



UNIVERSITY OF
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**PERSONAL AND INDOOR EXPOSURE TO
NANOPARTICLES AND ITS RELATIONSHIP TO
BIOLOGICAL MARKERS**

By

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ABSTRACT

Exposure to particulate matter (PM) and its components is associated with negative health outcomes and it is hypothesised that the channel through which these effects are expressed is via the mechanism of inflammation and oxidative stress. In order to assess the relationship between exposure and health related outcomes in different personal and indoor exposure environments, the association between exposure to PM/PM components and changes in local/systemic inflammation and oxidative stress (EBC-nitrate, EBC-nitrite, EBC-pH and urinary 8-oxodG), alterations in autonomic and lung functions respectively were determined using Spearman correlation. The acute exposures showed significant increases in EBC nitrate and nitrite immediately after exposure. While EBC-pH and urinary 8-oxodG, did not significantly change in post exposure measurements, some significant correlations were observed between the pollutants measured and the biomarkers in the different exposure scenarios. For example, in one of the exposure scenarios in this study- exposure to Japanese style cooking “Teppanyaki” fumes; there was a significant correlation between the UFP concentration measured during the exposure to Japanese style cooking “Teppanyaki” fumes and the 8-oxodG. In the acute exposure, a decrease in the ratio of EBC-nitrate/nitrite was observed with the presence of localised oxidative stress of the airways. Busy road side exposure, showed that EBC-Nitrate significantly correlated with UFP, while EBC-Nitrite, EBC-pH, 8-oxodG and FEV6 correlated with inhaled load estimate. In the comparison between personal exposure and reference fixed site measurements, using personal sensors, EBC-nitrite was significantly correlated with the fixed site BC measurement for the daily exposure, while EBC-nitrite was significantly correlated with particle size measured during cooking and BC personal sensor measurement during indoor Others. In the indoor particle characterisation, 8-oxodG was significantly correlated with the < 250 nm size fraction measurements and various PM bound metals were also correlated with the biomarkers

measured. Urinary metals were also analysed and compared with biomarkers and PM/PM components. PM bound Cu significantly correlated with its urinary counterpart Cu. Urinary Mn significantly correlated with EBC-pH. Indoor (homes) PM were characterised and it showed that in the mass-size distribution, indoor PM prevailed in the 250 – 500 nm and the 2500 – 5000 nm size range. The elements Na, Fe, Mn, Ca and Mg were prevalent in the coarse mode (2500 – 5000 nm) and toxic metals such as Mn, Cu, V, Ni, Pb and Sn were measured in the homes of the subjects. Si, S, K and Fe were the most prevalent elements observed in the particles collected on > 250 nm size fraction impactor plate and analysed with TEM/EDS. During commuter modes, lung function parameters FEV₁, FEV₆, FEV₁/FEV₆ ratio and FEF_{25-75%} decreased during the 5 days of the cycling mode. An inverse significant correlation was observed between PM_{2.5} and FEV₁ and FEV₆ respectively during the cycling mode. In the train mode, only FEV₁ and FEV₆ decreased during the 6 days of train travel. The increases observed in post exposure measurements and significant correlations observed between the markers of exposure and the measured PM and PM bound components measured in the different environments, alterations in lung function, suggests that exposure to PM and PM bound components have the ability to cause inflammation/ oxidative stress thereby affecting human health. Also, this study shows that biomarker expression after exposure can be used to measure acute responses.

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ABBREVIATIONS

8-OHdG	8-hydroxydeoxyguanosine
8-OHGua	8-hydroxyguanine
8-oxodG	8-oxo-7,8- dihydro-2'-deoxyguanosine
Al	Aluminium
Bal	Bronchoalveolar lavage
BC	Black Carbon
BP	Blood pressure
Ca	Calcium
CNTs	Carbon nanotubes
COD	Coefficient of divergence
CPC	condensation particle counter
Cr	Chromium
Cu	Copper
CV	Coefficient of variance
DE	Diesel exhaust
DEHS	Di-Ethyl-Hexyl-Sebacate
DM	DiSCmini
DMA	Differential mobility analyser
DNA	Deoxyribonucleic acid
eNO	exhaled Nitric Oxide
EBC	Exhaled breath condensate
EBC-NO ₂ ⁻	Exhaled breath condensate nitrite
EBC-NO ₃ ⁻	Exhaled breath condensate nitrate

EC	Elemental carbon
EDS	Energy dispersive spectroscopy
ETS	Environmental tobacco smoke
FDMS-TEOM	Filter Dynamics Measurement Systems-Tapered element oscillating microbalances
Fe	Iron
FEF ₂₅₋₇₅	Forced expiratory low
FeNO	Fractional exhale nitric oxide
FEV ₁	Forced expiratory volume in 1 second
FEV ₆	Forced expiratory volume in 6 second
FSs	Fixed sites
FVC	Forced vital capacity
GFAAS	Graphite furnace atomic absorption spectroscopy
hOGG1	8-oxoguanine DNA glycosylase
ICP MS	Inductively coupled plasma mass spectrometry
iNOS	Inducible nitric oxide synthase
K	potassium
LOD	Limit of detection
MEs	Microenvironments
Mg	Magnesium
MicroAeth	MicroAethalometer
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
Nm	Nanometer

NO	Nitric Oxide
NO ₂	Nitrogen dioxide
NPs	Nanoparticles
OLS	Ordinary least square
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead
PE	Personal exposure
PM	Particulate matter
PM ₁₀	Particulate matter with aerodynamic diameter size ≤ 10
PM _{2.5}	Particulate matter with aerodynamic diameter size ≤ 2.5
PNC	Particle number concentration
ppb	Parts per billion
ppm	Parts per million
PSs	Personal sensors
RFSs	Reference fixed sensors
RMA	Reduced major axis
ROS	Reactive Nitrogen species
RON	Reactive oxygen species
Sb	Antimony
SMPS	Scanning Mobility Particle Sizer
Sn	Tin
Sr	strontium
TEM	Transmission electron microscopy
UFP	Ultrafine particle
USEPA	United States Environmental Protection Agency

V	Vanadium
VOC	Volatile organic compounds
WHO	World Health Organisation
Zn	Zinc

1 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Various studies have concluded that exposure to air pollution especially the particles in their ultrafine state pose a major threat to health because not only do they have the ability to induce inflammation and oxidative stress, they are associated with high risk of respiratory, lungs, cardiovascular and other diseases (Buonanno et al., 2013). In addition to the size of these particles, their surface area and chemical composition play a role in their cumulative toxicity (Weichenthal et al., 2006). The World Health Organisation (WHO) estimates seven million deaths were attributable to the joint effects of household and ambient air pollution in 2012 (WHO, 2014). Researches involved in toxicological effects of exposure to air pollution are still in progress. In fact Researchers are still working on quantitative data to confirm these associations especially because of the ubiquitous nature of these pollutants (especially Particulate Matter (PM)) people are possibly exposed on a daily basis.

Many research studies on particulate exposure have focused on the outdoor environment, neglecting the indoors where majority of people spend a vast proportion of their time (Hollowell, 2011). People spend about 85 -90 % of their time indoors (Lazaridis, 2011, Höppe and Martinac, 1998) which includes their homes, work places, leisure and transport (Santos *et al.*, 2011). Unfortunately there is limited knowledge of airborne particles, limited information on the association between the concentrations of indoor particles, exposure, doses and health effects (Franck *et al.*, 2011, Gwinn and Vallyathan, 2006). The United States Environmental Protection Agency (USEPA) stated that “the concentrations of some pollutants indoors are often 2 to 5 times higher than typical outdoor concentrations”. This

may be because the concentration of pollutants can be intense when trapped indoors or within a closed areas/structure. Therefore people who are often most susceptible to the adverse effects of air pollution (e.g., children, elderly, people with cardiovascular or respiratory disease) tend to spend even more time indoors are therefore even more exposed to the effects of these particles.

Thus more researchers are now focused on measuring personal exposure (PE) in the indoor environment because, It is now widely recognized that a significant proportion of PE to particles occurs in the indoor environment (Vinzents et al., 2005), particles of indoor sources may be more bioactive than the particles of ambient origin (Zhang and Gutterman, 2007) and people spend a greater amount of time indoors as compared to the outdoors. Therefore to get an accurate PE profile, the indoor concentrations has to be included in the measurements (Weichenthal et al., 2006).

Most PE studies are usually based on measurements of pollutant concentrations in the ambient air, measured with outdoor single fixed-site monitors or monitoring stations in a city. However, PE can be measured with personal sensors (PS) (Vinzents et al., 2005). With the use of the PS, a more individual pollutant concentration experienced by a person during activities and or in a particular environment can be made (Gietl et al., 2010). Thus short term acute sporadic changes of air pollutants in the breathing zone of an individual can be accounted.

In recent times, the use of biomarkers has been employed in order to enhance research on the health effects of air pollution. The measurement of biomarkers in relation to air pollution, are used for increasing the understanding of mechanisms of health effect exposure outcome,

investigation of individual susceptibility to exposure and the use of changes in concentration levels of biomarkers of exposure can help relate exposure to PM to biological and health effects. In addition to biomarkers, another way of assessing health effect of exposure is the use of health risk assessment method. This method, estimates the occurrence of adverse health effects resulting from the inhalation of PM_{2.5} (particles with aerodynamic diameter size ≤ 2.5) particles that are emitted from indoor and outdoor sources (Lombardi-Boccia et al., 2005).

This study proposes to measure PE pollutant concentrations to ultrafine particle (UFP), PM_{2.5}, black carbon (BC) and Nitrogen dioxide (NO₂) in different exposure scenarios, determine mass concentration and elemental composition of five different particle size fractions and characteristics of particles in the indoors, measure biomarkers of exposure and health risk associated with these pollutants in exposed asymptomatic individuals.

1.1.1 Content and structure of thesis

Figure 1.1 shows the schematic representation of the thesis outline.

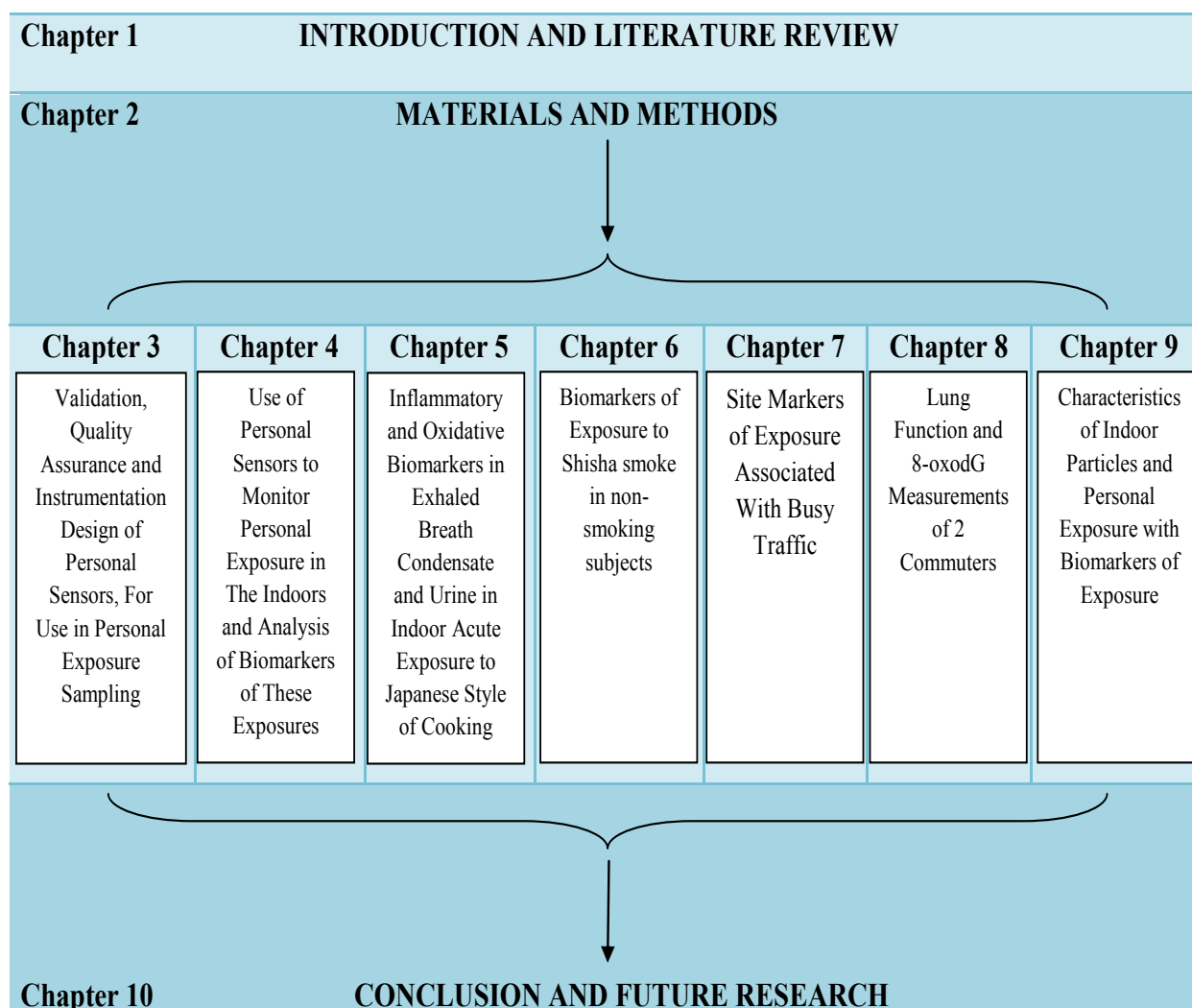


Figure 1.1 Schematic representation of the thesis outline

Based on the different projects in this study as outlined in Figure 1.1, the hypothesises of this study are

- 1) The PSs are suitable for indoor and PE measurement
- 2) Indoor and PE to air pollutants can induce inflammatory responses
- 3) Elevated pollutant exposure concentrations can induce airway inflammation and systemic oxidative stress, alter pulmonary and autonomic functions.

- 4) Indoor and PE to air pollution can augment metal concentrations in urine therefore urine in metals can be used as biomarkers of exposure to air particulates
- 5) Markers related to cardiovascular diseases pathophysiological pathways are sensitive to changes in the concentrations of air pollutants.
- 6) There are health risk associated with trace metals Cr, Ni and Pb in the particle size fractions measured in indoors (homes)

1.1.2 Nanoparticles and sources

These nano-scaled particles known as nanoparticles (NPs) are defined as, particles with a diameter $< 100\text{ nm}$ ” (Masciangioli and Zhang, 2003, Auffan et al., 2009) and have properties that are not Shared by their non-nano-scale or bulk samples with the same chemicals composition (Handy et al., 2008, Auffan et al., 2009). NPs is sometimes referred to as UFP, they frequently have been used interchangeably with “Nano” seen as a more recent nomenclature (Warheit et al., 2008). In this work NPs and UFPs are used interchangeably.

These NPs are formed through nucleation, gas to particle reaction or evaporation or during incomplete fuel combustion (Brouwer et al., 2004, Li et al., 2003). NPs are being generated continuously and in large numbers and different concentrations by both natural and anthropogenic sources in the urban and rural areas.

1.1.2.1 Natural sources of nanoparticles

NPs are abundant in nature because they exist in natural systems such as the soil, the water bodies, and in the atmosphere (MacMicking et al., 1997, Tsao et al., 2011, Dhawan and Sharma, 2010) and are produced during many natural processes such as photochemical reactions, volcanic eruptions, forest fires, erosions plants and animals for example when animals shed their skin and hair (Buzea Cristina et al. 2007).

1.1.2.2 Anthropogenic sources

NPs are produced as a result of human impact on nature which can be incidental or intentional (Dhawan and Sharma, 2010). Most of the time they are produced during combustion and or are produced intentionally when NPs are engineered/manufactured (Nowack and Bucheli, 2007). These sources include: transportation vehicle exhaust emission, within saturated vapour emission from processes such as welding, soldering, laser ablation, generated during mechanical process such as grinding, cutting, polishing (Bradford et al., 2009), cooking, heating, cigarette smoking (Hollowell, 2011) accidental discharge of engineered NPs (Nowack and Bucheli, 2007) and other processes.

1.2 Characteristics of nanoparitics associated with toxicity

NPs show their unique properties with reduction in size and these unique properties are because of the increases fraction of the surface atom associated with size reduction (Gubin et al., 2005). Therefore it is reasonable to assume that when exposed to these nano-scaled particles, that the biological effect /response will differ from those of their bulk (Warheit et al., 2008) because for a given particle type, as one moves down the nano-scale (i.e., as the particle size is decreased within the nano-scale range), basic physical and chemical properties appear to change often yielding completely new and different physical/chemical properties (Warheit, 2008). These characteristics of these nano-materials influence their fate and behaviour in the environment and their potential to induce toxicity in human and different environmental receptors (Stone et al., 2010) as there is a strong indication that their activity in a biological medium or environmental receptor will depend on their physicochemical properties and this is not usually the case for toxicity screening studies of bulk particles that mostly rely on dose (Oberdörster et al., 2005a). For example based on their size and chemical property, exposure to these particles through inhalation can be toxic as it has the ability to

deposited in the deep lung regions and maybe pass into the blood stream (Marra et al., 2010). Therefore in order to understand the potential toxic effect of NPs, it is important that these particles are characterised.

1.2.1 Size

The size of NPs may be the most important property for characterisation of a particle (Dhawan and Sharma, 2010) and its (size) is responsible for causing the deviation in a nano-material's behaviour from that of its bulk particle (Gavankar et al., 2012). For example, a study by Renwick et al. (2004) showed that ultrafine BC and titanium dioxide (TiO₂) particles administered to rats, induced inflammation and epithelial damages to a greater extent than their bulk particle counterpart (Renwick et al., 2004). Therefore size is important for determining the interactions of NPs in living cells (Dhawan and Sharma, 2010). In the sense that it modifies the physicochemical properties of the material as well as creates the opportunity for increased uptake and interaction with biological tissues (Nel et al., 2006). NPs, because of their size appear able to be inhaled deeply into the lungs, i.e. inhaled into the alveolar region (Choi et al., 2010); can be internalized by type II lung epithelium cells (Yang and Watts, 2005), causing lung injury (i.e. small size particle, a large surface area, and an ability to generate reactive oxygen species (ROS) thereby induce lung injury (Oberdörster et al., 2005a, Nel et al., 2006)) and affect extra-pulmonary organs (Bakand et al., 2012). These NPs, because of their size have higher deposition efficiency in the lower respiratory tract and slower clearance rates (Yang and Watts, 2005), they can diffuse through cell membranes and other biological barriers to gain access to the intra-nuclear area (Elsaesser and Howard, 2012) and translocation to the bloodstream, brain and other organs via the lungs and other entry routes (Gwinn and Vallyathan, 2006). Choi et al. (2010) in an in vivo experiment suggested that noncationic NPs with hydrodynamic diameter ≤ 34 nm are potentially the most dangerous

in that they rapidly traverse the epithelial barrier, enter regional lymph nodes, and, if ≥ 6 nm, are not cleared efficiently by the kidneys. Conversely, the potential of NPs to cross barriers can be exploited for drug delivery and pharmaceutical applications (Nyström and Fadeel, Gwinn and Vallyathan, 2006) but their deposition in the lung is still yet to be properly understood (Choi et al., 2010). Also based on the size of the particle, Elder et al. (2009) in their study, translocation of inhaled ultrafine manganese oxide particle to the central nervous system (CNS), concluded that olfactory neuronal pathway represents a significant exposure route of CNS tissue to inhaled solid Mn oxide UFPs and that in rats, translocation of inhaled nano-sized particles along neurons seems to be a more efficient pathway to the CNS than via the blood circulation across the blood–brain barrier (Elder et al., 2006). Even though size may be the most important factor for characterisation of NPs, size in combination with other factors and properties may influence their (NPs) interaction with biological and environmental systems and medium (Boverhof and David, 2010).

1.2.2 Particle Surface area

With a reduction in size of the particle to a nano-scale, large portion of the atoms or molecule become available on the surface of the particle when compared to their bulk counterpart (Gavankar et al., 2012, Warheit et al., 2008, Roco, 1999) thereby making chemical reactivity to increase rapidly and thus generating more effective catalysts in a variety of applications (Warheit et al., 2008, Warheit, 2008). This is an important factor in NPs health related toxicity because the interaction between the biological system and the NPs take place on the surface of the particle (Dhawan and Sharma, 2010). From a health implications perspective, reactive groups on a particle surface are likely to modify the biological (potentially toxicological) effects (Warheit, 2008). Thus the reactive group on the surface of the particle will interfere or influence biological response when compared with nonreactive groups

(Warheit et al., 2008); they may interfere or influence cellular metabolism by catalyzing specific reactions within the cytosol (Limbach et al., 2007). This therefore may be the reason why they are more toxic than their bulk counterpart; where a catalytically active material may repeat its chemical interaction with the host over and over again, a toxic bulk particle (agent) will depend on a dose- and mass-related effect on the corresponding organism (Limbach et al., 2007).

1.2.3 Chemical composition and surface characteristic

The chemical composition of a NP may affect its toxicity as well as the particle may be modified by any substance that absorbs or binds onto its surface. NPs of the same chemical composition but different intrinsic and extrinsic factor may show different behaviour and different environmental and health effects (Gavankar et al., 2012, Zhang, 1997). For example the different behaviour exhibited by fullerenes and carbon nanotubes (CNTs) in the environment despite their identical chemical composition (Klaine et al., 2008). Also the extent of change to the properties of the particle as it moves down to the nano-scale is strongly dependent on the chemical composition of the particle (Nel et al., 2006). That means that the surface composition can be hydrophilic or hydrophobic, lipophilic or lipophobic, or catalytically active or passive (Nel et al., 2006) depending on if the NP is metal/metal oxides, organics, compounds, polymers as well as biomolecules or perhaps a mixture/complex mixture of one or more of the before mentioned (Oberdörster et al., 2005a). Depending on the chemistry of the particle, the surface group can act as reactive sites thereby modifying biological effects (Oberdörster et al., 2005a, Nel et al., 2006). For example where surface properties can lead to toxicity is the interaction of electron donor sites with molecular dioxygen (O_2) leading to the formation of the superoxide radical ($O_2^{\cdot-}$), which through dismutation/Fenton chemistry can generate additional ROS (Nel et al., 2006).

1.2.4 Shape/Morphology

Nano-scaled materials can be spherical, tubular, cubes, pyramids or irregular in shape including aspect ratio where necessary (high-aspect ratio and low-aspect ratio) e.t.c. (Zhang, 1997, Buseck and Adachi, 2008, Oberdörster et al., 2005a). Following the fact or perhaps proposition that reduction of particle size to the nano-scale affects the characteristics of the particle, the shape may affect the characteristics of the particle because the shape or morphology may affect the fate and transportation of the particle causing the particle to behave differently in terms of kinetics of dissolution in the body/solubility, stability, absorption in the body e.t.c. (Gavankar et al., 2012, Maynard et al., 2006, Buseck and Adachi, 2008, Oberdörster et al., 2005a). For example Guang et al. (2005), in cytotoxicity study using different carbon materials: Single-wall nanotube, Multi-wall nanotube and C₆₀, concluded that nano-materials with different geometric structure (shape) exhibit different cytotoxicity and bioactivity. Also Jia et al. (2005), confirmed that nano-materials with different geometric structure (shape) exhibit different cytotoxicity and bioactivity in-vitro but this may not be reflected properly in-vivo cases (Jia et al., 2005). The shape of a NP can contribute to toxicity unrelated to its composition; for example, fibre like CNTs, with aspect ratios of 100 or more, have properties and hazards that seem similar to asbestos fibres (Borak, 2009).

However, much work is still needed to advance knowledge in the area of physicochemical characterisation of nano-materials because this will help understand the fate and behaviour of NPs in the environment and their potential to induce toxicity in human and different environmental receptors (Stone et al., 2010, Gubin et al., 2005).

1.3 Methods of characterising nanoparticles

Recent studies have suggested that to assess the potential toxicity of a NP in a biological milieu, a complete and accurate characterisation is essential (Powers et al., 2006, Boverhof and David, 2010, Dhawan and Sharma, 2010), to enable proper understanding of these nano-scale materials both within biological milieu and outside for scientist or researchers to properly assign mechanism and or causation of toxic effect of these materials (Powers et al., 2006).

There are different methods available for determining the size, shape and surface chemistry of NPs and these methods have been used by various researchers. Some of them are; dynamic light scattering (DLS) (Kato et al., 2009), Brunauer, Emmett and Teller (BET) (Baalousha et al., 2012), scanning electron microscopy (SEM) (Babiuch et al., 2012), small-angle X-ray angling (SAXS) (Goyal and Aswal, 2006), small angle neutron scattering (SANS) (Goyal and Aswal, 2006), wide-angle X-ray angling (WAXS), atomic force microscopy (AFM) (Baer et al., 2010), X-ray diffraction (XRD) (Šlouf et al., 2006), energy dispersive spectroscopy (EDS) and transmission electron microscopy (TEM) (Bello et al., 2012). Each method provides a reliable answer however they each have different principle, different merits and demerits and they provide slightly different outcome in terms of size distributions and averages (Baalousha et al., 2012). For example according to Baalousha et al. (2012), AFM, TEM, DLS, BET and XRD will give the same size measurement only for stable, monodisperse, single crystal and hard sphere but any deviation from the above mentioned criteria will result in differences in the measured sizes (Baalousha et al., 2012).

1.4 Other pollutants of interest

1.4.1 Black carbon and Elemental carbon

Many research studies often interchange the use of BC and elemental carbon (EC) but they are not the same thing. However they have both often been used as equivalent (Ballatori, 1994). In fact they are typically defined operationally by their measurement protocols (Kulshrestha et al., 2014). BC concentration can be measured by quantifying visible light transmitted through a filter while EC is measured by the thermal-optical method i.e. through sequential volatilisation of organic and elemental carbon (McNabola et al., 2008). However BC is produced by incomplete combustion of carbon containing fuels; coal, diesel engines, biofuels, and outdoor biomass burning that dominates particulate light absorption (Novakov et al., 2000, McNabola et al., 2008, Kulshrestha et al., 2014). EC represents thermally refractory carbon with a graphitic structure measured by oxidation and detection of the evolved gas (Kulshrestha et al., 2014). A major source of EC is the emissions from diesel heavy trucks. They both come from incomplete combustion of carbonaceous fuel (Kulshrestha et al., 2014). BC a marker of combustion PM is a valuable additional air quality indicator to evaluate the health risks because combustion-related particles are thought to be more harmful to health than PM that is not generated by combustion (Delgado-Saborit, 2012, Janssen et al., 2011, Katsouyanni et al., 2001). Generally BC and EC on their own are considered inert, but the combustion process from which BC is generated, results in the BC being coated by organic matter such as nitroso compounds and particle bound polycyclic aromatic hydrocarbons (PAH) semi volatile organic compounds and other compounds (McNabola et al., 2008, Ballatori, 1994). Ultrafine BC particles can be inhaled deep into the lungs (McNabola et al., 2008) and they have a greater potential of causing inflammation (Delfino et al., 2011, Briggs et al., 2008, Thompson et al., 1999, Grass et al., 2010). Delfino

et al. (2008) found significant positive associations for the biomarkers C-reactive protein (CRP), interleukin-6 (IL-6), TNF- α soluble receptor II (sTNF-RII), and soluble platelet selectin (sP-selectin) with outdoor and/or indoor concentrations of quasi-ultrafine PM ≤ 0.25 μm with EC, and BC amongst other pollutants from the current-day and multiday averages.

1.4.2 Particulate matter and Particulate Matter 2.5 (PM_{2.5})

PM is a heterogeneous mixture of many various substances with size ranging from a few nanometres to tens of micrometres. Toxicological studies suggest that it is the very small particles that may be responsible for the cause adverse health effects. PM_{2.5} is defined as PM with an aerodynamic diameter of < 2.5 μm (Chuang et al., 2007). PM_{2.5} can be generated from combustion and non combustion sources (Jarjour et al., 2013). Studies have shown association between exposure to PM_{2.5} and health related effects. PE to PM_{2.5} was associated with an increase in 8-oxodG in lymphocytes in humans (Barceloux and Barceloux, 1999). Acute exposure to low doses of PM_{2.5} can induce lung inflammation, oxidative stress and worsened lung impedance and histology in a dose-dependent pattern in mice (Sutcliffe et al., 2012). The health effects of PM_{2.5} are associated with the cardiopulmonary system (Mutlu et al., 2001).

1.5 Measuring biological responses to particulate matter exposure

From various studies conducted it is known that chemicals in their nano-size form may possess properties that may differ from their bulk form thus their interaction in the biological system may be different from their larger physical form (Nel et al., 2006). Therefore it is important to measure biological responses to NP exposure to enable development and validation of risk assessment.

1.5.1 The use of biomarkers

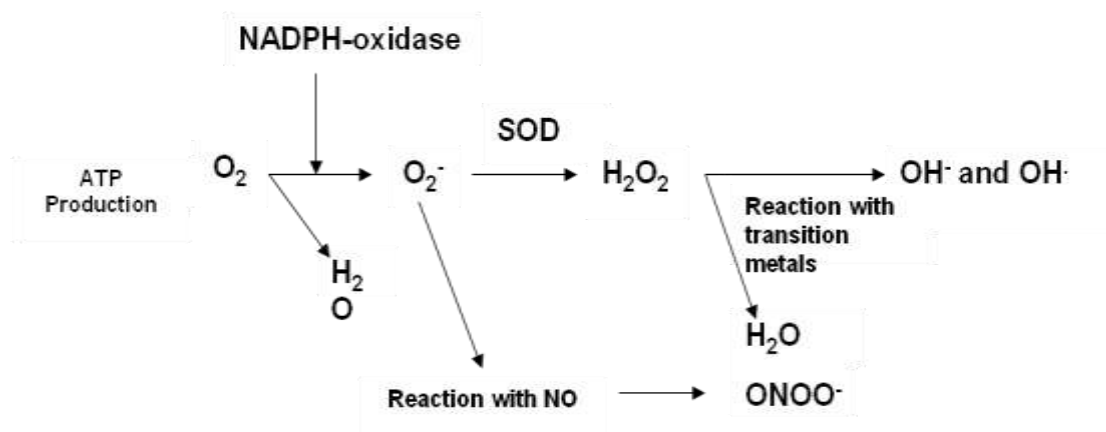
Biological markers (biomarkers) are indicators of signalling events in biological systems or samples i.e. they are “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids” (The Committee on Biological Markers of the National Research, 1987, Hulka, 1991). Biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Mateos and Bravo, 2007). The employment of biomarkers by epidemiologists, physicians, and scientists to study human disease include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease (Mayeux, 2004). Biomarkers can be used to measure exposure and have the ability to provide information on the absorption and metabolism of the exposure (Mayeux, 2004). Therefore products of oxidative stress, inflammation, or tissue damage can be used as biomarkers for early indication of adverse effects of PM exposure (Nel, 2005).

1.6 Biological production of reactive oxygen species

ROS is a collective term for both free radicals (any chemical species that contain unpaired electron (Wickens, 2001)) associated with the oxygen atom and some non-radical oxidizing agents (Toyokuni, 1999, Bayr, 2005). However, these ROS have some beneficial effects such as: at low concentrations they are involved in the defence against microbial pathogens (Montuschi et al., 2007). It is an important feature of many acute and chronic diseases and normal aging process (Montuschi et al., 2007).

1.6.1 Endogenous sources of reactive oxygen species production

ROS production occurs mainly in the mitochondria during the electron transport chain (ETC) also known as the respiratory chain process and then in the cell membranes (peroxidation reactions (Wickens, 2001)), phagosomes and the endoplasmic reticulum (Zhang and Gutterman, 2007, Ghio et al., 2011, Schröder and Krutmann, 2005). During normal cellular respiration in the mitochondria (Figure 1.1), there is a stepwise release of energy adenosine triphosphate (ATP) during which molecular oxygen (O_2) is reduced to produce water and reactive species such as superoxide (O_2^-) by NADPH-oxidase (Khansari et al., 2009, Wickens, 2001). SOD converts O_2^- to hydrogen peroxide (H_2O_2) and then H_2O_2 further reacts with the transition metals to form hydroxyl radical ($OH^\cdot$) are formed (Khansari et al., 2009, Wickens, 2001). These free radicals are toxic to the cells, however are tightly regulated in the cells by antioxidants (Wickens, 2001, Martins et al., 2016). Also hydrogen peroxide is toxic to the cells (Wickens, 2001).



NADPH-oxidase Nicotine adenine diphosphate oxidase, SOD-Superoxide dismutase, NO-Nitric oxide, $ONOO^-$ Peroxynitrite

Figure 1.2. Mitochondria ROS production during ETC illustration

Additional endogenous sources of cellular ROS are the neutrophils, eosinophils and macrophages (Khansari et al., 2009) which make up the innate immune system and are known as phagocytic leukocytes (Conner and Grisham, 1996). The immune system can be divided into the innate immune system (possessed by all kinds of multi-cellular organisms) and the acquired immunity (possessed by only vertebrates) (Sitzmann et al., 1999). In the innate immunity, the macrophages, neutrophils, dendritic cells and other phagocytic cells are important because they are involved in the recognition and response process (phagocytises) of foreign compounds (Sitzmann et al., 1999). During this antimicrobial process ROS can be generated in low concentrations or over production leading to high concentrations which can damage and abate normal lipid, protein and DNA function (Sitzmann et al., 1999, Bogdan et al., 2000, Khansari et al., 2009).

1.6.2 Pathogenic sources of ROS production

Chronic inflammatory conditions can increase the production of ROS/RNS (reactive nitrogen specie) and may subsequently increase the risk of cancer (Khansari et al., 2009, Risom et al., 2005). Metabolism of xenobiotics, aging and activation of inflammatory cells are also capable of generating ROS and RNS (Risom et al., 2005). That is, these inflammatory and cancer cells themselves produce free radicals and mediators such as metabolites of arachidonic acid, chemokines, angiogenic factors and cytokines, which act by further producing reactive species. (Federico et al., 2007, Khansari et al., 2009).

1.6.3 Exogenous sources of ROS production

In addition to the production of ROS by endogenous sources and pathogenic sources, free radicals can occur by environmental sources there by leading to oxidative stress (Schröder and Krutmann, 2005, Khansari et al., 2009). These sources include: pollution e.g. exposure to PM especially UFP, ozone, transitional metal, ultraviolet light, ionisation radiation, infrared

radiation and nutrition (Khansari et al., 2009, Lodovici and Bigagli, 2011, Ghio et al., 2011, Schröder and Krutmann, 2005). All these sources of free radicals and other reactive species have the ability to cause oxidative damage to different biomolecules when the oxidant species exceed the antioxidant defences in the body thereby causing oxidative stress (Mateos and Bravo, 2007).

The interest in this work is the production of ROS/RNS by NP/UFP which are the smaller size PM. Therefore, the harmful effect and biological damage caused by ROS is termed oxidative stress (Khansari et al., 2009). Oxidative stress is caused by response to an imbalance between exposure to excessive levels of free radicals and decreased antioxidant activity (ability to detoxify or repair damage) (Montuschi et al., 2007, Lodovici and Bigagli, 2011) or a biochemical imbalance where production of ROS exceeds the natural antioxidant capacity (Delfino et al., 2011). This oxidative stress may lead to the damage of the DNA, protein, lipids, disturbance of multiple signalling pathways, activation of cellular defence, aging, inflammation and cell death pathways (Lodovici and Bigagli, 2011, Montuschi et al., 2007).

1.6.3.1 Mechanism of oxidative stress induced by exposure to air pollution particles

PMs are known to be the primary cause of toxicity in the respiratory and cardiopulmonary system (Delfino et al., 2011). These PM have bound to them, a complex mixture of toxic and carcinogenic compounds, which when they gain access into the lung's alveolar region may cause oxidative stress (Delfino et al., 2011). It has been suggested that inflammation and oxidative stress appears to be an important pathway via which these health effects may occur (Ricciardolo et al., 2004b, Laumbach and Kipen, 2010, Lin et al., 2011). That is, inhaled pollutants can react fast with extracellular macromolecules or cell constituents in the airway

epithelium to generate ROS/RNS thereby inducing local and systemic oxidative or nitrosative stress and subsequent inflammation (McCafferty et al., 2004).

