (2018). In Vitro Human Placental Studies to Support Adenovirus-Mediated VEGF-D$^{\Delta N \Delta C}$ Maternal Gene Therapy for the Treatment of Severe Early-Onset Fetal Growth Restriction. *Hum Gene Ther Clini Dev*, *29*(1), 10–23.

Diapouli, E., Chaloulakou, A., & Spyrellis, N. (2007). Levels of ultrafine particles in different microenvironments - Implications to children exposure. *Sci Total Environ*, *388*(1–3), 128–136.

Díaz, P., Sibley, C. P., & Greenwood, S. L. (2016). Oxygen-sensitive K+ channels modulate human chorionic gonadotropin secretion from human placental trophoblast. *PLoS ONE*, *11*(2), 1–15.

Donaldson, K., & Stone, V. (2003). Current hypotheses on the mechanisms of toxicity of ultrafine particles. *Ann Ist Super Sanita*, *39*(3), 405–410.

Donohoe, M., & Keegan, A. (2008). *Air quality and health*. *J Complement Med* (Vol. 7).

Dunk, C., Huppertz, B., & Kingdom, J. (2008). *Development of the placenta and its circulation*. *Fetal Medicine* (Third Edit). Elsevier Ltd.

Durackova, Z. (2010). Some Current Insights into Oxidative Stress, *8408*, 459–469.

Dutta, A., Khramstova, G., Brito, K., Alexander, D., Mueller, A., Chinthala, S., Adu, D., Ibigbami, T., Olamijulo, J., Odetunde, A., Adigun, K., Pruitt, L., Hurley, I., Olopade, O., Ojengbede, O., Rana, S., & Olopade, C. O. (2018). Household air pollution and chronic hypoxia in the placenta of pregnant Nigerian women: A randomized controlled ethanol Cookstove intervention. *Science Total Environ*, *619–620* (November 2017), 212–220.

Dybdahl, M., Risom, L., Bornholdt, J., Autrup, H., Loft, S., & Wallin, H. (2004). Inflammatory and genotoxic effects of diesel particles in vitro and in vivo. *Mutat Res Genet Toxicol Environ Mutagen*, *562*(1–2), 119–131.

Dylos Corporation. (n.d.). DC1700 Air Quality Monitor. Available at: http://www.dylosproducts.com/ornodcairqum.html.

Edwards, R., Hasselholdt, C. P., Hargreaves, K., Probert, C., Holford, R., Hart, J., Tongeren, M. V., & Watson, A. F. R. (2006). Levels of second hand smoke in pubs and bars by deprivation and food-serving status: A cross-sectional study from North West England. *BMC Public Health*, *6*, 1–6.

English, F. A., Kenny, L. C., & McCarthy, F. P. (2015). Risk factors and effective management of preeclampsia. *Integr Blood Press Control*, *8*, 7–12.

Erickson, A. C., & Arbour, L. (2014). The shared pathoetiological effects of particulate air pollution and the social environment on fetal-placental development. *J Environ Public Health*, *2014*.

European Comission. (2007). *Scientific Committee on Health and Environmental Risks SCHER Opinion on risk assessment on indoor air quality Indoor air RA*.

European Commission. (2008). *Level 2 - Details on Indoor Air Quality*.

Familari, M., Nääv, Å., Erlandsson, L., de Iongh, R. U., Isaxon, C., Strandberg, B., Lundh, T., Hansson, S. R., & Malmqvist, E. (2019). Exposure of trophoblast cells to fine particulate matter air pollution leads to growth inhibition, inflammation and ER stress. *PLoS ONE*, *14*(7), 1–20.

Fecht, D., Fischer, P., Fortunato, L., Hoek, G., De Hoogh, K., Marra, M., Kruize, H., Vienneau, D., Beelen, R., & Hansell, A. (2015). Associations between air pollution and socioeconomic characteristics, ethnicity and age profile of neighbourhoods in England and the Netherlands. *Environ Pollut*, *198*, 201–210.

Fonseca, J., Slezakova, K., Morais, S., & Pereira, M. C. (2014). Assessment of ultrafine particles in Portuguese preschools: Levels and exposure doses. *Indoor Air*, *24*(6), 618–628.

Frank, H.-G. (2017). *Placental Development. Fetal and Neonatal Physiology* (Fifth Edit). Elsevier Inc.

Franken, R., Maggos, T., Stamatelopoulou, A., Loh, M., Kuijpers, E., Bartzis, J., Steinle, S., Cherrie, J. W., & Pronk, A. (2019). Comparison of methods for converting Dylos particle number concentrations to PM2.5 mass concentrations. *Indoor Air*, *29*(3), 450–459.

Franklin, P., Tan, M., Hemy, N., & Hall, G. L. (2019). Maternal exposure to indoor air pollution and birth outcomes. *Int J Environ Res Public Health*, *16*(8).

Fujimoto, A., Tsukue, N., Watanabe, M., Sugawara, I., Yanagisawa, R., Takano, H., Yoshida, S., & Takeda, K. (2005). Diesel exhaust affects immunological action in the placentas of mice. *Environ Toxicol*, *20*(4), 431–440.

Gaccioli, F., & Lager, S. (2016). Placental nutrient transport and intrauterine growth restriction. *Front Physiol*, *7*(FEB), 1–8.

Gardosi, J. (2006). New definition of small for gestational age based on fetal growth potential. *Horm Res*, *65*(SUPPL. 3), 15–18.

Gaspar, F. W., Maddalena, R., Williams, J., Castorina, R., Wang, Z. M., Kumagai, K., McKone, T. E., & Bradman, A. (2018). Ultrafine, fine, and black carbon particle concentrations in California child-care facilities. *Indoor Air*, *28*(1), 102–111.

Gee, I. L., Stewart, L., Waston, A. F. R., Fletcher, G., & Niven, R. (2002). Indoor air quality in smoking and non-smoking households. *Indoor Air*, 512–517.

Ghosh, J. K. C., Wilhelm, M., & Ritz, B. (2013). Effects of residential indoor air quality and household ventilation on preterm birth and term low birth weight in Los Angeles County, California. *Am J Public Health*, *103*(4), 686–694.

Giussani, V. (2013). *The UK Clean Air Act 1956: An Empirical Investigation*. *J Chem Inf Model* (53), 1689–1699.

Goncalves, J. M., Casart, Y. C., & Camejo, M. I. (2016). Nitric oxide and oxidative stress in placental explant cultures. *Syst Biol Reprod Medicine*, *62*(1), 11–16.

GOV.UK. (2013). Air pollution from farming: preventing and minimising. Available at: https://www.gov.uk/guidance/reducing-air-pollution-on-farms (Last accessed 16/12/2019).

Grevendonk, L., Janssen, B. G., Vanpoucke, C., Lefebvre, W., Hoxha, M., Bollati, V., & Nawrot, T. S. (2016). Mitochondrial oxidative DNA damage and exposure to particulate air pollution in mother-newborn pairs. *Environ Health*, *15*(1), 10.

Gude, N. M., Roberts, C. T., Kalionis, B., & King, R. G. (2004). Growth and function of the normal human placenta. *Thromb Res*, *114*(5–6 SPEC. ISS.), 397–407.

Gundacker, C., & Hengstschläger, M. (2012). The role of the placenta in fetal exposure to heavy metals, 201–206.

Guo, Y., Huo, X., Li, Y., Wu, K., Liu, J., Huang, J., Zheng, G., Xiao, Q., Yang, H., Wang, Y., Chen, A., & Xu, X. (2010). Monitoring of lead, cadmium, chromium and nickel in placenta from an e-waste recycling town in China. *Sci Total Environ*, *408*(16), 3113–3117.

Han, I., Symanski, E., & Stock, T. H. (2017). Feasibility of using low-cost portable particle monitors for measurement of fine and coarse particulate matter in urban ambient air. *J Air Waste Manage Assoc*, *67*(3), 330–340.

Hannam, K., McNamee, R., Baker, P., Sibley, C., & Agius, R. (2014). Air pollution exposure and adverse pregnancy outcomes in a large UK birth cohort: Use of a novel Spatio-Temporal modelling technique. *Scand J Work Environ Health*, *40*(5), 518–530.

Harmon, A. C., Cornelius, D. C., Amaral, L. M., Faulkner, J. L., Cunningham, M. W., Wallace, K., & LaMarca, B. (2016). The role of inflammation in the pathology of preeclampsia. *Clin Sci*, *130*(6), 409–419.

Harnly, M. E., Bradman, A., Nishioka, M., Mckone, T. E., Smith, D., Mclaughlin, R., Kavanagh-Baird, G., Castorina, R., & Eskenazi, B. (2009). Pesticides in dust from homes in an agricultural area. *Environ Sci Technology*, *43*(23), 8767–8774.

Harris, G., Thompson, W. D., Fitzgerald, E., & Wartenberg, D. (2014). The association of PM(2.5) with full term low birth weight at different spatial scales. *Environ Res*, *134*(6), 427–34.

Health Canada. (1994). *Polycyclic aromatic hydrocarbons*. *Food Analysis by HPLC, Third Edition*.

Heazell, A. E. P., & Crocker, I. P. (2008). Live and Let Die - Regulation of Villous Trophoblast Apoptosis in Normal and Abnormal Pregnancies. *Placenta*, *29*(9), 772–783.

Hirst, C. R. (2015). Placental Taurine Transport in.PhD. The University of Manchester.

Hobel, C., & Culhane, J. (2003). Role of Psychosocial and Nutritional Stress on Poor Pregnancy Outcome. *J Nutr*, *133*(5), 1709–1717.

Hougaard, K. S., Jensen, K. A., Nordly, P., Taxvig, C., Vogel, U., Saber, A. T., & Wallin, H. (2008). Effects of prenatal exposure to diesel exhaust particles on postnatal development, behavior, genotoxicity and inflammation in mice. *Part Fibre Toxicol*, *5*.

Howson, E. C., Kinney, M., & Lawn., J. (2012). *Born Too Soon, The Global Action Report on Preterm Birth. World Health Organization. Geneva* (Vol. 13).

Hsu, C. S., Liu, P. L., Chien, L. C., Chou, S. Y., & Han, B. C. (2007). Mercury concentration and fish consumption in Taiwanese pregnant women. *BJOG*, *114*(1), 81–85.

Huang, Y.-L., Chen, H.-W., Han, B.-C., Liu, C.-W., Chuang, H.-C., Lin, L.-Y., & Chuang, K.-J. (2014). Personal exposure to household particulate matter, household activities and heart rate variability among housewives. *PloS One*, *9*(3), e89969.