The oxidative stress caused by particulates may arise from;

Metals bound to PM are said to be toxic and can induce inflammation and oxidative stress in exposed subjects (Risom et al., 2005). They can induce inflammation and oxidative stress either as solid redox active particles or can solubilise (White et al., 2012, Lai et al., 2013). a)

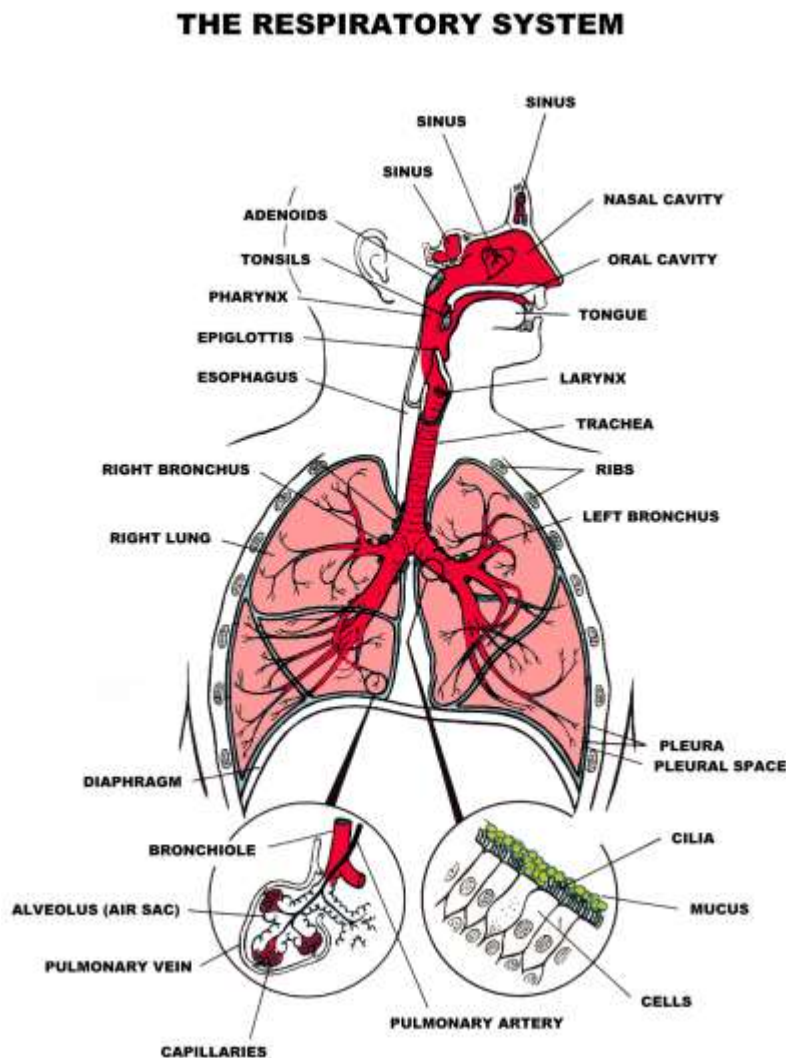
Soluble compounds such as transition metals on the surface of the particle leading to the formation of hydroxyl radical (Risom et al., 2005, Lodovici and Bigagli, 2011). For example particles may contain transition metals (Fe, Cu, Cr, V) on their surface, thus generate ROS through Fenton reaction by acting as a catalyst (Risom et al., 2005, Lodovici and Bigagli, 2011). $(\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{OH}^\bullet + \text{H}_2\text{O})$

Also particles may contain organic compounds such as PAHs absorbed on their surface and these compounds can also generate ROS through redox-cycling (Delfino et al., 2011). A study demonstrated that diesel exhaust particles (DEP) enhanced proinflammatory responses in human airway epithelial cells and induced the expression of the *CYP1A1*, a cytochrome P450 specifically involved in polycyclic aromatic hydrocarbons (DEP) metabolism (Bonvallot et al., 2001).

(b) Direct particle generation of ROS can occur through the presence of free radicals and oxidants (Risom et al., 2005). These ROS produced due to exposure to air pollution targets the DNA (Risom et al., 2005). This may serve as a biomarker for oxidative stress for important diseases caused by air pollution and may be involved in cancer risks (Risom et al., 2005). During the development of these diseases, oxidative stress and inflammatory response play an important role as these responses are provoked by PM and UFP (Müller et al., 2010). Therefore biomarkers of oxidative stress and inflammation, should serve as proxy measures to compare potential health effects of different sources (Møller and Loft, 2010).

1.7 Lung architecture and function

The main duty of the lung is gas exchange i.e. to bring oxygen to the bloodstream and to remove carbon dioxide from the bloodstream.



Adopted from the Saskatchewan Canadian lung association
https://www.lung.ca/children/grades7_12/respiratory/respiratory_system.html

Figure 1.3. Respiratory system

Figure 1.2 shows the respiratory system. The respiratory system is divided into three regions. The first is the head airways region which is made up of the nose, mouth, pharynx and larynx (Hinds, 1999). The function of this region is to warm, humidify and filter the air (Stocks and Hislop, 2002, Hinds, 1999). The second region is the lung airways or the tracheobronchial

region (Hinds, 1999). This region is the passage leading from the throat to the lungs and it consists of the trachea, bronchus and the bronchiole (Hinds, 1999). The trachea splits into two the left and the right bronchi, which further splits into tiny branches known as the bronchioles (Holmgren, 2011). Finally the third region known as the pulmonary or alveolar region, where gas exchange takes place (Hinds, 1999). This consists of the alveolus (air sacs), pulmonary vein and capillaries. The gas exchange in this region occurs by diffusion where oxygen is moved from the alveolar sacs to the capillary rich with red blood cells that carry oxygenated blood to the body and carbon dioxide is reabsorbed by the alveolar and exhaled through the lungs (Holmgren, 2011). Unlike the other regions of the respiratory system that have the protective mucus layer and or are ciliated to help clear the system from inhaled deposited particles, the alveolar region because of its gas exchange function does not have (Holmgren, 2011, Hinds, 1999). Rather inhaled particles deposited in this region may dissolve slowly or be engulfed and thus cleared out by phagocytic cells known as alveolar macrophages (Hinds, 1999).

1.7.1 Inflammatory mediators in the lung

Inflammation is a protective mechanism of response to destroy, dilute, or isolate an eliciting foreign and or injurious compound and promote the repair of injured tissue (Driscoll et al., 1997). It is a multistep process that includes] injury, repair and resolution with the help of inflammatory mediators such as cytokines, isoprostanes and leukotrienes (Khansari et al., 2009). They are the main immune system reaction to remove disease causing organisms or stimuli in to restore cell to its normal state or even replace destroyed cell (Khansari et al., 2009). Therefore following exposure to NPs, airway inflammation is initiated in response to the stimuli this then leads to the formation of ROS (Khansari et al., 2009, Ghio et al., 2012). This inflammatory response will continue until repair or replacement is completed but

continued active response can lead to apoptosis or over production of ROS from the inflammatory cells (inflammatory cells such as cytokines) (Khansari et al., 2009). The macrophages cell which are important members of the mononuclear phagocyte system (MPS) play a crucial role in inflammation because their activation secretes immune regulators: for example chemokine a type of cytokine that glues itself to circulating monocytes, trafficking MPS cells to inflammation site and generate immune response (Stevenson et al., 2011). Inflammatory process induces oxidative stress (that is, one of the initiating pathogenic effect of oxidative stress is inflammation (Wan et al., 2012)) and reduces cellular antioxidant capacity (Loft et al., 2012a, Khansari et al., 2009).

1.8 Analysis of biological responses to NP exposure

There are different techniques for assessing biomarkers of airway inflammation and oxidative stress (Malerba and Montuschi, 2012) . These techniques include: from sputum induction, bronchoscopy, bronchial biopsies, EBC (exhaled breath condensate) (Malerba and Montuschi, 2012, Horváth et al., 2005, Montuschi and Barnes, 2002) EBA (exhaled breath aerosol), blood and the urine which measures biomarkers of systemic inflammation and oxidative damage (Montuschi and Barnes, 2002, Svedahl et al., 2013, Almstrand et al., 2008). For this study we will discuss EBC and urine.

1.8.1 Exhaled Breath Condensate

EBC is a non-invasive technique for analysing inflammatory mediators present in the airway lining especially the lower airway secretions (Koczulla et al., 2009b, Horváth et al., 2005, Montuschi, 2007), i.e. collecting information on inflammation without affecting the function of the airway (Hoffmeyer et al., 2011). To collect the breath for analysis, exhaled air is passed through a condensing apparatus, the warm exhaled air cools below dew point by

exchange of heat between the air and the cold condenser surface and the accumulation of fluid due to this condensation of the warm exhaled breath is known as the EBC (John, 2002). Assuming respiratory rate remains constant, approximately 1 – 2 ml of EBC can be collected from an individual during a period of 10 mins (Dodig and Čepelak, 2013). The condensing apparatus used in collection of EBC could be home-made or commercially manufactured (Horváth et al., 2005). There are some commercial devices that have been manufactured and are widely used, they are; RTubeTM, (Respiratory Research Inc., Charlottesville, Virginia, USA), ECoScreen (Cardinal Health, Hochberg, Germany), Anacon (Biostec, Valencia, Spain) and TURBO-DECCS (ItalChill Pharma and Incofar Srl, Modena, Italy) (Hoffmeyer et al., 2009, Houspie et al., 2011) but they are quite expensive.

EBC is made up of mainly water vapour (almost 99 % water content (Bikov et al., 2010, McKenzie et al., 2012)), containing aerosolized airway lining fluid (non-volatile) and volatile compound that provide non-invasive indications of ongoing biochemical and inflammatory activities in the lung (John, 2002, Todor A, 2011). It is the respiratory air in liquid form. There are more than 3,500 compounds contained in the EBC, they include the following; oxygen, nitrogen oxides, leukotrienes, cytokines, peptides, isoprostanes, hydrogen peroxide, ethanol ammonia and volatile organic compounds (VOCs) which can be detected in healthy humans and those with different airway inflammatory disease (Nel, 2005, Horváth et al., 2005, Montuschi, 2007, John, 2002) and it has a very weak buffer capacity (Bikov et al., 2010). The concentration of these compounds and mediators are influenced by lung diseases and therapeutic interventions (Nel, 2005). It is believed that during tidal breathing, airway surface (epithelial lining) fluid is aerosolized during turbulent airflow (turbulence in airway is due to reopening of closed bronchioles and alveoli) and these particles in the condensate originates from the bronchioles and alveoli thereby reflecting the composition of

bronchoalveolar lavage fluid (BALF) (Rahman and Biswas, 2004, Dodig and Čepelak, 2013). Formation of these aerosolised droplets depend on the velocity of the flow of exhaled air and the surface tension (i.e. higher velocity/lower surface tension) (Dodig and Čepelak, 2013). According to the American Thoracic Society (2006), the two primary purpose for studying EBC are: the fluid can safely provide information about the airway lining fluid (ALF) and secondly, to provide evidence of airway disease especially in regards to inflammation. Different biomarkers in the EBC may reflect different aspects of inflammation or oxidative stress of the lungs of healthy subjects and of patients with different lung diseases (Montuschi, 2005) but for the purpose of this study, nitrates, nitrite and ECB-pH are the biomarkers of interest.

1.8.1.1 Advantages of using EBC

Advantages of EBC include; it is non-invasive, easy to use, allows repeated sampling within short intervals, and is suitable for analysis of children and patients with severe disease on mechanical ventilation (Nel, 2005, Koczulla et al., 2009b). It is also simple to collect samples, inexpensive and safe. EBC can also provide sequential and longitudinal sampling of the lower respiratory tract without the need for more invasive tests, such as bronchoscopy with BAL and induced sputum (Houspie et al., 2011, Nel, 2005, Horváth et al., 2005).

1.8.1.2 Disadvantages of using exhaled breath condensate

The results of mediators measurement is not in real time because EBC samples need to be assayed for different biomolecules (Horváth et al., 2005). It is not anatomic-site specific (Adams et al., 2001) and several and important methodological issues such as standardisation, validation of analytical methods for measuring each biomarker lead to variability of results and this needs to be addressed before the EBC can be used in the clinical setting or practice (Horváth et al., 2005, Nel, 2005, Montuschi, 2010, Kullmann et al., 2007).

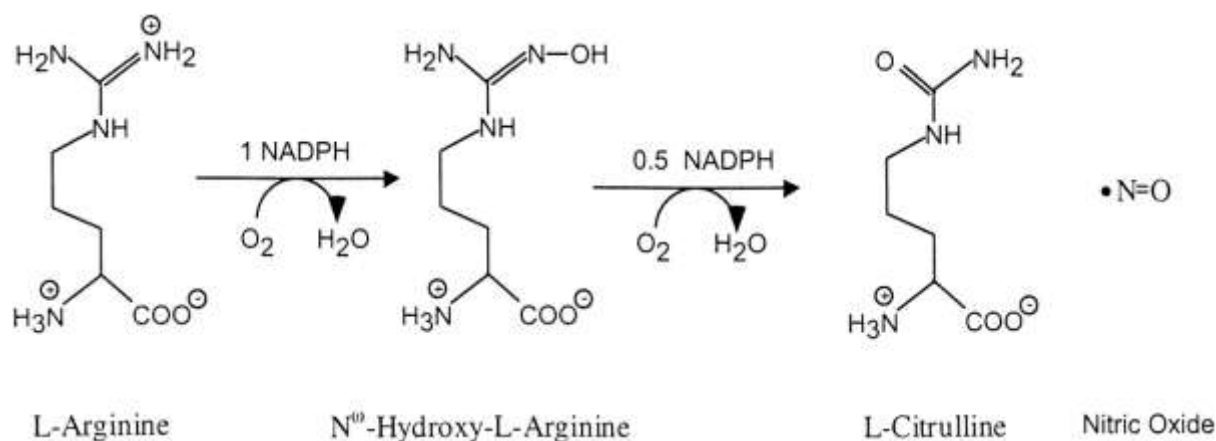
Factors such as food, medication, smoking, alcohol consumption, exercise, type and rate of breathing, nasal and salivary contamination, immunoassays used, environmental temperature and humidity can affect results of the mediators measured. (Elhefny et al., 2012, Kullmann et al., 2007, Dodig and Čepelak, 2013). The EBC is made up of large amount of liquid water, this liquid water dilutes the non-volatile solutes that are already present in very low concentration thereby making their concentration level to be at or < detection limits of the commercially available assays (McKenzie et al., 2012).

1.9 Nitrites and Nitrates

Breath biomarkers of airway inflammation involved in air pollution studies includes oxidised end product metabolites of nitric oxide (NO): nitrite (NO_2^-) and nitrate (NO_3^-) among others biomarkers making them surrogates markers of NO (Corradi et al., 2003, Laumbach and Kipen, 2010, Saijo et al., 2010). Nitrite and nitrate are relatively stable forms of NO that are non-volatile and reported to have been found in EBC (Marteus et al., 2005, Chladkova et al., 2006). Previously, NO_2^- and NO_3^- were assumed to be inert oxidative end products of endogenous NO (Lundberg et al., 2008). However in recent times, research has shown that NO_2^- and NO_3^- are physiologically recycled in the blood and tissue to produce NO (Lundberg et al., 2008). This NO- NO_2^- - NO_3^- cycle may implicate nitrate and nitrite in physiological processes that are known to be regulated by NO and are therefore markers for airway inflammation (Lundberg et al., 2008, Marteus et al., 2005). Also NO has been involved in many metabolic functions of which vascular tone, inflammation and role in immune system is included (Moorcroft et al., 2001). Therefore measurement of NO_2^- and (NO_3^-) provides a more reliable measurement of the unstable NO thus used when assessing inflammatory responses as especially the level of nitrite correlates with the degree of injury (Moorcroft et al., 2001).

1.9.1 Synthesis of Nitrite and nitrate

L-arginine is a precursor to NO and its end products NO_2^- and NO_3^- (Stuehr, 2004) (Figure 1.3). NO is produced endogenously from various sources, however the most important source is through L-arginine (Ellis et al., 1998). Where arginine is oxidised by nitric oxide synthase (NOS) to give citrulline and NO (Ellis et al., 1998, MacMicking et al., 1997, Lundberg et al., 2008, Miranda et al., 2001). The oxidation of arginine happens in the presence of NADPH and another cosubstrate O_2 (Stuehr, 2004). In humans there three isoenzymes of NOS and they are thought to catalyze the production of NO by the same biochemical pathway (MacMicking et al., 1997). However, the isoenzyme which is important to this study is the isoenzyme called inducible nitric oxide synthase (iNOS). The iNOS is produced in macrophages and many other cell types in response to inflammation or infection (Ellis et al., 1998, MacMicking et al., 1997). Upon activation of the immune and inflammatory signal the iNOS is expressed by the macrophages, neutrophils, PMNs and other cell types that respond during inflammation (Miranda et al., 2001) The iNOS hydroxylates arginine in the presence of the cosubstrates to generate N-hydroxy-L-arginine (NOHA) as an enzyme bound intermediate and then NOHA is then oxidized further by the enzyme iNOS to generate NO plus citrulline (Stuehr, 2004).



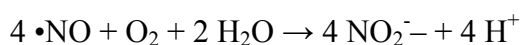
The NADPH and oxygen requirements of each reaction are shown adopted from Stuehr, D. J. (2004).

Figure 1.4. Synthesis of NO The two reactions of NO synthesis as catalyzed by NOS

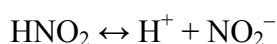
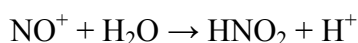
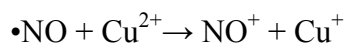
Under physiologic conditions NO is oxidised to form NO₂⁻ or NO₃⁻ is reduced to for NO₂⁻ (Lundberg et al., 2008, Bergamaschi, 2012). In the tissue NO reacts with water to form NO₂⁻, the reaction for the formation of NO₂⁻ is represented in the reaction below (Ellis et al., 1998, Miranda et al., 2001). This is a slow non-enzymatic reaction but in the plasma it is a rapid reaction and it is catalysed by multicopper oxidase and NO oxidase ceruloplasmin (Lundberg et al., 2008).

1.9.2 Nitrite formation

Auto-oxidation of NO

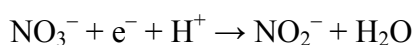


Ceruloplasmin



Also bacteria in the oral groove and gastrointestinal tract contribute to nitrite formation via a one-electron reduction of nitrate, as seen in the reaction below (Lundberg et al., 2008).

Bacterial nitrate reductase



Finally nitrite is then oxidised in the blood in the presence of heme to generate nitrate and methaemoglobin (Met-Hb). (Lundberg et al., 2008, Ellis et al., 1998).

Nitrite oxidation

Haemoglobin



Under pathological and physiological conditions where oxygen is depleted (hypoxia), NO_2^- and NO_3^- are recycled in the blood to produce NO (Ellis et al., 1998, Lundberg et al., 2008, Shiva, 2013). This can be seen as a form as backup for the production of NO as this process does not depend on oxygen to generate NO and NOS (Lundberg et al., 2008, Ellis et al., 1998).

Another source of NO_2^- and NO_3^- in the human body is via diet (Lundberg et al., 2008). In Plasma, NO_2^- and NO_3^- can be produced by bacteria, enzymatic production and Dietary sources (Lundberg et al., 2008). Dietary source of nitrate is green leafy vegetables and water and nitrite is cured meat (Shiva, 2013, Lundberg et al., 2008).

1.9.3 Nitrite and nitrates as markers of lung inflammation

Particles inhaled into the lungs due to exposures results in increased ROS and RNS production and in turn add to oxidative stress in the lungs through inflammatory response (Donaldson and Borm, 2006, Bergamaschi, 2012). Also this increased ROS and RNS production could as result of oxidants generated from the inhaled particles or and from the lung phagocytes or epithelial cell generated to produce oxidants. In particle-induced nitric oxide formation: on exposure to particles, ROS and RNS generation is increased (Donaldson and Borm, 2006), because the lung cells are stimulated especially the alveolar macrophages

(AM), (PMNs) polymorphonuclear neutrophils and type II cells activating the production of ROS through the NADPH oxidase and the mitochondrial ETC and induce the release of the enzyme iNOS (Donaldson and Borm, 2006). This enzyme leads to the production of NO in the presence of a substrate L-arginine and then under physiologic conditions unstable NO reacts with oxygen to form NO_2^- and NO_3^- (Bergamaschi, 2012, MacMicking et al., 1997, Lundberg et al., 2008). NO is a mediator of inflammatory responses in the lung, which plays an important role in physiological regulation of the airway (Guo and Erzurum, 1998). However because of its unstable nature, its stable metabolites NO_2^- and NO_3^- are measured (Miranda et al., 2001). EBC-Nitrate and EBC-Nitrite, biomarkers of inflammation and local oxidative stress of the airways have been measured in association with air pollution exposure (Bogdan et al., 2000).

1.9.3.1 Analysis of nitrite and nitrate in EBC

Various analytical methods have been used by different researchers to analyse NO_2^- and NO_3^- in EBC. Some of these methods are tabled below:

Table 1.1. Nitrite and nitrate analysis table

Mediator	Technique	Detection limit	Detection range (healthy subject)	Reference
NO_2^-	Greiss	0.025 μM	(0.45 $\pm$ 0.06 μM) (Mean $\pm$ SEM*)	(Corradi et al., 2001)
NO_3^-	Ion chromatography	2 μM	[9.6 (2.6 - 119.4) μM] [Median (range)]	(Corradi et al., 2003)
$\text{NO}_2^- / \text{NO}_3^-$	Greiss	0.1 μM	0.63, 0.20 – 0.41 μM Mean, 95% CI	(Ganas et al., 2001)
NO_2^- $\text{NO}_2^- / \text{NO}_3^-$	DAN*	10 nM (Misko et al., 1993)	(2.4 (0.3) μM) (Mean (SE)) (20.2 (2.8) (Mean (SE))	(Balint et al., 2001)
$\text{NO}_2^- / \text{NO}_3^-$	High performance Liquid chromatography		NO_2^- (0.42 - 11.8 μM) NO_3^- (0.72 - 47.2 μM) (Range)	(Chladkova et al., 2006)

*DAN = 2,3-diaminonaphthalene *SEM = Standard error of mean *SE = Standard error

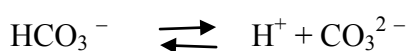
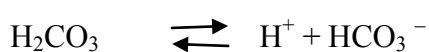
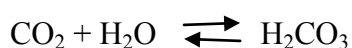
1.10 pH as a marker of airway inflammation

EBC-pH is believed to reflect the acid-base balance of the airways (Kullmann et al., 2007). Acidification of the EBC-pH has been suggested as a biomarker of inflammation associated with airways diseases (Gessner et al., 2003, Do et al., 2008). The production and release of acids and bases such as ammonia in the different buffer systems of the airways helps to regulate the airway pH (Do et al., 2008) therefore, disturbance of the airway pH homeostatic regulatory system may lead to excessive acidification (Ricciardolo et al., 2004b) even though the disturbance of the airway pH may be a host defence mechanism (Ricciardolo et al., 2004b). The disturbance of the airway pH may be due to diseases such as asthma (Nel, 2005), cystic fibrosis (CF) (Nel, 2005), COPD where various studies have shown increased acidification of the airway (Nel, 2005), whereas the EBC-pH of a Healthy subject is slightly alkaline; pH of 7.5 – 7.8 (Ricciardolo et al., 2004b, Walsh et al., 2006). A study has suggested that airway acidification is associated with exposure to UFP and indicator pollutant

of combustion PM elemental carbon in subjects with either mild or moderate asthma (McCreanor et al., 2007). Another study showed increase pH in workers exposed to welding fumes (Boyce et al., 2006).

1.10.1 Measurement of EBC-pH

EBC-pH can be measured with pH electrode (Nel, 2005, Bikov et al., 2010), indicator dyes (Nel, 2005, Bikov et al., 2010) and Blood-gas analyser (Bikov et al., 2010). Various studies have suggested that neat pH (pH immediately tested after collection) tends to be unstable (Bikov et al., 2010, Nel, 2005, Kullmann et al., 2007) because of the presence of dissolved CO₂. This CO₂ in the sample, when it comes in contact with ambient air causes an increase in the EBC-pH (Bikov et al., 2010). Therefore it has been suggested by many researchers that to enhance the stability of the EBC-pH, de-aeration or gas standardisation with CO₂ free gas should be carried out (Bikov et al., 2010, Nel, 2005, Kullmann et al., 2007, Walsh et al., 2006). CO₂ free gasses such as argon (Do et al., 2008), oxygen free nitrogen, helium have been used by researchers to remove CO₂ from EBC (Nel, 2005). The essence of de-aeration is to remove almost all of the dissolved CO₂ (which is the major volatile component of the EBC (Bikov et al., 2010)) in the EBC, thereby making it more stable and increasing its reproducibility (Nel, 2005, Walsh et al., 2006). This is because CO₂ is a precursor to the formation of carbonic acid (H₂CO₃) which drives acidification but has nothing to do with the airway lining fluid (Walsh et al., 2006). That is, in an aqueous environment, CO₂ forms H⁺ and HCO₃²⁻ thereby affects the pH of a dilute solution as in the case of EBC (Bikov et al., 2010).



However some EBC-pH analysis have been carried out without de-aeration for example the study by McCafferty et al. (2004) (McCafferty et al., 2004).

1.11 Urine metabolite analysis

The urine is used as a biological sample for investigation because of its rich metabolite composition (Saude et al., 2007). Urine contains small molecule metabolites in various concentrations and the different levels of these different molecules at different concentration may be associated with different conditions or exposure. That is it can contain chemical compounds or their metabolites or biomarkers which should not be there normally due to exposure to these foreign compound or indication of a condition (Kozłowska et al., 2003). The collection of urine is non-invasive; collection can be repeated as often as possible and relatively large amounts can be collected. In this study Urinary 8-oxo-7,8- dihydro-2'-deoxyguanosine (8-oxodG) and metals in urine due to exposure were analysed.

1.11.1 Urinary 8-oxo-7,8- dihydro-2'-deoxyguanosine

During physiological and pathological processes in living cells, free radicals/ROS are generated, these free radicals/ROS have physiologic importance (Valavanidis et al., 2009). However the excessive and continuous production of these ROS/RNS by inflammatory cells, can cause oxidative damage to DNA, lipids of cellular membrane and proteins because of their reactive nature (Lin et al., 2004a, Valavanidis et al., 2009). In other words it leads to oxidative modification of macromolecules (such as proteins and lipids) and DNA bases (İncelenmesi, 2009). Also in the absence of oxidative stress, some of these ROS/RNS escape the antioxidant defence resulting in a background level of damage (Cooke et al., 2002). In addition to the endogenous factors, exogenous factors such as UV light, asbestos, tobacco smoke, exposure to PM may lead to the production of ROS under different conditions

(Valavanidis et al., 2009, Risom et al., 2005). When these free radicals/ROS/RNS interact with DNA, it results in alteration of gene sequence such as point mutations, deletions, or rearrangements (Coussens and Werb, 2002). Among the damaged DNA products is 8-oxodG which is probably the most abundant and studied oxidation product due to its relative ease of measurement and premutagenic potential (Lunec et al., 2002, Risom et al., 2005). The excess ROS/RNS react with the purine and pyrimidine bases of the DNA, they oxidize them thereby resulting in oxidative products such as 8-oxodG, 8-OHdG (8-hydroxydeoxyguanosine), 8-OHGua (8-hydroxyguanine), thymine glycol, 5-hydroxymethyluracil, 5-hydroxycytosine and others (İncelenmesi, 2009, Cooke et al., 2002, Valavanidis et al., 2009).

8-oxodG is a biomarker of oxidative DNA damage and repair product which cannot be metabolised but rather it is carried through then blood and excreted in the urine (Valavanidis et al., 2009, Wu et al., 2004). In literature 8-OHdG and 8-oxodG are used for the same compound and this have caused a lot of confusion among readers (Wu et al., 2004, Valavanidis et al., 2009). It is a tautomer of 8-OHdG (Valavanidis et al., 2009)

It has also been shown that it is a biomarker for diseases such as arthritis, chronic hepatitis, most studies involving cancer tissue or other samples from patients with malignant diseases or diseases associated with an increased risk of cancer show signs of an increased rate of oxidative DNA modification or in some instances deficient repair (Loft and Poulsen, 1996, Cooke et al., 2002, Roszkowski et al., 2011).

Urinary 8-oxodG has been measured as a systematic oxidative stress associated with air pollution (Suzuki et al., 1995, Loft et al., 1999, Sørensen et al., 2003b). A study observed increased levels of urinary 8-oxodG in children exposed to ETS (Svecova et al., 2009b) and others have also shown increased levels of urinary 8-oxodG repeatedly in

smokers (Sørensen et al., 2003b). Various studies over the past years have shown that exposure to airborne ultrafine/fine/respirable PM, transitional metals, PAHs and even exposure to DEP have significantly increased the levels of urinary 8-oxodG in both in-vivo and in-vitro studies (Loft et al., 1999, Sørensen et al., 2003b). For example, A study observed that, 8-oxodG excretion was significantly higher in bus drivers from central areas compared with bus drivers from suburban/rural areas of Copenhagen (Loft et al., 1999). Short term exposure to traffic pollution in three subjects in Tokyo resulted in an increase in oxidative DNA damage estimated from excretion of 8-oxoguanine, a repair product of this lesion in DNA (Suzuki et al., 1995, Loft et al., 1999).

1.11.2 Structure of Urinary 8-oxo-7, 8- dihydro-2'-deoxyguanosine

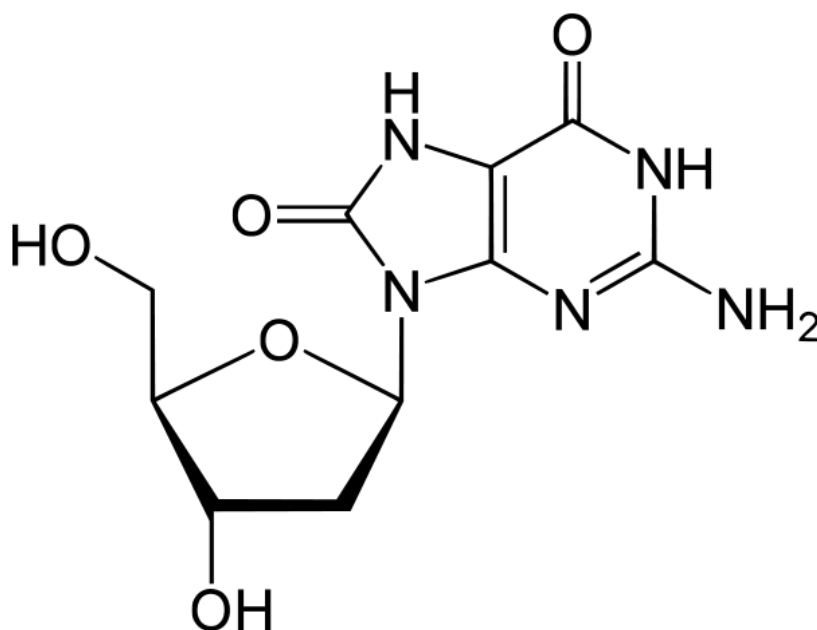
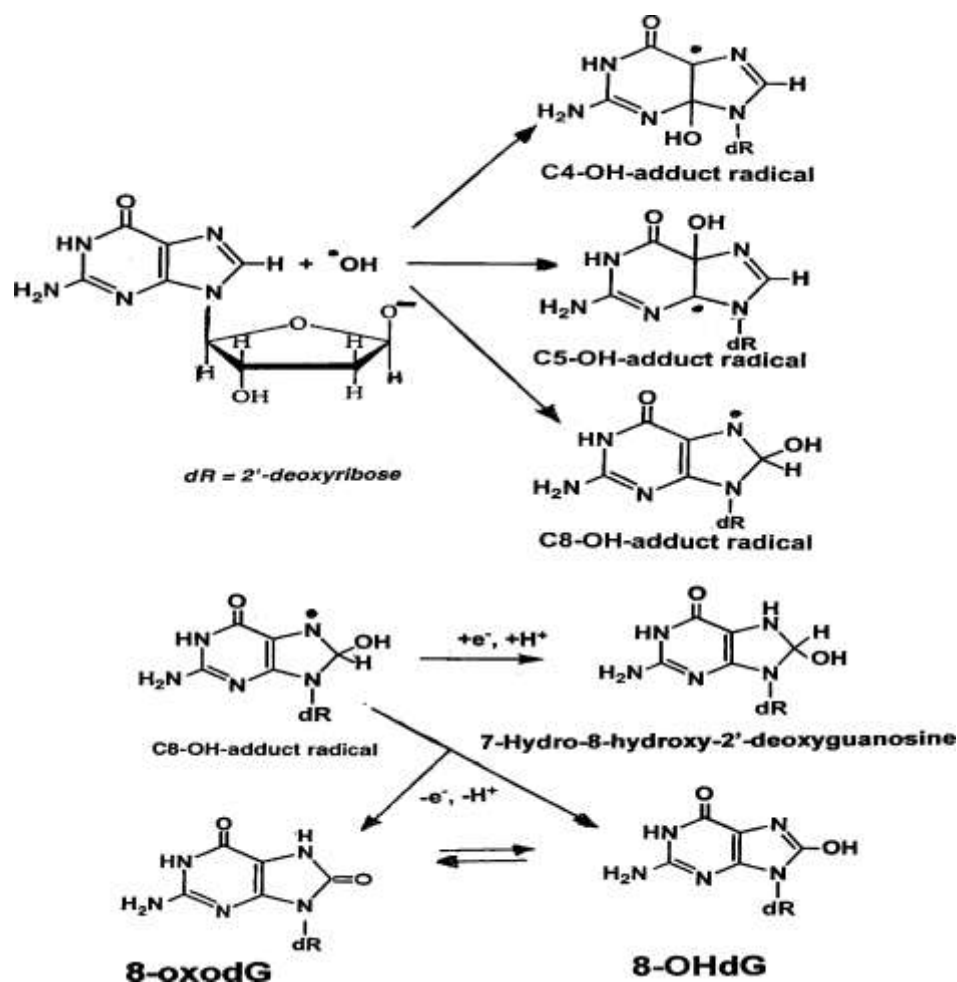


Figure 1.5. Structure of Urinary 8-oxo-7, 8- dihydro-2'-deoxyguanosine

It has the formula $C_{10}H_{13}N_5O_5$ and molecular weight 283.24072 g/mol. 8-oxodG has a carbonyl group at C8 and is protonated on N7 (Krahn et al., 2003) (Figure 1.4). 8-oxodG is a

deoxynucleoside of 8-oxo-guanine (8-oxoG, also referred to as 8-oxo-7,8-dihydroguanine), which is derived from guanine (Cooke et al., 2002).

1.11.3 Synthesis of 8-oxo-7,8- dihydro-2'-deoxyguanosine



Reaction of 2-deoxyguanosine with hydroxyl radicals, radical adducts followed by reduction to 7-hydro-8-hydroxy-2-deoxyguanosine, and by oxidation to 8-hydroxy-2-deoxyguanosine (8-OHdG) or its tautomer 8-oxo-7-hydro-2-deoxyguanosine (8-oxodG) (Valavanidis et al., 2009).

Figure 1.6. Synthesis of 8-oxodG

As previously mentioned, 8-oxodG is a biomarker of oxidative DNA damage and repair product (Valavanidis et al., 2009). During the damage to biomolecules (proteins, membrane lipids, and DNA), active oxygen species, generated by ionizing radiation, air pollution, other exogenous sources and by endogenous oxidation processes, react with deoxyguanosine (dG) residues in DNA to form 8-oxodG (Valavanidis et al., 2009, Tchou et al., 1991, Loft et al.,

2008). The hydroxyl radical ($\text{HO}\bullet$) is the most important oxygen-free radical causing damage to basic biomolecules (Valavanidis et al., 2009). Guanosine (2'-dG) is the DNA base that is most easily oxidized because of its redox potential (Buettner, 2011). The $\text{HO}\bullet$ reacts with guanine (DNA strand) and forms C8-hydroxyguanine (8-OHGua) or its nucleoside form deoxyguanosine 8-OHdG (Valavanidis et al., 2009). Firstly, the reaction of the $\text{HO}\bullet$ addition leads to the generation of radical adducts or intermediates, then by one electron abstraction, the 8-OHdG is formed (Figure 1.8) (Buettner, 2011, Valavanidis et al., 2009). The 8-OHdG undergoes tautomerism, which favours the oxidized product 8-oxodG (Valavanidis et al., 2009). This 8-oxodG is implicated in mutagenesis, carcinogenesis hence they have premutagen potentials (Mateos and Bravo, 2007). The damaged strand is then repaired i.e. removed or cleaved by the action of endonucleases and glycosylases enzyme (oxoguanine DNA glycosylase 1 (OGG1)) resulting in the release of 8-oxodG for urine excretion (Mateos and Bravo, 2007, Loft et al., 2012b).

1.11.4 Analysis of 8-oxo-7, 8- dihydro-2'-deoxyguanosine

There are various methods that have been used for the analysis of urinary 8-oxodG some of them are: HPLC with electrochemical detection (EC) (HPLC-EC), gas chromatography-mass spectrometry (GC-MS), HPLC tandem mass spectrometry and ELISA/EIA (Mateos and Bravo, 2007, Wu et al., 2004, Valavanidis et al., 2009, Chiou et al., 2003).

1.12 Metals in urine

Metals and other elements have been assessed in urine for various reasons and assessment for occupational and environmental exposure is one of them (Kozłowska et al., 2003). Urinary metal analysis has been employed in occupational exposure monitoring in order to measure changes in levels of urinary metals in relation to inhalation exposure. For example, Mutti et

al. (1979) monitored Cr in urine for exposure to Cr in welders. Another study measured Cd in urine as an indicator of Cd exposure (Fitzpatrick et al., 2009).

Occupational exposed workers showed a significantly higher excretion of urinary As than the control and also up to two fold increases of urinary As excretion was observed in exposed rats compared with control rats were also observed in animals dosed with various forms of arsenicals (Prahalad et al., 1999). Monitoring of trace elements in human health risk assessment is important because it helps to evaluate levels of exposure to different chemical compounds. These trace elements can be monitored in body fluids. These trace elements in human can be affected by various environmental and physiological factors such as air, dietary style/habits, water because they are found everywhere and it almost impossible to avoid exposure (ChandrakalaAluganti et al., 2012, Jomova and Valko, 2011, Valko et al., 2007).

Table 1.2. Some dietary sources of some metals

Metals	Dietary sources and comments
Cu	shellfish, whole grains and organ meats (kidney, liver), dark leafy greens
Mn	grains, beans and nuts and in heavy tea drinkers
Ni	oatmeal, dried beans, peas, nuts and soya products
Cr	meat and whole grain products however in very small quantities
Sb	Very low concentrations in meats and fishes
V	seafood products
Sn	vegetables and fruit and through consuming canned foods
Na	Salt
Zn	Beverages

Adopted from (Jamaludin, 2014).

Dietary sources of metals have been discussed in various studies (Arnich et al., 2012, Barceloux and Barceloux, 1999, Badmaev et al., 1999, Gibson, 2012). However Table 1.7 is adopted from Jamaludin et al. (2014).

Quantifying the concentrations of certain trace metals present in human biological system by using environmental measurements to estimate the extent and magnitude of exposure is growing but this estimation can be difficult because the sources and route of exposure are usually unknown (ChandrakalaAluganti et al., 2012). However, trace element monitoring has

been used in several air pollution studies to measure exposure to elements. Such measurement of Cr in urine of 24 chrome-plating workers (Federico et al., 2007) and monitoring 29 municipal waste incinerator workers for metals in their urine due to exposure (Rava et al., 2012).

1.13 Other parameters and markers of interest to this study

1.13.1 Pulmonary and autonomic functions

Other parameters and markers of exposure to air pollution of interest to this study include: lung function parameters such as forced expiratory volume in one second (FEV₁), forced expiratory volume in six seconds (FEV₆), ratio of FEV₁/ FEV₆ and forced expiratory flow (FEF_{25-75%}), Blood pressure (BP): systolic and diastolic BP, pulse rate, minute ventilation (V_E) and lung dose/inhaled load estimate.

Studies have reported that short-term exposures to traffic related air pollutants on or near roadways have been associated with increased respiratory tract inflammation, decreased lung function, and changes in heart rate variability (HRV) (Hollowell, 2011, Huang et al., 2012). Blood pressure (BP) measured as systolic and diastolic blood pressure and heart rate (HR) measured as the pulse rate are well-established risk factors for cardiovascular health (Walsh et al., 2006). Acute indoor and outdoor pollution were positively associated with BP measured on the same day in an elderly study population (Saude et al., 2007). Increases were found in systolic and diastolic BP and HR in association with particles with 1- 3 hours moving averages of particles with a size range of 0.02 – 1 µm in patients with lung function impairment (Lin et al., 2004a). Short-term exposure to urban roadside diesel traffic led to consistent and significant reductions in lung function, accompanied by airway acidification for persons with asthma (Malerba and Montuschi, 2012). Studies have reported that increases

in PM_{2.5}, NO₂, and O₃ are associated with decreases in FEV₁, forced vital capacity (FVC), and/or PEF rate in healthy subjects (Hambach et al., 2013).

When particles are inhaled into the airways, in order for the pollutants to elicit their effect, factors such as dose concentration, reactivity, solubility and the physical properties of the particle are all involved (Batista et al., 2009). The dose that reaches the target organ may be affected by ventilation i.e. the volume of inhaled air or expiratory volume or minute ventilation V_E (Batista et al., 2009, Hoet et al., 2013). Therefore in ventilation, a measure of the frequency and depth of breathing, is an important determinant of the amounts of indoor and outdoor air pollutants that enter the respiratory tract (Jamaludin, 2014). Thus various physical activities in the day will determine the ventilation variation and the amount of inhaled air and in turn affect the doses of inhaled air pollutants delivered to the respiratory tract (Jamaludin, 2014, Hoet et al., 2013). It has now been suggested that in order to estimate the dose of inhaled particles, V_E alongside particle and exposure condition and duration data should be gathered (Jamaludin, 2014). Various studies have developed methods for determining V_E (LPM) by applying the HR to regression analysis models (Arnich et al., 2012, Batista et al., 2009, Hoet et al., 2013). For this study the regression model method developed by Mermier et al. (1993) will be used.

$$V_E = \text{Exp}(0.022\text{HR} + 1.148) \quad (1.1)$$

Where HR is the measured heart rate i.e. pulse rate, 0.022 is the slope and 1.148 is the intercept values from Mermier et al. (1993) regression model. The essence of calculating V_E is to enable calculation of inhaled load estimate (lung dose) i.e. quantify the amount of inhaled pollutants. A study reported that whereby the bus passenger was exposed to higher concentrations than the cyclist following the same route into and out of the city centre but when the breathing rate and respective time of commute between transport modes are taken

into consideration, they observed that the cyclist inhaled a slightly greater mass of pollutants per journey than the bus passenger (Carraro et al., 2005).

Dose concentration of a pollutant that reaches the target organ is an important factor to be considered in exposure measurements because the dose concentration is associated with the effects caused by the pollutants amongst other factors such as exposure time.

In order to quantify the dose concentration inhaled into the lungs in the after-exposure exercise, the inhaled dose estimate factor will be calculated using the formula from Cozza et al. (2015).

$$\text{Estimated inhaled load } (\mu g) = \left(\frac{\text{Obtained concentration}}{1000} \mu g/m^3 \right) \times \text{estimated ventilation (L/min)} \times \text{Time (mins)} \quad (1.2)$$

Where obtained concentration is the pollutant concentration measured during exposure in $\mu g/m^3$, estimated ventilation gotten from equation (1.1). Estimated ventilation is the V_E and Time is the exposure time in minutes.

1.13.2 Health risk assessment

Health risk assessment is tool used to estimate the occurrence of adverse health effect resulting from the inhalation of fine particles that are generated both from indoor and outdoor sources (Lombardi-Boccia et al., 2005, Taner et al., 2013).

Cr, Ni and Pb are the known toxic and carcinogenic metals. These metals can be bound to the PM and the subjects were exposed to these metals via the inhalation pathway, thus posing a risk of adverse effect to humans. Cr is corrosive, cytotoxic, and carcinogenic for humans and can bring about acute and chronic lung tissue toxicity (Federico et al., 2007). Chronic exposure to Cr (VI) leads to ulcerations and perforations of the nasal septum, chronic

bronchitis, decreased pulmonary function, pneumonia, and other respiratory effects (Federico et al., 2007, Lodovici and Bigagli, 2011). Cr (III) has limited toxicological effects (Federico et al., 2007, Zhang et al., 2013). Cr (III) is classified group D therefore not for human carcinogenicity or carcinogenic potential while Cr (VI) is classified as Group A indicating human carcinogenicity. The general knowledge for the Cr (III) to Cr (VI) ratio is 6:1 (Sørensen et al., 2003a) As used in a previous study, the concentration of Cr (VI) used for the carcinogenic risk assessment was calculated as one seventh of the total concentration (Pope III and Dockery, 2006).

Ni exposure causes formation of free radicals in various tissues in both human and animals which can then lead to various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulphydryl homeostasis (Zuurbier et al., 2011). There are different types of nickel of which these is included nickel refinery dust, nickel subsulfide (Ni_3S_2), nickel carbonyl and nickel-soluble salts (nickel chloride, nickel sulphate). The US EPA has classified nickel refinery dust and nickel subsulfide as group A materials, indicating that it is a known human carcinogens. The IARC (2006, 2012) has classified Ni as Class 1 carcinogenic elements and Pb as Class 2B carcinogenic element but human evidence is inadequate in IRIS thus, values for IUR were selected from California Environmental Protection Agency. Therefore in this study, the excess cancer risk (ECR) will be calculated for these carcinogenic metals even though Pb human evidence is inadequate for Pb carcinogenicity and the USEPA is amending its unit risk (Pope III and Dockery, 2006). The essence of calculating the ECR from the particles measured in the indoor (homes) is essential in order to assess the total particulate exposure and the related toxicity evaluation of the carcinogenic metals like Cr, Ni and Pb.

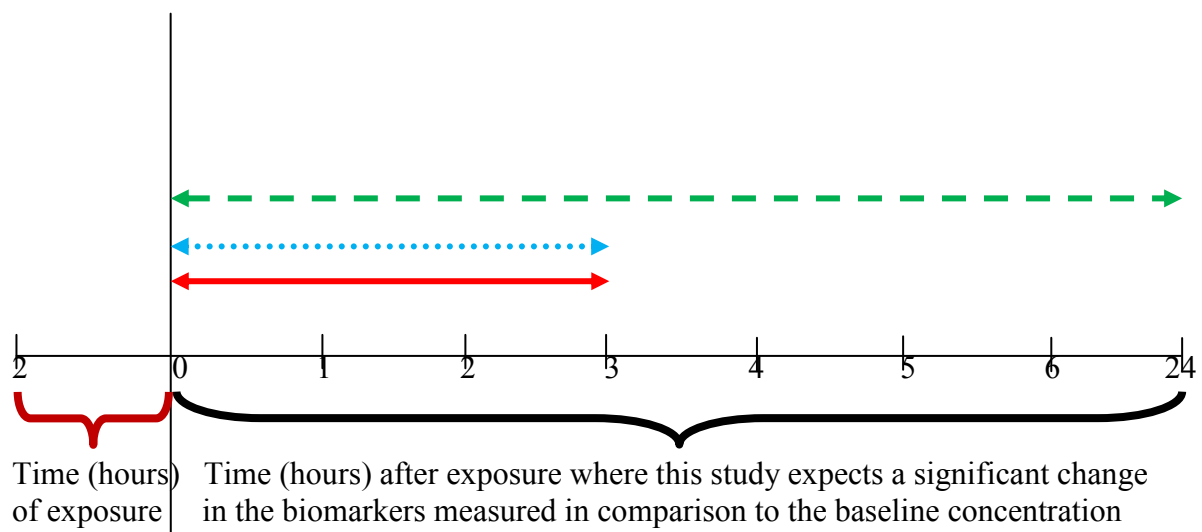
1.14 Acute versus chronic responses

Acute exposure response is the initial stage of inflammation where the immune system is activated to respond to stimuli such as exposure to PM, infection, allergy and injury (Héroux et al., 2010, Buonanno et al., 2009, Nitta et al., 1994). For example in the inhalation of air borne particles, the innate immunity during the initial stage of inflammation is activated in order to protect the airways. Acute inflammation should persist for a short time (Buonanno et al., 2009). Some of the innate immune cells are macrophages, mast cells, dendritic cells and these cells can initiate the inflammatory response by releasing cytokines, chemokines, ROS/RNS, NO, nitrite and nitrate thereby leading to the elimination of the stimuli and repair of the damaged tissues at the site of injury (the lungs in this study). Therefore acute exposure responses is an indicator of a challenge to a particular tissue in this case the lung caused by inhalation of PM, thereby leading to inflammation or localised oxidative stress. Such biomarkers showing acute exposure response effects are increase in nitrate, nitrite and change in pH of the airways and they can be measured in the EBC.

However where exposure continues and the immune system response fails to control the production of reactive species (i.e. an imbalance between reactive species and antioxidant) thereby also causing inflammation to last for a longer period of time then this could lead to systemic oxidative stress. Also known as the second stage of inflammation (Buonanno et al., 2009). That is these reactive species interact with macromolecules and DNA causing DNA damage, modify cellular proteins and cause apoptosis either directly or indirectly (Valavanidis et al., 2009, Héroux et al., 2010). 8-oxodG is a biomarker of DNA damage and repair (Risom et al., 2005), a biomarker of exposure effect leading to chronic responses such as systemic oxidative stress. Therefore 8-oxodG is an indicator of harm to the entire system

while acute exposure response biomarkers nitrate, nitrite and change in pH of the airways is localised to affected tissue. Table 1.3 shows the timeline of physiological effects of the biomarkers (relevant to this study) measured in EBC and urine in other studies. It shows that EBC-pH, nitrate and nitrite mostly show significant changes in concentration immediately after exposure showing that the innate immune systems defending the airways indicating that the airways is challenged with foreign compounds. Therefore they are biomarkers of acute exposure response. While significant changes in 8-oxodG biomarker of effects is mostly measured after long and or constant exposure, suggesting harm to the entire system because DNA has been interfered with. Based on literature and results from other studies such as those listed in Table 1.3, this study expects to observe significant changes to biomarker concentrations in comparison to the baseline concentrations at the timeline displayed in Figure 1.7.

Figure 1.7. Expected physiological timeline for changes in biomarkers



← - - - → 8-oxodG (significant change is expected anytime within 24 hours after exposure. Moller et al. (2010) summarised that from their experience in their various studies, that oxidized DNA is observed within a lag period < 24 hr after the exposure to PM.

← → EBC-pH (significant decrease is expected immediately after exposure at 0 hours and expected to remain decreased until 3 hours after exposure).

← ——— → EBC-nitrate and EBC-nitrite (significant increase is expected immediately after exposure at 0 hours and expected to remain decreased until 3 hours after exposure).

Table 1.3 Physiologic effect timeline of biomarkers (relevant to this study) measured in EBC and urine in other studies

Exposure scenario	Body fluid collection time	Biomarker	Reference
18 healthy never smoked subjects exposed to secondhand smoke for 1 hour at bar/restaurant level in a chamber.	EBC was collected before exposure and then after exposure at 0 mins (immediately), 30 mins, 60 mins, 120 mins, 180 mins and 240 mins.	EBC-pH decreased significantly compared to the before at 0, 30, 60, 120 and then returned to baseline by 180 mins. No changes in EBC Total nitrite/nitrate was observed	(Maynard et al., 2006)
10 healthy subjects exposed in intermittent exercise protocol to ozone concentration at 240 ppb and 40 ppb (sham). That is 2 hours and 4 hours with intermittent 20 mins exercise intervals	EBC was collected before exposure and then at 0 mins, 4 hours and 16 hours after exposure.	EBC-pH increased at 0 mins and 4 hours after exposure and returned to baseline at 16 hours after exposure. Exercise may have enhanced EBC-pH and thus compensated for the acidification	(Chithra and Nagendra, 2013)
16 healthy volunteers exposed to ethyl acrylate at 5 ppm and 0.05 ppm (sham) for 4 hours.	EBC was collected before and immediately after exposure.	EBC-pH decreased in the 5 ppm after exposure as compared to the before exposure concentration.	(Okada and Heintzenberg, 2003)
15 swine confinement (SC) workers and 9 respiratory healthy non-smoking volunteers for 5 hours.	EBC was collected before and immediately after exposure and after one working week for the SC workers	EBC-pH significantly decreased in after 5 hours exposure for the volunteers and significantly increased after one working week for the SC workers.	(Long et al., 2000)
60 adult non-smokers (31 mild and 29 moderate asthma) exposed on Oxford street where only diesel powered buses and taxi cabs are permitted for 2 hours. Then on separate time exposed in Hyde park as the control	Urine and lung function measurements were collected before and after exposure in each scenario.	EBC-pH significantly decreased in the Oxford street exposure compared to that of Hyde park. Likewise FEV ₁ and FVC.	(Wan et al., 2012)

16 healthy non-smokers were exposed to acute sulphur dioxide in concentrations of 0 (clean air), 0.5, 1 and 2 ppm in a repeated measure cross over design for 4 hours, with 2 periods of 15 mins exercise (beginning and end)	EBC was collected before and immediately after exposure	EBC-pH significantly increased in the after exposure for 0 and 0.5 ppm of clean concentration in SO ₄ .	(Li et al., 1993)
EBC nitrate and nitrite			
21 young adults exposed to traffic related particles during rush hour traffic. For 1.5 hours unfiltered and 1.5 hours filtered air for control.	EBC was collected before and immediately after exposure.	EBC nitrite and EBC nitrite + nitrate increased in the after unfiltered exposure.	(Laumbach et al., 2014)
15 healthy smokers and 14 non-smokers as the control.	Before sample was collected after 4 hours cigarette abstinence. After sample was taken at 30 mins and 90 mins after smokers smoked 2 cigarettes.	EBC nitrite + nitrate increased at 30 mins, returned to normal at 90 mins and no change in EBC nitrite for the smokers. Suggesting acute increase of nitrate.	(Balint et al., 2001)
12 healthy non-smoking young volunteers, 2 hours on-highway rush hour car ride with and without filter 26 healthy non-smoking subjects inhaled freshly generated 200 µg/m ³ diesel exhaust (DE) and clean air separately for 2 hours in controlled chamber 17 asthmatic subjects inhaled freshly generated 300 µg/m ³ DE	Before exposure and immediately after exposure. Also 5 to 6 hours after exposure.	EBC nitrite increased after traffic exposure in unfiltered air and decreased in the filtered air exposure in the 12 healthy subjects. EBC nitrite increased after DE exposure and decreased after the clean air exposure for the chamber protocol 5 to 6 hours after exposure EBC nitrite returned to normal for all	(Knaapen et al., 2002)

and clean air separately for 1 hours in controlled chamber		subjects.	
8-oxodG			
Exposure scenario	Body fluid collection time	Biomarker	Reference
18 healthy non-smoking subjects were exposed in a chamber for 3 hours to DE at 276 $\mu\text{g}/\text{m}^3$ from a passenger car and to filtered air at 2 $\mu\text{g}/\text{m}^3$, with co exposure to traffic simulated noise at 48 and 75 Db (A) in four separate random sequences.	Urine was collected before, immediately after and 20 hours post exposure	8-oxodG in the after exposure was unaltered.	(Martin et al., 2015)
Ten adult subjects with metabolic syndrome were exposed on separate days for two hours to filtered air or to DE.	Urine collected before and at 3 hours, 22 hours after initiation of exposure	8-OHdG in the after exposure concentrations did not significantly change	(Allen et al., 2009)
32 bus depot workers exposed to particles (PM ₄ (25 -71 $\mu\text{g}/\text{m}^3$) and pollutants for an entire shift for two consecutive days	Urine was collected before and immediately after work shift daily	8-OHdG (tautomer of 8-oxodG) increased at the end of each work shift	(Wall et al., 1988)
67 cooks and 43 service staff as control in a Chinese restaurant.	Morning after urine	Significantly higher 8-oxodG in cooks as compared to non exposed service staff	(Yang and Watts, 2005)
13 healthy subjects exposed for 4 hours to wood smoke in a chamber.	Urine was collected from bedtime after exposure until the following morning	No change in 8-oxodG was observed	(Danielsen et al., 2008)

PM₄-particulate matter with aerodynamic diameter < 4 μm

1.15 Personal exposure assessment using personal sensors

Exposure to air pollutants can occur in the indoors, outdoors and during commuter modes. Measured exposure concentration to these pollutants may vary based on sampling technique. Numerous studies have shown that PE monitoring has been carried out using the concentrations measured at the fixed site (FS)/central monitoring networks (Buonanno et al., 2014b, Avery et al., 2010, Gulliver and Briggs, 2004). This because there is an assumption that the data obtained from these FS represents the entire population. Although the use of FS monitoring for PE are very important and useful, the flaws with using these FS are that short personal peak exposures which contribute to average daily exposure will not be accounted for (Gulliver and Briggs, 2004). Different spatio-temporal mobility patterns and inter-individual variations in behaviours influencing exposure may not also be accounted for (Boudet et al., 2001, Avery et al., 2010, Steinle et al., 2013b). Thereby leading to a bias, loss of statistical power or both in health effects estimates (de Nazelle et al., 2013). The use of FS could be a good indicator of average PE concentrations of population living in close proximity to the monitoring site but on individual bases it may not be representative (Gu et al., 2015).

People spend most of their time indoors (Lazaridis, 2011, Höppe and Martinac, 1998), and there are various indoor air pollution sources (Olsen et al., 2014, Wallace and Ott, 2011, Weichenthal et al., 2006, Taner et al., 2013, Rim et al., 2010, Chen and Zhao, 2011) and the major source of the ambient PM pollution is road traffic (Gulliver and Briggs, 2004), this may make assessment of health risks using outdoor measurement obtained from the FS for exposure estimate not reflect true population exposure to UFP and BC (Ghio et al., 2011). Thus with the advent of the new portable/personal sampling sensors with high sampling response time, PE monitoring is now more ideal. These PSs in collaboration with time-

activity diaries make PE even more accurate because with the help of the diaries, the concentrations representative of where, when and how the subject spent their time can be identified. That is with the diaries the concentrations recorded by the portable sensor can be linked to an activity, where the activity took place, when the activity took place and how long the subject was exposed during the activity. PE to UFPs measurements should include the various activities/microenvironments an individual is exposed to and also the sources of the UFP in the microenvironment (ME).

1.16 Indoor particles

Studies have shown that the outdoor has a lot of air pollution sources both from the natural and anthropogenic effect. However recent studies now focusing on the indoors have suggested that PE in a non-smoking suburban indoor can be higher than the outdoor (Wallace and Ott, 2011). Some of the different indoor environments identified are homes, leisure and cellar rooms, working room/places in buildings, public buildings, restaurants, hotels/inns, shisha lounge, community facilities such as schools as well as spaces in motor vehicles, aeroplanes, other public transportation systems (Fromme, 2012, Raulf-Heimsoth et al., 2010) and in fact any enclosed space. PEs to indoor airborne particles may arise from indoor sources such as: cooking (Dennekamp et al., 2001a), other combustion devices, use of aerosol products and cleaning (Ogulei et al., 2006, Fromme, 2012). Nano-size particles emitted from printers and photocopiers (Lazaridis, 2011). Other sources also include penetration from the outdoors (which can be removed by deposition, adsorption, and other mechanisms) from appliances that are operated by electric motors and re-suspension of particulate matter from house dust by humans and pets (Ogulei et al., 2006, Fromme, 2012, Hänninen et al., 2004, Szymczak et al., 2007, Wallace and Ott, 2011). UFP are increasingly studied because they

have been considered to be associated with toxicological health effects: especially respiratory effects and ability to generate oxidative stress and inflammation to the lungs (Oberdörster et al., 2005b, Pope, 2000).

1.17 Indoor and personal exposure microenvironment in this research

The indoor and PE assessments in this research were carried out in the following MEs: home, office, University, commuter mode, cooking fumes in the Japanese restaurant, shisha lounge and any other environment in which the subject visited during sampling.

1.18 Aims and objectives

The aim of this work will be to characterise PEs and indoor concentrations of NPs/UFP, PM and BC in different indoor and personal exposure scenarios and to study their associations with biological markers of inflammation and oxidative stress. Secondly to characterise the

PM concentrations and metal composition in nine Birmingham UK homes, study their associations with biological markers of inflammation and oxidative stress and quantify their toxicity evaluation of carcinogenic trace metals Cr, Ni and Pb. Thirdly to investigate if urinary metals can be used as biomarkers of exposure to PE.

Specific Objectives are:

- To measure PE to UFP, PM and BC in different exposure scenarios: elevated and normal exposure
- To measure biological markers of inflammation and oxidative stress in different PE and indoor exposure scenarios
- To measure changes in pulmonary and autonomic functions in different traffic/commuter related exposure scenarios
- To measure urinary metals and their correlation to personal exposure
- To assess the correlation between biomarkers and different exposure metrics, such as measured exposure and estimated inhaled load.
- To characterise the concentration, size ranges and physicochemical signatures of UFP and PM (Cr, V, Zn, Ni, Cu, Sb, Ca, Mg, Mn, Sr, Na, Pb, Sn and Fe) in indoor (home) environments.
- To quantify the toxicity evaluation of carcinogenic trace metals Cr, Ni and Pb.

2 MATERIALS AND METHODS

2.1 Design history

Originally 30 healthy non-smoking adult volunteers were to be recruited to conduct exposure measurement at the personal level and in indoor microenvironments (both home and workplace), i.e. 30 summer/warm and 30 cold/winter period. Each subject would have a nano-MOUDI sampler that will collect size segregated NPs onto PTFE filters at 10L/min in size ranges <10nm, 18-32nm, 32-56nm, 56-100nm, 100-180nm, 180-320 nm, 320-560nm, 560-1000 and 1000-10,000 nm. Samples will be collected at the subjects home (N=30 per size fraction) and workplace (N=30 per size fraction) 7 days for home and 7 days for workplace each subject. Also, PSs will be given to each subject participating in the study to collect samples over 15 days, which corresponds with the same length of time that the indoor microenvironment sampling will take place.

During the pilot study it was observed that the nano-MOUDI was not indoor friendly, for the following reasons listed below:

- The noise generated by the nano-MOUDI and the oil pump was > 40db above background noise in the different indoor environment where noise level was measured.
- To alleviate this problem, a soundproof cabinet was built but that did not solve the noise problem. The noise level became 30 to 40 db above background noise.
- The odour from the oil pump was so much and it became unbearable to breathe in the indoor environment. We tried purchasing a vacuum pump but none was appropriate for use with the nano-MOUDI.

- Temperature was too high inside the soundproof cabinet even with fans installed in the cabinet it was so high that it blew up the thermometer that was placed inside to monitor temperature.
- It was decided that the nano-MOUDI be placed outside and then the inlet tubing passed into the house through holes bored in the walls. However volunteers did not accept because their landlords refused.
- It was then decided that the nano-MOUDI should be placed outside but inlets tubing be passed through vents/extractor fan holes. The pressure of the instrument dropped once the tubing was raised very high above the ground level, especially on the first floor.

With all these bottle necks that were encountered, it was decided that we buy a more indoor and PE friendly instrument. Thus we decided on the SIOUTAS personal impactor.

While the nano-MOUDI pilot study was going on, we came up with other projects that involved only the use of the PSs; the validation of the PSs and PE exposure studies. Then we also had to wait for the approval of the fund for the SIOUTAS. When the SIOUTAS arrived the original study was carried, however, only 10 people were samples (10 warm/summer period and 10 cold/winter period) because of time constrain and six different projects had already been carried out.

However, with the new project design, the aims and objectives still remains the same. In choosing the new projects, projects with the same health outcome measurements were selected.

2.2 Study design and subjects

The validation of the PSs used for the indoor and PE measurements and six other different yet similar projects were carried out in this study and they all have their relatively different design and methodology. Details of each materials and methods are found in each individual chapter. However they have some similar instrumentation, sample collection and analysis. The similar characteristics will be discussed in this chapter.

2.2.1 Subjects

All subjects that volunteered for the entire study were healthy and non-smoking. Ethical approval was granted for the entire study. The ethical approval was granted by the Humanities and Social Sciences Ethical Review Committee University of Birmingham. The ethical approval stipulates that the Subjects be notified of what is required of them and what samples will be collected from them. If they understand and agree they consented by signing a consent form saying they understand that their homes and PE measurements will be collected alongside GPS data for their location during sampling time, urine, EBC, BP and lung function measurements. How their urine and EBC samples will be treated after use was also mentioned to them and confidentiality of the data was guaranteed. Also for their inconveniences, financial compensations were paid. Prior to selection of subject a screening questionnaire were giving to people who responded to the subject recruitment advert in order to select the appropriate subject for the study. Only those that meet the screening criteria were selected (See questionnaire template and details in the appendix). A baseline questionnaire that describes the characteristic of the subject's home and office was given to all subjects in the chapter 4 and chapter 9 because the PE sampling included their homes and offices. A set activity diary and questionnaires which included the following: activity diary, dietary questionnaires, (environmental tobacco smoke) ETS questionnaire location sheet for in

transit, location sheet for static location and sampling questionnaires was given to all subjects when they were sampled. The questionnaires were then analysed and the information obtained was used alongside the PS data for PE analysis.

2.3 Summary of individual study design

2.3.1 Chapter 3

This is an introductory study where the PSs that were to be used for the personal and indoor exposure study were validated using the reference instruments/sensors.

Table 2.1. Personal and reference sensors

Variables or metrics	Personal Sensors (number used in this study)	Reference sensors	Unit
PM_{2.5}	RTI MicroPEM: (4 sensors)	FDMS-TEOM	µg/m ³
UFP (0.3 µm)	Testo DiscMini: (3 sensors)	Condensation Particle Counter TSI 3022A (CPC) Scanning Mobility Particle Sizer Spectrometer 3080 (SMPS)	Particles/cm ³
Black Carbon	AethLabs MicroAeth AE-51: (4 sensors)	Aethalometer AE-22	µg/m ³
NO₂	Aeroqual 500 Sensor (6 sensors)	Chemiluminescence API M200E	µg/m ³

The validation exercise was done in two categories, the laboratory and the field/outdoor. The field exercise was done in two seasons: summer and winter period. In both seasons the PSs were placed alongside the reference sensors (RFSs) in 4 different monitoring sites:

Birmingham Tyburn urban Background Site (Tyburn), London Marylebone Road

(Marylebone), London North Kensington (North Kensington) and Harwell Observatory Site (Harwell). The PSs were tested for Accuracy (calculated as percentage bias) measured as difference between the reference and test instrument, precision, limit of detection (LOD), and linearity. All PSs and RFSs are discussed in details in chapter three.

2.3.2 Personal sensor Instrument description

2.3.2.1 Personal sensors

2.3.2.1.1 RTI Micro-miniature Personal Exposure Monitoring- MicroPEM

MicroPEM Model v3.2, developed by RTI International ([RTI 2008](#)), is a small light weighted (weighs under 300 g) nephelometer (using light-scattering at 780 nm for periods as short as 1 s). It measures PM_{2.5} concentration in real-time at 0.5 LPM as well as simultaneously collecting particles on a gravimetric filter (25 mm Teflon filter). It is fixed with cut-off size of 2.5 µm impactor, which allows for the detection of the particle size of interest. It has an optical bench consisting of a supporting circuit, laser and a highly sensitive photodiode (Gao, 2012). Particles filter through the impactor into the testing chamber, where they pass through a laser beam causing light scattering. The scattered light is then sensed by the photodiode and then the light intensity change is transferred into a voltage change (Gao, 2012). The supporting circuit will amplify the electrical signal and then process it. The corresponding particle concentration can be determined using the calibration curve from the optical output.

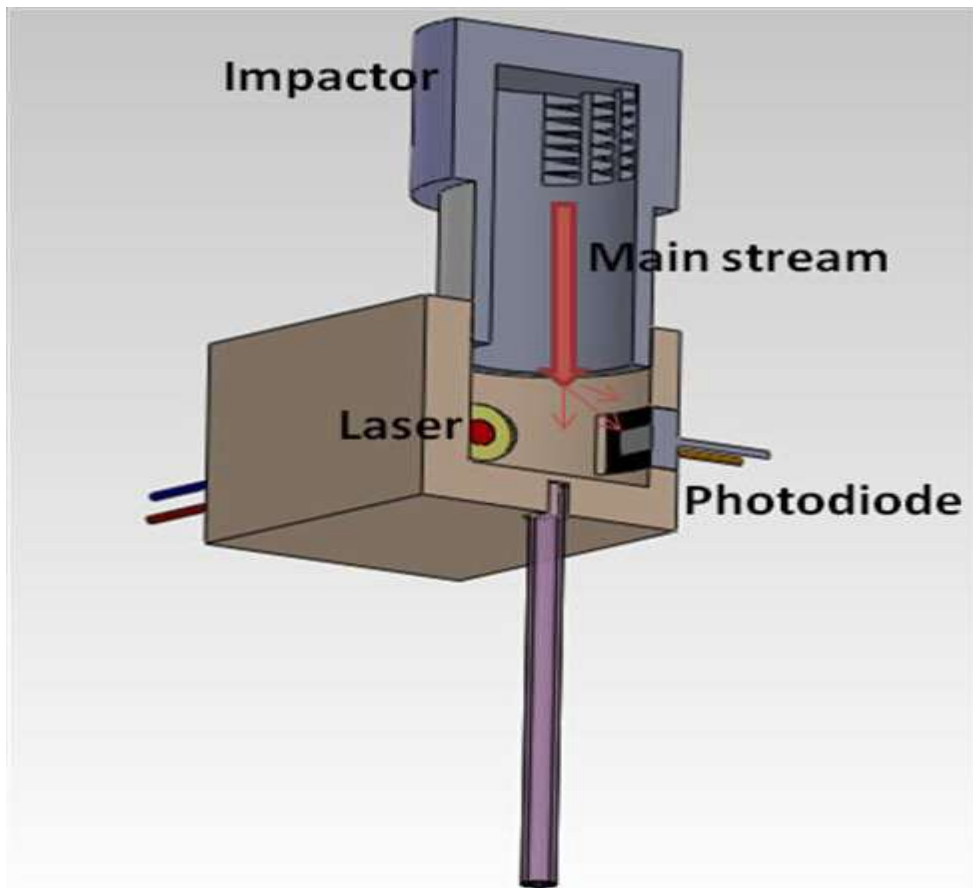
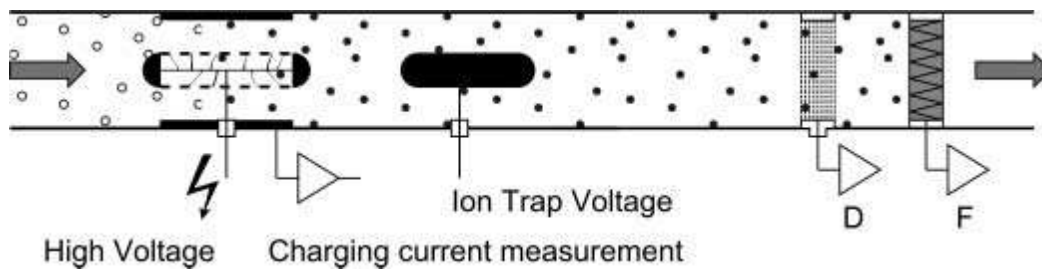


Figure 2.1 Detecting principle diagram of MicroPEM sampler (Gao, 2012)

2.3.2.1.2 Miniature Diffusion size classifier

The miniature diffusion size classifier is commercially known as the DiSCmini and available from Matter Aerosol, Wohlen, Switzerland). The DiSCmini is a handheld device. When particles get drawn into the DiSCmini, the particles get charged in a unipolar diffusion charger, the excess ions are removed in an ion trap before they are passed on to a two stage particle deposition system (Fierz et al., 2011). The first stage known as the diffusion stage (i_D current) contains a diffusion screen, where particles are deposited with a size-dependent efficiency and then to the second stage known as the filter stage (i_F current) contains a high efficiency filter: HEPA filter, on which all particles that penetrated the first stage are captured. (Kaminski et al., 2013). Both stages are connected to an electrometer that

continuously measures the current, induced by the deposited particles on the two different stages (Asbach et al., 2012, Fierz et al., 2011, Kaminski et al., 2013). From the current from these two independent measurements, the PNC (total current $i_F + i_D$) and the average particle size (ratio of the current i_F/i_D) are derived (Kaminski et al., 2013, Asbach et al., 2012). The DM measures the modal diameter. Figure 3.2 show the schematic overview of the DiSCmini. The DiSCmini is equipped with an impactor which has a cut-off of about 700 nm with an optimal measurement size range from 10 nm to 300 nm and a concentration range from 10^3 to 10^6 #/cm³. (Kaminski et al., 2013, Asbach et al., 2012). It weighs 670 g measures 4 x 9 x 18 cm.



D = diffusion stage and F = filter stage which contains the electrometer to measure current generated. Adopted from (Fierz et al., 2011).

Figure 2.2 Schematic overview of the DiSCmini:

2.3.2.1.3 MicroAethalometer AE-51 (MicroAeth)

The MicroAeth measures BC also known as EC or soot. The BC concentrations were measured using an Aethalometer AE51 (Magee Scientific), which determines BC concentration based on the Beer Lambert law, by measuring continuously light absorbed by optically-absorbing particles (Buonanno et al., 2013). The AE-51 has an 880 nm wavelength beam of light produced by an LED light source aimed at a photo diode detector (Buonanno et al., 2013). As the light travels, the light beam passes through the aerosol sample collected on a quartz filter. The attenuation of the light is linearly proportional to the amount of BC deposited on the filter (Delgado-Saborit, 2012). This device is small and portable (250 g) and

can run on battery for about 20 hours when logging on a 5 min interval and has a pump speed been set at a rate of 100 ml per minute.

When sampling at low concentrations or high time-resolution with the AE-51, instrumental and optical noise can cause the ATN to remain unchanged or slightly reduced from one time period to the next and this will lead to a negative BC concentration data as the calculation of BC concentration is dependent on the successive attenuation (Hagler et al., 2011). Find equation in the appendix. This noise may also cause a very low value at one time and then followed by a very high value at the next or vice versa (Cheng and Lin, Hagler et al., 2011). to This algorithm Optimised noise-reduction averaging (ONA) was developed by Hagler et al. (2011) eliminate the noise and negative values from the data (Hagler et al., 2011). The ONA algorithm was used in this validation study to remove the noise and the negative values from the data and then the Apte et al. (2011) correction factor was applied to eliminate the underestimation of BC concentration as filter BC mass increases, especially when sampling highly light-absorbing particles (Apte et al., 2011).

The formula is

$$BC_{Corrected} = BC_{Instrument\ reported} (0.88Tr + 0.12)^{-1} \quad (2.1)$$

Where $Tr = \exp(-ATN/100)$ is the aethalometer filter transmission that is calculated from the instrument-reported attenuation coefficient (ATN) (Apte et al., 2011).