Hudda, N., Simon, M. C., Zamore, W., Brugge, D., & Durant, J. L. (2016). Concentrations in the Greater Boston Area, *50*(16), 8514–8521.

Huppertz, B. (2008). The anatomy of the normal placenta. *J Clin Pathol*, *61*(12), 1296–1302.

Huppertz, B. (2011). Trophoblast differentiation, fetal growth restriction and preeclampsia. *Pregnancy Hypertens*, *1*(1), 79–86.

Iyengar, G. V., & Rapp, A. (2001). Human placenta as a "dual" biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: Toxic trace elements in placenta and placenta as a biomarker for these elements. *Sci Total Environ*, *280*(1–3), 221–238.

Jackson-Morris, A., Bleymann, K., Lyall, E., Aslam, F., Bam, T. S., Chowdhury, I., Daouda, E. J., Espinosa, M., Romo, J., Singh, R. J., & Semple, S. (2016). Low-cost air quality monitoring methods to assess compliance with smoke-free regulations: A multi-center study in six lowand middle-income countries. *Nicotine Tob Res*, *18*(5), 1258–1264.

Janssen, B. G., Munters, E., Pieters, N., Smeets, K., Cox, B., Cuypers, A., Fierens, F., Penders, J., Vangronsveld, J., Gyselaers, W., & Nawrot, T. S. (2012). Placental mitochondrial DNA content and particulate air pollution during in utero life. *Environ Health Perspec*, *120*(9), 1346–52.

Jantunen, Matti, Eduardo O., Paolo C. S. K. (2011). *Promoting actions for healthy indoor air (IAIAQ)*.

Jantzen, K., Roursgaard, M., Desler, C., Loft, S., Rasmussen, L. J., & Møller, P. (2012). Oxidative damage to DNA by diesel exhaust particle exposure in co-cultures of human lung epithelial cells and macrophages. *Mutagenesis*, *27*(6), 693–701.

Jedrychowski, W. A., Majewska, R., Spengler, J. D., Camann, D., Roen, E. L., & Perera, F. P. (2017). Prenatal exposure to fine particles and polycyclic aromatic hydrocarbons and birth outcomes: a two-pollutant approach. *Int Arch Occup Environ Health*, *90*, 255–264.

Jedrychowski, W. A., Perera, F. P., Pac, A., Jacek, R., Whyatt, R. M., Spengler, J. D., Dumyahn, T. S., & Sochacka-Tatara, E. (2006). Variability of total exposure to PM2.5 related to indoor and outdoor pollution sources. Krakow study in pregnant women. *Sci Total Environ*, *366*(1), 47–54.

Jedrychowski W, Perera F, Flak, E., Spengler, J., Budzyn-mrozek, D., & Kaim, I. (2013). Impact of Barbecued Meat Consumed in Pregnancy on Birth Outcomes Accounting for Personal Prenatal Exposure to Airborne Polycyclic Aromatic Hydrocarbons. Birth Cohort Study in Poland. *Nutrition*, *28*(4), 372–377.

Jeyabalan Arun. (2014). Epidemiology of preeclampsia, *71*(0 1), 1–14.

Jones, N. C., Thornton, C. A., Mark, D., & Harrison, R. M. (2000). Indoor/outdoor relationships of particulate matter in domestic homes with roadside, urban and rural locations. *Atmos Environ*, *34*(16), 2603–2612.

Jones, S., Anthony, T. R., Sousan, S., Altmaier, R., Park, J. H., & Peters, T. M. (2017). Concentrations in a Swine Building, *60*(5), 597–607.

Jones, S. M. (2015). Evaluation of a low-cost aerosol sensor to assess occupational exposures. Available at: http://ir.uiowa.edu/etd/1858

Jorquera, H., Barraza, F., Heyer, J., Valdivia, G., Schiappacasse, L. N., & Montoya, L. D. (2018). Indoor PM2.5 in an urban zone with heavy wood smoke pollution: The case of Temuco, Chile. *Environ Pollut*, *236*, 477–487.

Kaiser, L. L., & Allen, L. (2002). Nutrition and lifestyle for a healthy pregnancy outcome. *Journal Acad Nutr Diet*, *102*(10), 1479–1490.

Kalejaiye, H. A., Adeyemi, M. F., Akinshipo, A., Sulaiman, A. O., Braimah, R. O., Ibikunle, A. A., & Taiwo, A. O. (2016). Prevalence of Dental Caries and Pattern of Sugar Consumption among Junior Secondary School Students in Northcentral Nigeria. *Nigerian J Exp Clin Biosci*, *4*, 13–18.

Kannan, S., Misra, D. P., Dvonch, J. T., & Krishnakumar, A. (2006). Exposures to airbone particulate matter and adverse perinatal outcomes: A biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect*, *114*(11), 1636–1642.

Karottki, D. G., Bekö, G., Clausen, G., Madsen, A. M., Andersen, Z. J., Massling, A., Ketzel, M., Ellermann, T., Lund, R., Sigsgaard, T., Møller, P., & Loft, S. (2014). Cardiovascular and lung function in relation to outdoor and indoor exposure to fine and ultrafine particulate matter in middle-aged subjects. *Environ Int*, *73*, 372–381.

Karottki, D. G., Spilak, M., Frederiksen, M., Jovanovic Andersen, Z., Madsen, A. M., Ketzel, M., Massling, A., Gunnarsen, L., Møller, P., & Loft, S. (2015). Indoor and outdoor exposure to ultrafine, fine and microbiologically derived particulate matter related to cardiovascular and respiratory effects in a panel of elderly urban citizens. *Int J Environ Res Public Health*, *12*(2), 1667–86.

Khan, M. N., Nurs, C. Z. B., Islam, M. M., Islam, M. R., & Rahman, M. M. (2017). Household air pollution from cooking and risk of adverse health and birth outcomes in Bangladesh: A nationwide population-based study. *Environ Health*, *16*(1), 1–8.

Kim, C. W., Lee, H. M., Lee, K., Kim, B., Lee, M. Y., & Choi, K. C. (2017). Effects of cigarette smoke extracts on cell cycle, cell migration and endocrine activity in human placental cells. *Reprod Toxicol*, *73*, 8–19.

Kim, S.-Y., Yi, S.-J., Eum, Y. S., Choi, H.-J., Shin, H., Ryou, H. G., & Kim, H. (2014). Ordinary kriging approach to predicting long-term particulate matter concentrations in seven major Korean cities. *Environ Health Toxicol*, *29*, 1-8.

Kippler, M., Hoque, A. M. W., Raqib, R., Öhrvik, H., Ekström, E. C., & Vahter, M. (2010). Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol Lett*, *192*(2), 162–168.

Kittelson, D. B. (1998). Engines and nanoparticles: A review. *J Aerosol Sci*, *29*(5–6), 575–588.

Klepeis, N. E., Hughes, S. C., Edwards, R. D., Allen, T., Johnson, M., Chowdhury, Z., Smith, K. R., Boman-Davis, M., Bellettiere, J., & Hovell, M. F. (2013). Promoting Smoke-Free Homes: A Novel Behavioral Intervention Using Real-Time Audio-Visual Feedback on Airborne Particle Levels. *PLoS ONE*, *8*(8).

Korevaar, T. I. M., Steegers, E. A. P., de Rijke, Y. B., Schalekamp-Timmermans, S., Visser, W.

E., Hofman, A., Jaddoe, V. W. V., Tiemeier, H., Visser, T. J., Medici, M., & Peeters, R. P. (2015). Reference ranges and determinants of total hCG levels during pregnancy: the Generation R Study. *Eur J Epidemiology*, *30*(9), 1057–1066.

Kowaltowski, A. J., & Vercesi, A. E. (1999). Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med*, *26*(3–4), 463–471.

Krivoshto, I. N., Richards, J. R., Albertson, T. E., & Derlet, R. W. (2008). The Toxicity of Diesel Exhaust: Implications for Primary Care. *J Am Board Fam Med*, *21*(1).

Kurt-karakus, P. B. (2012). Determination of heavy metals in indoor dust from Istanbul, Turkey: Estimation of the health risk. *Environ Int*, *50*, 47–55.

Lawn, J. E., Blencowe, H., Waiswa, P., Amouzou, A., Mathers, C., Hogan, D., Flenady, V., Frøen, J. F., Qureshi, Z. U., Calderwood, C., Shiekh, S., Jassir, F. B., You, D., McClure, E. M., Mathai, M., & Cousens, S. (2016). Stillbirths: Rates, risk factors, and acceleration towards 2030. *Lancet*, *387*(10018), 587–603.

Lee, P. C., Roberts, J. M., Catov, J. M., Talbott, E. O., & Ritz, B. (2013). First trimester exposure to ambient air pollution, pregnancy complications and adverse birth outcomes in Allegheny County, PA. *Matern Child Health J*, *17*(3), 545–555.

Li, H., Qian, X., & Wang, Q. (2013). Heavy metals in atmospheric particulate matter: A comprehensive understanding is needed for monitoring and risk mitigation. *Environ Sci Technol*, *47*(23), 13210–13211.

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J., & Nel, A. (2003). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ Health Perspect*, *111*(4), 455–460.

Li, R. J., Kou, X. J., Geng, H., Dong, C., & Cai, Z. W. (2014). Pollution characteristics of ambient PM2.5-bound PAHs and NPAHs in a typical winter time period in Taiyuan. *Chin Chem Lett*, *25*(5), 663–666.

Li, R., Zhou, R., & Zhang, J. (2018). Function of PM2.5 in the pathogenesis of lung cancer and chronic airway inflammatory diseases. *Oncol Lett*, *15*(5), 7506–7514.

Lim, M., Myagmarchuluun, S., Ban, H., Hwang, Y., Ochir, C., Lodoisamba, D., & Lee, K. (2018). Characteristics of indoor pm2.5 concentration in gers using coal stoves in ulaanbaatar, mongolia. *Int J Environ Res Public Health*, *15*(11).

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2009). Free radicals, antioxidants and funtional foods: Impact on human health. *Int J Shoulder Surg*, *3*(4), 94–97.

Lundborg, M., Dahlén, S. E., Johard, U., Gerde, P., Jarstrand, C., Camner, P., & Låstbom, L. (2006). Aggregates of ultrafine particles impair phagocytosis of microorganisms by human alveolar macrophages. *Environ Res*, *100*(2), 197–204.