2.3.2.1.4 Aeroqual 500

PE to nitrogen dioxide (NO₂) was performed using an Aeroqual handheld monitor Series 505 with a NO₂ NW GSS64 sensor head. This is an electrochemical sensor, consisting of: electrodes, electrolyte, and a membrane. The NO₂ gas diffuses through the membrane and reacts at the electrolyte–catalyst interface, which creates a current. Figure 3.3 show the schematic of an electrochemical cell used in electrochemical sensors. The instrument measures the current in the electrodes and translates it into gas concentration because current

output by the sensor is directly proportional to the concentration of NO₂ gas (Mead et al., 2013). The sensor operation range is 1–200 ppb. The sensor head is calibrated by the manufacturer. According to the manufacturers, the accuracy is $< \pm 1$ pbb in the range 0–100 ppb and is $< \pm 10\%$ in the range 100–200 ppb (Delgado-Saborit, 2012). It weights 460 g and has a battery that runs for 5 hours plus.

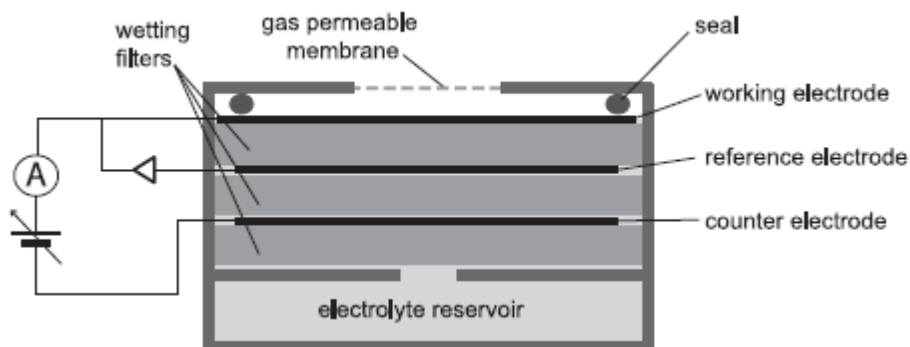


Figure 2.3. Schematic of an electrochemical cell used in electrochemical sensors (Mead et al., 2013).

2.3.3 Reference sensor description

The description of the reference sensors used in the validation study can be found in the appendix.

2.3.4 Chapter 4

A single non-smoking healthy subject was selected for this study. This was a pilot study. The UFP sensor used for this study was the Philips Aerasense Nano Tracer. This instrument was used because we wanted to buy it and thus it was being tested in this pilot study, unfortunately after this study it was discontinued and thus we did not buy it rather the DiSCmini was bought. The PSs were placed in a backpack and given to the subject to carry at all time for 9 days. The morning after each exposure measurement day, EBC and urine samples were collected (Table 2.2). The subject was given a set activity diary and questionnaires to carry around. This was to enable subject record every

activity/microenvironment entered. Subject was also given GPs receiver data logger during sampling to provide temporo-spatial data and voice recorder to facilitate the activity logging. The aims and objectives of this study and details are found in chapter 4.

2.3.5 Chapter 5

12 subjects were selected. Each of the 12 subjects was each sampled within 24 hours during their own sampling day. PSs were placed in a backpack and given to the subject to carry at all times during the sampling day. EBC and urine before and after Japanese style cooking fumes exposure were collected for biomarker analysis to enable comparison. The subjects were given a set activity diary and questionnaire to carry around during their sampling day, in order to fill in their activities. This was to enable subject record every activity/microenvironment entered, in order to identify when subjects were in the Japanese restaurant. Aims, objectives and details of this study are found in chapter 5.

2.3.6 Chapter 6

4 subjects were selected, of the 4 subjects one was the control. Only one was given the backpack containing the PE to carry. Before subjects were exposed to shisha fumes in the shisha lounge, EBC and urine samples were collected from all 4 subjects. After exposure another set of EBC and urine samples was collected for biomarker analysis to enable comparison. The PS were turned on immediately after before-exposure EBC- and urine samples were collected and turned off after subjects left the shisha lounge. Activity diary and questionnaire was filled for only when the PSs were on. This was to enable subjects record shisha exposure start time and when subjects were in the shisha lounge and when they left. The aims and objectives of this work and details are found in chapter 6.

2.3.7 Chapter 7

Fifteen subjects were selected. They were sampled in threes or two. The sampling was divided into two. The first part, each group was taken to a point close to the sampling point. They were kept indoors to relax for at least 30 mins. Then their blood pressure, lung capacity, EBC and urine samples were collected. They were each given a cardio watch to monitor their heart rate. The group was then given a back pack with the PS. They were then taken to the busy roadside: Bristol road for two hours. Their lung capacity was monitored every hour. At the end of two hours they were taken back to the rest point and after-exposure samples were then collected.

For the second part each group was taken to Calthrope Park as the control to the busy roadside and the same sample collection process was repeated. The control exposure was done on a different day from the busy road side exposure. The aims and objectives of this work and details are found in chapter 7.

2.3.8 Chapter 8

Two subjects were selected.

2.3.8.1 Subject 1

Subject 1 was sampled for a period of three weeks. The PSs were placed in a backpack and given to the subject. The subject was given a set activity diary and questionnaires to carry around and fill in activity detail. This was to enable subject record every activity/microenvironment entered, in order to identify when subject was in the transportation mode and enable analysis of the various transportation modes. Subjects were also given GPS receiver data logger during sampling to provide temporo-spatial data and voice recorder to facilitate the activity logging.

2.3.8.2 Subject 2

Subject 2 was sampled for 6 days of daily train travel. Journey start time and end time was recorded in the activity diary and questionnaire. PM_{2.5} cyclone head attached to a pump to measure PM_{2.5} and BC was given to the subject to carry all through the journey time.

Morning after exposure urine was collected from subject 1, while for subject 2, morning after exposure urine and EBC samples were collected. Lung function parameters were measured for both Subject 1 and subject 2 before, during and after the journey. The aims and objectives of this work and details are found in chapter 8.

2.3.9 Chapter 9

10 volunteers with their home free of tobacco were selected. Their homes were not higher than two storeys in order to avoid dilution of air pollution at higher levels. This sampling was done in the warm and cold season i.e 10 subjects warm/summer and the same in the cold/winter season. The SIOUTAS impactor was used as the fixed sampler that was placed in the homes of each subject.

The SIOUTAS impactor was used as the fixed sampler that was placed in the homes of each subject. The sampling was for 7 days maximum non-stop, except when not possible.

Then early morning EBC and urine samples were collected on the start day, the middle and at the end of the sampling. The aims and objectives of this chapter and details is found in chapter 9.

2.3.10 Description of the SIOUTAS impactor

The SIOUTAS personal cascade impactor (SKC, Inc.) was used to separate and collect particles in five size ranges from > 2.5 , 1.0 to 2.5 , 0.50 to 1.0 , 0.25 to 0.50 , and < 0.25 micron. Particles above each cut-point are collected on a 25-mm PTFE filter (with PTFE support, $0.5\text{ }\mu\text{m}$, product code 225-2708, SKC) in each appropriate stage and then the particles $<$ the 0.25 -micron cut-point of the last stage, are collected on a 37-mm PTFE (with PMP support ring, $2\text{ }\mu\text{m}$, Product code 225-1709, SKC) after-filter. This last stage $< 0.25\text{ }\mu\text{m}$, in this study may be referred to as after-filter or outlet stage. The SIOUTAS Cascade impactor in this sampling was used with the Leland Legacy® Sample Pump (SKC Cat. No. 100-3002), but the SIOUTAS impactor can be used with any other pump capable of maintaining 9LPM flow rate.

The particles in the SIOUTAS cascade impactor are classified into aerodynamic sizes. A jet of particle-laden air is drawn in through the nozzle in stage A, where the lighter particles remain in the air flow stream (i.e. particles smaller than a certain size defined as the impactor's cutpoint) and the larger particles ($\geq$ to the cut-point) due to inertia are collected on the collection stage.

The Size-fractionated samples obtained from this impactor were analyzed gravimetrically, chemically, and with the TEM. Figure 2.4 show the schematics of the SIOUTAS impactor.

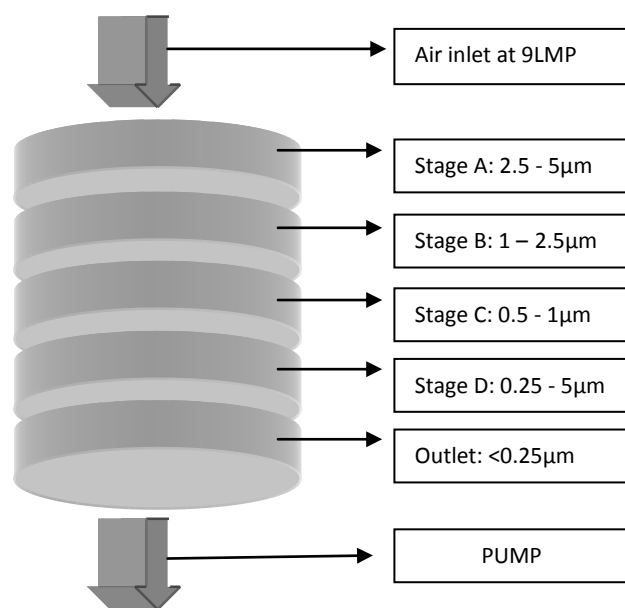


Figure 2.4. Schematic representation of the Personal cascade SIOUTAS impactor

Table 2.2 Table summarising all studies cohort size and sampling time

Thesis Chapter	Sampling population size	Markers analysed	Marker collection time
4	1	EBC: nitrite, nitrate and pH. Urine: 8-oxodG	Morning after exposure
5	12	EBC: nitrite, nitrate and pH. Urine: 8-oxodG	Before and after exposure
6	4	EBC: nitrite, nitrate and pH. Urine: 8-oxodG	Before and after exposure
7	15	EBC: nitrite, nitrate and pH. Urine: 8-oxodG and metals in urine Lung function: FEV ₁ , FEV ₆ , FEV ₁ /FEV ₆ , FEF25-75%, BP, pulse rate, Estimated V _E and estimated inhaled load.	Before and after exposure
8	2	Urine: 8-oxodG Lung function: FEV ₁ , FEV ₆ , ratio FEV ₁ /FEV ₆ , FEF25-75%	Urine was collected morning after exposure while lung function parameters were measured before, during and after exposure
9	10	EBC: nitrite, nitrate and pH. Urine: 8-oxodG and metals in urine	First sample collected at the beginning of sampling. Second sample collected midway sampling. Third sample collected on the final day. (all samples were collected early morning)

See Figure 1.1 for chapter title. (FEV₁) - forced expiratory volume in one second; (FEV₆) - forced expiratory volume in six second; (FEF25-75%) - forced expiratory flow; (BP) – Blood pressure

2.4 Other instrument of interest

2.4.1 Sunset organic carbon/elemental carbon (OC/EC) analyser

The Sunset OC/EC was used for EC analysis under the EUSAAR_2 protocol (European supersite for atmospheric aerosol research). The OC/EC instrument uses the thermal optical analysis technique. A punched area of the quartz filter is placed in a quartz oven, where the organic and elemental carbon components of the particulates on the filter is analysed by a thermal-optical analysis. The thermally desorbed carbon components are converted to CO₂ and then methane which is then quantified by the flame ionisation detector (FID). In the first stage of analysis based on filter absorbance measurement, external standard (sucrose solution) and internal standard (methane) are used for the FID. The pyrolysis step; the second stage, most of the OC tends to come out at a lower temperature in an oxygen free atmosphere (non-oxidising carrier gas like He); therefore, it can be separated from EC. What is left on the filter; the EC is then removed from the filter under oxidising carrier gas He/O₂ optically carbon is removed at high temperature.

2.4.2 The UFP sensor: Philips Aerasense NanoTracer

This instrument was used only in chapter 4. The Philips NanoTracer (NT) measures is a small, handheld or portable sensor that measures UFP particle number count from 0 - 10⁶ cm³ in the particle size range of 10 -300nm (Mazaheri et al., 2012, Buonanno et al., 2014a). In this study the sensor was set in the advanced mode so that it can log data for both PNC and average particle size simultaneously and it logged data at 16s intervals. Mode of operation of this instrument is found in the appendix.

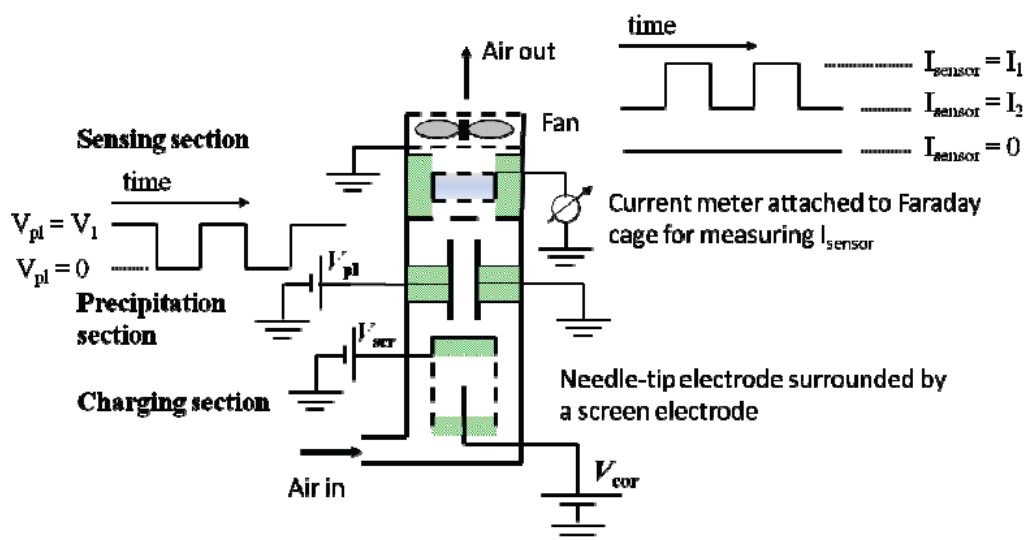


Figure 2.5 The schematics of the Aerasense Nano Tracer adopted from Marra et al. (2011)

2.5 Exhaled breath condensate sample collection

EBC was collected for measurement of NO_2^- , NO_3^- and EBC-pH using a system developed in Birmingham. It was adopted from the RUPIOH (Relationship between Ultrafine and fine Particulate matter in Indoor and Outdoor air and respiratory Health) study in Birmingham (Manney et al., 2012). This comprised of Teflon tubes of 7.9 mm diameter (Du Pont, Wilmington, Delaware, USA) submerged in a polystyrene container of ice/ice packs and water. The Teflon tube was attached to a mouthpiece that was attached to a two-way non-rebreathing valve (Intersurgical Ltd Berkshire, England). Subjects were asked to breathe through the mouthpiece while wearing a nose clip for 15 mins. Within the 15 mins breathing, whenever subject felt discomfort they were allowed to stop and then continue almost immediately. At the end of the 15 mins sampling period, samples were tipped from the end of the tubes into 4 ml polypropylene tubes, which were kept on ice or field freezer and returned as soon as possible to the laboratory where they were kept at -80°C . This method produces an average volume of 1.5 ml of condensate in laboratory studies agreeing with other studies

like Manney et al. (2012). Saliva contamination was a problem for some subjects. Therefore by visual examination, all samples that contained saliva (frothy, opaque appearance with more viscous properties) were discarded (Manney et al., 2012).

2.5.1 Exhaled breath condensate analytical method

2.5.1.1 Ion chromatography

Ion chromatography with a conductivity measurement analyser (Dionex ICS-2100) was used to measure the concentration of NO_2^- and NO_3^- in the EBC. The method used has been previously used by other studies (Corradi et al., 2003). Briefly, the EBC was brought out of the freezer and allowed to thaw. Then 0.2 ml of EBC was diluted to 4 ml in a 5 ml vial with deionised water. These are then loaded into the autosampler. The analyser uses an anion exchange column (AS11HC) which separates the anions which are then detected using the conductivity analyser. The chromeleon software post processes the resulting chromatograms. It then determines the anion concentration by reference to the calibration standard (0.1 – 10 ppm). The data was then retrieved and used for comparative study in the different projects. The nitrate and nitrite concentrations were expressed in ppm (parts per million) and then converted micromole. Each EBC samples was run in triplicates and the average was obtained and used as the final concentration.

2.5.1.2 pH analysis

Thawed EBC was transferred to 2ml vial and then de-aerated with nitrogen gas (>90%) purity bubbling through the EBC for 5 mins. pH analysis was performed with a thin and sensitive glass electrode (Thermo scientific Orion semi micro glass probe) and pH meter (Thermo Orion 3 star). De-aeration of EBC samples removes CO_2 from the sample, thus allowing standardization of measurements of EBC samples. The hydrogen concentration of each

sample was measured in triplicates and the average was obtained and then used for the analysis.

2.6 Urine sample collection and analysis

All urine samples were collected in a urine specimen container. The urine was then transferred to 5 ml vial (5 aliquots were made for each subject) and then place on ice packs or field freezer. It is then transferred to the laboratory as soon as possible where it is stored at -80°C. All the urine samples as a single batch was then shipped to the laboratory at the Chung Shan Medical University, Taichung Taiwan for the 8-oxodG and creatinine analysis by on-line solid-phase extraction (SPE) and isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) which gives an automated measurement of 8-oxodG (Hu et al., 2006). This method has been described in details in a previous study (Hu et al., 2006). The creatinine analysis was done in the same laboratory at Taiwan using a reported high performance liquid chromatography with ultraviolet detector (HPLC-UV) method (Pant and Harrison, 2013). The urine analysis was done by Profs. Chiung-Wen Hu and Mu-Rong Chao from Chung Shan Medical University and the LOD and methodology have been reported in their previous study (Hu et al., 2006).

The urinary metal analysis for the urine collected from the subjects were analysed by Dr. Nicholas J. Miller of Biolab Medical Unit London UK using the Inductively Couple Plasma Mass Spectrometry (ICP-MS) method. The urinary metals analysed are sodium (Na), Magnesium (Mg), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), tin (Sn), antimony (Sb) and lead (Pb).

2.7 Lung function and blood pressure measurement and instrument

The vitalograph lung monitor was used to measure the lung function. The subject exhaled into the disposable mouth piece attached to the monitor and the monitor display and also saves the different lung function parameters used and discussed in chapter 7 and chapter 8.

The Blood pressure was measured with a digital monitor arm-type blood pressure monitor by Value health. This monitor also measured the pulse-rate.

3 VALIDATION, QUALITY ASSURANCE AND INSTRUMENTATION DESIGN OF PERSON SENSORS FOR USE IN THE PERSONAL EXPOSURE SAMPLING

3.1 Introduction

The general notion is that exposure of humans to air pollution especially in the fine to ultrafine range have the ability to evoke deleterious health effects associated with morbidity and mortality. Epidemiological studies of the impact of these exposures on human health has been studied using the ambient air PM concentrations (McCreddin et al., 2015) among other pollutants. Air quality is affected by pollutants such as nitrogen dioxide (NO₂), PM which include UFP, fine particles and PM marker pollutants like BC and EC (Steinle et al., 2013a, Delgado-Saborit, 2012, Wheeler et al., 2011) and they in turn affect health quality (Wheeler et al., 2011).

Mostly PE of individuals to air pollutants has been measured by estimating population-wide exposure via networks of fixed monitoring sites of which were created to evaluate compliance with air quality standards and not to examine population exposure (Boudet et al., 2001, Steinle et al., 2013a). The majority of the epidemiological studies on impact of air pollution exposure have been studied using surrogate, ambient and or modelled exposure from central/fixed sites measured concentrations (Laden et al., 2006, Pope et al., 2002). The advantage of these fixed network sites usually provide a large quantity of data for a wide range of pollutant but from a particular spatial jurisdiction (Steinle et al., 2013a). However using FS data as exposure estimates ignores the impact of individual mobility patterns, time spent at home and other indoor environments such as offices and in-vehicle environment. Some studies have argued that the central FS do not represent the entire population exposure to ambient PM because there could be different spatio-temporal mobility patterns among various individuals (Boudet et al., 2001, Steinle et al., 2013a). Also due to some differences

between pollutants measured at a FS and PE monitoring, some of the epidemiological results may be considered as a weak representative thereby may lead to a biased conclusion in terms of health outcomes (McCreddin et al., 2015). However, the estimations obtained from these FS have been the basis for a significant body of epidemiological evidence on the links between air pollution exposure and health impacts and have significant results in the field of air pollution science (McCreddin et al., 2015, Dockery et al., 1993).

While the use of surrogate or proxy ambient or modelled exposure still have numerous strength today, the use of personal and wearable sensors can be used to alleviate the limitations involved in characterising exposure with the surrogate/proxy ambient or modelled exposure. Most of these personal and wearable sensors provide continuous real-time information i.e. providing information on where and when exposures occurred. Thereby providing temporal and spatial information on PE which will help explain factors that affect exposure i.e. activities/environment. They are small, easy to use and therefore may require less professional attention during sampling. However in reality, these sensors may show partial failures such as bias, drifting, complete failure or partial degradation which may affect accuracy and reliability of the data generated by these sensors thus resulting in a biased interpretation of the results (Liu et al., 2011). Also complete unavailability of data for download and analysis due to sensor malfunction (Liu et al., 2011).

3.2 Aim and objectives

The aim of this chapter is to validate the PSs to be used for the PE and indoor concentrations characteristics against the recognised reference methods, in order to confirm their accuracy and reliability.

Therefore the specific objective of this study is to test the measurements generated by these PSs for the following

- accuracy
- Precision
- Limit of detection (LOD)
- Linear fit (Linearity)

3.3 Materials and methods

3.3.1 Study Design

This validation study took place in 2013 – 2014. For the field study, four outdoor monitoring stations with contrasting traffic were selected for the instruments to be tested over the warm season (May - September) and the cold season (November - February) so as to expect different air pollutant concentrations and meteorological conditions. The selected sites are:

Birmingham Tyburn Background Site (Tyburn) an urban background site, London

Marylebone Road (Marylebone) a kerbside station, *London North Kensington (North*

Kensington), an urban background site and *Harwell Observatory Site (Harwell)*, a rural site.

Detailed description of sites is found in the appendix.

The PSs sampled alongside the RFSs for 14 days at each of the four sites during the cold and the warm seasons, except in cases where either the PS or RFS broke down and where some data could not be downloaded from either sensor because of system malfunction. The PS and RFS used in this study are listed in Table 2.1.

3.3.2 Instrumentation

Instrumentations used for the validation study are described in chapter 2.

3.3.3 Off-line validation Tests

Off-line validation tests were carried out for the PM_{2.5} and BC sensors.

The *PM_{2.5} MicroPEM* sensor was further validated by comparing the integrated PM_{2.5} sensor readings with the concentrations determined by gravimetric analysis. The gravimetric values were provided by the sensor's own downstream filter (integrated filter) and by a second Teflon filter collected on separate sampling equipment consisting of a PM_{2.5} cyclone/filter holder/pump sampling at 2 L/min continuously for 7 days.

The *microaethalometer* was further validated by comparing the integrated mass of BC measured by the microaethalometer with the EC concentrations determined by chemical analysis using a Sunset OC/EC analyser of a quartz filter sample collected on separate gravimetric sampling equipment consisting of a filter holder and a pump continuously sampling for 7 days at 3 L/min.

3.3.4 Laboratory validation experiment

For the laboratory study, laboratory experiments were implemented to further validate the UFP sensors by comparing the UFP concentrations and average diameter measured by the DISCmini sensor with those measured by a SMPS (with a DMA- differential mobility analyser connected to a CPC after being challenged with Di-Ethyl-Hexyl-Sebacate (DEHS). In, the following performance characteristics were tested:

- Accuracy (calculated as percentage bias) measured as difference between the reference and test instrument
- Precision
- limit of detection (LOD)
- Linear fit (linearity)

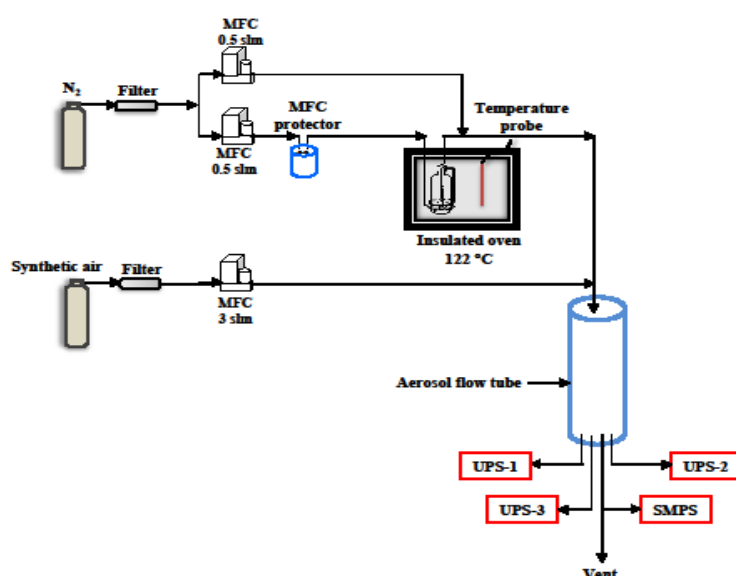
The laboratory experiments process includes:

The *DISCmini UFP sensors* were validated in the laboratory by comparing the UFP concentrations and average diameter measured by the DISCmini sensor with those measured by a SMPS (with a DMA connected to a CPC) after being challenged with DEHS (also known as Bis-(2-ethylhexyl) sebacate) particles generated in the lab.

Figure 3.1 shows schematics of the set up used to generate DEHS aerosol. The setup generate the aerosol via nucleation after heating the DEHS liquid in an oven and passing a stream of air or N₂ over the liquid surface to generate vapour condensation of DEHS. The DISCminis

and the SMPS (with a DMA connected to a CPC) instruments were connected to the set up to measure the particle counts and average size diameter.

The concentration of particle counts and average size diameter measured by the DISCmini sensor were compared against those measured by the SMPS (with a DMA connected to a CPC) instruments.



MFC: mass flow controller; **SMPS:** Scanning mobility particle sizer; **slm:** standard litre per minute; **UFPs:** Ultrafine particle sensor. Schematics representation designed by Suad Alkindi.

Figure 3.1. Experimental setup for the UFP sensor calibration

3.3.4.1 Laboratory calibration of the ultrafine particle sensors – Setup:

5 ml of DEHS were introduced into an organic bubbler, which was subsequently inserted in a polydisperse aerosol generation oven reaching a temperature of 122°C (+/-3°C). The aerosol generated was carried by a stream of N₂ gas (0.5 L/min). An additional flow of N₂ gas (0.5 L/min) was introduced at the exit of the oven to dilute the generated aerosol. Further dilution was achieved by introducing 3 L/min of synthetic air into the line prior to entrance into the top of the sampling chamber. This consisted of a glass sealed tube of 15 cm diameter and 100

cm length. At the bottom of the chamber four inlets were connected: one for the SMPS/CPC system, and the other three for the three UFP sensors. Five experiments were conducted consisting each in two cycles of on/off generation of UFP as follows:

- 42 minutes of aerosol generation
- 24 minutes where the sampling chamber or aerosol flow tube was disconnected from the aerosol generator to allow a null particles atmosphere.
- 24 minutes of aerosol generation
- 24 minutes where the sampling chamber was disconnected from the aerosol generator to allow measurement of the decay of particles concentrations to null.

3.3.5 Data analysis

3.3.5.1 Laboratory validation

For the laboratory validation of the DiSCmini, various parameters were checked:

3.3.5.1.1 Bias percentage

In this study accuracy of the PS DiSCmini were calculated as the bias percentage of the sensors from the RFS (SMPS/CPC system). This bias was calculated by subtracting the measurement of the RFS known as the True value T from the measurement of the PS known as actual value A and then dividing by the True value T. In this case the median measurements for the entire sampling period for each cycle of each instrument (both PSs and the RFS) were used. The median was used in order to eliminate outliers. This method was adopted from previous studies (Wallace et al., 2011, Wheeler et al., 2011).

$$Bias = \frac{A-T}{T} * 100 \quad (3.1)$$

3.3.5.1.2 Precision

For the precision since we had to check three instruments placed side by side and sampling under the same condition, we adopted the wheeler et al. (2011) approach, known as the Bias-corrected precision. The instruments were first corrected for bias before precision was calculated. Details of this method of precision calculation for multiple sensors can be seen in Wheeler et al. (2011).

$$Precision = \frac{Abs[A' - T]}{T} \quad (3.2)$$

A' is the bias-corrected value of the PS. True value T is the median of the reference instrument. The bias was corrected by: (a) calculating the median of each co-located PS during the sampling session (b) ratio of the mean of all of the PSs' medians to the mean of each PS (c) the ratio multiply by the mean of each instrument to get the corrected bias then (d) apply corrected bias to equation 3.2 above to get precision (Wheeler et al., 2011, Wallace et al., 2011).

3.3.5.1.3 Limit of detection (LOD)

The DM is continuous sampler and it is not integrated with a filter based system which allows particle masses collected on the filter to be weighed and used to determine LOD. Fierz et al. (2011) suggested that using the standard convention that LOD is given by 3σ and that a signal of $\sim 1\text{fA}$ on the two electrometer stages of the DM is required for a sensible measurement. In order to measure the LOD, particles were generated and then the system was turned off for the particles to decay. As the particles decayed, it came to a point where the DM steadily measured PNC was $< 10^1 \text{ \#}/\text{cm}^3$ and the measured particle size was 0 nm. That is the DM could no longer detect particles. Note that the DM cannot detect particles less than 5 nm (Fierz et al., 2011) (remember that optimal detection is between 10 nm - 300nm) therefore in

the instrument data output any particle less than 5 was reported as 0 nm. Also the particles < 5 nm may not be able to generate enough current to allow the DM to give a sensible PNC reading. Thus based on this it was assumed that the air was relatively free from particles. The standard deviation (σ) value of the diffusion stage current i_D and filtration stage current i_F for 20 mins, for each DM and each decay sessions of the 5 cycles were calculated. Then 3 multiplied by σ of i_D and σ of i_F respectively to get the LOD. Then the 50 percentile of the values obtained from all the cycles of the 3 DMs was considered the practical LOD of the DMs.

To compare with the SMPS the average concentrations of all the DMs in each decay session of the 5 cycles were measured. A simple bar graph was used to illustrate which instrument was more sensitive to the PNC during the decay.

3.3.5.1.4 Linearity fit

The Regression analysis ordinary least square (OLS) was used to study the comparison between the PNC of the PSs and the RFS SMPS while the Reduced major axis (RMA) was used for the particle size inter-comparison. The RMA was chosen for the size comparison because it was assumed that deviation from the fitted regression line could be due to the y-axis (DM) or the x-axis (SMPS). Therefore to minimise this deviation RMA was used. For the size inter-comparison, the sizes were compared when particles were generated i.e., when there were peaks in each cycle and is called 1st and 2nd generation. Also size comparison was done for the entire cycle i.e., both when particles were generated and decayed for each of the 5 cycles and was called cycle 1 through to cycle 5 respectively.

OLS regression was used to comparison between all three DMs and RMA was used for the size comparison of all three DMs.

3.3.5.2 Field validation

For the MicroPEM and Aeroqual sensors, obvious outliers, negative values and invalid data-points were eliminated from the data-set. Then Bias percentage as accuracy and Linearity fit were calculated in reference to the FDMS-TEOM and chemiluminescence AP1 M200E respectively.

The MicroPEM can also be used to collect integrated PM samples using a Teflon filter. The gravimetric PEMs i.e. the integrated PM collection was measured. The Gravimetric measurements were compared with the online using spearman's correlation and difference of means using the Mann-Whitney U test.

The data from the microAethalometers were corrected as described in section 2.3.2.1.3 only the linearity fit was analysed with the RFSs.

3.3.5.3 Off-line validation

The gravimetric result from the Teflon filter that was placed in the cyclone were obtained and then compared with the PEM using OLS.

The integrated BC collected by the MicroAeth were then compared (using OLS) against the EC obtained from samples collected on a quartz filter.

3.4 Results

3.4.1 Laboratory validation

3.4.1.1 Concentration trend

The three DMs were simultaneously tested alongside the SMPS. They were challenged with DEHS and the resulting concentration profiles are shown in Figure 3.5. The DMs and the SMPS show very similar result pattern however difference in concentration count is due to the way the instrument functions. That is the DM measures particle size optimally at 10 – 300nm, so for measurements > 300 nm, it assigns a larger charge thereby lowering the size and then overestimating PNC. It also shows that the time response of the DM is fast enough to follow the changes during the generation and decay of particles. Figure 3.5 also shows that with concentrations $> 1.5 \times 10^5 \text{ \#/cm}^3$, as in the case in cycle 1 and cycle 2, the DM sensors showed decreasing accuracy and precision. While Asbach et al. (2012) in their study indicated that the DiSCmini show decreasing accuracy for lower concentration, which they attributed to signal to noise ratio in the electrometer.

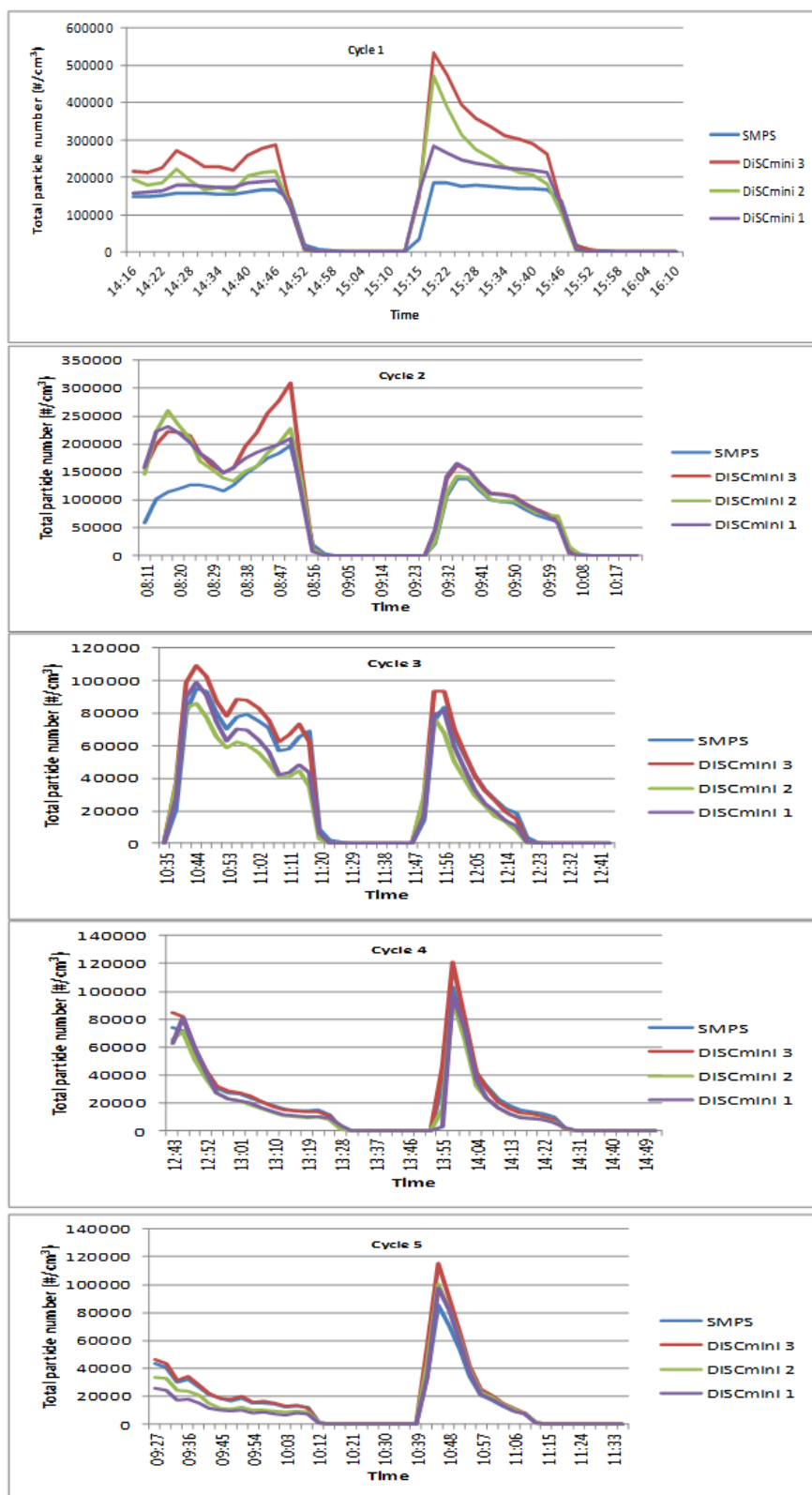


Figure 3.2. Particle number concentrations (PNCs) measured by the DiSCmini sensors and SMPS during the 5 cycles run

3.4.1.2 Linearity, bias and precision

The R^2 value for the concentration comparison between DM and SMPS was calculated for each cycle based 3 mins average. A cycle represents 2 sets of particle generation and decay. Regression analyses were carried out as shown in Table 3.1. The idea was to get a linear fit for the concentrations of the DM and RFS. The data fit, i.e., the regression coefficient is used to describe the comparability of the measurement. Ideally the comparability of all the data points should fall into the 1:1 line, i.e., giving a R^2 value of 1 and intercept zero. However a 1:1 was not achieved but a high linear relationship between all three DMs and the SMPS was achieved. The R^2 values in Table 3.1 are very similar to values observed by other researchers. For example a study showed the R^2 value for DM against SMPS for poly-dispersed metal and NaCl particles to be 1 and mono-dispersed NaCl particle to be 0.99 but the particles in this study are DEHS poly-dispersed particle (Borak, 2009). Fierz et al. (2011) in a near busy road study in Zurich, DM was calibrated using sample with specific particle size distribution diameter range of 10 – 300 nm with Geometric standard deviation (GSD) (σ_g) = 1.7 and σ_g =2, they arrived at a comparison of PNC with R^2 = 0.97. In another study the correlation of the DM and SMPS PNC in adapted size range (16 – 311 nm) was r =0.98 which is R^2 =0.96 and size range of (10 -1110 nm) was R^2 = 0.90 (Meier et al., 2013). Where as in this case the particle size generated was not restricted and the DM was used in the size range calibrated by the manufacturer i.e., with a cut-off of about 700 nm and with a most favourable measurement size range of 10 nm to 300 nm and a concentration range from 10^3 to 10^6 #/cm³. However the σ_g measured by the SMPS for the DEHS particles that were generated during this study for all the 5 cycles were between 1.46 - 1.62. The particle size range for the SMPS setup in this study was 15.1 – 661 nm. The slope observed was between 0.808 to 1.802 (Table 3.1) suggesting variations between the DMs. These variations in the DM can also be seen in Figure 3.1, where cycle 1 and cycle 2 have a broader size distribution while cycle 3 to

cycle 5 shows a narrower size distribution due to the GSD of the particle size that was measured. Thus cycle 1 and cycle 2, show similar size distribution and then cycle 3 to cycle 5 show similar narrower distribution.

Table 3.1. Regression analysis of the UFP sensors against the SMPS during the laboratory calibration experiment

Cycle	Min – Max SMPS concentration	Min – Max DM concentration	DISCmini ID	Regression coefficients Slope(x)	R ²
1	$7 * 10^0 - 1.8 * 10^5$	$7 * 10^0 - 5.3 * 10^5$	DISCmini 1	$1.217x + 534$	0.92
			DISCmini 2	$1.407x - 3537$	0.81
			DISCmini 3	$1.802x - 6181$	0.86
2	$9 * 10^0 - 1.9 * 10^5$	$4 * 10^0 - 3.0 * 10^5$	DISCmini 1	$1.236x + 4041$	0.87
			DISCmini 2	$1.196x + 3650$	0.83
			DISCmini 3	$1.431x - 2387$	0.92
3	$5 * 10^0 - 0.9 * 10^5$	$9 * 10^0 - 1.1 * 10^5$	DISCmini 1	$0.919x - 1243$	0.96
			DISCmini 2	$0.808x - 310$	0.93
			DISCmini 3	$1.116x - 456$	0.99
4	$3 * 10^0 - 1.0 * 10^5$	$5 * 10^0 - 1.2 * 10^5$	DISCmini 1	$0.943x - 1888$	0.94
			DISCmini 2	$0.915x - 1701$	0.98
			DISCmini 3	$1.147x - 1524$	0.99
5	$1 * 10^0 - 0.8 * 10^5$	$6 * 10^0 - 1.2 * 10^5$	DISCmini 1	$0.983 x - 3027$	0.91
			DISCmini 2	$1.026 x - 2165$	0.89
			DISCmini 3	$1.189 x - 1477$	0.90

Regression coefficient x is the SMPS

The precision in this study ranged from 19 to 37% as shown in Table 3.2 below.

Table 3.2 also shows the bias ranging from – 39% to 43%. With DM3 in cycle 1, DM1 and DM2 in cycle 4 and DM1 in cycle 5 above the manufacturers $\pm 30\%$ specified accuracy range.

This means that DM 3 and DM2 80% of the time were within the $\pm 30\%$ of the reference value and DM1 was 60% of the time within the reference value for the number concentration.

The three DMs show different level of correlation with the SMPS, however this laboratory study also agrees with other studies that the DM accuracy is usually in the $\pm 30\%$ range

agreement of the manufacturer but in few exceptions were noted (Asbach et al., 2012,

Kaminski et al., 2013). Asbach et al. (2012) measured the performance of DM against CPC

with NaCl, DEHS and soot. They observed that DEHS concentration was underestimated

$\sim 8\%$ as compared to NaCl and soot. They therefore suggested that the accuracy of the DM is

likely dependent on particle size and not shape because the 3 substances compared had

different shapes. Thus the higher biases may be artefacts of the particle size measured by the

DM, i.e. the size distribution of the DEHS particle sizes generated were above the 10 – 300

nm optimal DM measurements (Table 3.3). Remember PNC of the DM is inferred from size

(equation 3.3 to 3.7). The upper DEHS size generated by the set up was > 300 nm (Table

3.3), therefore the DM underestimated those sizes reducing them to 300 nm (i.e. the > 300

nm particles carry more charge, thus the DM has more count thereby overestimating

concentration), thus could be the reason for the bias deviations observed. The ± 30 accuracy

is higher than the ± 20 DQC which was the target for this study. Looking at our study target

might have been overambitious for the DM.

Table 3.2. Instrument bias and precision for concentration

Cycle	Instrument	Bias (%)	Precision (%)
1	DISCmini 1	7	19
	DISCmini 2	10	19
	DISCmini 3	43	19
2	DISCmini 1	26	24
	DISCmini 2	18	24
	DISCmini 3	29	24
3	DISCmini 1	-16	37
	DISCmini 2	-14	37
	DISCmini 3	17	37
4	DISCmini 1	-35	26
	DISCmini 2	-34	26
	DISCmini 3	-11	26
5	DISCmini 1	-39	21
	DISCmini 2	-25	21
	DISCmini 3	1	21

When the DM is not underestimating size i.e. capping sizes > 300 nm to 300 nm (in comparison with the sizes measured with the SMPS) and/or measuring particles ≤ 100 nm it shows a fair to strong linear relationship (R^2 values coloured yellow) (Table 3.3). Thus, the correlation between DM and SMPS measured size maybe affected by the DM behaviour with size measurement. Therefore leading to a size bias as reported in Table 3.4. Asbach et al. (2012) in their study used a specific size of 30 nm – 180nm DEHS to challenge and

suggested that the DM fits with the CPC and other instruments when within this range. However when larger amount of particle size exceeds the upper limit it shows significant deviations (Asbach et al., 2012). Fierz et al. (2011) recommended, the DM should be used for aerosols with a mean diameter near 100 nm, another study agreed that the DM operates best with aerosols having a mean diameter near 100 nm, but is also effective at measuring particle sizes near 30 nm (Kato et al., 2009). Mills (2013) suggested that test aerosol that included more > 300 nm particles, show uncertainty with the DM thereby affects the size measurement capabilities. Mills (2013), therefore recommend caution when measuring aerosols with a substantial number of particles > 300 nm. Table 3.3 show that size measured by the DM is in a wide range with minimum diameter below 100 nm and maximum beyond 300 nm indicating that the average is above Fierz et al. (2011) and Mills (2013) recommendations. This therefore may have caused the size bias reported in this study (Table 3.4).

Table 3.3.Reduced major axis regression for size comparison of the UFP sensors against the SMPS during the laboratory calibration experiment (size in nm)

Cycle ^SMPS Min – Max size	Min – Max size DM measurement	DISCmini ID	Regression coefficients	R ²
1 st generation of cycle 1 ^215 - 347	41 -299	DM1	1.487x - 211	0.89
	111 – 300	DM2	1.351x - 161	0.39
	179 - 300	DM3	1.679x - 306	0.26
2 nd generation of cycle 1 ^206 - 450	87 – 240	DM1	0.615x + 0.509	0.29
	118 – 300	DM2	0.884x - 90	0.17
	103 - 300	DM3	0.846x - 95	0.29
Cycle 1 ^164 - 450	0 - 299	DM1	1.542x - 277	0.65
	0 – 300	DM2	1.625x - 301	0.43
	0 – 300	DM3	1.433x - 267	0.31
1 st generation of cycle 2 ^180 - 467	107 – 226	DM1	0.478x + 49	0.14
	41 - 300	DM2	0.154x + 95	0.00
	24 – 300	DM3	0.873x - 48	0.33
2 nd generation cycle 2 ^132 - 312	101 – 221	DM1	0.894x - 20	0.31
	164 – 295	DM2	1.153x - 28	0.08
	195 -298	DM3	1.313x - 62	0.31
Cycle 2 ^132 - 467	0 – 225	DM1	1.099x - 131	0.43
	0 – 300	DM2	1.409x - 179	0.47
	0 – 300	DM3	1.404x - 178	0.45
1 st generation cycle 3 ^182 -279	100 – 273	DM1	1.837x - 169	0.54
	300 – 300	DM2	4.029x - 624	0.05
	150 - 281	DM3	1.489x - 88	0.39
2 nd generation cycle 3 ^227 - 318	273 – 300	DM1	3.219x - 517	0.02
	200 – 300	DM2	3.615x - 622	0.46
	265 – 299	DM3	4.666x - 857	0.27
Cycle 3 ^63 - 318	0 – 300	DM1	2.630x - 398	0.37
	0 – 300	DM2	2.931x - 417	0.04
	0 - 299	DM3	2.669x - 396	0.40
1 st generation cycle 4 ^221 - 264	100 – 300	DM1	7.691x - 1696	0.68
	200 – 300	DM2	5.663x - 1165	0.27
	100 – 300	DM3	7.525x - 1643	0.56
2 nd generation cycle 4 ^209 - 236	100 – 300	DM1	4.369x - 741	0.65
	100 – 300	DM2	4.237x - 716	0.93
	300 -300	DM3	5.046x - 908	0.09
Cycle 4 ^33 - 287	0 – 300	DM1	2.513x - 379	0.35
	0 – 300	DM2	2.638x - 440	0.26
	0 - 300	DM3	2.509x - 371	0.38
1 st generation cycle 5 ^180 - 194	100 – 300	DM1	15.105x - 2608	0.93
	200 – 300	DM2	7.80x - 1225	0.77
	200 – 217	DM3	1.466x - 71	0.43
2 nd generation cycle 5 ^167 - 193	84 – 195	DM1	3.074x - 399	0.66
	134 – 300	DM2	3.711x - 493	0.40
	129 - 201	DM3	1.963x - 184	0.76
Cycle 5 ^47 – 194	0 – 300	DM1	3.178x - 384	0.46
	0 – 300	DM2	3.037x - 361	0.49
	0 - 217	DM3	2.433x - 284	0.51

When the DM gives particle size as zero it is below the DM detectable limit therefore instrument software reports that as 0 in the data output, ^ Minimum – Maximum SMPS size range, 1st and 2nd generation means only generation peaks, Cycle includes generation peaks and decay, Regression coefficient X is the SMPS

The manufacture reports a size accuracy of $\pm 30\%$. A study reported that accuracy for polydisperse NaCl and metal particles, the average particle diameter measured with the miniDiSC were found to be within $\pm 35\%$ of the reference value (Goyal and Aswal, 2006, Kato et al., 2009). Another study reported a deviation of $\pm 27\%$ of the reference value for the particle diameter measured for soot particles (Asbach et al., 2012). However in this study, bias or accuracy for particle size ranged from -100 to 40% (Table 3.4). DM2 out of the 5 cycles run, deviated from the manufacturer's $\pm 30\%$ reported accuracy 2 times, i.e. 40 % of the time and DM3 deviated only 1 time i.e. 20 % of the time based on this study. Using the entire DMs in all the cycles, 80% of the time did not deviate from within $\pm 35\%$ of the reference value observed by Mills et al. (2013).

Table 3.4. Instrument bias for particle size

Cycle	Instrument	Bias (%)
1	DISCmini 1	-30
	DISCmini 2	-35
	DISCmini 3	-45
2	DISCmini 1	-16
	DISCmini 2	2
	DISCmini 3	0.41
3	DISCmini 1	9
	DISCmini 2	40
	DISCmini 3	8
4	DISCmini 1	14
	DISCmini 2	-100
	DISCmini 3	21
5	DISCmini 1	-4.6
	DISCmini 2	-3.5
	DISCmini 3	-5

3.4.1.3 Limit of detection (LOD)

LOD was calculated based on the charges (iF and iD) generated by the DM when it was assumed that it was measuring particle free air i.e. during the decay session. Detailed description is found in section 3.3.5.1.3. The charges (iF and iD) for DM2 in cycle 2 and cycle 3 were removed during calculation of the LOD because they were considered outliers. The 50 percentile of iF 1.65 and iD 1.15 in Table 3.5 was considered the LOD and the values obtained are in agreement with Fierz et al. (2011).

Table 3.5. DiSCmini limit of detection (LOD)

Cycle	DM ID	iF (3σ)	iD (3σ)
Cycle 1	DM1	1.65	0.35
	DM2	1.35	0.4
	DM3	3.65	1.15
Cycle 2	DM1	0.5	4.35
	DM3	1.65	1.75
Cycle 3	DM1	4.95	2.45
	DM3	1.45	0.4
Cycle 4	DM1	2.2	3.2
	DM2	5.4	4.85
	DM3	1	0.7
Cycle 5	DM1	2.9	2
	DM2	1.95	1.1
	DM3	0.9	0.45
	50 percentile	1.65	1.15

Both charges on the 2 electrometers must be approximately up to the practical LOD calculated in Table 3.5 in order for the DM to measure up to $10^3 \text{ \#}/\text{cm}^3$ which is the DM lower limit PNC range. From a polynomial regression performed using the ratio of the current (i_F/i_D) against the particle diameter (see Figure 6 (Sørensen et al., 2005)), a_i was derived.

$$y = -1\text{E-}11x^6 + 6\text{E-}09x^5 - 1\text{E-}06x^4 + 1\text{E-}04x^3 - 0.0036x^2 + 0.0675x - 0.0033 \quad (3.3)$$

$a_i = y$ and a_i is a calibration constant already stored in the DM. In equation 3.3, $x = i_F/i_D$.

$$d = a_i \cdot (i_F/i_D) + a_i \cdot (i_F/i_D)..... \quad (3.4)$$

Then in order to calculate the PNC, equations 3.5 and 3.7 adopted from Fierz et al. (2011) will then be applied

$$I_{\text{total}} = N \cdot Q(dp) \cdot \phi \quad (3.5)$$

$$N = \frac{i_F + i_D}{Q(dp) \cdot \phi} \quad (3.6)$$

Where I_{total} is $(i_F + i_D)$, ϕ is the instrument flow rate 1 LPM, $Q(dp)$ is the electrical charge of a given size of a particle.

$$Q(dp) = C \cdot d^x \quad (3.7)$$

Where C is constant already stored in the instrument derived from theoretical charge per particle with Fuchs theory (Fierz et al., 2011). That is Fierz et al. (2011), multiplied the Fuchs charging efficiency with the calculated charger loss and they arrived at a power law fit where $C = y = 0.015 \cdot d^{1.1}$. d is the measured diameter and superscript x is the charger. Fierz et al. (2011)

According to this validation, if $(i_F$ and $i_D)$ measured $> i_F$ 1.65 and i_D 1.15 (applying these values to the equation) concentration obtained was $1400 \text{ \#/cm}^3$, therefore agreeing with the manufacturer. However, if the $(i_F$ and $i_D)$ values are less than the DM cannot detect the particles thus will give concentration below $1000 \text{ \#/cm}^3$ thereby not making a sensible measurement as suggested by Fierz et al. (2011).

The concentration during the particle decay was measured. Figure 3.3 shows that during the decay, the DM was measuring concentration lower than the study LOD. Therefore concentrations were less than $1000 \text{ \#/cm}^3$. These concentrations are not considered sensible. However, Figure 3.3 shows that the SMPS is more sensitive than the DMs because it is

measuring higher concentrations during the decay except during cycle 4. The SMPS/CPC system, can detect particles as low as 5nm therefore can detect more particles thereby measuring higher concentration as seen in Figure 3.3. While all particles > 5 nm is outside the detection limit of the DM.

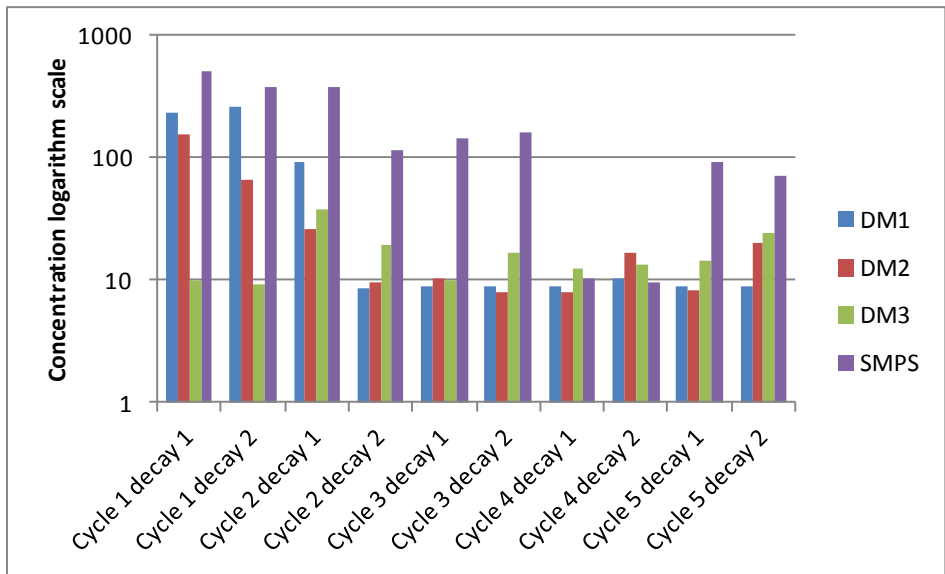


Figure 3.3.DM and SMPS PNC comparison during decay

3.5.1.4. Comparison of the three DiSCminis

Table 3.6 show the linearity fit between the DMs. Cycle 1, 2, 5 and DM2 of cycle 4 show very strong agreement 0.89 – 0.99. Cycle 3 show a good agreement 0.60 – 0.68, while DM1 in cycle 4 show weaker agreement of 0.35. The reason for the weaker agreement shown by DM1 in cycle 4 is unclear.

Table 3.6. Particle number concentration comparison of DiSCmini 3 against the other two DiSCminis

Cycle	DiSCmini ID	Regression coefficients	R ²
1	DiSCmini 1	0.6367x -10987	0.95
	DiSCmini 2	0.797x -1349	0.97
2	DiSCmini 1	0.6782x + 4624	0.95
	DiSCmini 2	0.8549x + 3600	0.95
3	DiSCmini 1	0.651x + 6637	0.60
	DiSCmini 2	0.6159x + 5125	0.68
4	DiSCmini 1	0.5007x + 6092	0.35
	DiSCmini 2	0.8638x – 946	0.99
5	DiSCmini 1	0.8134x - 1808	0.96
	DiSCmini 2	1.026 x – 2165	0.89

Table 3.7 show the linearity fit between the DMs for size. The inter-DM relationship, show from good to very strong agreement with the R² value ranging from 0.60 – 0.95.

Table 3.7. Reduced major axis Size comparison of DiSCmini 3 against the other two DiSCminis

Cycle	DiSCmini ID	Regression coefficients	R ²
1	DiSCmini 1	1.0757x + 10.20	0.69
	DiSCmini 2	1.1342x +2.201	0.83
2	DiSCmini 1	0.7806x + 10.56	0.91
	DiSCmini 2	1.0107x + 7.382	0.95
3	DiSCmini 1	0.9730x + 2.792	0.68
	DiSCmini 2	1.1603x – 0.882	0.76
4	DiSCmini 1	0.9650x + 6.888	0.77
	DiSCmini 2	1.1016x – 36.70	0.60
5	DiSCmini 1	1.3058x – 14.05	0.89
	DiSCmini 2	1.2539x – 7.035	0.95

3.4.2 Field validation

3.4.2.1 DiSCmini field validation

Studies have investigated the behaviour of the DM in the field in relation to RFSs and they found the DM to correlate with the RFSs with one of such example being Meier et al. (2013). However in this study a maximum of 336 hours (i.e. 2 weeks) of nonstop sampling for the purpose comparison between the DM and RFSs (SMPS and CPC) in the field was carried out. The DMs were placed alongside the reference instrument SMSP and CPC in the various national sampling sites. The sampling times of the DMs were averaged to 15 mins and the compared with the RFSs. Also both the RFSs and DMs were averaged to 24 hours and also compared. Table 3.8 show the sampling time summary of the DMs and RFSs. The periods were no data was inputted means that the DM was not available or the RS had no data due to instrument malfunction. Also lesser sampling periods were due to problems with instrument.

For the field validation of the DMs against RFS the linearity regression fit and accuracy (measured as bias) were calculated.

Table 3.8. Sampling location season and sampling time for ultrafine particles

Season	Location	DM ID	Sampling time (Hr)	Validated sampling time (Hr)
Summer	Tyburn Birmingham	DM1	336	336
		DM2	336	336
		DM3	336	336
	Harwell Observatory site	DM1	-	-
		DM2	336	168
		DM3	336	96
	North Kensington London	DM1	336	336
		DM2	336	336
		DM3	-	-
	Marylebone London	DM1	336	336
		DM2	336	336
		DM3	-	-
Season	Location	DM ID	Sampling time (Hr)	Validated sampling time (Hr)
Winter	Tyburn Birmingham	DM1	-	-
		DM2	-	-
		DM3	-	-
	Harwell Observatory site	DM1	336	336
		DM2	336	120
		DM3	336	336
	North Kensington London	DM1	336	336
		DM2	336	336
		DM3	-	-
	Marylebone London	DM1	240	240
		DM2	240	240
		DM3	192	192

In Table 3.9, the values in red indicate that the DMs had technical faults (error messages were showing on the screen) and therefore measurements were extremely higher or lower than the RFSs however data were still collected. The error messages were in relation to the corona wire and sometimes temperature error messages. This affected the R^2 for DM1 and DM2 based on 15 mins average for both the SMPS and CPC at North Kensington in the summer. It also affected the R^2 for DM2 based on both 15 mins and 24 hrs averages at Marylebone in the winter.

Also in Table 3.9 the comparison fit equations for the DMs and the SMPS and the DMs and the CPC reveal a very good inter-comparability of the devices based on 24 hours average over a maximum of 2 weeks sampling for both summer and winter. Showing R^2 values of 0.82 – 0.99 for SMPS and 0.63 – 0.99 for the CPC. Except for DM2 at Marylebone during the winter due to system malfunction of the DMs. However for the 15 mins average over a maximum of 2 weeks in both seasons except in DM1 and DM2 in North Kensington, show a fair to very good inter-comparability R^2 values between 0.54 – 0.87 for the SMPS and 0.51 – 0.98 for the CPC. The wider spread of data in the 15 mins average is the reason for a lesser correlation. While the data in the 24 hours average is cleaned up reducing the outliers that were more obvious in the 15 mins average. The variability between the slopes of the regression equation for both the SMPS and CPC was not too wide but it could be due to σ_g of the particle size distribution across the different sites (urban background, kerbside and rural) and during the different season. However there is a good correlation between the DM and RFSs except for periods associated with errors (Table 3.9) under real world conditions.

Table 3.9. Regression analysis for particle number concentration

Season	Location	DM ID	Linear fit equation SMPS (15 mins average)	Linear fit equation CPC (15 mins average)	Linear fit equation SMPS (24 hrs average)	Linear fit equation CPC (24 hrs average)
Summer	Tyburn Birmingham	DM1	-	0.902x + 1542 R ² = 0.60	-	1.083x + 121 R ² = 0.98
		DM2	-	0.625x + 1626 R ² = 0.56	-	0.694x + 1077 R ² = 0.92
		DM3	-	0.945x + 1923 R ² = 0.56	-	1.033x + 1238 R ² = 0.93
	Harwell Observatory site	DM2	0.964x + 703 R ² = 0.87	0.478x + 2169 R ² = 0.66	0.988x + 599 R ² = 0.98	0.804x + 499 R ² = 0.81
		DM3	1.515x + 1424 R ² = 0.57	1.734x + 341 R ² = 0.98	1.229x + 1575 R ² = 0.99	1.501x + 279 R ² = 0.99
	North Kensington London	DM1	1.018x + 3518 R ² = 0.40	0.859x + 4469 R ² = 0.24	1.573x + 540 R ² = 0.94	2.228x - 2488 R ² = 0.92
		DM2	0.716x + 2578 R ² = 0.44	0.587x + 3349 R ² = 0.24	0.958x + 1267 R ² = 0.93	1.296x - 256 R ² = 0.85
	Marylebone London	DM1	0.431x + 4107 R ² = 0.68	0.684x + 6057 R ² = 0.66	2.044x - 5342 R ² = 0.97	1.268x - 3934 R ² = 0.98
		DM2	1.224x + 1059 R ² = 0.69	0.757x + 1800.2 R ² = 0.67	1.597x - 5048 R ² = 0.98	0.898x - 2446 R ² = 0.90
Season	Location	DM ID	Linear fit equation SMPS (15 mins average)	Linear fit equation CPC (15 mins average)	Linear fit equation SMPS (24 hrs average)	Linear fit equation CPC (24 hrs average)
	Harwell Observatory site	DM1	1.095x + 767 R ² = 0.61	1.035x + 282 R ² = 0.63	1.183x + 597.2 R ² = 0.82	1.307x - 392 R ² = 0.95
		DM2	1.033x + 704 R ² = 0.72	0.887x + 454 R ² = 0.66	1.127x + 461 R ² = 0.93	0.928x + 311 R ² = 0.95
		DM3	1.025x + 890 R ² = 0.54	0.927x + 538 R ² = 0.51	1.205x + 546 R ² = 0.82	1.317x - 423 R ² = 0.94
	North Kensington London	DM1	1.665x + 1830 R ² = 0.82	1.102x - 1281 R ² = 0.86	1.586x + 2299 R ² = 0.98	1.184x - 2280 R ² = 0.99
		DM2	0.871x - 2152 R ² = 0.87	1.3895x - 131 R ² = 0.93	1.434x - 397.12 R ² = 0.99	1.044x - 4223 R ² = 0.96
	Marylebone London	DM1	1.879x + 3294 R ² = 0.72	1.453x - 2581 R ² = 0.73	2.509x - 6871 R ² = 0.95	1.926x - 14645 R ² = 0.91
		DM2	0.272x - 1915.2 R ² = 0.58	0.195x - 2502 R ² = 0.50	0.184x - 892 R ² = 0.43	0.112x - 833 R ² = 0.24
		DM3	1.946x + 7541 R ² = 0.69	1.492x + 1147 R ² = 0.66	2.759x - 4123 R ² = 0.93	1.913x - 9028 R ² = 0.63

Inter-comparison between all 3 DMs for PNC was from 0.91 – 0.96 and for diameter size from 0.94 – 0.97 in the field validation. Except in Marylebone summer where DM1 against DM2 was 0.66 for PNC and for diameter size value of the SMPS and DM for Harwell, North Kensington and Marylebone for the summer showed R² value of 0.72 – 0.85 and in Marylebone where R² value of inter-comparison between diameter value of the SMPS and DM2 was 0.38 in the winter. The reason was due to the DM fault.

Table 3.10. Instrument bias for particle number concentration in the field study

Season	Location	DM ID	Average Bias from CPC (%)	Average Bias from SMPS (%)
Summer	Tyburn	DM1	10	-
		DM2	-17	-
		DM3	19	-
	Harwell	DM2	5	22
		DM3	64	98
	North Kensington	DM1	70	68
		DM2	24	21
	Marylebone	DM1	8	64
		DM2	-20	22
Season	Location	DM ID	Bias from CPC (%)	Bias from SMPS (%)
Winter	Harwell	DM1	15	20
		DM2	7	40
	North Kensington	DM1	-1	97
		DM2	-31	37
	Marylebone	DM1	35	109
		DM2	-91	-86
		DM3	54	146

Larger deviations from the $\pm 30\%$ and $\pm 35\%$ accuracy as suggested by the manufacture and some other studies respectively were observed (Table 3.10). This may be due, to wider range of particle sizes (i.e. modal diameter of < 30 and > 300 nm) and aerosol of various chemical composition mixed together measured in the various location in the field study.

3.4.2.2 MicroPEM field validation

There were 4 MicroPEMs setup for validation at each site. However at some sites as seen in Table 3.11 the MicroPEMs were not complete and or sampling hours were not up to 336 hours because the missing MicroPEMs developed faults, and were sent to the manufacturer

for repairs. There were no measurements for Harwell in the summer because of the RFS system malfunction. There were no data for Marylebone in the winter because the MicroPEM developed a system error (date and time were inconsistent i.e. time stamp malfunction) therefore the data was invalid. Table 3.11 also show the sampling summary of the MicroPEMs at the various locations. Validated time is the time that the instrument recorded valid date and time of sampling. Then from the validated time the data was cleaned up by removing negative values, zero values, obvious outliers and timestamp malfunction that could not be reconciled.

Table 3.11.Sampling location season and sampling time for PM2.5

Season	Location	MicroPEM ID	Intended Sampling time (Hr)	Validated sampling time (Hr)
Summer	Tyburn Birmingham	MicroPEM1	336	168
		MicroPEM2	336	336
		MicroPEM3	336	336
		MicroPEM4	336	168
	North Kensington London	MicroPEM2	336	336
		MicroPEM3	336	312
		MicroPEM4	336	312
	Marylebone London	MicroPEM3	336	336
		MicroPEM4	336	336
Season	Location	MicroPEM ID	Sampling time (Hr)	Validated sampling time (Hr)
Winter	Tyburn Birmingham	MicroPEM2	336	216
		MicroPEM3	336	192
		MicroPEM4	336	336
	Harwell Observatory site	MicroPEM1	336	168
		MicroPEM2	336	168
		MicroPEM3	336	168
		MicroPEM4	336	168
	North Kensington London	MicroPEM2	336	240
		MicroPEM3	336	240
		MicroPEM4	336	240

3.4.2.3 Error in MicroPEM validation results

After the MicroPEM summer and winter validation data were downloaded and analysis commenced, some inconsistencies were observed in some of the results especially with the downstream filter. These inconsistencies were due to improper placement of the O-ring. Therefore the sampling was not sealed thus affecting results. The O-ring is found within the testing chamber of the instrument (Figure 3.1). In some instance even when the O-ring was properly placed, before the end of the sampling period it was found to have loosened. These loosened O-rings were observed whenever the instrument was opened to retrieve the downstream filter.

Having experienced this measures were put in place to make sure that further results obtained from this instrument for the indoor and PE study were reliable.

- Sent to the manufacturer to make sure the place the O-ring sits on is fitted properly and instrument serviced and upgraded.
- O-ring was double checked to make sure it was fitted properly before sampling commenced
- Another study was carried out by other researchers in the team (USE OF REAL-TIME SENSORS TO ASSESS MISCLASSIFICATION AND TO IDENTIFY MAIN SOURCES CONTRIBUTING TO PEAK AND CHRONIC EXPOSURES. 5-MONTH YEAR 2 PROGRESS REPORT. Author: Dr Juana Maria Delgado-Saborit, University of Birmingham, September 2014) to make sure data obtained in further studies using the MicroPEM were reliable.

Table 3.12 show that at higher ambient PM as in the case of Marylebone London, the MicroPEM and the TEOM-FDMS show a good to strong correlation for both 1hour average and 24 hours average with R^2 ranging from 0.63 – 0.92. In Tyburn, Birmingham, PEM1 and

PEM4 show a good correlation with R^2 ranging from 0.51 – 0.68. However, PEM2 and PEM3 show a weak correlation for both 1 hour and 24 hours average (0.16 – 0.47). North Kensington showed a weak correlation for both 1 hour and 24 hours average (all $R^2 \leq 0.28$), except MicroPEM2 in the 24 hours average with an R^2 value of 0.57. The slopes of the MicroPEM against the FDMS-TEOM in Table 3.12 indicated that at a factor of ≥ 0.41 the MicroPEM makes measurements that correlate with the FDMS-TEOM nearly 70 % of the entire sampling at the different sites.

The y intercepts for the 1hr averages (ranging from - 4.252 to + 5.819) and 24 hrs averages (ranging from - 1.685 to + 5.428) may be indicative of a systematic offset between the two sampling methods, owing to the MicroPEM as the FDMS-TEOM is the reference standard.

As far as I know, this is the only study that has measured the accuracy of the MicroPEM for $PM_{2.5}$ against the FDMS-TEOM in a real-life field study scenario validation. The accuracy of the instrument was tested as bias percentage and it ranged from -74 to 19% in reference to FDMS-TEOM for all 4 MicroPEMs in the various locations. However validation of the MicroPEM against another instrument has been measured. The accuracy of the MicroPEM against a pseudo-referee sampler MSP personal environmental monitor (MSP PEM) during simulated solid fuel cookstove showed a bias of +3.8% for $PM_{2.5}$ (Chartier et al. 2013). This +3.8 % < the values obtained in this study and the MicroPEM agreement with other instruments has also been measured. Measurement of cookstove fuels in the indoor the MicroPEM against Dustrak in a 1 min average interval showed linearity fit equation: equation below (Chatier, 2014).

$$Y = 1.0201x + 19.167 \quad (3.8)$$

$$R^2 = 0.999$$

3 MicroPEMs were tested against the GRIMM in the outdoor environment at 5 mins average and the R^2 values obtained were 0.57 – 0.87, with slopes ranging from 1.1094 – 1.619 (USEPA, 2014). The correlation with the GRIMM were similar to what was obtained in this study and it also showed that MicroPEM was measuring at a factor > 0.41 to have a good to strong correlation with the GRIMM.

Table 3.12. Statistics, regression analysis and accuracy for PM2.5 MicroPEM sensor in the summer

SUMMER					
Location	Average (Min – Max) FDMS-TEOM ($\mu\text{g}/\text{m}^3$)	MicroPEM ID Average (Min – Max) ($\mu\text{g}/\text{m}^3$)	Linear fit equation (1hr average)	Linear fit equation (24hrs average)	Accuracy (%)
Tyburn	16 (3 – 42)	PEM1 6 (3 – 24)	$0.414x - 0.669$ $R^2 = 0.58$	$0.473x - 0.981$ $R^2 = 0.51$	-67
		PEM2 6 (3 – 21)	$0.331x - 0.379$ $R^2 = 0.23$	$0.604x + 2.810$ $R^2 = 0.47$	-74
		PEM3 7 (3 – 24)	$0.173x - 4.252$ $R^2 = 0.16$	$0.177x + 4.523$ $R^2 = 0.16$	-50
		PEM4 11 (4 – 39)	$0.715x - 0.121$ $R^2 = 0.58$	$0.721x + 0.547$ $R^2 = 0.67$	-31
North Kensington	8 (2 – 19)	PEM2 7 (3 – 23)	$0.349x + 4.522$ $R^2 = 0.28$	$0.563x + 2.619$ $R^2 = 0.57$	-2
		PEM3 9 (2 – 31)	$0.449x + 5.819$ $R^2 = 0.14$	$0.437x + 5.789$ $R^2 = 0.25$	18
		PEM4 7 (4 – 21)	$0.267x + 4.406$ $R^2 = 0.13$	$0.134x + 5.428$ $R^2 = 0.21$	-15
Marylebone	23 (2-96)	PEM3 23 (6– 113)	$1.020x - 0.082$ $R^2 = 0.85$	$1.089x - 1.162$ $R^2 = 0.92$	1
		PEM4 12 (5 – 85)	$0.579x - 0.858$ $R^2 = 0.63$	$0.638x - 1.685$ $R^2 = 0.79$	-47

All the MicroPEM sensors in Tyburn and Marylebone show strong correlation (ranging from 0.81 – 0.94) (Table 3.13). However in North Kensington the MicroPEM show a very weak correlation (0.05 -0.19) with each other. The reason why the MicroPEMs in North Kensington did not agree is unclear, there might have been a fault with the instruments, as the correlation with the FDMS-TEOM was also weak (Table 3.12) in North Kensington. Table 3.13 also shows that Y intercepts ranging from 0.05 – 6.63 indicated that there were offsets between the MicroPEMs. MicroPEM2 against MicroPEM3 and MicroPEM2 against MicroPEM4 in North Kensington show the larger intercept indicating greater offset which may have resulted in the weak correlation as displayed in Table 3.13.

Table 3.13. Relationship between the MicroPEM sensors in the field study for the summer season based on hourly average

Location	Relationship between MicroPEM	Regression coefficient	R ²
Tyburn	PEM1 against PEM2	0.374x + 0.049	0.89
	PEM1 against PEM3	0.954x + 0.602	0.94
	PEM1 against PEM4	1.666x + 1.565	0.92
North Kensington	PEM2 against PEM3	0.378x + 6.633	0.05
	PEM2 against PEM4	0.477x + 3.039	0.19
Marylebone	PEM3 against PEM4	0.591x – 1.181	0.81

The online measurement in each location was for 2 weeks. However at the end of each sampling week the MicroPEM integrated filter was changed. Therefore, resulting in 2 Teflon filters for each sampling site/location. Table 3.14 show the average weekly concentrations of MicroPEM real-time and integrated filter measurements for the summer. Using the data in Table 3.14, difference of mean and the correlation between the real-time and integrated filter was measured. The result indicated that there was no significant differences between the real-

time and gravimetric measurements (Mann Whitney U $P = 0.147$) and weak correlation was observed $R^2 = -0.47$.