Manchester Evening News. (2019). Warning of high levels of air pollution in Greater Manchester on Wednesday. Available at: https://www.manchestereveningnews.co.uk/news/greater-manchester-news/warning-high-levels-air-pollution-15890218) (Last accessed: 20/12/2019).

Madsen, C., Håberg, S. E., Aamodt, G., Stigum, H., Magnus, P., London, S. J., Nystad, W., & Nafstad, P. (2019). Preeclampsia and hypertension during pregnancy in areas with relatively low levels of traffic air pollution. *Physiol Behav*, *176*(3), 139–148.

Magsumbol, M. S., Singh, A., Ghosh, A., Kler, N., Garg, P., Thakur, A., Beg, A., Srivastava, A., & Hajat, S. (2014). Association of air pollution on birth outcomes in New Delhi - a pilot study on the potential of HMIS data for environmental public health tracking. *Indian J Med Inform*, *8*(2), 52–56.

Mangia, C., Cervino, M., & Gianicolo, E. A. L. (2015). Secondary particulate matter originating from an industrial source and its impact on population health. *Int J Environ Res Public Health*, *12*(7), 7667–7681.

Mannelli, C., Ietta, F., Avanzati, A. M., Skarzynski, D., & Paulesu, L. (2015). Biological tools to study the effects of environmental contaminants at the feto–maternal interface. *Dose-Response*, *13*(4), 1–11.

Mannelli, C., Ietta, F., Carotenuto, C., Romagnoli, R., Szostek, A. Z., Wasniewski, T., Skarzynski, D. J. & Paulesu, L. (2014). Bisphenol A alters β-hCG and MIF release by human placenta: An in vitro study to understand the role of endometrial cells. *Mediators Inflamm*, *2014*.

Mayhew, T. M. (2014). Turnover of human villous trophoblast in normal pregnancy: What do we know and what do we need to know? *Placenta*, *35*(4), 229–240.

McCowan, L., & Horgan, R. P. (2009). Risk factors for small for gestational age infants. *Best Practice and Research: Clinic Obstet Gynaecol, 23*(6), 779–793.

McDermott, S., Salzberg, D. C., Anderson, A. P., Shaw, T., & Lead, J. (2015). Systematic Review of Chromium and Nickel Exposure during Pregnancy and Impact on Child Outcomes. *J Toxicol. Environ Health Part A, 78*(21–22), 1348–1368.

Meyrueix, L., Adair, L., Norris, S. A., & Ideraabdullah, F. (2019). Assessment of placental metal levels in a South African cohort. *Environ Monit Assess*, *191*(8).

MHCLG. (2019). *The English Indices of Deprivation 2019 - Statistical Release*. United Kingdom.

Miller, R. K., Genbacev, O., Turner, M. A., Aplin, J. D., Caniggia, I., & Huppertz, B. (2005). Human placental explants in culture: Approaches and assessments. *Placenta*, *26*(6), 439–448.

Moll, S. J., Jones, C. J. P., Crocker, I. P., Baker, P. N., & Heazell, A. E. P. (2007). Epidermal growth factor rescues trophoblast apoptosis induced by reactive oxygen species. *Apoptosis*, *12*(9), 1611–1622.

Moore, K. L., Persaud, T. V. N., Torchia, M. G. (2003). Placenta and fetal membranes: The Developing Human: Clinically Orientated Embryology. Saunders, Elsevier science, pp. 120–126

Mørck, T. J., Sorda, G., Bechi, N., Rasmussen, B. S., Nielsen, J. B., Ietta, F., Rytting, E., Mathiesen, L., Paulesu, L., & Knudsen, L. E. (2010). Placental transport and in vitro effects of Bisphenol A. *Reprod Toxicol*, *30*(1), 131–137.

Mostofsky, E., Schwartz, J., Coull, B. A., Koutrakis, P., Wellenius, G. A., Suh, H. H., Gold, D. R., & Mittleman, M. A. (2012). Modeling the association between particle constituents of air pollution and health outcomes. *Am Journal Epidemiol*, *176*(4), 317–326.

Muchemi, O. M., Echoka, E., & Makokha, A. (2015). Factors associated with low birth weight among neonates born at Olkalou District Hospital, Central Region, Kenya. *Pan Afr Med J, 20*, 108.

Muhihi, A., Sudfeld, C. R., Smith, E. R., Noor, R. A., Mshamu, S., Briegleb, C., Bakari, M., Masanja, H., Fawzi, W., & Chan, G. J. Y. (2016). Risk factors for small-for-gestational-age and preterm births among 19,269 Tanzanian newborns. *BMC Pregnancy Childbirth*, *16*(1), 1–12.

Münzel, T., Gori, T., Al-Kindi, S., Deanfield, J., Lelieveld, J., Daiber, A., & Rajagopalan, S. (2018). Effects of gaseous and solid constituents of air pollution on endothelial function. *Eur Heart J*, *39*(38), 3543–3550.

Myatt, L., & Cui, X. (2004). Oxidative stress in the placenta. *Histochem Cell Biol*, *122*(4), 369–382.

Nardozza, L. M. M., Caetano, A. C. R., Zamarian, A. C. P., Mazzola, J. B., Silva, C. P., Marçal, V. M. G., Lobo, T. F., Peixoto, A. B., & Araujo Júnior, E. (2017). Fetal growth restriction: current knowledge. *Arch Gynecol*, *295*(5), 1061–1077.

Nethery, E., Brauer, M., & Janssen, P. (2009). Time-activity patterns of pregnant women and changes during the course of pregnancy. *J Expo Sci Environ Epidemiol*, *19*(3), 317–324.

NHS. (2017a). Premature labour and birth. Available at: https://www.nhs.uk/conditions/pregnancy-and-baby/premature-early-labour/ (Last accessed 12/01/2020).

NHS. (2018a). Symptoms pre-eclampsia. Available at: https://www.nhs.uk/conditions/pre-eclampsia/symptoms/ (Last accessed 21/12/2019).

NHS. (2018b). Causes pre-eclampsia. Available at: https://www.nhs.uk/conditions/pre-eclampsia/causes/ (Last accessed 21/12/2019).

NHS. (2018c). Complications pre-eclampsia. Available at: https://www.nhs.uk/conditions/pre-eclampsia/complications/ (Last accessed 21/12/2019).

NHS. (2019). Causes stillbirth. Available at: https://www.nhs.uk/conditions/stillbirth/causes/ (Last accessed 21/12/2019).

National Institute for Health and Care Excellence, NIEHS. (2019). Indoor air quality at home. Available at: https://www.nice.org.uk/guidance/indevelopment/gid-ng10022 (Last accessed 21/12/2019).

NICVA. (2015). Air Quality Monitoring: Automatic Urban and Rural Network. Available at: http://data.nicva.org/dataset/air-quality-monitoring-automatic-urban-and-rural-network-ni (Last accessed 21/12/2019).

NIEHS. (2019). Air pollution. Available at: https://www.niehs.nih.gov/health/topics/agents/air-pollution/index.cfm (Last accessed 12/01/2020)

NIST. (2013). *Certificate of Analysis Certificate of Analysis*: *Standard Reference Materials 1650b*

NIST. (2014). *Certificate of Analysis Certificate of Analysis: Standard Reference Materials 2585*

Nordenhäll, C., Pourazar, J., Blomberg, A., Levin, J. O., Sandström, T., & Ädelroth, E. (2000). Airway inflammation following exposure to diesel exhaaust: A study of time kinetics using induced sputum. *Eur Respir J*, *15*(6), 1046–1051.

Norris, W., Nevers, T., Sharma, S., & Kalkunte, S. (2011). Review: HCG, preeclampsia and regulatory T cells. *Placenta*, *32*(SUPPL. 2), S182–S185.

Northcross, A. L., Edwards, R. J., Johnson, M. A., Wang, Z. M., Zhu, K., Allen, T., & Smith, K. R. (2013). A low-cost particle counter as a realtime fine-particle mass monitor. *Environ Sci Process Impacts*, *15*(2), 433–439.

O'Neill, M., King, K., Horth, C., Twigg-Purcell, R., Greenen, C., Elliott, L.,Rose, B., Brown, S., Mongan, G., & Pollitt, R. (2017). *2016 Air Quality Annual Status Report (ASR) for Greater Manchester*.

Office for National Statistics. (2019). People living in deprived neighbourhood. Available at: https://www.ethnicity-facts-figures.service.gov.uk/uk-population-by ethnicity/demographics/people-living-in-deprived-neighbourhoods/latest (Last accessed 15/12/2019).

Ogden, T. L. (2010). Handling results below the level of detection. *Ann Occup Hyg*, *54*(3), 255–256.

Olsson, D., Mogren, I., & Forsberg, B. (2013). Air pollution exposure in early pregnancy and adverse pregnancy outcomes : a register-based cohort study. *BMJ Open*, *3*.

Osman, K., Åkesson, A., Berglund, M., Bremme, K., Schütz, A., Ask, K., & Vahter, M. (2000). Toxic and essential elements in placentas of Swedish women. *Clini Biochem*, *33*(2), 131–138.

Padula, A. M., Mortimer, K. M., Tager, I. B., Hammond, S. K., Lurmann, F. W., Yang, W., Stevenson, D. K. & Shaw, G. M. (2014). Traffic-related air pollution and risk of preterm birth in the San Joaquin Valley of California. *Ann Epidemiol*, *24*(12), 888–895.e4.

Patelarou, E., & Kelly, F. (2014). Indoor Exposure and Adverse Birth Outcomes Related to Fetal Growth, Miscarriage and Prematurity—A Systematic Review. *Int J Environ Res Public Health*, *11*(6), 5904–5933.

Paulesu, L., Rao, C. V., Ietta, F., Pietropolli, A., & Ticconi, C. (2018). HCG and its disruption by environmental contaminants during human pregnancy. *Int J Mol Sci*, *19*(3), 1–11.

Pedersen, M., Halldorsson, T. I., Olsen, S. F., Hjortebjerg, D., Ketzel, M., Grandström, C., Raaschou-Nielsen, O., & Sørensen, M. (2017). Impact of road traffic pollution on pre-eclampsia and pregnancy-induced hypertensive disorders. *Epidemiol*, *28*(1), 99–106.

Pereira, G., Bell, M. L., Lee, H. J., Koutrakis, P., & Belanger, K. (2014). Sources of fine particulate matter and risk of preterm birth in connecticut, 2000-2006: A longitudinal study. *Environ Health Perspect*, *122*(10), 1117–1122.

Peters, A., Veronesi, B., Calderón-Garcidueñas, L., Gehr, P., Chen, L. C., Geiser, M., Reed, W., Rothen-Rutishauser, B., Schürch, S., & Schulz, H. (2006). Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Part Fibre Toxicol*, *3*, 1–13.

Pihlava, T., Uuppo, M., & Niemi, S. (2013). Health Effects of Exhaust Particles. *Proceedings of the University of Vaasa, Reports*, *187*, 1–33.

Pleil, J. D., Vette, A. F., & Rappaport, S. M. (2004). Assaying particle-bound polycyclic aromatic hydrocarbons from archived PM2.5 filters. *J Chromatogr A*, *1033*(1), 9–17.

Polivka, B. B. J. (2018). A series in collaboration with the Alliance of Nurses for Healthy Environments The Great London Smog of 1952, *118*(4), 57–61.

Pruneda-Álvarez, L. G., Pérez-Vázquez, F. J., Ruíz-Vera1, T., Ochoa-Martínez, Á. C., Orta-García, S. T., Jiménez-Avalos, J. A., & Pérez-Maldonado, I. N. (2016). Urinary 1-hydroxypyrene concentration as an exposure biomarker to polycyclic aromatic hydrocarbons (PAHs) in Mexican women from different hot spot scenarios and health risk assessment. *Environ Sci Pollut Res*, *23*(7), 6816–6825.

Qing, Y. M., Turpin, B. J., Korn, L., Weisel, C. P., Morandi, M., Colome, S., Junfeng, Z., Stock, T., Spektor, D., Winer, A., Zhang, L., Jong, H. L., Giovanetti, R., Cui, W., Kwon, J., Alimokhtari, S., Shendell, D., Jones, J., Farrar, C., & Maberti, S. (2005). Influence of ambient (outdoor) sources on residential indoor and personal PM2.5 concentrations: Analyses of RIOPA data. *J Expo Anal Environ Epidemiol*, *15*(1), 17–28.

Radaideh, J. A., & Shatnawi, Z. (2015). Correlation between indoor and outdoor air. *WIT Transactions on Ecology and the Environment*, *198*, 357–367.

Raghunath, R., Tripathi, R. M., Sastry, V. N., & Krishnamoorthy, T. M. (2000). Heavy metals in maternal and cord blood. *Sci Total Environ*, *250*(1–3), 135–141.

RCOG Guideline. (2013). The Investigation and Management of the Small for Gestational Age Fetus. *Green-Top Guideline No. 31*, (31), 1–34.

Redline, R. W. (2004). Placental inflammation. *Semin Neonatol*, *9*(4), 265–274.

Rich, D. Q., Liu, K., Zhang, J., Thurston, S. W., Stevens, T. P., Pan, Y.,Kane, C., Weinberger, B., Ohman-Strickland, P., Woodruff, T. J., Duan, X., Assibey-Mensah, V., & Zhang, J. (2015). Differences in birth weight associated with the 2008 Beijing olympics air pollution reduction: Results from a natural experiment. *Environ Health Perspect*, *123*(9), 880–887.

Ritz, B., & Wilhelm, M. (2010). Air Pollution Impacts on Infants and Children. *Southern California Environmental Report Card- Fall 2008*.

Ritz, B., Yu, F., Chapa, G., & Fruin, S. (2000). Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. *Epidemiology, 11*(5), 502–511.

Roberts, S. M., Kehrer, J. P., & Klotz, L. O. (2015). Studies on experimental toxicology and pharmacology. *Stud Experiment Toxicol Pharmacol*, 1–493.

Rosen, L., Zucker, D., Hovell, M., Brown, N., Ram, A., & Myers, V. (2015). Feasibility of measuring tobacco smoke air pollution in homes: Report from a pilot study. *Int J Environ Res Public Health*, *12*(12), 15129–15142.

Rull, K., & Laan, M. (2006). Expression of β -subunit of human chorionic gonadotropin genes during the normal and failed pregnancy. *Hum Reprod.*, *20*(5796), 3360–3368.

Sajani, S. Z., Trentini, A., Rovelli, S., Ricciardelli, I., Marchesi, S., MacCone, C.,Bacco, D., Ferrari, S., Scotto, F., Zigola, C., Cattaneo, A., Cavallo, D. M., Lauriola, P. Poluzzi, V., & Harrison, R. M. (2016). Is particulate air pollution at the front door a good proxy of residential exposure? *Environ Pollut*, *213*, 347–358.

Sakamoto, M., Yasutake, A., Domingo, J. L., Man, H., Kubota, M., & Murata, K. (2013). Relationships between trace element concentrations in chorionic tissue of placenta and umbilical cord tissue : Potential use as indicators for prenatal exposure. *Environ Int*, *60*, 106–111.

Sawicka-Kapusta, K., Bydłoń, G., Zakrzewska, M., Nowicka-Połeć, A., Zadrożna, M., & Nowak, B. (2007). Heavy Metal Concentration in Human Placenta from Southern Poland, *4*(Table 1), 3–6.

Sbrana, E., Suter, M. A., Abramovici, A. R., Hawkins, H. K., Moss, J. E., Patterson, L., … Kjersti Aagaard-Tillery. (2011). Maternal Tobacco Use Is Associated With Increased Markers Of Oxidative Stress In The Placenta. *Am J Obstet Gynecol*, *205*(3), 1–7.

Semple, S., Apsley, A., & MacCalman, L. (2013). An inexpensive particle monitor for smoker behaviour modification in homes. *Tob Control*, *22*(5), 295–298.

Semple, S., Ibrahim, A. E., Apsley, A., Steiner, M., & Turner, S. (2015). Using a new, low-cost air quality sensor to quantify second-hand smoke (SHS) levels in homes. *Tob Control*, *24*(2), 153–158.

Semple, S., & Latif, N. (2014). How long does secondhand smoke remain in household air: Analysis of PM2.5 data from smokers' homes. *Nicotine Tob Res*, *16*(10), 1365–1370.

Seo, S., Kim, D., Min, S., Paul, C., Yoo, Y., & Choung, J. T. (2016). GIS-based association between PM10 and allergic diseases in Seoul: Implications for health and environmental policy. *Allergy Asthma Immunol Res*, *8*(1), 32–40.

Shah, P. S., & Balkhair, T. (2011). Air pollution and birth outcomes: A systematic review. *Environ Int*, *37*(2), 498–516.

Sharma, M., Kumar, R., Bhatla, N., & Dhingra, R. (2012). A Comparative Study of Apoptosis in Placentas of Normal and Preeclamptic Indian Pregnant Women by TUNEL Assay and M30 Immunostaining. *J Clin Lab Anal*, *26*(6), 459–466.

Shimada, A., Yamamoto, E., Morita, T., Yoshida, M., Suzuki, J. S., Satoh, M., & Tohyama, C. (2004). Ultrastructural Demonstration of Mercury Granules in the Placenta of Metallothionein-Null Pregnant Mice after Exposure to Mercury Vapor. *Toxicol Pathol*, *32*(5), 519–526.

Shin, S. W., Bae, D. J., Park, C. S., Lee, J. U., Kim, R. H., Kim, S. R., Chang, H. S., & Park, J. S. (2019). Effects of air pollution on moderate and severe asthma exacerbations. *J Asthma*, *0*(0), 1–11.

Simán, C. M., Sibley, C. P., Jones, C. J. P., Turner, M. A., & Greenwood, S. L. (2001). The functional regeneration of syncytiotrophoblast in cultured explants of term placenta. *Am J Physiol Regul Integr Comp Physiol*, *280*(4 49-4), 1116–1122.

Sippola, M. R., Sextro, R. G., & Thatcher, T. L. (2014). Measurements and modeling of deposited particle transport by foot traffic indoors. *Environ Sci Technology*, *48*(7), 3800–3807.

Smith, R. B., Fecht, D., Gulliver, J., Beevers, S. D., Dajnak, D., Blangiardo, M., Ghosh, R. E., Hansell, A. L., Kelly, F. J., Ross Anderson, H., & Toledano, M. B. (2017). Impact of London's road traffic air and noise pollution on birth weight: Retrospective population based cohort study. *BMJ (Online)*, *359*, 1–13.

Sponder, M., Fritzer-Szekeres, M., Marculescu, R., Mittlböck, M., Uhl, M., Köhler-Vallant, B., & Strametz-Juranek, J. (2014). Blood and urine levels of heavy metal pollutants in female and male patients with coronary artery disease. *Vasc Health Risk Manag*, *10*, 311–317.

Steiner, S., Bisig, C., Petri-Fink, A., & Rothen-Rutishauser, B. (2016). Diesel exhaust: current knowledge of adverse effects and underlying cellular mechanisms. *Arch Toxicology*, *90*(7), 1541–1553.

Steinle, S., Reis, S., Sabel, C. E., Semple, S., Twigg, M. M., Braban, C. F.,Leeson, S. R., Heal, M. R., Harrison, D., Lin, C., & Wu, H. (2015). Personal exposure monitoring of PM2.5 in indoor and outdoor microenvironments. *Sci Total Environ*, *508*, 383–394.

Stieb, D. M., Chen, L., Hystad, P., Beckerman, B. S., Jerrett, M., Tjepkema, M., Crouse, D. L., Omariba, D. W., Peters, P. A., van Donkelaar, A., Martin, R.V., Burnett, R. T., Liu, S., Smith-Doiron, M., & Dugandzic, R. M. (2016). A national study of the association between traffic-related air pollution and adverse pregnancy outcomes in Canada, 1999-2008. *Environ Res*, *148*, 513–526.

Tagiyeva, N., Anua, S. M., Semple, S., Dick, F., & Devereux, G. (2012). The "take home" burden of workplace sensitizers: Flour contamination in bakers' families. *Environ Int*, *46*, 44–49.

Takeda, K., Tsukue, N., & Yoshida, S. (2004). Endocrine-disrupting activity of chemicals in diesel exhaust and diesel exhaust particles. *Environ Sci International J Environ Physiol Toxicol*, *11*(1), 33–45.

Taleban, S., Gundogan, F., Chien, E. K., Degli-Esposti, S., & Saha, S. (2015). Placental inflammation is not increased in inflammatory bowel disease. *Ann Gastroenterol*, *28*(4), 457–463.

Tenório, M. B., Ferreira, R. C., Moura, F. A., Bueno, N. B., De Oliveira, A. C. M., & Goulart, M. O. F. (2019). Cross-Talk between Oxidative Stress and Inflammation in Preeclampsia. *Oxid Med Cell Longev*, *2019*.