Table 3.14. Average weekly concentrations of MicroPEM real-time and integrated filter measurements ($\mu\text{g}/\text{m}^3$) for the summer

Site	Week	MicroPEM ID	Real-time	Gravimetry
Tyburn	Week 1	MicroPEM1	6	7
		MicroPEM2	8	9
		MicroPEM3	6	15
		MicroPEM4	11	8
	Week 2	MicroPEM2	3	14.2
		MicroPEM3	8	3
North Kensington	week 1	MicroPEM2	7.9	9.81
		MicroPEM3	9.83	8.98
		MicroPEM4	6.8	8.81
	week 2	MicroPEM2	6.7	6.05
		MicroPEM3	9.12	4.03
		MicroPEM4	6.43	3.5
Marylebone	week 1	MicroPEM3	15.59	3.15
		MicroPEM4	8.88	3.32
	week 2	MicroPEM3	30.57	3.57
		MicroPEM4	16.26	3.6

Table 3.15 show the linearity fit equation and R^2 value for 1 hour and 24 hours average for the MicroPEM against the FDMS-TEOM for the winter season. 24 hours average showed better and consistent good to strong correlation in comparison to 1 hour average. The accuracy of the MicroPEM in reference to the FDMS-TEOM throughout the winter sampling ranged from 12 % to 201 %. The upper end of this bias is high indicating a massive deviation from the RFS. The winter deviation from the RFS was higher (Table 3.15) as compared to the summer (Table 3.12).

Table 3.15. Statistics, regression analysis and accuracy for PM_{2.5} in the winter

WINTER					
Location	Average (Min – Max) FDMS- TEOM (µg/m ³)	MicroPEM ID Average (Min – Max) (µg/m ³)	Linear fit equation (1hr average)	Linear fit equation (24hrs average)	Bias (%)
Tyburn	7 (0.3 – 13)	PEM2 10 (4 – 20)	0.735x + 5.912 R ² = 0.38	0.679X + 5.776 R ² = 0.77	97
		PEM3 9 (4 – 19)	0.625x + 5.686 R ² = 0.35	0.609x + 5.734 R ² = 0.79	88
	7 (0.3 – 30)	PEM4 12 (4 – 42)	0.855x + 6.469 R ² = 0.54	0.777x + 6.876 R ² = 0.62	103
Harwell	3 (0.10 - 11)	PEM1 7 (2 – 12)	0.074x + 6.702 R ² = 0.01	1.175x + 3.315 R ² = 0.35	167
		PEM2 8 (3 – 16)	0.6522x + 6.312 R ² = 0.20	2.815x – 0.374 R ² = 0.54	201
		PEM3 6 (3 – 9)	0.416x + 4.407 R ² = 0.27	1.304x + 1.677 R ² = 0.61	106
		PEM4 8 (4 – 16)	0.539x + 6.471 R ² = 0.16	1.819x + 2.529 R ² = 0.54	194
North Kensington	14 (2-96)	PEM2 14 (4 – 48)	0.663x + 5.539 R ² = 0.82	0.701x + 4.801 R ² = 0.97	26
		PEM3 13 (4 – 39)	0.414x + 7.123 R ² = 0.56	0.411x + 7.136 R ² = 0.69	12
		PEM4 16 (4 – 50)	0.688x + 6.772 R ² = 0.84	0.738x + 6.025 R ² = 0.97	40

Table 3.16, show the R² value for all cohorts of all MicroPEMs validated in the winter ranged from 0.57 – 0.96, except in Harwell MicroPEM2 against MicroPEM1 that indicated a weak correlation of 0.28. Again like in Table 3.13, Table 3.16 show that the Y intercepts varied among MicroPEMs which may be indicative of system offset between MicroPEMS.

MicroPEM2 against MicroPEM1 in Harwell show the greatest intercepts between their comparisons this again could affect the correlation between those 2 MicroPEMs resulting in a weak agreement.

It is worthy to note that before the start of the 336 hours (2 weeks) sampling at each site, the MicroPEMs were calibrated and sensor voltage corrected in order to remove any offsets that may affect the readings. However offsets were still identified based on the y intercepts as seen in Table 3.13 and Table 3.16.

Table 3.16. Relationship between the MicroPEM sensors in the field study for the winter based on hourly average

Location	Relationship between MicroPEM	Regression coefficient	R ²
Tyburn	PEM2 against PEM3	0.7855x + 1.5608	0.8052
	PEM2 against PEM4	0.793x + 2.730	0.5120
Harwell	PEM2 against PEM1	0.3119x + 4.322	0.2752
	PEM2 against PEM3	0.4251x + 2.115	0.6754
	PEM2 against PEM4	0.6542x + 2.584	0.5724
North Kensington	PEM2 against PEM3	1.005x + 1.489	0.9601
	PEM2 against PEM4	0.6249x + 3.769	0.7014

Table 3.17 show the average weekly concentrations of MicroPEM real-time and integrated filter measurements for the winter. Using the data in Table 3.17, difference of mean and the correlation between the real-time and integrated filter was measured. The result indicated that there was no significant differences between the real-time and gravimetric measurements ($P = 0.160$) but a weak correlation was observed $R^2 = 0.45$.

The weak correlations observed from the Table 3.14 and Table 3.17 i.e. between the real-time and gravimetric measurements is as a result of improper placement of the O-ring i.e as air is

drawn in particles are lost because the O-ring is not placed properly, therefore the sampling was not sealed. Also, as the air enters the instrument, it makes real-time measurement and also collects in the downstream filter but as more air flows in, it may blow off semi volatile $PM_{2.5}$ therefore resulting in the filter having less weight than collected.

Table 3.17. Average weekly concentrations of MicroPEM real-time and integrated filter measurements ($\mu\text{g}/\text{m}^3$) for the winter

Site	Week	MicroPEM ID	Real-time	Gravimetry
Tyburn	week 1	PEM2	9.73	7.28
		PEM3	9.19	3.52
		PEM4	10.1	2.41
	week 2	PEM4	14.1	5.59
Harwell	week 1	PEM1	6.91	4.56
		PEM2	8.62	4.58
		PEM3	5.77	1.86
		PEM4	8.18	0.67
North Kensington	week 1	PEM2	11.92	10.21
		PEM3	10.88	6.85
		PEM4	15.9	2.69

Figure 3.4 show the concentration trend of the MicroPEM and FDMS-TEOM over time. Four random concentration profiles were selected to show that the MicroPEMs follow the same pattern as the FDMS-TEOM. When there is a rise in concentration both instruments rise and vice versa. However there is a difference in the average concentration of both instruments as already shown in the bias (Table 3.12 and Table 3.15).



Figure 3.4. Concentration trend of MicroPEM and FDMS-TEOM over time

3.4.2.4 MicroPEM integrated filter and standalone filter and FDMS-TEOM

Standalone measurements were collected using the cyclone as described in section 3.3.3. The gravimetric analyses obtained were compared against the MicroPEM integrated filter, against the MicroPEM real-time and against the RFS FDMS-TEOM. Concentrations from each MicroPEM integrated for week 1 and week 2 were pulled together. Table 3.18 show the relationship between MicroPEM integrated filter and the stand-alone filter measurements. Weak correlations were observed. This is partly because the O-ring was not placed properly as previously explained and also difference in sampling method. While the MicroPEM sampled at 0.5 LPM, the standalone sampled at 2LPM. Table 3.19 show the relationship between MicroPEM real-time and stand-alone filter. The real-time showed a better relationship with the standalone filter. Table 3.20 show very weak correlation between MicroPEM integrated filter and the FDMS-TEOM. This again may have been due to the O-

ring placement. In addition to the O-ring, the difference in sampling style could have affected the outcome of the relationship. That is, loss of semi volatiles due to heating up of the MicroPEM during sampling and evaporation of these volatiles as constant air is drawn into the MicroPEM. While the FDMS-TEOM accounts for both the semi-volatile and non-volatile PM_{2.5} components. Even with the improper placement of the O-ring observed in some of the sampling, the results in Table 3.18 to Table 3.20, suggest that the real-time MicroPEM measurements provides a better measurement than the downstream filter collection-the integrated filter of the MicroPEM. It also suggests that the MicroPEM integrated filter was more affected by the improper placement of the O-ring.

Table 3.18. Relationship between MicroPEM integrated and stand-alone filter measurement

Summer						
Location	Week	MicroPEM ID	Integrated	Stand-alone	R ²	Mann-Whitney U
TY	week 1	PEM 1	7	26.42	0.13	0.00
TY	week 1	PEM 2	9	26.42		
TY	week 2	PEM2	14.2	10.26		
NK	week 2	PEM2	6.7	12.9		
TY	week 1	PEM 3	15	26.42		
NK		PEM3	8.98	26.04		
MY		PEM3	3.15	49.53		
TY	week 2	PEM 3	3	10.26		
NK		PEM3	4.03	12.9		
MY		PEM3	3.57	52.64		
TY	week 1	PEM4	8	26.42		
NK		PEM4	8.81	26.04		
MY		PEM4	3.32	49.53		
NK	week 2	PEM4	3.5	12.9		
MY		PEM4	3.6	52.64		
Winter						
Location	Week	MicroPEM ID	Integrated	Stand-alone	R ²	Mann-Whitney U
TY	week 1	PEM2	7.28	10.36	0.21	0.00
HW		PEM2	4.58	6.45		
NK		PEM2	10.21	12.86		
TY	week 2	PEM2	2.79	7.69		
NK		PEM2	4.03	11.19		
TY	week 1	PEM 3	3.52	10.36		
HW		PEM3	1.86	6.45		
NK		PEM3	6.85	12.86		
TY	week 2	PEM3	2.00	7.69		
NK		PEM3	4.23	11.19		
TY	week 1	PEM4	2.41	10.36		
HW		PEM4	0.67	6.45		
NK		PEM4	2.69	12.86		
TY	week 2	PEM4	5.59	7.69		
NK		PEM4	0.99	11.19		

Ty = Tyburn, HW = Harwell, NK = North Kensington, MY = Marylebone

Table 3.19. Relationship between MicroPEM real-time and stand-alone filter

Summer						
Location	Week	MicroPEM ID	Real-time	Stand-alone filter	R ²	Mann-Whitney U
TY	week 1	PEM1	6	26.42	0.51	0.00
TY	week 1	PEM2	8	26.42		
NK		PEM2	7.9	26.04		
TY	week 2	PEM2	3	10.26		
NK		PEM2	6.7	12.9		
TY	week 1	PEM3	6	26.42		
NK		PEM3	9.83	26.04		
MY		PEM3	15.59	49.53		
TY	week 2	PEM 3	8	10.26		
NK		PEM3	9.12	12.9		
MY		PEM3	30.57	52.64		
TY	week 1	PEM4	11	26.42		
NK		PEM4	6.8	26.04		
MY		PEM4	8.8	49.53		
NK	week 2	PEM4	6.43	12.9		
MY		PEM4	16.26	52.64		
Winter						
Location	Week	MicroPEM ID	Real-time	Stand-alone filter	R ²	Mann-Whitney U
HW	week 1	PEM1	6.91	6.45	0.35	0.20
TY	week 1	PEM2	9.73	10.36		
HW		PEM2	8.62	6.45		
NK		PEM2	11.92	12.86		
TY	week 2	PEM2	11.85	7.69		
NK		PEM2	19.37	11.19		
TY	week 1	PEM3	9.19	10.36		
HW		PEM3	5.77	6.45		
NK		PEM3	10.88	12.68		
TY	week 2	PEM3	7.39	7.69		
NK		PEM3	15.66	11.19		
TY	week 1	PEM4	10.1	10.36		
HW		PEM4	8.18	6.45		
NK		PEM4	15.9	12.86		
TY	week 2	PEM4	14.1	7.69		
NK		PEM4	22	11.19		

Ty =Tyburn, HW = Harwell, NK = North Kensington, MY = Marylebone

Table 3.20. Relationship between MicroPEM integrated filter and FDMS-TEOM

Summer						
Location	Week	MicroPEM ID	Integrated	FDMS-TEOM	R ²	Mann-Whitney U
TY	week 1	PEM 1	7	15	0.05	0.01
TY	week 1	PEM 2	9	15		
TY	week 2	PEM2	14.2	16		
NK	week 2	PEM2	6.7	7.68		
TY	week 1	PEM 3	15	15		
NK		PEM3	8.98	8		
MY		PEM3	3.15	18		
TY	week 2	PEM 3	3	16		
NK		PEM3	4.03	7.68		
MY		PEM3	3.57	27		
TY	week 1	PEM4	8	15		
NK		PEM4	8.81	8		
MY		PEM4	3.32	18		
NK	week 2	PEM4	3.5	7.68		
MY		PEM4	3.6	27		
Winter						
Location	Week	MicroPEM ID	Integrated	FDMS-TEOM	R ²	Mann-Whitney U
TY	week 1	PEM2	7.28	5.46	0.00002	0.03
HW		PEM2	4.58	3.23		
NK		PEM2	10.21	10.04		
TY	week 2	PEM2	2.79	8.01		
NK		PEM2	4.03	20.88		
TY	week 1	PEM 3	3.52	5.46		
HW		PEM3	1.86	3.23		
NK		PEM3	6.85	10.04		
TY	week 2	PEM3	2.00	8.01		
NK		PEM3	4.23	20.88		
TY	week 1	PEM4	2.41	5.46		
HW		PEM4	0.67	3.23		
NK		PEM4	2.69	10.04		
TY	week 2	PEM4	5.59	8.01		
NK		PEM4	0.99	20.88		

Ty =Tyburn, HW = Harwell, NK = North Kensington, MY = Marylebone

3.4.2.5 Aeroqual sensor field validation

Table 3.21 show the sampling location, season and time of the Aeroqual sensor and the RFS chemiluminescence API M200E. Validated time is as explained in section 3.4.2.2.

Table 3.21.Sampling location season and sampling time for NO2

Season	Location	Aeroqual ID	Intended Sampling time (Hr)	Validated sampling time (Hr)
Summer	Tyburn Birmingham	AQ3	336	205
		AQ4	336	336
		AQ5	336	96
		AQ6	336	205
	North Kensington London	AQ1	336	336
		AQ2	336	336
		AQ3	336	336
		AQ4	336	336
		AQ5	336	336
		AQ6	336	336
	Marylebone London	AQ1	336	336
		AQ2	336	336
		AQ3	336	336
		AQ4	336	336
Season	Location	MicroPEM ID	Sampling time (Hr)	Validated sampling time (Hr)
Winter	Harwell Observatory site	AQ1	336	336
		AQ3	336	336
		AQ4	336	336
		AQ5	336	336
		AQ6	336	336
	North Kensington London	AQ1	336	336
		AQ3	336	336
		AQ4	336	336
	Marylebone	AQ1	288	288
		AQ3	288	288
		AQ4	288	288

NO₂ can be readily detected by the Aeroqual sensor especially where the sensor current is proportional to the gas abundance (considering the measurement technique) i.e., where the gas NO₂ diffuses through the membrane and reacts with the electrolyte-catalyst interface which then creates a current.

In Table 3.22 there were no data for Harwell because there were no data from the RFS therefore, no data available for comparison with the Aeroqual. In Table 3.22, for the 1 hour average AQ6 for Tyburn, AQ1 and AQ2 for North Kensington and AQ1 for Marylebone showed good correlation (0.49 – 0.58) with the RFS. For the 24 hours average, the AQ5 and AQ6 in Tyburn showed good and strong correlation (0.64 and 0.93) respectively with the RFS but the sampling times were for 264 and 96 hours respectively. Still for the 24 hours average AQ1 and AQ2 for North Kensington and AQ2 and AQ4 for Marylebone against the RFS showed a good correlation ranging from 0.67 – 0.88. Marylebone is an urban roadside monitoring site with very heavy traffic flow. The good to strong correlation may be because of the higher concentration of NO₂ related to the heavy traffic. Owing to the common knowledge that NO₂ is primarily formed by reactions of exhaust emissions of internal combustion engines.

Table 3.22. Regression analysis and accuracy for NO2 Aeroqual sensor in the summer

SUMMER					
Location	Average (Min – Max) API M200E ($\mu\text{g}/\text{m}^3$)	Aeroqual ID Average (Min – Max) ($\mu\text{g}/\text{m}^3$)	Linear fit equation (1hr average)	Linear fit equation (24hrs average)	Accuracy (%)
Tyburn	21 (5 – 48)	AQ3 15 (7 – 25)	$0.114x + 13.07$ $R^2 = 0.12$	$0.131x + 12.69$ $R^2 = 0.18$	-10
		AQ4 8 (1 – 27)	$0.265x + 3.042$ $R^2 = 0.21$	$0.639x - 5.078$ $R^2 = 0.28$	-56
		AQ5 7 (2 – 13)	$0.325x + 0.457$ $R^2 = 0.30$	$1.127x - 11.72$ $R^2 = 0.93$	-55
		AQ6 2 (2 – 6)	$0.105x - 1.287$ $R^2 = 0.58$	$0.721x + 0.547$ $R^2 = 0.68$	-93
North Kensington	25 (6 – 84)	AQ1 16 (1 – 43)	$0.453x + 3.714$ $R^2 = 0.53$	$0.522x + 1.992$ $R^2 = 0.75$	-26
		AQ2 17 (2 – 42)	$0.441x + 4.352$ $R^2 = 0.52$	$0.534x + 2.032$ $R^2 = 0.75$	-23
		AQ3 15 (1 – 44)	$-0.097x + 16.97$ $R^2 = 0.04$	$0.186x + 10.41$ $R^2 = 0.36$	-35
		AQ4 26 (1 – 80)	$-0.522x + 40.16$ $R^2 = 0.19$	$-0.674x + 45.57$ $R^2 = 0.12$	-15
		AQ5 24 (2 – 80)	$-0.207x + 28.85$ $R^2 = 0.06$	$0.291x + 17.10$ $R^2 = 0.12$	8
		AQ6 7 (2 – 27)	$-0.007x + 7.5032$ $R^2 = 0.001$	$0.097x + 2.782$ $R^2 = 0.08$	-67
Marylebone	82 (12 – 213)	AQ1 51 (11 – 130)	$0.381x + 19.68$ $R^2 = 0.49$	$0.485x + 11.96$ $R^2 = 0.88$	-36
		AQ2 449 (19 – 2097)	$5.531x - 76.75$ $R^2 = 0.29$	$5.082x - 6.439$ $R^2 = 0.70$	334
		AQ3 16 (2 – 57)	$0.155x + 2.915$ $R^2 = 0.43$	$0.111x + 6.437$ $R^2 = 0.67$	-81
		AQ4 29	$0.388x - 6.697$ $R^2 = 0.42$	$0.460x - 11.66$ $R^2 = 0.78$	-71

AQ=Aeroqual

In Table 3.23 there were no data for Tyburn because there were no data from the RFS therefore no data was available for comparison with the Aeroqual. Table 3.23 shows that all through the sampling period there were no correlation between the RFS and the PS Aeroqual. Also the accuracy in reference to the API M200E ranged from -95 % - 771 %.

The Aeroqual showed the same concentration trend i.e capturing temporal concentration variations. However, all through the NO₂ validation for both summer and winter, the Aeroqual measured at lower concentration than the RFS. Delgado-Saborit et al. (2012) in their 7days (within July and October) validation study of the Aeroqual NO₂ observed R² of 0.6321 for 1hour average, similar to the findings in the summer for this study. However they advised that validation should include longer periods in order to account for temperature and relative humidity (RH) effect on the instrument. From the result in Table 3.22 and Table 3.23, it is apparent that environment and meteorological conditions affects the measurements made by the Aeroqual sensor. Seeing that the RFS show inconstant correlation in the summer while in the winter, very poor correlations with the RFSs were observed. Bart et al. (2014) in their study suggested that gas sensitive semiconductors of which the Aeroqual is one, are sensitive to RH. However another study observed limited RH bias and imprecision effect on the Aeroqual but observed cross interference or co-sensitivity due to ozone (O₃) (Nadif et al., 2014). Therefore they suggested that a co-deployment of ozone monitors for correction of the co-sensitivity. Another study also demonstrated that electrochemical sensors have cross-sensitivity problems with other gases, for example O₃ is the most significant gas that interferes with the sensing of NO₂ (Austin et al., 2006) . Therefore O₃ interference in the sensing of NO₂ by the aeroqual sensor could an example of environmental factor affecting the measurement generated by the sensor. This was reported to the manufacturer who agreed that temperature and relative humidity may have some effects on the performance of the Aeroqual

sensor. Therefore a temperature and RH correction will be needed in other to get a consistent correlation between the two instruments. Also performance of the AQ sensor may have been affected by sampling time, e.g. the winter sampling was done after the summer sampling and perhaps the instrument is not built for long time and harsh condition sampling.

Table 3.23. Regression analysis and accuracy for NO₂ Aeroqual sensor in the winter

WINTER					
Location	Average (Min – Max) API M200E (µg/m ³)	Aeroqual ID Average (Min – Max) (µg/m ³)	Linear fit equation (1hr average)	Linear fit equation (24hrs average)	Accuracy (%)
Harwell	9 (2 – 35)	AQ1 31 (12 – 55)	0.111x + 30.23 R ² = 0.01	0.408x + 27.46 R ² = 0.11	351
		AQ3 31 (13 – 45)	0.092x + 30.36 R ² = 0.01	-0.109x + 32.33 R ² = 0.01	354
		AQ4 3.5 (1 – 11)	-0.051x + 4.152 R ² = 0.01	0.093x – 0.015 R ² = 0.07	-79
		AQ5 32 (13 – 57)	-0.111x + 33.08 R ² = 0.01	-0.690x + 38.38 R ² = 0.27	364
		AQ6 60 (23 – 86)	-0.151x + 61.77 R ² = 0.01	-1.292x + 72.15 R ² = 0.27	771
North Kensington	47 (7 – 136)	AQ1 53 (32 – 96)	-0.057x + 55.99 R ² = 0.01	-0.051x + 55.83 R ² = 0.01	20
		AQ3 3 (1 – 8)	-0.002x + 3.509 R ² = 0.05	-0.001x + 0.895 R ² = 8 * 10 ⁻⁵	95
		AQ4 10 (1 – 42)	-0.037x + 12.01 R ² = 0.02	-0.029x + 11.56 R ² = 0.01	80
Marylebone	96 (32 -183)	AQ1 78 (37– 138)	0.029x + 76.27 R ² = 0.002	0.027x + 103.9 R ² = 0.07	-21
		AQ3 5 (1 – 17)	0.001x + 4.67 R ² = 0.01	0.002x + 5.99 R ² = 0.0002	95
		AQ4 24 (2 – 62)	0.036x + 21.08 R ² = 0.001	-0.032x + 27.38 R ² = 0.0002	76

Table 3.24 show the correlation between the Aeroqual sensors at the different sites during the summer. The sensors at Tyburn show good inter-sensor correlation (0.64 – 0.79). All the sensors at North Kensington show very weak correlation (0.002 – 0.08) except AQ2 against AQ1 that show very strong correlation. At Marylebone all but AQ1 against AQ3 (with R^2 value of 0.12) show good to strong correlation (0.60 – 0.80).

Table 3.24. Relationship between the Aeroqual sensors in the field validation in the summer

Location	Relationship between AQ sensors	Regression coefficient	R^2
Tyburn	AQ4 against AQ3	$0.514x + 10.54$	0.79
	AQ4 against AQ5	$1.119x + 3.851$	0.64
North Kensington	AQ2 against AQ1	$0.999x - 0.129$	0.99
	AQ2 against AQ3	$-0.038x + 14.43$	0.002
	AQ2 against AQ4	$-0.514x + 39.14$	0.05
	AQ2 against AQ5	$-0.173x + 24.59$	0.02
	AQ2 against AQ5	$0.123x + 4.541$	0.08
Marylebone	AQ1 against AQ2	$6.061x + 103.53$	0.12
	AQ1 against AQ3	$0.325x - 0.986$	0.60
	AQ1 against AQ4	$0.963x - 26.19$	0.80

Table 3.25 show the correlation between the Aeroqual sensors at the different sites during the winter. The measurements made by the Aeroqual sensors in Harwell and North Kensington showed from very weak correlation amongst themselves (R^2 value ranged from $7 * 10^{-5}$ – 0.33), except Aeroqual sensor 1 against Aeroqual sensor 4 in North Kensington that show a strong correlation ($R^2 = 0.91$). In Marylebone the sensors show a good to strong correlation

between themselves (0.70 -0.91). The intercepts in Table 3.24 and Table 3.25 show the different offset amongst the Aeroqual sensor.

Therefore, because of the inconsistencies observed between the Aeroqual sensors and the RFS and especially the inter-Aeroqual comparison observed in this study, the Aeroqual sensor was not validated and therefore not used in further studies.

Table 3.25. Relationship between the Aeroqual sensors in the field validation in the winter

Location	Relationship between AQ sensors	Regression coefficient	R ²
Harwell	AQ1 against AQ3	0.009x + 30.92	7 * 10 ⁻⁵
	AQ1 against AQ4	0.306x – 9.184	0.33
	AQ1 against AQ5	-0.256x + 40.19	0.06
	AQ1 against AQ6	-0.491x + 75.73	0.06
North Kensington	AQ1 against AQ3	0.028x + 4.544	0.009
	AQ1 against AQ4	0.529x – 18.25	0.91
Marylebone	AQ1 against AQ3	0.202x – 12.55	0.70
	AQ1 against AQ4	0.526x -16.92	0.91

3.4.2.6 MicroAethalometer (AE-51)

This instrument has been studied extensively by various researchers however it was validated in this study before the use for personal and indoor exposure measurements.

The R² between the AE-51 and the RFS AE-22 comparison for the summer in Tyburn ranged from 0.50 – 0.64, in Harwell the R² ranged from 0.54 – 0.59, in North Kensington the R² ranged from 0.61 – 0.69 showing a good correlation and in Marylebone the R² ranged from 0.50 – 0.66 all of which showed good correlation.

In the winter season, there were no data for the Tyburn site because there was a problem with the RFS data. However, the R^2 between the AE-51 and the RFS AE-22 comparison in Harwell ranged from 0.79 – 0.84, in North Kensington the R^2 ranged from 0.81 – 0.90 and finally in Marylebone the R^2 value ranging from 0.59 – 0.63. All of which showed a good to strong correlation. AE51 BC PS and other reference Aethalometer in other studies have shown strong correlation with R^2 ranging between 0.83 and 0.95 (Delgado-Saborit, 2012, Ducret-Stich, 2014, Nel et al., 2001). However AE-22 automatically changes the filter ticket before optical saturation is reached while the AE-51 manual changing was required. Therefore even though change of the filter ticket was often a slight delay in addition to instrument noise and vibrations could affect attenuation, thus affecting AE-51 measurements but correction factors were applied. Differences were observed in intercept show zero-off setting between the AE-51 sensors (Table not displayed).

3.4.2.7 Relationship between the AE-51 and Elemental carbon measurements

The average of the AE-51 measurements, were compared with the average EC values collected at the various sites. The EC-values were obtained from the quartz filter that was attached to a pump. Table 3.26 show the linearity fit equation and the difference in the intercept is due to difference in methods. However the both measurements show very strong correlation and show no significant difference in their measurements. The summer correlation is based on 4 concentrations for week 1 and 4 concentrations for week 2 making it 8 concentrations. Then the winter is based on 6 concentrations i.e. 3 concentrations for week 1 and 3 concentrations for week 2.

Despite strong correlation between the EC and the BC, there are variations in the different FS and shifts in slopes and intercepts. This may be because the light absorption property of BC is different from that of EC as a result of difference in their physicochemical properties (i.e.

chemical composition and particle size distribution). Also the mixing state due to the wind speed and direction as a result of the weather condition and other meteorological conditions can also be factors. For example wood smoke particles had lower light absorptivity than traffic generated particles as shown by Jeong et al. (2004). High wind speed can bring different EC particles e.g from wood and diesel with their different structures and in turn affect EC concentrations and furthermore result in variations with BC. Atmospheric processing changed the absorptivity where EC collected within 2 m of a roadway was twice as light absorbing as that collected a 1 km from a large interstate highway (Garçon et al., 2006).

Table 3.26. Relationship between the AE-51 measurements and EC values

SUMMER			
Sensor	Regression coefficient	R²	Mann-Whitney U
AE-51-1	1.186x + 0.29	0.91	0.410
AE-51-2	1.324x + 0.67	0.89	0.606
AE-51-3	1.221x + 0.67	0.88	0.401
AE-51-4	1.265x + 0.74	0.86	0.442
WINTER			
Sensor	Regression coefficient	R²	Mann-Whitney U
AE-51-3	0.727x - 0.86	0.89	0.827
AE-51-4	0.750x - 0.83	0.89	0.827

2-tailed probability test

3.4.3 Use of outdoor reference fixed sites to calibrate sensors for indoor and PE and challenges with this

The fixed reference sites were used in this study because the reference sites contain the European reference and regulatory methods under 2008/50/EC Directives requirements for measuring air particulates and gases. The FSs are used to monitor the pollutants to make sure that they do not exceed the guidelines. Thus comparing the PS measurements against the reference methods validates their performance and ability to cope in any environment. The reference sites are making important contribution in providing data to assess trends and background concentrations for the large population but the PSs can make spatial, temporal, personal and indoor measurements. One of the major challenges is for the PS measurement to match the reliability and robustness of the reference sensors at the fixed sites. Therefore PSs are required to show that they can reasonably measure ambient concentrations of the particulates and the gases across an adequate range with good sensitivity. Hence, the reason why different reference sites representing different concentration levels and particle characteristics i.e., background, urban background, urban busy roadside and rural background sites during the warm and the cold season were used to test range and sensitivity of the PSs against FSs. Also with the reference sites, other factors such as meteorological condition effects on the PSs compared to FSs can be measured. Another reason for using fixed reference sites is that, these reference sensors cannot be placed inside a home because of their size or method of operation, thus the PSs are placed by the reference sensors to make sure that the PSs can provide a degree of quality data that can be used for PE analysis before deploying them to the indoors or for PE assessments. As observed, the Aeroqual NO₂ PSs were not validated because the instrument did not consistently have a good correlation with the reference method and showed poor and inconsistent inter-aeroqual correlation especially in the winter. Suggesting that the temperature and relative humidity due to the meteorological

conditions may have affected the measurements made by the Aeroqual (Table 3.22 to Table 3.25). Another example in this study, the DM showed good correlation with the PNC made with the SMPS and CPC from both the RFS and the laboratory. However, for particles < 10 nm the DM could not reliably detect these particles and for > 300 nm it underestimates the size of the particle thereby overestimating the PNC. Various studies have used fixed reference sites to validate PSs to be used for indoor and or PE measurements (Ricciardolo et al., 2004a, Brant et al., 2014, Lima et al., 2013, Mead et al., 2013). Other problems faced with the PSs as compared to the RFSs are battery replacement problems, longevity of the instrument like in the case of the DM where the corona wire sensitivity starts to deteriorate (before the end of the summer season validation) and this affects outcome therefore frequent maintenance was also needed. Frequent data download from the PSs was required due to limited sensor storage capacity so as to prevent overwriting of new measurement over the old and allow easy reconciliation of timestamp malfunction like in the case of the MicroPEM. During deployment of the PSs at the RFS for comparison, making sure that the PS inlets are placed in the same area as the RFs so that relatively the same air is sampled and that the flow inlets are not blocked was also a challenge. Also availability and access to all the required RFS can be found at a reference fixed site.

3.5 Conclusion

This study constituted a laboratory study for the DM validation and a field study for the DM, MicroPEM, MicroAethalometer and Aeroqual analysers. In the laboratory study, it was observed that the DM and the SMPS/CPC system showed good correlation with R^2 value ≥ 0.83 based on the sampling time in Figure 3.1. The accuracy calculated as % bias in the laboratory validation for the PNC of the 3 DMs was mostly within $\pm 35\%$ of the reference value obtained from the SMPS. Except for DM3 in cycle 1 with and DM1 cycle 5 with accuracy of 43% and -39% respectively. This is in agreement with other studies that suggest that the DM falls within and the manufacturers almost all the time (Šlouf et al., 2006, Mills et al., 2013, Asbach et al., 2012). Precision of the DMs were measured as bias-corrected precision ranging from 19 to 37% across all 5 cycles. The major discovery in the laboratory that has not been identified by other studies is that for $PNC \geq 1.5 \times 10^5$ number/cm³, the DM loses accuracy in reference to the SMPS for the poly dispersed particles that was studied. However $PNC \geq$ that concentration were not observed in the field validation therefore this could not be further explored. The DM can detect particles as low as 10 nm to 300 nm comfortably. However for particle sizes < 10 nm and > 300 nm, a reference instrument should be used along side. This is because the DM assigns a larger charge to particles > 300 nm thereby underestimating the size of the particle and then overestimating the PNC. While the DM cannot detect sizes < 5 nm suggesting that size measurements with the DM may not be so reliable for sizes outside this range but can be used to give size estimates in exposure measurements. LOD of the DMs were measured and the values obtained were in line with the manufacturer's value; i_D and i_F must both be at least 1 fA to make a concentration measurement of $\geq 10^3$. The value obtained in this laboratory study for i_D and i_F are 1.15 fA

and 1.65 fA respectively are in agreement with the manufacturer but are slightly high than 1 fA, giving a PNC of 1400 numbers/cm³.

The field validation allowed us to investigate the response of DM over wider range of sizes, mixture of different aerosols over a period of 336 hours nonstop in a real life scenario.

Despite strong correlation with the CPC and SMPS the accuracy of the DMs at some sampling sites were $> \pm 35 \%$ observed by other studies. This may be because 1) the instrument may not be built for very long hours of nonstop sampling especially in with the outdoor harsh conditions. 2) Whenever the instrument shows the error sign (high voltage) the data outcome of the DM measurement is affected. 3) Particle size is mostly outside the optimal range and therefore the σ_g of the size distribution are quite different from the σ_g 1.7 used by the manufacturers to calibration the instrument then deviations are possible.

The MicroPEM was developed to measure PM_{2.5} for both personal and indoor exposures, including strong source indoor air pollution according to Chartier et al. (2013) but not exactly for longer than 24 hours outdoor monitoring. Nevertheless the MicroPEM was validated in this study over long sampling hours (~336 hours) using the outdoor air. There was a problem with the placement of the O-ring during some of the study and factors outside the capacity of the MicroPEM such as meteorological factors may have influenced the measurement.

Generally the concentrations trend match i.e. they both respond to the same temporal variations during measurements. About 70 -80 % of the time the instrument showed good to strong correlation with the FDMS-TEOM especially over 24 hours average. In reference to the RFS FDMS-TEOM, the MicroPEM showed a bias of -67 % to 18% in the summer and higher bias in the winter than the summer ranging from 12 % to 201%. Indicating that in the winter the MicroPEM at all times made higher PM_{2.5} concentration measurements than the FDMS-TEOM. This may be due to the effect of temperature and RH, however these

parameters were not measured so this is merely a speculation. The MicroPEM real-time showed better correlation with the FDMS-TEOM and stand-alone as compared to the MicroPEM integrated filter. Thus suggesting that the MicroPEM real-time makes better measurement than the integrated especially in this case where there was problems with the O-ring during some of the sampling.

The major issue with the MicroPEM is with the raw data, where obvious outliers, negative values, blank and 0 values were observed. However these values were removed during data processing. Also, the loss of timestamp, or the timestamp reverting to default, especially when there is loss of power in the instrument is another problem. Variability observed in the slopes and intercepts where due to off sets associated with the zeroing of the instruments during calibration, i.e. over long sampling periods offsets starts to occur as seen in this study therefore requires shorter sampling times so that zeroing/calibration can be more frequent. These offsets may have affected the outcome of the comparison at between the RFS and the MicroPEM results. Removal of the entire data anomaly can be cumbersome and can lead to losses that may affect final result outcome or loss of data. It is easy to use however requires high maintenance (change of the filter, nephelometer zero corrections, calibrations, batteries). Laboratory validation of the MicroPEM is strongly recommended in other to check various factors such as environmental conditions, volatile and non-volatile particles and perhaps interference of other pollutants. Also indoor validation is also advised. Unfortunately we could not perform these validations due to time constrains but another researcher in the team did an indoor validation.

However based on the good to strong correlations with the reference instrument that were observed ≥ 70 % of the time in this study and despite using it for ambient monitoring for

several hours more than the recommendations of the manufacturer. The MircoPEM is a good instrument to use for PE and indoor air sampling. This is because results that are plausible and comparable to the FDMS-TEOM can be obtained. The outcome in this study is generally supported by other research work by other authors.

This is one of the few studies that have tried to validate the Aeroqual sensor. The plus side of this instrument is that it can detect NO_2 in the environment where it is measuring. Therefore episodes or sources of NO_2 can be identified. However the validity of the measurement can be questioned owing to the measurements and results seen in this study. Performance of this instrument is affected by environmental and meteorological conditions as seen in the results obtained at Marylebone in the summer and during the winter campaign as compared to the summer. Also inter-Aeroqual sensor comparison was not consistent. . Therefore correction factors may be applied to correct the effects of these environmental and meteorological conditions. Thus a laboratory validation is required in order to test these conditions.

Unfortunately due to time factor this study could not go into that. Therefore the Aeroqual sensor was eliminated from the subsequent personal and indoor exposure measurements.