The Lancet. (2016). Ending preventable stillbirths: an Executive Summary for The Lancet's Series. *The Lancet*, 1–8. Available at: http://www.thelancet.com/pb/assets/raw/Lancet/stories/series/stillbirths2016-exec-summ.pdf

Tong, S., Wallace, E. M., & Rombauts, L. (2006). Association between low day 16 hcg and miscarriage after proven cardiac activity. *Obstet Gynecol*, *107*(2), 300–304.

Tsukue, N., & Dep, P. M. (2002). Diesel Exhaust Affects The Abnormal Delivery In Pregnant Mice And The Growth Of Their Young

Tsukue, N., Tsubone, H., & Suzuki, A. K. (2002). Diesel Exhaust Affects The Abnormal Delivery In Pregnant Mice And The Growth Of Their Young. *Inhal Toxicol*, *8378*(14), 635–651.

Turco, M. Y., & Moffett, A. (2019). Development of the human placenta. *Development (Cambridge)*, *146*(22), 1–14.

Turner, M. A., Roulstone, C. J., Desforges, M., Cretney, M., Champion, E., Lacey, H., & Greenwood, S. L. (2006). The extent and variability of effects of culture conditions on the secretion of human chorionic gonadotrophin and interleukin-6 by human, term placental explants in culture. *Placenta*, *27*(1), 98–102.

Tyagi, A., & Singh, P. (2013). Applying Kriging Approach on Pollution Data Using GIS software. *Int J Environ Eng Manag*, *4*(3), 185–190.

UNICEF. (2004). *Low Birthweight: Country, regional and global estimates*. *UNICEF*.

USEPA. (2019a). Transboundary Air Poluution. Available at: https://www.epa.gov/international-cooperation/transboundary-air-pollution (Last accessed 02/01/2020)

USEPA. (2019b). Introduction to Indoor Air Quality. Available at: https://www.epa.gov/indoor-air-quality-iaq/introduction-indoor-air-quality (Last accessed 12/01/2020)

USEPA. (2018). Particulate Matter (PM) Basics. Available at: https://www.epa.gov/pm-pollution/particulate-matter-pm-basics#PM (Last accessed 03/01/2020)

US National Research Council. (1981). Monitoring and Modeling of Indoor Air Pollution. *Indoor Pollutants*, (National Academies Press US), 30. Available at: https://www.ncbi.nlm.nih.gov/books/NBK234059/

Vähäkangas, K., Loikkanen, J., Sahlman, H., Karttunen, V., Repo, J., Sieppi, E.,Kummu, M., Huuskonen, P., Myöhänen, K., Storvik, M., Pasanen, M., Myllynen, P., & Pelkonen, O. (2014). *Biomarkers of toxicity in human placenta*. *Biomarkers in Toxicology*.

Valentino, S. A., Tarrade, A., Aioun, J., Mourier, E., Richard, C., Dahirel, M., Rousseau-Ralliard, D., Fournier, N., Aubrière, M. C., Lallemand, M. S., Camous, S. Guinot, M., Charlier, M., Aujean, E., Al A., H. Fokkens, P. H., Agier, L., Boere, J. A., Cassee, F. R., Slama, R., & Chavatte-Palmer, P. (2016). Maternal exposure to diluted diesel engine exhaust alters placental function and induces intergenerational effects in rabbits. *Part Fibre Toxicol*, *13*(1), 1.

Valero De Bernabé, J., Soriano, T., Albaladejo, R., Juarranz, M., Calle, M. E., Martínez, D., & Domínguez-Rojas, V. (2004). Risk factors for low birth weight: A review. *Eur J Obstet Gynecol Reprod Biol*, *116*(1), 3–15.

van den Hooven, E. H., Pierik, F. H., de Kluizenaar, Y., Hofman, A., van Ratingen, S. W., Zandveld, P. Y. J., Russcher, H., Lindemans, J., Miedema, H. M. E., Steegers, E. A. P., & Jaddoe, V. W. V. (2012). Air Pollution Exposure and Markers of Placental Growth and Function: The Generation R Study. *Environ Health Perspect*, *120*(12), 1753–1759.

van Eeden, S. F., Tan, W. C., Suwa, T., Mukae, H., Terashima, T., Fujii, T., Qui, D., Vincent, R., & Hogg, J. C. (2001). Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). *Am J Respir Crit Care Med*, *164*(5), 826–30.

Vattanasit, U., Navasumrit, P., Khadka, M. B., Kanitwithayanun, J., Promvijit, J., Autrup, H., & Ruchirawat, M. (2014). Oxidative DNA damage and inflammatory responses in cultured human cells and in humans exposed to traffic-related particles. *Int J Hyg Environ Health*, *217*(1), 23–33.

Vega, F., Alonso-Fariñas, B., Baena-Moreno, F. M., Rodríguez, J. A., & Navarrete, B. (2019). *Technologies for control of sulfur and nitrogen compounds and particulates in coal combustion and gasification*. *New Trends in Coal Conversion*.

Veldurthy, V., Wei, R., Oz, L., Dhawan, P., Jeon, Y. H., & Christakos, S. (2016). Vitamin D, calcium homeostasis and aging. *Bone Res*, *4*(8), 1–7.

Verbovšek, T. (2011). A comparison of parameters below the limit of detection in geochemical analyses by substitution methods. *RMZ - Materials and Geoenvironment*, *4*(58), 393–404.

Volesky, K. D., Maki, A., Scherf, C., Watson, L., Van Ryswyk, K., Fraser, B., Weichenthal, S. A., Cassol, E., & Villeneuve, P. J. (2018). The influence of three e-cigarette models on indoor fine and ultrafine particulate matter concentrations under real-world conditions. *Environ Pollut*, *243*, 882–889.

Waalkes, M. P., Poisner, A. M., Wood, G. W., & Klaassen, C. D. (1984). Metallothionein-like proteins in human placenta and fetal membranes. *Toxicol Appl Pharm*, *74*(2), 179–184.

Wang, L., & Choi, H. (2014). Using semiparametric-mixed model and functional linear model to detect vulnerable prenatal window to carcinogenic polycyclic aromatic hydrocarbons on fetal growth. *Biom. J, 56*(2), 243–255.

Wang, Y. & Zhao, S. (2010a). Chapter 2 Placental Blood Circulation. In *Vascular Biology of the Placenta* (pp. 1–10).

Wang, Y. & Zhao, S. (2010b). Chapter 3 Structure of the Placenta. In *Vascular Biology of the Placenta* (pp. 2010–2013).

Wang, C., Yang, J., Hao, Z., Gong, C., Tang, L., Xu, Y., Lu, D., Li, Z., & Zhao, M. (2017). Suppression of progesterone synthesis in human trophoblast cells by fine particulate matter primarily derived from industry. *Environ Pollut*, *231*, 1172–1180.

Weldy, C. S., Liu, Y., Liggitt, H. D., & Chin, M. T. (2014). In Utero exposure to diesel exhaust air pollution promotes adverse intrauterine conditions, resulting in weight gain, altered blood pressure, and increased susceptibility to heart failure in adult mice. *PLoS ONE*, *9*(2).

Whitehead, T., Metayer, C., Gunier, R. B., Ward, M. H., Nishioka, M. G., Buffler, P., & Rappaport, S. M. (2011). Determinants of polycyclic aromatic hydrocarbon levels in house dust. *J Expo Sci Environ Epidemiol*, *21*(2), 123–32.

WHO. (1990). *Indoor air quality: biological contaminants*.

WHO. (2006). WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide: global update 2005: summary of risk assessment. *Geneva: World Health Organization*, 1–22.

WHO. (2010). *WHO guidelines for indoor air quality: selected pollutants*. *WHO Regional Office for Europe*.

WHO. (2011). *WHO recommendations for: Prevention and treatment of pre-eclampsia and eclampsia*. *World Health Organization*.

WHO. (2013). Zinc supplementation during pregnancy. Available at: https://www.who.int/elena/bbc/zinc_pregnancy/en/. (Last accessed 15/12/2019)

WHO. (2014a). *Global nutrition targets 2025: Low birth weight policy brief*. *World Health Organization*.

WHO. (2014b). Indoor Air Quality Guidelines: Household Fuel Combustion. *World Health Organization*, 1–172. Available at: http://www.who.int/iris/bitstream/10665/141496

WHO. (2015). *WHO recommendations on interventions to improve preterm birth outcomes*. Available at: www.who.int/reproductivehealth

WHO. (2016). *Ambient air pollution: A global assesment of expsoure and burden of disease*.

WHO. (2019a). Air pollution. Available at https://www.who.int/health-topics/air-pollution#tab=tab_1 (Last accessed 12/01/2020)

WHO. (2019b). Ambient air pollution: Pollutants. Available at https://www.who.int/airpollution/ambient/pollutants/en/ (Last accessed 12/01/2020)

WHO. (2019c). Stillbirths. Available at: https://www.who.int/maternal_child_adolescent/epidemiology/stillbirth/en/ (Last accessed 21/12/2019)

WHO. (2019d). The neglected tragedy of stillbirths. Available at: https://www.who.int/reproductivehealth/topics/maternal_perinatal/stillbirth/en/ (Last accessed 21/12/2019)

WHO. (2020). Causes of child mortality. Available at https://www.who.int/gho/child_health/mortality/causes/en/ (Last accessed 09/01/2020)

Wick, P., Malek, A., Manser, P., Meili, D., Maeder-Althaus, X., Diener, L., Diener, P. A., Zisch, A., Krug, H. F., & Von Mandach, U. (2010). Barrier capacity of human placenta for nanosized materials. *Environ Health Perspect*, *118*(3), 432–436.

Williams, I. D., & Bird, A. (2003). Public perceptions of air quality and quality of life in urban and suburban areas of London. *J Environ Monit*, *5*(2), 253–259.

Williams, Kaufman, R., Garvey, A., & Sam. (2015). *Dylos DC1100 Citizen Science Operating Procedure*. *Citizen Science Operating Procedure* (Vol. EPA/600).

Williams, J. L. R. (2008). Function and expression of K^+ channels in human placental trophoblast. PhD. The University of Manchester.

Woodruff, T. J., Parker, J. D., Darrow, L. A., Slama, R., Bell, M. L., Choi, H., Glinianaia, S., Hoggatt, K. J., Karr, C. J., Lobdell, D. T. & Wilhelm, M. (2009). Methodological issues in studies of air pollution and reproductive health. *Environ Res, 109*(3), 311–320.

Wu, F., Tian, F. J., & Lin, Y. (2015). Oxidative stress in placenta: Health and diseases. *BioMed Res Int*, *2015*.

Xia, T., Kovochich, M., & Nel, A. E. (2007). Impairment of mitochondrial function by particulate matter (PM) and their toxic components: Implications for PM-induced cardiovascular and lung disease. *Front Biosci*, *12*(4), 1238–1246.

Xia, W., Hu, J., Zhang, B., Li, Y., Wise, J. P., Bassig, B. A.,Zhou, A., Savitz, D. A., Xiong, C., Zhao, J., Du, X., Zhou, Y., Pan, X., Yang, J., Wu, C., Jiang, M., Peng, Y., Qian, Z., Zheng, T., & Xu, S. (2016). A case-control study of maternal exposure to chromium and infant low birth weight in China. *Chemosphere*, *144*, 1484–1489.

Yang, J. M., Jiang, X. Z., Chen, Q. Y., Li, P. J., Zhou, Y. F., & Wang, Y. L. (1996). The distribution of HgCl2 in rat body and its effects on fetus. *Biomed Environ Sci, 9*(4), 437–442. Journal Article.

Yatim, N. M. (2018). Air pollution, placental DNA damage and repair and pilot study of effects on pregnancy outcomes. PhD. The University of Manchester.

Yu, M., Zheng, X., Witschi, H., & Pinkerton, K. E. (2002). The role of interleukin-6 in pulmonary inflammation and injury induced by exposure to environment air pollutants. *Toxicol Sci*, *68*(2), 488–497.

Zhang, J.-M., & An, J. (2009). Cytokines, Inflammation and Pain. *Int Anesthesiol Clin.*, *69*(2), 482–489.

Zhang, Y., Cao, S., Xu, X., Qiu, J., Chen, M., Wang, D., … Zhang, Y. (2016). Metals compositions of indoor PM2.5, health risk assessment, and birth outcomes in Lanzhou, China. *Environ Monit Assess*, *188*(6).

Zheng, G., Zhong, H., & Guo, Z. (2014). Levels of Heavy Metals and Trace Elements in Umbilical Cord Blood and the Risk of Adverse Pregnancy Outcomes : a Population-Based Study, 437–444.

Zhu, L., Takahashi, Y., Amagai, T., & Matsushita, H. (1997). Highly sensitive automatic analysis of polycyclic aromatic hydrocarbons in indoor and outdoor air. *Talanta*, *45*(1), 113–118.

Zusterzeel, P. L. M., Rütten, H., Roelofs, H. M. J., Peters, W. H. M., & Steegers, E. A. P. (2001). Protein carbonyls in decidua and placenta of pre-eclamptic women as markers for oxidative stress. *Placenta*, *22*(2–3), 213–219.

APPENDIX 1A: Research Ethics Approval (Research Ethic Committee)

Health Research Authority

East Midlands - Leicester South Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

25 October 2018

Dr. Reena Perchard
School of Medical Sciences, Faculty of Biology, Medicine & Health
5th Floor (Research), Royal Manchester Children's Hospital
University of Manchester
M13 9WL

Dear Dr. Perchard

Study title:	**The Manchester Pregnairclean Study**
REC reference:	**18/EM/0317**
Protocol number:	**NHS001450**
IRAS project ID:	**250365**

Thank you for your letter of 16/10/18, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

APPENDIX 1B: Research Ethics Approval (Health Research Authority)

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr. Reena Perchard
School of Medical Sciences, Faculty of Biology, Medicine & Health
5th Floor (Research), Royal Manchester Children's Hospital
University of Manchester
M13 9WL

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

29 October 2018

Dear Dr. Perchard

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**The Manchester Pregnairclean Study**
IRAS project ID:	**250365**
Protocol number:	**NHS001450**
REC reference:	**18/EM/0317**
Sponsor	**The University of Manchester**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

APPENDIX 2: Participant information sheet (PIS)

THE MANCHESTER PREGNAIRCLEAN STUDY

PARTICIPANT INFORMATION SHEET (PIS)

This PIS should be read in conjunction with The University privacy notice.

You are being invited to take part in a research study [as part of student project aimed at examining how feasible it is to collect information on indoor air quality (IAQ)]. The result of this study will help design a future study looking at the association between indoor air quality and pregnancy outcomes.

Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like to know more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Who will conduct the research?

The research will be conducted by Mrs. Norhidayah Binti Ahmad, Dr. Andrew Povey and Dr. Adam Stevens from the Faculty of Biology, Medicine and Health, University of Manchester.

What is the purpose of the research?

The main purpose of this research is to determine the feasibility of collecting information on indoor air quality (IAQ) from the homes of pregnant women.

Why have I been invited?

We are inviting you to take part in this study because you are pregnant and attending antenatal care session at Manchester Placenta Clinic, St. Mary's Hospital, Manchester. We aim to have 50 participants join in this study.

Do I have to take part?

It is up to you to decide. Your participation in this study is completely voluntary. Whatever your decision, the standard of care you receive will be the same.

What would I be asked to do if I take part?

If you are agree to take part in this study, the written consent procedure will be taken by a member of research team. You will be asked to sign the participant consent form (PCF) at the end of your clinic appointment to confirm your willingness to participate in this study. You are also being asked to complete the consent to contact form (CCF) after signing the PCF.

At the end of your clinic appointment, the research team will explain to you the complete research process and your roles in this study. In brief, this will involve the following:

a. **Clinic appointment:**

i. An indoor air sampling device "DYLOS air quality monitor (DC1700)" will be given to you at the end of your clinic appointment by the research team. You will be asked to take this home, and given instructions regarding how to set up the device in your living room and run it for 24 hours together with standard operating procedure (SOP) and a sampling diary. You are able to request the indoor air particles result of your home and the result will be sent to you at the end of data collection process (after a delivery process). We would encourage you to inform other household members about the indoor air pollution monitoring that is being carried out in your home. We suggest that you keep the SOP and instructions next to the DYLOS and inform other household members. If they have any questions, they can contact us on the number below.

i. You will also be given a 60 millilitres (mL) sampling pot and asked to fill this with dust from your household hoover.

ii. A questionnaire regarding your demographic and socioeconomic information, smoking habits and indoor air quality of your home will be given and you will be asked to complete it at your home. Some of the questions in a questionnaire may be sensitive to some people and you are free to not answer that question if you wish to do so.

iii. Lastly, 10mls of your blood (maternal blood) will also be collected during your clinic visit, by a member of the clinical team.

iv. At the end of the session, the research team will arrange a date for collection of the research materials (DYLOS, sampling pot, sampling diary and questionnaire) and will be at least two days after the clinic visit.

b. **Collection of research materials:**

i. A day before the home visit, and if you agree, a member of research team will send you a reminder (via email or SMS) to collect the research materials at your home. Please note that this is optional. The researcher will only visit at the date and time agreed by you, irrespective of whether you agree to this reminder or not.

ii. At a collection date and time agreed, a member of the research team will visit your home to collect the research materials. This will not require the team member to enter your residential home, only to collect the research materials at the doorstep.

iii. Then, all research materials will be collected, coded and sealed. All the collected research materials except house dust will be will be stored in stand-alone, locked cabinets in secure facilities located in the Centre for Occupational and Environmental Health, Ellen Wilkinson Building, The University of Manchester until further analysis. The house dust samples will be sent to the Biomarker's Laboratory, Stopford's Building, The University of Manchester and it will be kept in the freezer at -20^{0}C until further processing.

c. **Collection of biological samples:**

i. At delivery, your placental tissue and a sample of cord blood (as much as can be extracted) will be collected by the clinical staff at St. Mary's Hospital, using standard procedures that are carried out routinely.

ii. The biological samples will be analysed in the laboratory to see if we can detect certain chemicals that may vary depending on the quality of the indoor air. It is including the analysis of heavy metals and polycyclic aromatic hydrocarbons (PAHs) in the biological samples and house dust. The level of biomarkers in the biological samples will be compared with the chemical contents in the house dust by using appropriate statistical analysis. The indoor air particles measured by DYLOS also will be used to explain this association. Finally, the analysis of DNA adduct in the biological samples will be conducted to explain the biological mechanism of indoor air pollution and pregnancy outcomes.

What are the possible benefits of taking part?

The main advantage of taking part is that you will be helping us to understand more about indoor air pollution and pregnancy outcomes, which may help in the future.

What are possible disadvantages and risks of taking part?

You may be inconvenienced by the indoor air sampling device (DYLOS DC1700) in your home, which will produce a low pitched humming sound.

You may not want a member of research team to visit your home to collect the research materials, but this will be at a pre-agreed date and time. The researcher will be at your doorstep and will not enter your home.

The collection of maternal blood will potentially cause discomfort or bruising to you, but this will be carried out by trained experienced clinical staff.

What will happen to my personal information?

Your personal information is strictly confidential. Only the research team has the right to access information gained and related to this study. In order to undertake the research project we will need to collect the following personal information in the participant consent form (PCF), consent to contact form (CCF) and questionnaire which includes:

- Name
- Email address
- Phone number
- Demographic and socioeconomic information
- Smoking information
- Indoor air quality information

We are collecting and storing this personal information in accordance with the General Data Protection Regulation (GDPR) and Data Protection Act 2018 which legislate to protect your personal information. The legal basis upon which we are using your personal information is "public interest task" and "for research purposes" if sensitive information is collected. For more information about

the way we process your personal information and comply with data protection law please see our Privacy Notice for Research Participants.

The University of Manchester, as Data Controller for this project, takes responsibility for the protection of the personal information that this study is collecting about you. In order to comply with the legal obligations to protect your personal data, the University has safeguards in place such as policies and procedures. All researchers are appropriately trained and your data will be looked after in the following way:

- The research team at University of Manchester will have access to your personal identifiable information, that is data which could identify you, but they will anonymise it, to protect your identity. All data gained from the CCF will be kept in order to contact you regarding this study and for the collection of research materials only. This data will be stored in stand-alone, locked cabinets in secure facilities in the Centre for Occupational and Environmental Health, Ellen Wilkinson Building, The University of Manchester.
- When your biological samples are collected, all your personal and medical information will be removed and replaced with a code number so your confidentiality will be preserved. Only the research team will have access to the code.
- All the anonymised study data will be retained for a period of 10 years as per The University of Manchester policy and the chief investigator will be fully responsible for data maintenance after completion of this study. The biological samples may enter the Human Tissue Authority (HTA) Biobank of Maternal and Fetal Health Research Centre, St. Mary's Hospital, Manchester and used for future research. However, if you do not wish for these to be stored, please indicate this on the consent form and your samples will be discarded at the end of this study.