In all most all the measurement in this study AE-51 show good to strong correlation with the AE-22. It also showed a very strong agreement with the EC values. The AE-51, as long as the filter tickets are changed frequently to avoid overloading, correction factors are applied to remove the negative values due to noise and filter overloading, it is a good instrument to use for indoor and PE. Other researchers have also supported this outcome.

The small size and light weight of these PSs means that they can be easily carried around by subjects during activities/MEs. This can help in identification/estimation of corresponding

exposure levels in the different MEs. The PE measurements that will be made by these sensors can help epidemiological and toxicological studies gain knowledge on environmental (both indoor and outdoor) drivers of air pollution in an activity/ME. Thus help decision making for public health purposes for example applying control measures to activities/MEs where air pollution exceeds acceptable limits. Furthermore the degree of adverse effect associated to these exposures can be identified. That is by connecting PE measurements in precise activity/ME to biomarkers of exposure like will be applied in the rest of this study (chapters 4 to chapter 9). Combining these PE datasets with clinical information to obtain pathophysiological correlation can improve results of clinical decision-making and care on a more individualised basis (Folino et al., 2009). Epidemiological studies can then use the PE information gathered by these PSs, to raise awareness of which activities/MEs result in potential exposure. Furthermore, the PE data obtained from the use of PSs can be used for developing modeling and then the models developed can then be “upscaled” to larger populations and applied to epidemiological studies. Concentration levels of pollutants measured in PE in different MEs using PSs, that significantly correlated with biological responses can be applied as guideline concentrations in epidemiological studies. Also these PSs can be used to collect PM different MEs during PE monitoring and then these PM concentrations and other physicochemical properties of the particulates can be used in toxicological studies. That is using the particulates collected in airway model studies and studying the effects of single pollutants and synergistic effect between different pollutants on airway models.

In summary PSs will reduce drawbacks associated with the FSs during measurements in the breathing zone without losing information about the impact of the activities/ME on exposure. However, the exposure assessment conducted using data collected by fixed monitoring stations, has the advantage of relying on a very large number of data with minimal effort in

terms of time, human, and economic resources (Maestrelli et al., 2011). This method is therefore suitable for studies on large populations, although inevitably affected by a series of assumptions, sometimes critical, compared to the real exposure scenario (Maestrelli et al., 2011).

The values obtained from the PSs will be used as absolute values i.e. no errors and or range will be used for the entire studies in this thesis. Correction values as described in chapter 2 will only be applied to MicroAeth BC data while the rest of the measured pollutants will have no correction values applied. Except reasonable removal of obvious outliers, negative values and other invalid data as described in this chapter 3. Correction factor was applied only to MicroAeth BC data because the corrections factor is available, validated and peer-reviewed. While for the others, deriving of correction factors were not part of my own work, and correction factors have not yet been published during my validation study therefore I am unable apply any correction factors and thus used the absolute values instead for the PE study. Also for the data collection all through the studies in this thesis, the same PSs will be used and I am mainly looking at correlations between the pollutants (BC, PM_{2.5} and UFP) concentrations and the biomarkers, the fact that the data is not corrected and only absolute values have been used do not interfere with the results obtained in the pollutant-biomarker correlations

It is essential that the data obtained by the PSs are robust. That is, they can make valid, reasonable and comparative measurement to the RFSs. The validation study shows that the measurements made by these PSs were robust. This is because, of the good to strong correlation observed between the PSs and the RFSs especially the DM and MicroAeth. The O-ring of the MicroPEM was not placed properly hence the sampling line was not sealed thus

affecting the measurements during the validation study. The MicroPEM on the other hand even with the problem with the O-ring, most of the 24 hours real-time PM_{2.5} measured showed to be reasonable as compared to the MicroPEM integrated filter. Therefore the use of the MicroPEM real-time instead of the integrated will be used for the rest of the research. However, the problem with O-ring as previously explained was rectified and two other researchers in the team carried out another validation of the MicroPEM before it was used in the rest of this research. This can be found in “USE OF REAL-TIME SENSORS TO ASSESS MISCLASSIFICATION AND TO IDENTIFY MAIN SOURCES CONTRIBUTING TO PEAK AND CHRONIC EXPOSURES; 5-MONTH YEAR 2 PROGRESS REPORT. Author: Dr Juana Maria Delgado-Saborit, University of Birmingham, September 2014”.

The Aeroqual NO₂ PS showed in both reference and inter-Aeroqual comparison to be very inconsistent thus was not validated and will not be used in the rest of the PE and indoor sampling. However, the DM, AE-51 and MicroPEM show that reasonable estimate values can be obtained using them. The values they measured can be used in calculating association with health effects, which is one of the objectives of this research. Thus the validated DM, MicroPEM and MicroAetherlometer will then be used for the PE measurements for the rest of this study i.e. chapter's 4 to chapter 8.

4 USE OF PERSONAL SENSORS TO MONITOR PERSONAL EXPOSURE IN THE INDOORS AND ANALYSIS OF BIOMARKERS OF THESE EXPOSURES

4.1 Introduction

Exposure to UFP are associated with increased morbidity and mortality of cardiovascular, pulmonary diseases and even cancer (Valavanidis et al., 2011, Huang et al., 2009, Müller et al., 2010, Baalousha et al., 2012, Valavanidis et al., 2008, Risom et al., 2005). Bound to these particles are components such as the transition metals and polycyclic aromatic hydrocarbon that have the potential to induce oxidative damage, inflammatory injury and other biological effects (Valavanidis et al., 2008, Li et al., 2003). BC is a harmful component of UFP (Ng and Moore, 1998) which is said to be an indicator of adverse health effect (Janssen et al., 2011). Studies have shown that BC has the ability to induce oxidative stress (Nyhan et al., 2014) and synergistic effect of BC and iron oxide particles also induced oxidative stress (Seaton et al., 2005). Another study observed Increases in BC and PM_{2.5} were associated with increases in oxidative stress (Brunekreef et al., 1997).

PM generation moves in time and space. It is generated when people are in a mobile mode, in different ME such as home, work, restaurants, in-vehicle, school e.t.c. As the PM is generated in the different ME/activities, people become surrounded with PM in their personal breathing zone. Thus PM concentrations may vary across breathing zones, regions and time depending on activity. Hence using the PM measurements from the fixed central site may not be representative of personal exposure. Bearing in mind people spend most of their time indoors, PE measurement using PSs gives an in depth measurement of the indoor environment. In order to monitor the health effects associated with exposure to pollutants, relationship between biomarkers/health effects and PE concentrations of pollutants are measured.

4.2 Aims and objectives

The aim of this study is

- 1) To measure PE using PSs with activity diary then compare the concentrations measured with central FS measurements in order to check spatial variability
- 2) To analyse the biomarkers of exposure to these PM and measure relationship between PS PM measurement and FS PM measurement to the biomarkers.

The main objectives are

- Description of concentrations of all the pollutants (UFP and BC) in different activity/MEs and time contribution
- Description of average UFP diameter in different activity/MEs
- Compare the daily concentrations of UFP and BC measured by the PS to the FS for each activity/ME.
- Relationship between UFP, BC, size in the different activities/MEs
- To measure biomarker of PE (urinary 8-oxodG, EBC-nitrate, nitrite and pH) and assess its relationship with PE levels of UFP and BC for both PS and FS measurements.

4.3 Methodology and materials

4.3.1 Study design

In this study, the PE to UFP and BC of a subject was measured for 9 days in June 2012 in Birmingham UK of which 2 days were bank holidays, 4 days weekend and the rest were work days. The subject was given a backpack containing UFP sensor (Aerasense Philips nanotracer) and BC sensor (Micro-Aethalometer AE-51) and was asked to carry the backpack or place the bag beside the subject for the entire sampling period even when sleeping except when bathing or absolutely not possible. The subject was also asked to carry a GPS receiver data logger during the sampling period in order to help in location identification. A set of questionnaires as described in chapter 2 was also given to the subject to enable collection and interpretation of relevant data. A voice recorder was also given to the subject to facilitate the activity logging. The data from the fixed central site: Tyburn Birmingham urban background site was obtained from the AURN site and staff. Morning after the previous sampling day EBC and urine samples were taken from the subject in order to analyse for biomarkers of exposure. Method of collection and analysis of these samples have been described in chapter 2. All instrumentations used in this study have been discussed in details in chapter 2. In the results and discussion PNC was used as a surrogate for UFP therefore the words were interchanged.

4.3.2 Biomarker analysis

Urine and EBC samples were collected from the subject starting from morning after Day 2 till the end of the sampling period. Morning after Day 1 urine and EBC samples were not properly stored therefore discarded. 9 urine samples were collected and all the 9 samples were measured for 8-oxodG and normalised with creatinine successfully. Only 8 were used in the comparison with exposure to pollutants because there were no pollutant measurements for

Day 10. The subject was not subjected to any food, drink or lifestyle restriction. The urine was analysed for 8-oxodG, while the EBC for NO_2^- , NO_3^- and pH. All 8-oxodG was corrected with creatinine.

Transport mode and work MEs were not used in the relationship with biomarkers because out of the 9 days the subject used the transport mode for only 4 days and work for only 3 days (2 days of bank holiday in the sampling week).

4.3.3 Data analysis

4.3.3.1 Descriptive statistics

Descriptive statistics of the activity/ME, which include the minimum, maximum, mean and standard deviation (SD) measurements were used in the data analysis and discussion. The concentrations for each pollutant and size were calculated for each activity/ME by averaging the concentrations and size measured overtime in each activity/ME as confirmed by the GPs and activity diary. The activity diary and GPs were used to reconcile the ME/activity to the time. Daily (24 hours) descriptive statistics measurements were also calculated for the PS and FS measurements based on 15 mins average.

The Unclear time ME was not included in the PE of the subject because the PS was not with the subject. Meaning PE measurement at the subject's actual location was not recorded. However unclear time was included in the statistical summary of measured exposure details.

Descriptive summary of the different biomarkers analysed in this study were also calculated. Variation between the biomarkers on the different sampling days, were calculated using coefficient of variation.

$$\text{CV} = \text{SD}/\text{mean} \quad (4.1)$$

4.3.3.2 Relationships

Relationships between various variables were calculated in this study.

4.3.3.2.1 Relationship using spearman's rank correlation

Spearman's rank correlation was used to determine the association between all variables in this study.

4.3.3.2.2 Other relationships

Ratio and % differences were calculated between the FS and the PS measurements.

Differences of means were tested with Mann-Whitney U test.

$$\% \text{ Difference} = ((\text{Fixed site} - \text{PS})/\text{Fixed site}) * 100 \quad (4.2)$$

$$\text{Ratio} = \text{FS/PS} \quad (4.3)$$

4.3.3.3 Spatial variability

Spatial variability was assessed by calculating the coefficients of divergence (COD). In this study, the COD was measured for the daily concentrations of the PS and the FS and also PS and FS according to activities/ME.

The COD provides information on the degree of uniformity between the PS and the FS over measurement time and is defined as (Wilson et al., 2005, Kearney et al., 2011)

$$COD_{yz} = \sqrt{\frac{1}{p} \sum_{i=1}^p [(x_{iy} - x_{iz})/(x_{iy} + x_{iz})]^2} \quad (4.4)$$

Where x_{iy} and x_{iz} are the activity/ME averaged concentration at a given time i at the two different sites y and z and p is the number of observations. Small COD values imply similarities between the concentrations measured at various sites i.e. COD closer to 0, while COD values approaching unity indicate vast differences between sites i.e. COD closer to 1 and an indication of absolute heterogeneity (Wilson et al., 2005).

4.4 Results and discussion

4.4.1 Personal exposure concentrations

Table 4.1. Description of concentrations and particle size of the pollutants in different microenvironments and activities measured by the PS per 15 mins observation

Activity/Microenvironment	Statistics	UFP (#/cm ³)	Size (nm)	BC (µg/m ³)
Sleeping	Min	562	30	0.09
	Max	6.96 x 10 ⁴	152	14.82
	Mean	3.72 x 10 ³	91	1.65
	SD	5196	20	2.24
Indoor others	Min	520	32	0.53
	Max	8.4 x 10 ⁴	148	31.79
	Mean	6.56 x 10 ³	78	1.79
	SD	9386	19	3
Transport Mode	Min	1.904 x 10 ³	42	0.07
	Max	3.38 x 10 ⁴	78	10.62
	Mean	1.71 x 10 ⁴	56	4.45
	SD	10019	8	3.50
Work	Min	1.99 x 10 ³	42	0.07
	Max	8.52 x 10 ³	87	1.78
	Mean	3.395 x 10 ³	69	0.62
	SD	1116	9	0.37
Cooking Activities	Min	1.09 x 10 ³	26	0.10
	Max	8.01 x 10 ⁵	94	37.19
	Mean	7.87 x 10 ⁴	62	2.76
	SD	143866	21	6.25
Housework	Min	1.683 x 10 ³	39	0.25
	Max	1.19 x 10 ⁵	85	23.79
	Mean	1.29 x 10 ⁴	62	1.07
	SD	22416	12	0.56
Unclear Time	Min	3.71 x 10 ³	49	0.14
	Max	3.69 x 10 ⁴	92	2.13
	Mean	9.275 x 10 ³	73	0.77
	SD	7327	12	0.396
Mixed Environment	Min	1.19 x 10 ⁴	47	0.68
	Max	2.98 x 10 ⁴	63	5.71
	Mean	2.08 x 10 ⁴	55	2.48
	SD	12674	11	2.80

Due to instrument malfunction some of the data were not included in this section. The activities/MEs were categorised into 8 groups as described in chapter 2.

The description of all the pollutants concentrations in the different activities/MEs (Table 4.1), showed that highest concentration for UFP was 8.01×10^5 numbers/cm³, BC: 37 µg/m³ and the smallest particle average size 26 nm were all measured during the cooking activity in the kitchen. The lowest UFP and BC concentrations were measured in the indoor others activity/ME (i.e. no exact activity was happening) 521 numbers/cm³ and 0.5 µg/m³ respectively. The largest average particle size 152 nm was measured in the sleeping activity/ME.

It was observed that UFP concentration had an inverse relationship with the particle size, which remained almost stable for some of the activities/MEs, making these MEs to have very similar mean particle size for the entire sampling period. The mean average particle size of 56 nm (minimum 42 nm and maximum 78 nm) for the transport mode in this study falls within the range measured in other transport exposure studies. A mean average particle size of 64 nm was reported for pedestrian and cyclist in traffic related route and 70 nm for traffic free route, 60 nm in bus and in car 70 nm ME using the DiSCmini (Spinazzè et al., 2015). 40 nm mean average particle size was reported for pedestrians also using the DiSCmini (Ragettli et al., 2013). The mean average particle size for cooking activities was also similar to the average sizes reported by other researchers both in real life and laboratory kitchens (Dennekamp et al., 2001b, Sjaastad, 2010, Wallace, 2006). A study demonstrated that the PNC resulting from cooking had number median diameter ranging from 22 to 940 nm (He et al., 2004) but style of cooking, condiments/foodstuff, cooking fuel/energy and ventilation system affect the concentration and particle size produced (Wallace, 2006, He et al., 2003, Koczulla et al., 2009a). In the sleeping ME, it is not surprising that the particle size increased because in the absence of no indoor activity, nucleation mode particles (< 30 nm) start to undergo processes like coagulation and accumulation (>110 nm (Ferrazzoni et al., 2009))

thereby increasing in size and decreasing in concentration as the accumulated particles settle out on surfaces.

Taking into consideration all the activity/MEs in this study, it is obvious that the cooking activities had the highest UFP concentration and this agrees with other studies that cooking activities contributes greatly to indoor pollution (Koczulla et al., 2009a, Watson et al., 1988, Gwinn and Vallyathan, 2006).

Figure 4.1 show the percentage time contribution spent by the subject in the various activity/ME. Over the 9days the subject spent, 38% of time at home sleeping, 36% indoor others, 8% work, 6% cooking activities and 4% housework. Cumulatively, the subject spent almost 93% of the entire sampling time in the indoor environment, suggesting that most of the subject's time was spent in the indoor thus agreeing with other researchers observations for PE study over different time frame (Buonanno et al., 2014b, Gu et al., 2015, McCreddin et al., 2015). The subject spent about 1% of time in the mixed environment. This environment is a combination of indoor and transport mode. In addition to the 93% percent spent indoors, the subject spent a little over 3% (if the 1% in the mixed environment is factored into the transport mode) in the transport mode and this similar to the study by McCreddin et al. (2015), where the subjects spent 92% of their time indoors on an average day and 3% in enclosed transit. However in this study, the 3% in the transport mode activity/ME was not necessarily enclosed. It was a combination of car-driving, car-taxi, walking on the street to and away from the bus stop and travelling in the bus during the entire sampling period. Another recent study observed around 5% time was spent outdoors of which included active transport (Bekö et al., 2015).

When indoors the subject spent 6% of the time doing cooking activities and 4% doing housework meaning the subject spent 10% of time doing household duties (Figure 4.2). During the household activities PNCs of the UFP were raised as compared to absence of activity/household duties (sleeping and indoor others) (Table 4.1). Similar result was observed by Gu et al. (2015) but in their study participants spent 13% of their time in the indoor environments doing these household duties.

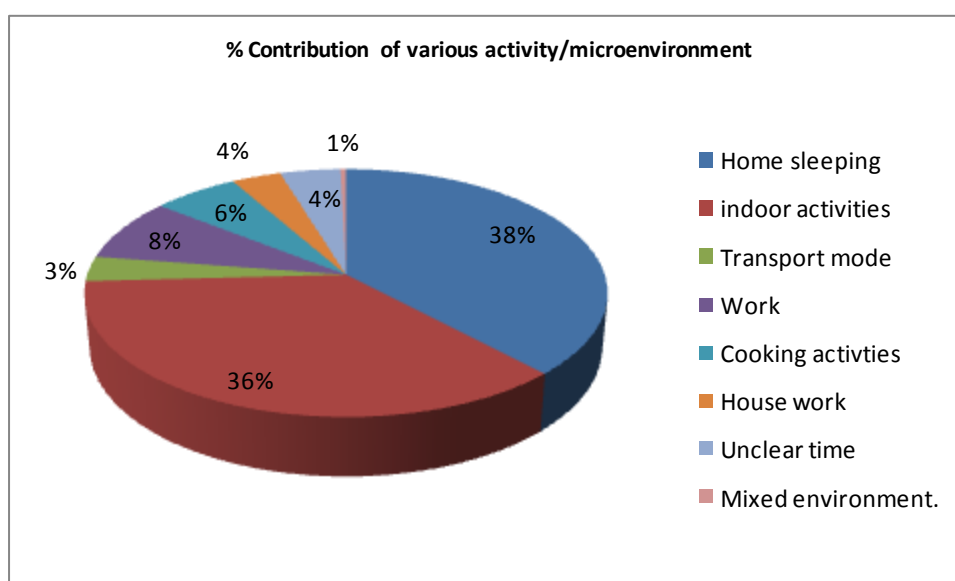


Figure 4.1. Percentage time contribution of the various activity/microenvironment for the entire sampling period

4.4.2 Fixed site measurement Vs Personal sensor measurement

Table 4.2 and Table 4.3 show the daily descriptive statistics UFP and BC for FS (Tyburn, Birmingham urban background site) and PS measurements respectively. Due to technical issues the FS did not have data from Day 1 until evening of Day 6 for UFP, thus only time with valid measurements for both FS and PS were used for the correlation calculation.

Table 4.2. Statistical daily summary for UFP (numbers/cm³) for fixed and PS measurement based on 15 mins average

		Descriptive statistics			
Day	Site	Minimum	Maximum	Mean	SD
Day 6	Fixed	1.89 x 10 ³	5.53 x 10 ³	3.79 x 10 ³	925
	PS	1.08 x 10 ³	3.30 x 10 ⁴	2.08 x 10 ³	581
Day 7	Fixed	1.34 x 10 ³	1.29 x 10 ⁴	4.81 x 10 ³	2394
	PS	521	8.52 x 10 ³	2.36 x 10 ³	1615
Day 8	Fixed	1.86 x 10 ³	2.98 x 10 ⁴	7.32 x 10 ³	6357
	PS	562	8.01 x 10 ⁵	1.51 x 10 ⁴	82439
Day 9	Fixed	3.86 x 10 ³	2.44 x 10 ⁴	1.34 x 10 ⁴	5487
	PS	1.74 x 10 ³	2.58 x 10 ⁴	5.59 x 10 ³	4203

The FS showed higher mean PNC except for Day 8 (Table 4.2). This showed that the activity/ME of the subject influenced the measurements of the PS measurement. On day 8 there was cooking activity (frying) for a minimum of 15 mins on gas while the other days did not have that type of cooking activity. They had only microwaving and boiling. Studies have reported that 80 - 90 % of cooking aerosol particles measured is UFP (Hussein et al., 2006). Cooking can raise indoor PNCs by a factor ranging from 1.5 $\geq$ 27 times (He et al., 2004) and oil based cooking such as frying generates even more UFPs (See and Balasubramanian, 2006). The cooking activity therefore drives the UFP concentration. In addition to the cooking activity, other indoor activities and the outdoor sources also contributed to the concentration indoors. This is the reason why the PS mean PNC measurement for Day 8 is higher than the FS.

Table 4.3. Statistical daily summary for BC ($\mu\text{g}/\text{m}^3$) for fixed and personal sensor measurement based on 15 mins average

		Descriptive statistics			
Day	Site	Minimum	Maximum	Mean	SD
Day 1	Fixed	0.28	1.34	0.89	0.30
	PS	0.34	4.20	1.19	0.63
Day 2	Fixed	0.11	0.95	0.39	0.18
	PS	0.13	31.79	3.51	5.47
Day 3	Fixed	0.11	4.25	0.79	0.83
	PS	0.05	37.19	2.22	4.68
Day 4	Fixed	0.39	1.70	0.90	0.26
	PS	0.33	3.58	1.24	0.81
Day 5	Fixed	0.12	5.50	1.98	1.28
	PS	0.07	11.15	1.43	1.81
Day 6	Fixed	0.28	3.31	1.23	0.56
	PS	0.28	10.28	1.28	1.44
Day 7	Fixed	0.14	1.84	0.91	0.53
	PS	0.08	8.24	0.65	1.11
Day 8	Fixed	0.13	7.68	1.29	1.67
	PS	0.13	17.74	1.35	2.10
Day 9	Fixed	0.14	6.04	2.47	2.14
	PS	0.35	7.20	3.69	2.49

The daily PS mean BC concentration measurement was higher for all the sampling days except Day 5 and Day 7 (Table 4.3). Day 2 accounts for the second highest 15 mins maximum $31.79 \mu\text{g}/\text{m}^3$ and the second highest mean $3.51 \mu\text{g}/\text{m}^3$ (Table 4.3). This is interesting because it occurred during indoor others ME (Table 4.1) with no exact activity happening. However there was barbeque in the outdoor but the subject's windows were

closed and it infiltrated into the house. This shows that with no indoor source, the indoor concentration is affected by the outdoor. Thus the outdoor is a contributing factor to indoor pollution. A study observed that barbecue in the summer increased $PM_{2.5}$ indoors, but did not specify if the home windows were open/closed during their indoor sampling (Fireman et al., 2008).

The PS 15 mins observed maximum of $31.79 \mu\text{g}/\text{m}^3$ and $37.19 \mu\text{g}/\text{m}^3$ during indoor others and cooking respectively (Table 4.1) and Day 2 and Day 3 respectively (Table 4.3), highlights much higher values than the FS thereby indicating that short high exposure peaks are not captured by the FS. Also increased BC during cooking indoors suggests that combustion related particles indoors are associated to cooking activities. The transport mode showed the highest mean BC concentration $4.45 \mu\text{g}/\text{m}^3$ (Table 4.1) and BC is a marker of diesel vehicular emissions (Ali et al., 2014).

4.4.3 Relationship between personal sensor and fixed sensor measurement for UFP

The daily mean between FS and PS (Table 4.4) showed that Day 6 to Day 8 were significantly correlated. FS concentrations for Day 6, 7 and 9 were significantly higher than PS ($p < 0.01$) and the opposite for Day 8. The COD values for Day 6 to Day 9 were all > 0.30 . This shows that there was spatial variation between the FS and PS measurements. Suggesting that the FS and PS are measuring concentration from different sources, thus they could have high correlation but not necessarily indicate uniformity between them. If the ratio of FS to PS measurements gave a value of 1 then it would mean that the same exposure concentration was measured by the FS and the PS. The ratio of FS/PS for Day 6, 7 and 9 show that the FS during the sampling days had a maximum of 2 fold concentration greater than the PS (Table 4.4). Day 8 the FS/PS ratio was < 0.5 , suggesting that the strong indoor

source on Day 8 affected the ratio. The ratio also shows that there are no strong indoor sources and that the FS measurements are higher in (Table 4.4). This was also reflected in Table 4.6 where the % difference between fixed and PS showed to be > 100 % on Day 8, while the rest sampling days were between 45 - 58 %.

Table 4.4. Daily statistics parameter between personal and FS UFP sensors

Day	Spearman correlation		Wilcoxon signed test <i>p</i> ***	COD	Ratio FS/PS*
	<i>r_s</i>	<i>p</i>			
Day 6	0.76	0.000**	0.000	0.30	1.82
Day 7	0.70	0.000**	0.000	0.41	2.04
Day 8	0.56	0.000**	0.000	0.51	0.48
Day 9	0.22	0.36	0.000	0.49	2.39

** Correlation between fixed and personal site sensors, $p < 0.01$ means significant correlation

*** Wilcoxon signed rank test between fixed and personal site sensors, $p < 0.05$ means significant difference

*FS/PS Fixed site/Personal sensor, 2-tailed probability

In Table 4.5 Day 1, 2, 3, 6 and 7 showed very weak or no correlation while in Day 4, 5, 8 and 9 show good correlations. There was a significant difference between PS and FS measurements for all the sampling days except Day 4 and Day 8. All the days show high COD (0.16 – 0.59) meaning that the spatial distribution of BC between the FS and the PS varied. The differences observed in the spatial variation could be due to activity/ME in which the subject was engaged in because the subject is exposed to the pollutant at its source.

Table 4.5. Daily statistics parameter between personal and FS BC sensors

Day	Spearman correlation		Wilcoxon signed test	COD	Ratio FS/PS*
	r_s	p			
Day 1	-0.02	0.869	0.003	0.30	0.75
Day 2	0.31	0.006**	0.000	0.59	0.11
Day 3	0.12	0.243	0.000	0.56	0.36
Day 4	0.62	0.000**	0.251	0.25	0.73
Day 5	0.57	0.000**	0.000	0.50	1.53
Day 6	0.27	0.008**	0.020	0.29	0.96
Day 7	0.36	0.000**	0.000	0.46	1.41
Day 8	0.65	0.000**	0.829	0.16	0.96
Day 9	0.79	0.000**	0.000	0.40	0.66

** Correlation between fixed and personal site sensors, $p < 0.01$ means significant correlation.

Wilcoxon signed rank test between fixed and personal site sensors where $p < 0.05$ means significant difference.

FS/PS* Fixed site /Personal sensor, 2-tailed probability

Wilson et al. (2005) used COD of 0.099 to indicate similar sources monitored concentrations and COD 0.230 to indicate different types of sources. Based on their conclusion, the COD for both for PNC and BC in this study were all > 0.230 suggesting heterogeneity of PS and FS. However, Kearney et al. (2011) did not quite agree with their criterion of using 0.230 to distinguish homogenous and heterogeneous spatial variability. They said that there is no formal scientific/regulative definition of what constitutes uniformity at intra-urban spatial scale but agree that COD is a relative summary of difference between sites (Kearney et al., 2011).

Based on activity/MEs for UFP and BC (Table 4.6 and Table 4.7), all COD values were between 0.18 – 0.66 meaning spatial distribution varied therefore concentration between PS and FS measurements sources may not be similar.

For UFP in Table 4.6, sleeping and work show fairly good correlation while the other activity/MEs were weak. Transport mode, unclear time and mixed environment had no values because the FS had no data on corresponding days therefore comparison were not possible. Cooking activities concentrations was significantly higher ($p < 0.05$) in PS measurement as compared to FSs and for the rest, the FS measurements were significantly higher but in housework there was no significant difference (Table 4.6). UFP for cooking activities and housework were greater than FS (Table 4.6). This shows that household duties have a lot of influence in production of UFP in the indoors and ventilation such as open windows/doors allow indoor PNCs to behave similar to PNCs of their outdoor counterparts (Kaji, 1994).

Table 4.6. Activities/microenvironment statistics parameter between personal sensor and Fixed site UFP (number/cm³)

Activity/ME	Fixed site	Personal sensor	Spearman correlation r_s	Wilcoxon signed test	% Difference ((FS – PS)/Fixed)*100 site	COD
Sleeping	9.45 x 10 ³	2.53 x 10 ³	0.73**	0.000	73.21	0.50
Indoor others	7.17 x 10 ³	6.04 x 10 ³	0.30**	0.000	15.75	0.45
Transport mode	-	-	-	-	-	-
Work	7.62 x 10 ³	3.66 x 10 ³	0.71**	0.000	51.98	0.42
Cooking activities	7.57 x 10 ³	6.33 x 10 ⁴	0.43	0.022	-735.58	0.49
Housework	6.64 x 10 ³	8.44 x 10 ³	0.36	0.686	-27.17	0.18
Unclear time	-	-	-	-	-	-
Mixed environment	-	-	-	-	-	-

** Correlation between personal and FS sensors, $P \leq 0.01$ means significant correlation

Wilcoxon signed rank test between personal and FS sensors where $P \leq 0.05$ means significant difference. 2-tailed probability

Table 4.7 also showed that for BC activity/ME for the entire sampling period, all PS measurements were greater than the FS measurements except at work and unclear time in terms of % difference. Sleeping and mixed environment show fair agreement while the rest of the activity/ME did not correlate for BC. PS measurements were significantly higher in sleeping, indoor others, transport mode and housework activity/ME. A study reported that particle concentrations were significantly higher during cooking activities, and could remain that high for as long as 14 hours (Chao et al., 2016). Therefore this build up could possibly affect the BC concentration in the indoors, thereby influencing the significant difference observed between PS and FS for sleeping, indoor others and housework. During transport mode subject is at the source of BC generation i.e. the vehicle. While in work ME, FS was significantly higher. In cooking activities, unclear time and mixed environment there were no

significant difference between FS and PS measurement but spatial difference and higher percentages between the PS and FS were observed. High percentage of BC during cooking activities show influence of elemental carbon produced due to incomplete combustion during cooking. A study observed that PM_{10} inside a car was $> 16\%$ during walking but no difference was observed for the $PM_{2.5}$ and the PM_1 (Gulliver and Briggs, 2004). Table 4.4 to Table 4.7 showed that PS helped to point out the spatial differences between the PS and FS measurement thereby making sure that the differences observed in the exposures due to location and activities are not under or overestimated.

Table 4.7. Activities/microenvironment statistics parameter between personal sensor and Fixed site BC ($\mu\text{g}/\text{m}^3$)

Activity/ME	Fixed site	Personal sensor	Spearman correlation r_s	Wilcoxon signed test P^{***}	% Difference ((FS – PS)/FS)*100	COD
Sleeping	1.23	1.65	0.61**	0.049	-34.14	0.37
Indoor others	1.13	1.79	0.16**	0.046	-57.67	0.43
Transport mode	1.18	4.45	-0.35	0.001	-277.67	0.66
Work	1.52	0.62	0.12	0.000	58.84	0.52
Cooking activities	0.79	2.76	-0.20	0.371	-249.68	0.54
Housework	0.72	1.08	0.09	0.018	-50.14	0.45
Unclear time	1.28	0.77	-0.12	0.229	39.89	0.44
Mixed environment	1.16	2.46	0.50	0.275	-112.81	0.54

** Correlation between personal and FS sensors, $P \leq 0.01$ means significant correlation.

*** Wilcoxon signed rank test between personal and FS sensors, $P \leq 0.05$ means significant difference, 2-tailed probability

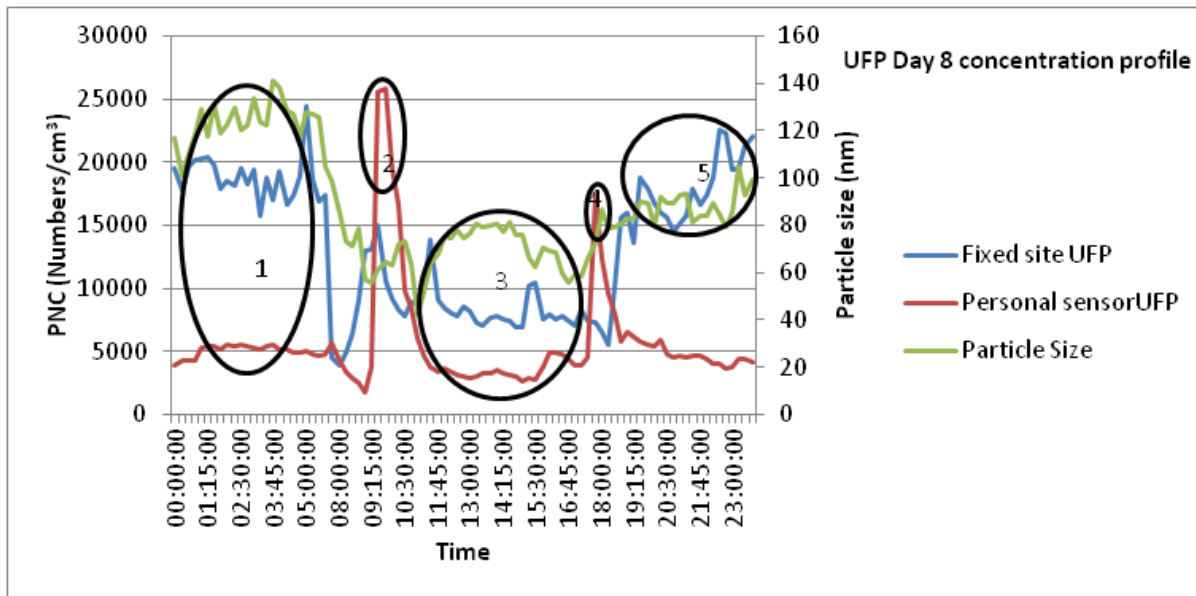
4.4.4 Relationship between pollutants in the different activity/microenvironment using personal sensor measurements

Table 4.8 show the relationship between the pollutants (UFP BC and size). UFP against BC show good to strong correlation in sleeping, indoor others, transport mode and work activity/ME, while in cooking activities it shows weak correlation of 0.35 however significant and in housework and unclear time it showed no significant correlation. UFP and BC are often well correlated however in urban air because they are from the same source (vehicular emission) and BC contribute to the chemical composition of UFP (Ng and Moore, 1998, Mutlu et al., 2001). UFP against size showed an inverse significant correlation in sleeping, indoor others, transport mode, work, cooking activities and unclear time, while housework showed no significant correlation. BC against size showed significant inverse correlation in sleeping and indoor others ME only. Work and unclear time show weak correlations that are not significant and the rest show inverse correlations that are not significant. The inverse correlation implies that the lower the concentration the bigger the particle size measured by the sensor and vice versa (Table 4.1).

Table 4.8. Spearman's correlation between UFP and BC in the different activity/microenvironment using the personal sensor measurements

Activity/microenvironment	UFP Vs BC	UFP Vs Size	BC Vs Size
Sleeping	0.82**	-0.69**	-0.50**
Indoor others	0.59**	-0.59**	-0.22**
Transport mode	0.74**	-0.58**	-0.32
Work	0.55**	-0.31****	0.22
Cooking activities	0.35****	-0.81**	-0.03
Housework	0.16	-0.33	-0.08
Unclear time	0.43	0.62****	0.14
Mixed Environment	-	-	-

****Correlation is significant at $P \leq 0.05$ level, **Correlation is significant at $P \leq 0.01$ level, 2-tailed probability



1 and 5 Sleeping ME, 2 cooking activities ME, 3 and 4 indoor others

Figure 4.2. Concentration profile for Day

Figure 4.2 shows the concentration profile for UFP on Day 8. Figure 4.2 showed that as the FS is far away from where the PS is measuring it may not be a good surrogate for the PE measurement. As seen during sleeping ME the concentration at the FS is 4 times higher than the PS measured concentration. For the cooking activities ME, as the PS concentration increased higher than the FS. Therefore an increase in one does not necessarily reflect in an increase in the other because of spatial difference. Also the inverse relationship between concentration and size is apparent (Figure 4.2 and Table 4.8). During the cooking activities, UFP (< 100) are generated and the PNC increase. The smaller the particle size the higher the PNC. While during sleeping and indoor others ME the opposite effect is seen. Number 4 (Figure 4.2) shows possible infiltration from the outdoors because no apparent activity was happening, the FS showed a drop in PNC (again reflecting the effect of distance between the FS and the PS) while the PS showed an increase in PNC. The distance between the PS and the FS on Day 8 was about 6 miles.

4.4.5 Biomarkers of personal exposure

Mechanism involving oxidative stress and inflammation has been proposed to explain association between UFP and cardiovascular diseases because UFP have the ability to induce oxidative stress and inflammatory responses.