You have a number of rights under data protection law regarding your personal information. For example you can request a copy of the information we hold about you. This is known as a Subject Access Request. If you would like to know more about your different rights, please consult our privacy notice for research and if you wish to contact us about your data protection rights, please email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie Building, University of Manchester, Oxford Road, M13 9PL. at the University and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner's Office, Tel 0303 123 1113.

Will my participation in the study be confidential?

Your participation in the study will be kept confidential to the study team and those with access to your personal information as listed above. All your personal information will be encrypted and identified based upon a code number (anonymization process) to protect your identity.

Individuals from the University of Manchester, NHS Trust or regulatory authorities may need to look at your and your baby's medical records and any data collected for this study to make sure the project is being carried out as planned. This may involve looking at identifiable data but all

individuals involved in auditing and monitoring the study, will have a strict duty of confidentiality to you as a research participant.

What happens if I do not want to take part or if I change my mind?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason and without detriment to yourself. However, it will not be possible to remove your data from the project once it has been anonymised and forms part of the dataset as we will not be able to identify your specific data. This does not affect your data protection rights.

Will my data be used for future research?

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation. The future research should not be incompatible with this research project and will concern about IAQ and pregnancy outcomes. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you regarding any other matter or to affect your care. It will not be used to make decisions about future services available to you.

Will I be paid for participating in the research?

The participants in this research study will not be received any financial benefit for participation in this study.

What is the duration of the research?

Each participant may be involved in this study for up to 6 months from the date of signing the consent form to the final stage of data collection. There is no further involvement after the delivery process (or after biological samples collection).

Where will the research be conducted?

The research will be conducted in the Manchester Placenta Clinic, St. Mary's Hospital, Manchester and in the participants' residential homes across Greater Manchester.

Will the outcomes of the research be published?

The outcomes will be presented at clinical and scientific meetings and published in journals read by doctors who care for women during pregnancy and children and researchers interested in indoor air quality. You will not be identified in any of our results.

Who has reviewed the research project?

The study has been reviewed by the East Midlands – Leicester South Research Ethics Committee who have given a favourable opinion.

What if I want to make a complaint?

If you wish to make a formal complaint or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact:

The Research Governance and Integrity Manager
Research Office
Christie Building
University of Manchester
Oxford Road
Manchester, M13 9PL, United Kingdom.
Tel : +44 (0)161 275 2674
Email : research.complaints@manchester.ac.uk

Thank you for reading this information sheet and for taking the time to consider our study. If you have any questions or concerns please contact us.

MRS. NORHIDAYAH BINTI AHMAD
PhD student
Centre for Epidemiology
Division of Population Health, Health Services Research and Primary Care
School of Health Sciences
Faculty of Biology, Medicine and Health
University of Manchester
Oxford Road
Manchester M13 9PL, United Kingdom.
Tel : +44 (0)161 306 3072
Email : norhidayah.ahmad@postgrad.manchester.ac.uk

DR. ANDREW POVEY
Reader in Molecular Epidemiology
Centre for Epidemiology
Division of Population Health, Health Services Research and Primary Care
School of Health Sciences
Faculty of Biology, Medicine and Health
University of Manchester
Oxford Road
Manchester M13 9PL, United Kingdom.
Tel : +44 (0)161 275 5232
Email : andy.povey@manchester.ac.uk

APPENDIX 3: Participant consent form (PCF)

THE MANCHESTER PREGNAIRCLEAN STUDY

PARTICIPANT CONSENT FORM (PCF)

A copy of this consent form will be retained for research purposes and will be treated confidentially. If you are happy to participate in this study, please complete and sign the consent form below:

Activities:	Initials:
1. I confirm I have read the attached participant information sheet (Version 4, Date 07/11/2018) for the above study and have had the opportunity to consider the information and ask questions and had these answered satisfactorily.	
2. I understand that my participation in this study is completely voluntary and I am free to withdraw my consent at any time without giving any reason and without detriment to myself. I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis.	
3. I agree to give a blood sample for this research. I also agree to donate a sample of placenta and umbilical cord blood (after it is no longer attached to me or baby) for this research. I understand that these samples will be analyzed for markers of indoor air quality.	
4. I understand that all the biological samples will be anonymized before storage and treated as confidential at all times to protect my identity.	
5. I agree to run indoor air sampling device "DYLOS air quality monitor (DC1700)" in my home and understand this device will be located in the living room for a sampling period of 24 hours and I also understand the standard operating procedure (SOP) of the device.	
6. I agree to provide a household dust sample taken from my vacuum cleaner/hoover for the research purpose as explained to me.	
7. I understand that the sponsors of this study may make my blood sample/DNA available to other researchers for future research and that this may include researchers working abroad. I give permission for these individuals to have access to my sample, (but not any personal identifying information about me. I offer my biological samples as a gift).	

8. I understand that after completion of this study, my and my baby's biological samples that have been collected purposely for this study, may enter the Human Tissue Authority Biobank of Maternal and Fetal Health Research Centre, St. Mary's Hospital, Manchester and all of these samples may be used for the future research or for long term curating if they are deemed to be of a rare or difficult to achieve demographic.	
9. I agree that any data collected may be published in anonymous form in academic books, reports or journals.	
10. I understand that relevant sections of my and my baby's medical notes and data collected during the study may be looked at by individuals from The University of Manchester, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
11. **Optional:** I wish to receive a copy of the indoor air particles result of my home and agree that the researchers may retain my contact details in order to provide me with a summary of the findings for this study.	
12. I agree to participate in this study.	

Data Protection

The personal information we collect and use to conduct this research will be processed in accordance with data protection law as explained in the Participant Information Sheet and the Privacy Notice for Research Participants.

_____	_____	_____
Name of participant	Signature	Date
_____	_____	_____
Name of person taking consent	Signature	Date

[When completed: 1 copy for the participant; 1 copy for study site file (original), 1 copy for medical notes]

APPENDIX 4: DYLOS standard operating procedure (SOP)

The Manchester Pregnairclean Study

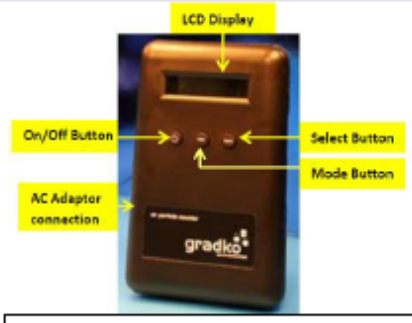
IRAS ID: 250365

DYLOS – STANDARD OPERATING PROCEDURE

THE MANCHESTER PREGNAIRCLEAN STUDY

GETTING STARTED WITH THE DYLOS IS SO SIMPLE!

- The DYLOS DC1700 is designed to measure air particles in the indoor environment.
- This device does not require an internet connection. The collected data can be downloaded later onto a computer using the company's software.
- While the DYLOS can detect levels of airborne particulates, it cannot determine the health impact for any individual.



SAMPLING POSITION

- Living room (please place the DYLOS at a height between 75 and 120 cm from the floor).
- Please avoid placing the DYLOS in front of any doors or windows (not within 2 metres).
- Do not expose the DYLOS directly to direct sunlight, fans, heaters or any air supply.

HOW TO OPERATE THE DYLOS?

- Plug the AC adaptor provided with the device into a power plug in the living room.
- Connect the AC adaptor to the DYLOS.
- Turn on the DYLOS by pressing the "On/Off button" and the device will start sampling. The DYLOS has a fan and it will produce a constant humming noise.
- No manual interaction with the DYLOS is required during its operation.
- Leave on for 24 hours and turn off by pressing the "On/Off button" again.
- If the DYLOS is turned off accidentally, please note down the time and restart it by pressing the "On/Off button"
- Record your indoor activities (smoking, cooking and windows opening) in the DYLOS sampling diary.

IMPORTANT SAFETY INSTRUCTIONS

- It is important to make sure the sampling activity will not obstruct your normal daily activities.
- Please do not open or disassemble the DYLOS. This may cause an electric shock or result in exposure to laser radiation.
- Please do not operate the DYLOS near any water sources (such as a sink or wash basin) or any source of heat.
- Please avoid spilling any liquids on the DYLOS.
- Please do not insert any objects into the openings of the DYLOS.
- Please operate the DYLOS with the provided AC adaptor only.
- Please refer to this website for more details: http://www.fijnstofmeter.com/index.html
- If you have any inquiry, please contact the researcher: Name : Mrs. Norhidayah Binti Ahmad
  Phone : +44 (0)161 306 3072
  Email : norhidayah.ahmad@postgrad.manchester.ac.uk

Version 2; Date 01/09/2018

Page 1 of 1

APPENDIX 5: Sampling diary

DYLOS – ENVIRONMENTAL SAMPLING DIARY

THE MANCHESTER PREGNAIRCLEAN STUDY

ENVIRONMENTAL SAMPLING DIARY			
Participant ID *(For researcher use only)*			
Date of Sampling :		Time Started :	
		Time Completed :	
Please specify the start and end times (if possible) for each activity in your home during the sampling period either performed by yourself or any household members.			
Smoking	(Please state your smoking area)	Start	End
Time 1	(Smoking area:_____)		
Time 2	(Smoking area:_____)		
Time 3	(Smoking area:_____)		
Time 4	(Smoking area:_____)		
Time 5	(Smoking area:_____)		
Cooking		Start	End
Time 1			
Time 2			
Time 3			
Time 4			
Time 5			
Window Opening (Living room only)		Start	End
Time 1			
Time 2			
Time 3			
Time 4			
Time 5			

FOR RESEARCHER USE ONLY

Average temperature (^{0}C) :
Average relative humidity (%) :
Estimate date for delivery :

APPENDIX 6: Questionnaire

THE MANCHESTER PREGNAIRCLEAN STUDY

PARTICIPANT QUESTIONNAIRE

- Thank you for agreeing to take part in this study which aims to investigate the feasibility of measuring indoor air quality in the residential homes of pregnant women.
- This questionnaire consists of 3 sections:

  Section 1: Demographic and socioeconomic information
  Section 2: Smoking information
  Section 3: Indoor air quality

- When answering each question, please tick the appropriate box (or boxes) or supply the requested information.
- Some of the questions may be sensitive to some people. If you do not wish to answer any particular question, please tick the 'Prefer not to answer' box.
- Please ask the researcher if you don't understand any particular question.
- This questionnaire will take approximately 15 minutes to complete.
- Once you have completed the questionnaire, please return it to the researcher during the collection of research materials session.

PARTICIPANT'S ID *(For researcher use only)*	
DATE OF COMPLETION	d d / m m / y y

SECTION 1: DEMOGRAPHIC AND SOCIOECONOMIC INFORMATION

SECTION 1 [PART A]: DEMOGRAPHIC DATA

1. Date of birth | m | m | / | y | y | y | y |

2. Height | | | | . | | | cm |

3. Weight | | | | . | | | kg |

4. Country of birth

(Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

5. What is your ethnic group?

☐ 1 = White (British, Irish, Gypsy or Irish Traveller, Other White)

☐ 2 = Mixed (White & Black Caribbean, White & Asian, White & Black African, Other Mixed)

☐ 3 = Asian/ Asian British (Indian, Pakistani, Bangladeshi, Chinese, Other Asian)

☐ 4 = Black/ African Caribbean/ Black British (African, Caribbean, Other Black)

☐ 5 = Other ethnic group (Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

SECTION 1 [PART B]: YOUR OCCUPATION

6. Which of the following describes your current situation? (You can select more than 1 answer)

☐ 1 = Unemployed **(Please proceed to question 9)**

☐ 2 = Caring for home and/or family (unpaid)

☐ 3 = Unable to work because of sickness or disability

☐ 4 = Student, further training, unpaid work experience

☐ 5 = Working for pay or profit (including paid apprenticeship/traineeship/sick leave)

☐ NN = None of the above

☐ DA = Prefer not to answer

7. If you are currently working, what is your job title?

(Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

8. In a typical week, how many hours do you spend at work?

(Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

SECTION 2: SMOKING

9. Have you ever smoked?

☐ 1 = No **(Please proceed to question 16)**

☐ 2 = Yes

☐ DA = Prefer not to answer

10. Do you smoke tobacco now?

☐ 1 = No **(Please proceed to question 14)**

☐ 2 = Yes, only occasionally

☐ 3 = Yes, on most or all days

☐ DA = Prefer not to answer

11. If yes, about how many cigarettes do you smoke on average each day?

(Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

12. What type of tobacco do you mainly smoke?

☐ 1 = Manufactured cigarettes

☐ 2 = Hand-rolled cigarettes

☐ 3 = Cigars or pipes

☐ UN = Do not know

☐ DA = Prefer not to answer

13. Do you use e-cigarettes (vaping)?

☐ 1 = No

☐ 2 = Yes, only occasionally

☐ 3 = Yes, on most or all days

☐ DA = Prefer not to answer

14. Did you smoke at the beginning of the first trimester of your current pregnancy (weeks 1 to 12)?

☐ 1 = No

☐ 2 = Yes, only occasionally

☐ 3 = Yes, on most or all days

☐ DA = Prefer not to answer

15. In the past, how often did you smoke tobacco?

☐ 1 = I have never smoked

☐ 2 = Just tried once or twice

☐ 3 = Smoked occasionally

☐ 4 = Smoked on most or all days

☐ DA = Prefer not to answer

16. Are you exposed to second hand tobacco smoke at your home?

☐ 1 = No

☐ 2 = Yes, only occasionally

☐ 3 = Yes, on most or all days

☐ DA = Prefer not to answer

17. If you, your partner or other household members are smokers, where do you smoke at home? (Please tick all that apply)

☐ 1 = Usually indoors

☐ 2 = Usually outdoors (e.g. on the balcony or in the garden)

☐ 3 = Always outdoors

☐ DA = Prefer not to answer

SECTION 3: INDOOR AIR QUALITY

18. How many years have you lived in your current home?

(Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

19. How would you describe your home?

☐ 1 = Flat/apartment

☐ 2 = Terraced

☐ 3 = Semi-detached

☐ 4 = Detached

☐ NN = None of the above

☐ DA = Prefer not to answer

20. How old is your current home? (number of years)

(Please specify :_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

21. Which area are you living in?

☐ 1 = Urban

☐ 2 = Suburban

☐ 3 = Semi rural

☐ 4 = Rural

☐ UN = Do not know

☐ DA = Prefer not to answer

22. Are there any of the following (within 200 metres) near to your home? (Please tick all that apply)

☐ 1 = Car parking

☐ 2 = Highway/motorway

☐ 3 = Busy traffic road

☐ 4 = Low traffic road

☐ 5 = Industrial area

☐ 6 = Farmland

☐ UN = Do not know

☐ NN = None of the above

☐ DA = Prefer not to answer

23. Have there been any renovations made to this home since you have been living here?

☐ 1 = No (Please proceed to question 25)

☐ 2 = Yes

☐ UN = Do not know

☐ DA = Prefer not to answer

24. If yes, what kinds of renovations have been carried out? (Please tick all that apply)

☐ 1 = Wall painting/ new wallpaper

☐ 2 = Ceiling repair/ replacement

☐ 3 = Floor repair/ polishing/ varnishing

☐ 4 = Water/ sewage system repair

☐ 5 = Window or door repair/ replacement

☐ 6 = Insulation repair/ replacement

☐ 7 = Wall construction/ removing

☐ 8 = Heating/ cooling system

☐ 9 = Building an extension to home

☐ 10 = Other (Please specify:_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

25. What is the floor type of your living room?

☐ 1 = Wood

☐ 2 = Concrete

☐ 3 = Stone/tile

☐ 4 = Linoleum

☐ 5 = Other (Please specify:_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

26. What is the main wall type of your home?

☐ 1 = Wallpaper

☐ 2 = Paint

☐ 3 = Wood/sealed cork

☐ 4 = Stone/tile

☐ 5 = Exposed concrete

☐ 6 = Plaster

☐ 7 = Other (Please specify: _____)

☐ UN = Do not know

☐ DA = Prefer not to answer

27. What is the main heating system in your home?

☐ 1 = Central heating with radiators

☐ 2 = Underfloor heating

☐ 3 = Wood burning stoves or fireplaces

☐ 4 = Other (Please specify: _____)

☐ UN = Do not know

☐ DA = Prefer not to answer

28. How is your home ventilated?

☐ 1 = Natural ventilation (windows/doors)

☐ 2 = Mechanical ventilation (assisted with fan)

☐ UN = Do not know

☐ DA = Prefer not to answer

29. How often do you have your living room windows open?

☐ 1 = Almost never

☐ 2 = Occasionally

☐ 3 = Only when weather is good

☐ 4 = Almost always open

☐ DA = Prefer not to answer

30. What type of glazing do you have in your living room?

☐ 1 = Single

☐ 2 = Double

☐ 3 = Other (Please specify:_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

31. Do your living room windows have any coverings such as curtains or blinds?

☐ 1 = No

☐ 2 = Yes

☐ DA = Prefer not to answer

32. Are you currently having any problems with home water leaks (e.g. plumbing, faucets or pipes leaking)?

☐ 1 = No

☐ 2 = Yes

☐ UN = Do not know

☐ DA = Prefer not to answer

33. Do you have any problems with damp in your home?

☐ 1 = No

☐ 2 = Yes

☐ UN = Do not know

☐ DA = Prefer not to answer

34. Do you have any problems with visible moulds in your home?

☐ 1 = No

☐ 2 = Yes

☐ UN = Do not know

☐ DA = Prefer not to answer

35. How often is the floor of your living room cleaned?

☐ 1 = Never (**Please proceed to question 37**)

☐ 2 = At least once a YEAR

☐ 3 = At least once every FEW MONTHS

☐ 4 = At least once a MONTH

☐ 5 = At least once a WEEK

☐ UN = Do not know

☐ DA = Prefer not to answer

36. How is your living room cleaned? (Please tick all that apply)

☐ 1 = Vacuum/Hoover

☐ 2 = Wet washing with a mop

☐ 3 = Dry sweeping with a cloth

☐ 4 = Dry sweeping with a broom

☐ 5 = Other (Please specify:_____)

☐ UN = Do not know

☐ DA = Prefer not to answer

37. Which best describes your family's habit regarding wearing shoes in the home?

☐ 1 = Shoes are taken off prior to entering the home

☐ 2 = Shoes are taken off right away after entering the home

☐ 3 = Shoes are taken off prior to entering certain rooms

☐ 4 = Shoes are not routinely taken off while in the home

☐ UN = Do not know

☐ DA = Prefer not to answer

38. What is your kitchen type?

☐ 1 = Indoor kitchen with partition (closed floor plan)

☐ 2 = Indoor kitchen without partition (open floor plan)

☐ 3 = Other (Please specify:_____)

☐ DA = Prefer not to answer

39. What kind of stove do you use in cooking? (Please tick all that apply)

☐ 1 = Electric

☐ 2 = Gas

☐ 3 = Other (Please specify:_____)

☐ DA = Prefer not to answer

40. Do you have a ventilation hood above the stove?

☐ 1 = No (Please proceed to question 42)

☐ 2 = Yes

☐ DA = Prefer not to answer

41. How often do you use the ventilation hood when cooking?

☐ 1 = Never/seldom

☐ 2 = Every now and then

☐ 3 = Regularly

☐ DA = Prefer not to answer

42. Do you currently have any indoor pets/animals?

☐ 1 = No

☐ 2 = Yes

☐ DA = Prefer not to answer

43. If yes, what type of pets do you have and how many of them do you have? (Please tick all that apply)

☐ 1 = Cats (Number:_____)

☐ 2 = Dogs (Number:_____)

☐ 3 = Rabbits (Number:_____)

☐ 4 = Rodents (Number:_____)

☐ 5 = Birds (Number:_____)

☐ 6 = Other (Please specify:_____) (Number:_____)

☐ DA = Prefer not to answer

You have now completed all the questions. Thank you very much for your time and participation in this study. It is highly appreciated.

If you have any further inquiries, please contact the researcher named below for more information.

Mrs. Norhidayah Binti Ahmad (PhD Student)
Centre for Epidemiology
Division of Population Health, Health Services Research and Primary Care
School of Health Sciences
Faculty of Biology, Medicine and Health
University of Manchester
Oxford Road
Manchester M13 9PL, United Kingdom.
Tel : +44 (0)161 306 3072
Email : norhidayah.ahmad@postgrad.manchester.ac.uk

APPENDIX 7: Temporal variation of $PM_{2.5}$ in pregnant women residential homes