4.4.5.1 8-oxodG (ng/mg creatinine)

Table 4.9 show the descriptive summary for 8-oxodG and CV for inter-day measurements. The CV value for 8-oxodG was 10.65 % indicating low variation between the daily 8-oxodG concentrations i.e., the levels of 8-oxodG were fairly consistent over the 9 days sampling period. The mean $\pm$ SD 8-oxodG concentration was 1.97 ± 0.21 (ng/mg creatinine) and the SD suggests that the variation between 8-oxodG concentration for the different days was not broad. Sorensen et al. (2003) in their PE to PM_{2.5} study also observed that there was no variation between daily measurement and suggested that was an indication of no apparent damage level during their entire sampling period.

Table 4.9. Descriptive summary and inter-day CV of the 8-oxodG

Samplingday	8-oxodG (ng/mg creatinine)
morning after Day 2	2.23
morning after Day 3	2.02
morning after Day 4	2.31
morning after Day 5	1.75
morning after Day 6	1.9
morning after Day 7	1.67
morning after Day 8	1.89
morning after Day 9	2.02
morning after Day 10	1.93
Mean	1.97
Median	1.93
SD	0.21
Minimum	1.67
Maximum	2.31
CV%	10.65

The mean 8-oxodG concentration observed in this study was ~ 2 (ng/mg creatinine). This is low compared to other studies (Hu et al., 2006, Lin et al., 2004b) but above the minimum concentration found in another studies for both smokers and non-smokers concentrations (Shimoi et al., 2002).

Table 4.10. Relationship between 8-oxodG (ng/mg creatinine) concentrations and exposure to UFP and BC personal sensor and FS

Biomarker	Spearman's correlation		
	UFP Personal sensor	BC Personal sensor	BC Fixed site
8-oxodG	0.39	0.38	-0.48

** Correlation significant at $P \leq 0.01$, ****Correlation significant at $P \leq 0.05$, 2-tailed probability

8-oxodG showed no significant correlation with any of the PS or FS daily measurements (Table 4.10). Similar result has been observed in other studies. For example, Sorensen et al (2003) in their study did not observe any significant correlation between urinary 8-oxodG and exposure concentration. Hemmingsen et al. (2015), did not observe any change in urinary 8-oxodG in 18 healthy non-smoking subjects (immediately and 20 hours) after exposure to DE for 3 hours in a chamber. In another study with 10 subjects with metabolic syndrome after exposure for 2 hours to filtered air or DE (at 200 μ g/m³) on 2 different days, did not observe any change in urinary 8-oxodG 3 hours and then 22 hours after initiation of exposure (Allen et al., 2009). However, in the Copenhagen driver study by Loft et al. (1999), observed significant higher urinary 8-oxodG in central bus drivers as compared to the rural bus drivers. Also Wang et al. (2011), in their study measured significantly higher 8-oxodG concentrations in cooks as compared to non-cooks in a Chinese restaurant. A possible explanation is that due to their occupation, the central bus drivers and the cooks were exposed to continuous higher concentrations of diesel-emitted PM (for the drivers) and cooking fumes (for the cooks) than the subjects in this study, which could lead to a more extensive PAH exposure and then oxidative stress. But in another study with 194 subjects, rural dwellers showed higher

concentration of lymphocyte 8-oxodG compared to the Athens city dwellers (Gong et al., 2014). Their suggested that even though the rural dwellers were exposed to a minimal burden of air pollution, the particles in their air showed to be more genotoxic because of factors like ETS as compared to the city dwellers. Therefore constituents of the air particulates may be a predictor of 8-oxodG. However the rural dwellers were constantly exposed to the air in their community.

8-oxodG correlation with the activity/ME was measured to check if activity/MEs would be a predictor of 8-oxodG. Table 4.11 showed that the UFP and BC concentration measured with the PS for sleep ME were significantly correlated with 8-oxodG. As this was not expected, lag effect was calculated in order to understand if the significant correlation observed was due to the effects from previous exposure during other activities/ME in the day before. The lag concentration was calculated as the concentrations during activities/ME from 6 am in the morning, which is the average waking time of the subject till the average sleeping time 8 pm of the subject. No significant correlation between the lag and the concentrations except with the BC-fixed site measurement that showed a significant inverse correlation. Thus the significant correlation observed in the sleeping ME (Table 4.11) is a statistics suggesting association and not a totality of causation therefore may be due to chance. However, production of 8-oxodG is associated with damage and repair of the DNA and may not be due to irritative effect due to exposure like in the case of EBC nitrate and nitrite. That is 8-oxodG is associated with continuous exposure and then imbalance between ROS/RNS and the antioxidants is reached, whereby the ROS/RNS starts to interact with DNA and other macromolecules. Thus alteration in the expression of 8-oxodG may not be measured during or immediately after exposure but the changes may appear later depending on the length of exposure, level of exposure and antioxidant and ROS/RNS interaction.

Table 4.11. Relationship between activity/microenvironment, Lag and 8-oxodG

	Spearman correlation	UFP personal sensor	BC personal sensor	BC fixed site	Average particle Size Personal sensor
Sleeping	r_s	0.91**	0.78****	0.16	0.57
Indoor others	r_s	0.02	-0.29	-0.62	-0.03
Cooking activities	r_s	0.42	-0.06	-0.19	-0.68
Housework	r_s	0.29	0.37	-0.98**	-0.23
Lag	r_s	0.24	0.42	-0.73***	0.06

** Correlation significant at $P \leq 0.01$, ****Correlation significant at $P \leq 0.05$, 2-tailed probability

Lag is all concentration before sleep at night i.e between 6 am to 8 pm

4.4.5.2 Exhaled breath condensate biomarkers

Table 4.12. Descriptive summary and inter-day CV of the Exhaled breath condensate NO_2^- , NO_3^- and pH concentrations

Sampling day	Nitrate (μM)	Nitrite (μM)	pH
morning after Day 2	5.92	0.40	7.44
morning after Day 3	8.84	0.38	8.35
morning after Day 4	6.44	0.50	7.74
morning after Day 5	18.78	0.92	8.21
morning after Day 6	10.21	0.46	8.82
morning after Day 7	15.86	0.44	8.68
morning after Day 8	20.62	0.49	7.96
morning after Day 9	7.11	0.71	7.86
morning after Day 10	8.16	0.47	8.20
Mean	11.33	0.53	8.14
Median	8.84	0.47	8.20
SD	5.60	0.17	0.44
Minimum	5.92	0.38	7.44
25%	7.11	0.44	7.86
75%	15.86	0.50	8.35
Maximum	20.62	0.92	8.82
CV%	49.45	32.81	5.45

NO_2^- and NO_3^- were detectable in the EBC samples collected from the subject. The NO_3^- mean concentration was 11.33 μM inter-day CV was almost 50 % (Table 4.12). The concentration of NO_3^- in this study is in the same range as found in other studies for healthy subjects; median and range concentration [9.6 (2.6-119.4) μM using the same ion chromatography method as this study (Corradi et al., 2003) and another study with median and 25; 75 percentile was 16.0 (7.2; 23.9) μM (Griese et al., 2003).

The NO_2^- were very low in concentration in comparison to the NO_3^- ; mean was 0.53 μM and a 33 % inter-day CV. Another study observed that the concentration of nitrate is usually 2 to 3 times higher than that of nitrite and the variability between nitrates was also higher than the nitrite (Chladkova et al., 2006). However the concentrations were in agreement with other studies; NO_2^- levels in a study showed median and range value (0.36 (0 – 2.10)) μM (Ho et al., 1998), 0.5 μM NO_2^- in healthy children (Cunningham et al., 2000) and 0.45 μM mean (Corradi et al., 2001).

The pH in this study had a mean of 8.14, median of 8.20 and CV of about 5.5 %. This is the normal range of EBC pH for healthy subjects and has been confirmed by different studies. A study of 400 healthy subjects showed that the median EBC pH of healthy subjects was 8.0 and they concluded that EBC pH is maintained in a modestly alkaline and tight range in asymptomatic subjects (Paget-Brown et al., 2006). The inter-day CV of 5.5 % showed that there was a very low variation of pH concentration between sampling days, thus suggesting no obvious change in airway pH.

Table 4.13. Relationship between biomarkers and daily concentrations for PS and FS

Biomarker	Spearman's correlation			
	UFP Personal sensor	BC Personal sensor	Size Personal sensor	BC Fixed site
EBC-Nitrate	-0.17	-0.33	0.24	0.55
EBC-Nitrite	-0.33	-0.05	0.57	0.81****
EBC-pH	-0.48	-0.48	-0.07	0.19

****Correlation significant at the 0.05 level 2-tailed probability

In Table 4.13, 8-oxodG shows an inverse significant correlation with EBC-nitrate. EBC-nitrite and EBC-pH showed an inverse correlation but not significant. Acidic pH can enhance oxidative stress i.e. low airways pH is responsible for the induction of nitric-oxide synthase in macrophages thereby enhancing oxidative stress (Wei et al., 2009). Also studies were relationship between pH and nitrite in asthmatic subjects were measured, have suggested that acid-base equilibrium may influence the concentration of EBC-nitrite/EBC-nitrate i.e., airway acidity favours the protonation of EBC-nitrite, as seen in asthmatic subjects, leading to the formation unstable nitrous acid (HNO_2), subsequently leading to the production NO (Corradi et al., 2001, Jaques and Kim, 2000, Chaouachi, 2009, Roco, 1999). In our study the EBC-pH (mean 8.14) was from a healthy subject. Thus a low EBC-nitrite and high EBC-pH will result in the inverse correlation. Perhaps sampling size may have affected significance. EBC-nitrite showed a significant correlation with FS BC (Table 4.13). A study observed a significant correlation between FS coarse particles and EBC-nitrate + nitrite but not with fine particles (Manney et al., 2012). This suggests that outdoor BC is a predictor of oxidative stress. The BC outdoor is a biomarker of vehicular emission related to diesel engines (Ali et al., 2014), which is now suggested to be an indicator of adverse health effects (Janssen et al., 2011). Exposure to BC was associated with acute respiratory inflammation in schoolchildren (Kaur et al., 2007).

Table 4.14. Relationship between sleep activity/microenvironment exposure and EBC biomarker

Biomarker	Spearman's correlation			
	UFP Sleep Personal sensor	BC Sleep Personal sensor	Size Sleep Personal sensor	BC Sleep Fixed site
EBC-Nitrate	-0.88**	-0.71**	0.56	0.14
EBC-Nitrite	-0.14	0.14	0.58	0.59
EBC-pH	-0.71****	-0.67	0.14	-0.12

** Significant at $P \leq 0.001$, **** Significant at $P \leq 0.05$, 2-tailed probability

EBC-nitrate and EBC-pH markers of localised oxidative stress and inflammation showed an inverse significant correlation with sleep ME (Table 4.14). PNC and $PM_{2.5}$ concentrations were not related to EBC nitrate + nitrite for any of the evaluated locations and lags (0, 1, 2 days) in the study carried out by Manny et al. (2012). However, in their study the 133 subjects used suffered from pulmonary diseases. EBC-nitrate and EBC-nitrite are biomarkers of effect, thus it is likely that they show their effects immediately after exposure and return to normal 6 hours after exposure according to the study by Laumbach et al. (2014). In fact the results from more study suggests that the effect of biomarker of airway acidification EBC-pH and metabolic end product of NO: EBC-nitrate and nitrite were seen within 24 hours of exposure (i.e. changes in concentration levels were observed and returned to normal) while those of DNA damage like 8-oxodG were seen slightly later (Bogdan et al., 2000, Maynard et al., 2006, Chithra and Nagendra, 2013). In this study the EBC samples from which nitrate and nitrites were measured were taken morning after exposure day. Thus Lag was not calculated for nitrate, nitrite and EBC-pH. An inverse significant correlation was observed between UFP concentration in the sleep ME and EBC-pH (Table 4.14) suggesting that a decrease in UFP concentration like during sleep may result in as increase in EBC-pH of the exposed individual. A study of 18 healthy non-smoking subjects, observed a decrease in EBC-pH immediately after exposure to secondhand smoke and EBC-pH returned to baseline

by 180 mins after exposure (Maynard et al., 2006). Therefore an inverse correlation maybe observed between concentration and EBC-pH as seen in Table 4.14.

Table 4.15. Relationship between indoor other activity/microenvironment exposure and EBC biomarker

Biomarker	Spearman's correlation			
	UFP Indoor others Personal sensor	BC Indoor others Personal sensor	Size Indoor others Personal sensor	BC Indoor others Fixed site
EBC-Nitrate	0.19	0.42	0.06	0.57
EBC-Nitrite	0.07	0.89**	0.35	0.69
EBC-pH	-0.09	0.01	0.04	0.52

** Significant at $P \leq 0.001$, 2-tailed probability test

Table 4.15 show that EBC-nitrite was significantly correlated with BC PS concentration measured in indoor others ME, while in Table 4.16, EBC-nitrite was significantly correlated with size of the particle measured in the cooking activities. This agrees with the studies suggesting that particles especial those < 100 nm have the ability induce inflammatory responses in the airway leading to the production of NO which further oxidizes to nitrite (Risom et al., 2005, Donaldson et al., 2001). That is, UFP < 100 nm contribute less in mass but have high alveolar deposition fraction, high surface area, chemical bound to them can induce inflammation, and it has been suggested that they can translocate into the circulatory system (Donaldson et al., 2001, Risom et al., 2005, Liu et al., 2009). Studies have associated exposure to BC to airway oxidative stress (Bogdan et al., 2000, Žunić et al., 2009). Nitrate did not show any significant correlation it may be that nitrite is a better indicator of airway oxidation than nitrate. Moorcroft et al. (2001) suggests that nitrite concentration correlates with degree of injury. No significant correlation was found between the EBC measured biomarkers and housework (Table 4.17).

Table 4.16. Relationship between cooking activity/microenvironment exposure and EBC biomarker

	Spearman's correlation			
Biomarker	UFP Cooking Personal sensor	BC Cooking Personal sensor	Size Cooking Personal sensor	BC Cooking Fixed site
EBC-Nitrate	-0.17	0.41	0.35	0.62
EBC-Nitrite	-0.45	0.12	0.72****	0.52
EBC-pH	-0.24	0.14	0.08	0.55

* Significant at $P \leq 0.05$, 2-tailed probability test

Table 4.17. Relationship between housework activity/microenvironment exposure and EBC biomarker

	Spearman's correlation			
Biomarker	UFP Housework Personal sensor	BC Housework Personal sensor	Size Housework Personal sensor	BC Housework Fixed site
EBC-Nitrate	-0.14	-0.03	0.66	0.71
EBC-Nitrite	-0.43	-0.26	0.20	0.54
EBC-pH	0.14	-0.03	0.14	0.65

** Significant at $P \leq 0.001$, * Significant at $P \leq 0.05$, 2-tailed probability test

4.4.6 Confounding implications

Confounding from smoking and cardiovascular diseases is a major concern in this study because they both can cause increase in expression of EBC nitrate and nitrite, airway acidity and urinary 8-oxodG. However the subject used in this study, was a non-smoker with no cardiovascular or pulmonary diseases. Since it was a single young subject, age and gender was not an issue therefore no adjustment for bias was needed. Studies have suggested that food/diet and antioxidant have limited effect on oxidative DNA damage and repair biomarker like 8-oxodG and 8-OHGua (Hunt et al., 2000, Cooke et al., 2002, Suzuki et al., 1995). Also seen in Table 4.9 the inter-day CV for 8-oxodG was low indicating low variability between sampling days, therefore suggesting that diet may not be responsible for the variations observed. Rava et al. (2012) in their study using 964 people observed, that diet did not affect nitrate and nitrite in EBC rather the nitrate and nitrite in blood was affected. However, the

EBC sample collected for the analysis of nitrate, nitrite and pH were collected early in the morning after an all night fast. In a study where 19 healthy subjects were tested for acute effect of food on EBC-pH, no effect of food consumption on EBC-pH was observed (Fonseca et al., 2016). Cooke et al. (2002) also suggested that cell turnover did not affect 8-oxodG concentration. Based on these, adjustments to these factors were not applied and these factors may not be responsible for the variations observed.

Sampling site could be a confounding factor but in this study both measurement from FS and PS were taken. Therefore the PS measurement accounts for PE concentration i.e. concentration of pollutant in the breathing space of the subject.

4.5 Conclusion and limitations

The results show the benefits of using PS for measuring exposure and calculating the relationship between PE and health effect outcomes. The health outcomes measured in this chapter are biomarkers of exposure EBC NO_3^- , NO_2^- , pH and urinary 8-oxodG. The use of PSs breached spatial variability bias that may have occurred due to using FS measurements to measure PE. In fact the PS was able to detect infiltration into the home during a barbeque even when the windows and doors of the home were closed. This helped to also establish that in the course of no indoor activity, outdoors influence the particle concentration indoors. The PSs made activity/ME specific measurement and these sites specific measurements were used to explore association with the biomarkers of PE measured.

The cohort-size of this study is small; only a single subject was used in this pilot study. The size of this study may have affected significant biological outcomes i.e. the measured biomarkers, especially in low exposure concentration. Thus may have resulted in lack of sufficient statistical power to identify significant positive correlations between PE exposure and biomarker outcomes. In fact, since a single individual was used in this study, the activities/MEs in which this subject was exposed may not represent a larger group of people.

However, this chapter demonstrates that biomarkers of exposure can associated with exposures in the indoor: homes and other site specific and the PSs can be used to elucidate this association. This can help validate human health studies to PE in different MEs.

Significant correlation between EBC-nitrite and particle size during cooking, EBC-nitrite and BCPS during cooking, EBC-nitrite and indoor others ME, EBC-nitrite and BCFS and EBC-pH and UFP sleep, suggests that indoor particles in addition to the outdoors particles can induce oxidative stress and inflammation which agrees with the hypothesis of this study.

5 INFLAMMATORY AND OXIDATIVE BIOMARKERS IN EXHALED BREATH CONDENSATE AND URINE IN INDOOR ACUTE EXPOSURE TO JAPANESE STYLE OF COOKING (TEPPANYAKI)

5.1 Introduction

The Japanese cooking style “Teppanyaki” offers the traditional Japanese cuisine cooked (grilling, frying and broiling) on an iron griddle or hot steel plate in front of guests at restaurants. Several indoor air studies have identified cooking as one of the most pertinent indoor activities that generate fine (PM with aerodynamic size $2.5\ \mu\text{m}$ ($\text{PM}_{2.5}$)) UFP, $\text{PM} > 2.5\ \mu\text{m}$ and several other agents such as BC and other organic compounds (Höppe and Martinac, 1998, Santos et al., 2011, Sjaastad, 2010, Franck et al., 2011, Gwinn and Vallyathan, 2006, Long et al., 2001, Watson et al., 1988, Bradford et al., 2009, Zhang et al., 2010, Koczulla et al., 2009a). In terms of size, a study on contribution of indoor sources to particle number and mass concentration demonstrated that the particle number concentration (PNC) resulting from cooking had number median diameter ranging from 22 to 940 nm (He et al., 2004). Another study observed particle distribution profiles obtained during frying operations to have a modal diameter between 10 and 25 nm (Becker et al., 2005). Also, Abdullahi et al. (2013), in their cooking emission review, showed that different studies have measured particle diameter mode during cooking activities and it ranged from 10 to 320 nm.

The term “Cooking fumes” can be used to describe visible emissions generated during cooking (Sjaastad, 2010). During food cooking under high temperature, chemical processes such as degradation of sugars, pyrolysis of proteins and amino acids and the degradation of fats occur (Betha and Balasubramanian, 2011). Also, during cooking, PM is created by the cooling of hot vapour which is formed when cooking oil is heated above its boiling point i.e.

during frying and grilling/charbroiling (IARC 2010). Aerosol oil droplets, combustion products, organic gaseous pollutants and steam from the water content of the food being cooked are generated during cooking (IARC 2010). There are different chemical substances that researches have identified in cooking fumes. Some of them are: aldehydes, alkanolic acids, polycyclic aromatic hydrocarbons, and heterocyclic compounds, benzene, formaldehyde, elemental carbon, other organic and inorganic compounds (Betha and Balasubramanian, 2011, Li et al., 2003, Santos et al., 2011, Höppe and Martinac, 1998, Tsao et al., 2011, Oberdörster et al., 2005a, Gavankar et al., 2012).

The synergy between the unique physical properties of the UFPs with their ability to carry adsorbed chemical compounds give them an enhanced ability of triggering production of reactive oxygen species (ROS) (Li et al., 2003) thereby leading to oxidative stress (Li et al., 2003, Gubin et al., 2005) allergic and inflammatory reactions to a greater degree than coarser particles (Warheit, 2008, Stone et al., 2010).

Previous studies have shown that cooks appear to have an increased episode of respiratory distress, increased mortality from airway diseases (Handy et al., 2008) and increased risk of ischemic heart diseases (Warheit et al., 2008). Exposure to cooking fumes has been linked to respiratory cancer (Brouwer et al., 2004, Tsao et al., 2011). Several studies have measured oxidative and inflammatory responses to cooking fumes and different style of cooking for both occupational, commercial restaurants and laboratory/model/under controlled cooking. Some of these studies as shown in Table 5.1, show elevated levels of oxidative DNA damage and inflammation in people exposed to cooking fumes.

Table 5.1 Summary of some markers of exposure to cooking fumes

Type of exposure	Number of subjects	Length of exposure	Sample collection time	Marker of exposure measured	Reference
Modelled kitchen: Pan frying of beef	Group A -12 Group B - 12	Group A - 2 hours Group B - 4 hours	After exposure 6 hours after exposure 24 hours after exposure	Change in forced expiration time (FET) after exposure	(Bakand et al., 2012)
Chinese restaurant worker	Kitchen staff – 202 Service staff – 185 (control)	12 hours daily for 2 days	Morning after exposure urine for 2 days	8-OHdG and 1-OHP significantly higher in kitchen staff compared to service staff (control).	(Harper et al., 1983)
Chinese military cooks	Cooks – 61 Admin personnel – 37 the control		Pre shift urine first work day of the week Post exposure urine on the fifth work day	1-OHP significantly increased post exposure in the cooks After adjusting for confounding factors 8-OHdG and isoprostane were also significantly increased	(Nyström et al., 2010)
Chinese restaurant worker	Cooks – 67 Service staff – 43 the control		Morning after urine and fasting venous blood	Significantly higher median 1-OHP, 8-oxodG, BNMNs, tail length and MDA in the exposed kitchen staff	(Yang and Watts, 2005)
Restaurant workers	Cooks - 169 Service staff – 67 the control	12 hours daily for 2 days	Pre shift urine first day of work week Post shift urine last day of work week	Significantly higher 1-OHP, 8HOdG and MDA in the exposed than in the control	(Vilcassim et al., 2014)
Modelled kitchen: Pan frying of beef	Group A -12 Group B - 12	Group A - 2 hours Group B - 4 hours	Before exposure After exposure 24 hours after exposure	d-dimer increased significantly 24 hours after exposure compared to before	(Svedahl et al., 2013)

				exposure. Blood IL-6 and EBC IL-1 β significantly increased immediately after exposure. Ethane in exhaled air significantly increased immediately after exposure and 24 hours after exposure. Minor variation in exhaled NO and fibrinogen in blood	
Kitchen workers	Exposed (workers preparing hot meal) – 35 Control (mostly students) - 27		Expectorants were collected from the subjects at the end of the working day at three different days	Higher number of alveolar macrophages in exposed compared to unexposed.	(Nyström and Fadeel)
Chinese restaurant worker	45 workers from six different restaurant exposed to cooking oil fumes		Before and after installation of the embracing air curtain device (EACD)	Reduced level of urinary 8-OHdG and MDA after installation of the EACD in the exposed compared to before installation	(Nel et al., 2006)

MDA: malondialdehyde, 8-OHdG: 8-hydroxy- 2' -deoxyguanosine, 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine, NO: Nitric oxide, IL-6: Interleukin 6, IL1 β : Interleukin 1 beta, 1-OHP : 1-hydroxypyrene, BNMNs: binucleated micronuc

Toxicological research studies suggests that inflammation and oxidative stress play a central role in the mechanism of adverse health effects due to exposure air pollutants both in non-cooking (Laumbach and Kipen, 2010, Risom et al., 2005) and cooking scenarios (Saffari et al., 2013). Air pollutants induced ROS is said to occur when are particles are inhaled, they react with the airway lining/cells/cell constituents to produce ROS and or reactive nitrogen species (RNS) and these radicals can then induce inflammation and oxidative stress (Donaldson et al., 2001, Massey et al., 2012, Risom et al., 2005, Nitta et al., 1994). In this present study, EBC nitrate and nitrite were chosen to be measured instead of eNO as biomarker of inflammatory response because they are a more stable form of NO (Martens et al., 2005, Chladkova et al., 2006) and are used as surrogate markers of NO (Corradi et al., 2003). EBC-pH was also analysed as a biomarker of inflammation to the airways to see if exposure to cooking fumes emitted from Japanese style cooking “Teppanyaki” will cause acidification of the airways. 8-oxodG was measured as an oxidative biomarker of DNA breakdown. Figure 5.1 shows the Japanese cooking technique with the customers.

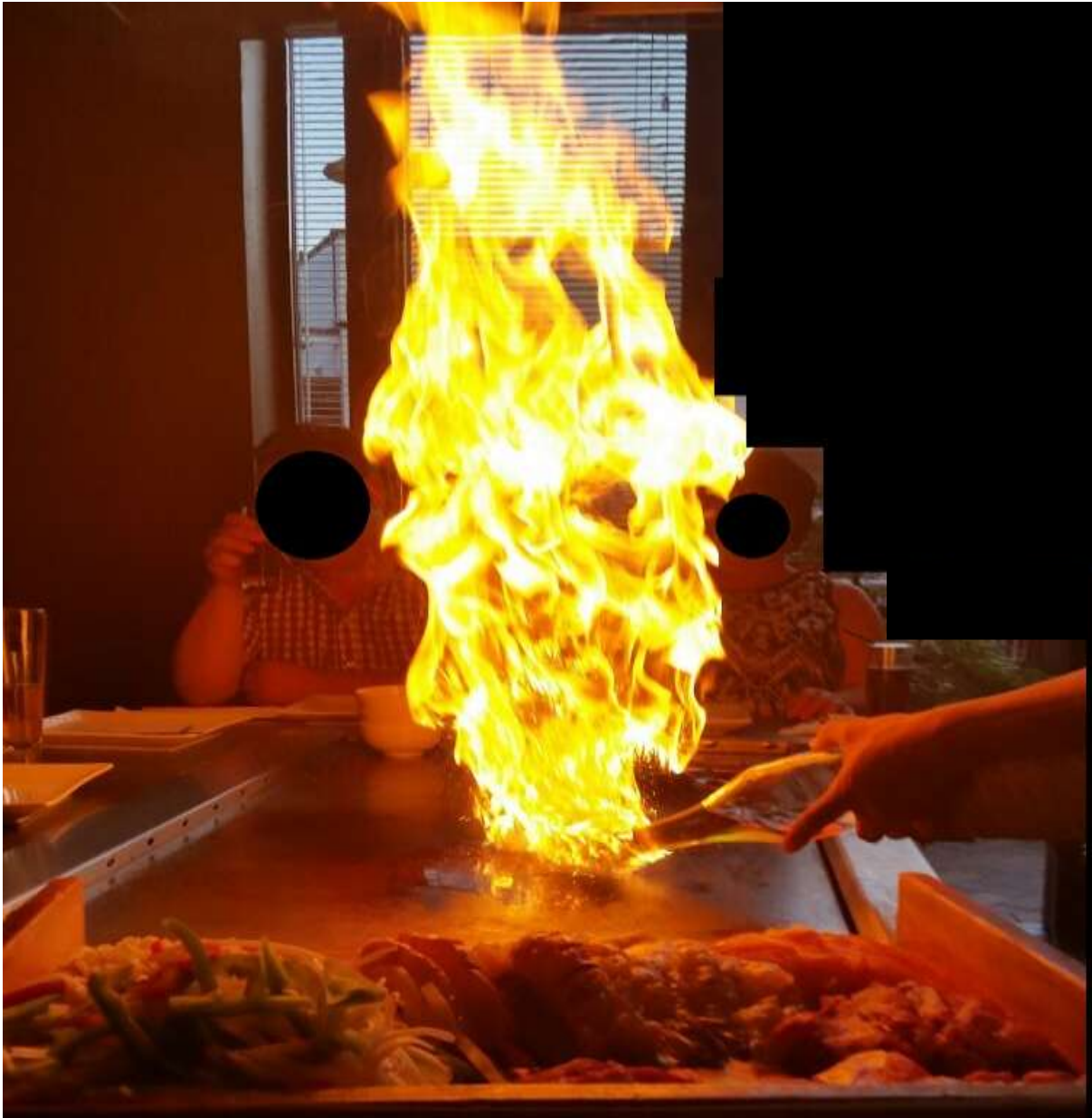


Figure 5.1. Japanese cooking technique (Teppanyaki) with the guest

5.2 Aims and objectives

Several studies have shown possible connections between exposure to cooking fumes and inflammatory and oxidative biomarkers. This study hypothesised that short term exposure to elevated concentration of respirable particles due to exposure to Japanese style cooking fumes will lead to changes in concentration of biomarkers of exposure as compared to before exposure. Furthermore this study hypothesised that changes in biomarkers would be associated with concentrations of pollutants measured in the Japanese exposure. Based on this, the aims of this study are:

- 1) To investigate the PE of customers to cooking fumes from the Japanese style of cooking “Teppanyaki” in a Japanese restaurant
- 2) To measure the changes in the expression of the biomarkers of inflammatory response (NO_3^- , NO_2^- and pH) in EBC and oxidative stress biomarker of DNA damage and repair in urinary 8-oxodG.

Specific objectives include:

- Description of pollutants concentrations (UFP, BC and $\text{PM}_{2.5}$) during the acute exposure in the Japanese restaurant and the other microenvironments visited by the subjects.
- Compare the levels of the biomarkers in the pre and the post acute exposure.
- Assess the relationship between measured pollutants and biomarkers of inflammation and oxidative stress

5.3 Methodology

5.3.1 Characteristics of the subjects

12 healthy non-smoking subjects consented to this study. None of the subjects suffered any form of discomfort due to this study. Ethical review criteria is as explained in chapter 2.

5.3.2 Instrumentation

The PE data from all subjects were acquired by the use of PSs. These PSs were placed in a backpack and given to each subject to carry at all times during the sampling period on their individual sampling days. Sampling was done in pairs. At the Japanese restaurant the backpack was placed on a seat beside the subject. The sensors were started at 9 am and lasted until after the exposure at the Japanese restaurant, except when the instrument malfunctioned or had power issues. The PSs used were: UFP sensor- DISCMini, PM_{2.5} sensor-MicroPEM and BC sensor-MicroAeth. The modes of operation of the PSs used are discussed in chapter 2.

5.3.3 Urine and exhaled breath condensate (EBC) collection

EBC and urine samples were collected from each subject 15 mins before exposure to the cooking fumes in Japanese restaurant. Another set of EBC and urine samples were collected immediately within 15 mins after exposure from each subject. The biomarker concentration in the before exposure samples were used as the baseline for each subject and the post samples were used to measure changes in the biomarker concentration level following the exposure. Also morning after exposure urine was collected from 5 subjects. In the Japanese restaurant each subjects spent 2 hours and also had a meal and a drink. Urine samples were analysed for 8-oxodG and EBC for NO₃⁻, NO₂⁻ and pH. The EBC and urine samples collection and analytical methods are as described in chapter 2.

5.4 Data analysis

5.4.1 Descriptive statistics

Descriptive statistics of the pollutant concentrations in each ME for each subject was calculated. Descriptive statistics of the measured concentration of biomarkers which include the minimum, mean, maximum and SD measurements were used in the data analysis and discussion.

5.4.2 Statistical analysis

The data were not normally distributed and therefore non-parametric tests were chosen for the analysis of the pre and post biomarker concentrations. The Wilcoxon's test for paired samples was used to compare the groups and the analysis was done using the IBM SPSS Statistics 21.

5.4.3 Relationships between pollutants and biomarkers

5.4.4 Spearman's correlation

Spearman's rank correlation was used to determine the association between the different variables.

5.4.4.1 Nitrate and nitrite ratio

NO_3^- was divided by NO_2^- in order to measure the change between the two biomarkers in the before and after exposure scenario.

5.5 Results and discussion

5.5.1 Description of pollutant concentration in the different microenvironment

Table 5.2 displays the mean (SD) concentration of the different MEs of the 12 subjects on the day of exposure showing concentrations of acute exposure at the Japanese restaurant. The concentration before exposure includes all activities until immediately pre exposure EBC and urine samples were collected. Exposure to cooking fumes at the Japanese restaurant is the exposure scenario for this study. Table 5.2 shows that the mean UFP concentration at the Japanese restaurant to be the highest concentration and it also applied to the PM_{2.5} and BC. The PS also measured the smallest particle size diameter in the Japanese restaurant exposure as compared to the before MEs. Based on the average concentration of the pollutants measured during the 2 hours cooking fumes exposure in the Japanese restaurant, the implication in terms of health impact may be relevant for the cooks and staff that work in the Japanese restaurant. The PNC a surrogate for the UFP concentration in this study during the exposure showed a mean diameter of 41.50 nm, a 19 % decrease in size in comparison to the before-exposure average size. Studies have revealed that smaller sized particles, such as ultrafine and nanoparticles (PM $\leq$ 100 nm), can be deposited in all regions of the respiratory tract (Chattopadhyay et al., 2003, Pant et al., 2015) and particle deposition increased as particle size decreased (Chattopadhyay et al., 2003).

Table 5.2. Mean exposure concentration in the different microenvironment

Concentrations				
	UFP (#/cm ³)	Size (nm)	PM _{2.5} (µg/m ³)	BC (ng/m ³)
Concentration before exposure	20.86 x 10 ³ (43.04 x 10 ³)	51.33 (15)	14.67 (10.2)	1.31 x 10 ³ (3.50 x 10 ²)
Concentration during exposure	12.54 x 10 ⁴ (95.81 x 10 ³)	41.50 (14)	192.25 (148)	57.43 x 10 ³ (41.28 x 10 ³)

5.5.2 Summary of biomarker concentration in the before and after exposure

Table 5.3 shows the minimum, mean, median, maximum and SD concentrations of the biomarkers in the before and after exposure to the cooking fumes at the Japanese restaurant. Mean NO₂⁻, NO₃⁻, pH and 8-oxodG were higher in the after exposure samples. Also the median NO₂⁻, NO₃⁻ and 8-oxodG are higher in the after or post exposure samples. While the EBC-pH and 8-oxodG did not significantly change

Table 5.3. Summary of inflammatory and oxidative biomarkers in the before and after exposure to cooking fumes from a Japanese restaurant

Subject Before Exposure N=11	NO₂⁻ (μM)	NO₃⁻ (μM)	pH	8-oxodG (ng/mg creatinine)	Subject After Exposure N=11	NO₂⁻ (μM)	NO₃⁻ (μM)	pH	8-oxodG (ng/mg creatinine)	Morning after 8-oxodG (ng/mg creatinine) N=5
Minimum	0.83	1.44	5.64	0.95	Minimum	3.31	17.23	6.98	0.83	1.03
Mean	4.18	11.48	7.40	2.88	Mean	10.35	28.82	7.51	2.88	2.26
Maximum	9.19	27.26	7.81	4.14	Maximum	36.96	59.68	8.05	4.31	3.85
Median	3.61	12.02	7.55	3.15	Median	7.01	21.94	7.50	3.00	2.16
STDEV	2.44	8.72	0.64	1.02	STDEV	10.14	15.61	0.21	1.15	1.22

N=subject number

5.5.3 Difference of mean between biomarkers

Table 5.4. Comparison between the pre and post exposure biomarkers levels

Biomarker	T-test (Wilcoxon) Pre and post exposure	T-test (Wilcoxon) Pre and morning after exposure	T-test (Wilcoxon) Post and morning after exposure
8-oxodG (ng/mg creatinine)	1	0.138	0.225
NO₃⁻	0.02****	-	-
NO₂⁻	0.03****	-	-
pH	0.678	-	-

****significant at $P < 0.05$, 2-tailed probability test

In this study, we evaluated the effects of exposure to Japanese style cooking fumes in healthy subjects that visited the Japanese restaurant by measuring the after-exposure biomarker concentrations against the baseline before-exposure biomarker concentrations. The results show that after-exposure biomarkers EBC nitrate and nitrite concentrations were significantly ($P < 0.05$) increased as compared to the before-exposure concentrations (Table 5.4). This adds weight to the hypothesis that particles fine and UFP (especially UFP despite low mass burden) on entering the respiratory tract can induce inflammatory cells which can be monitored in the EBC. This result (Table 5.4) also shows that the exposure to the pollutants at the Japanese restaurant is a predictor of the inflammatory markers NO₃⁻ and NO₂⁻ in this study. There was no significant difference between the Pre and post 8-oxodG and morning after exposure 8-oxodG (Table 5.4). There were no results for the morning after exposure NO₃⁻ and NO₂⁻ because those samples were not collected.

On inhalation of particles, inflammatory cells like the mast cells and macrophages are recruited at the site of injury (in this case the lungs) (Buonanno et al., 2009, Grahame and Schlesinger, 2010) as part of the immune system to protect the lungs. This then leads to an increase in oxygen uptake and increased production of ROS leading production of iNOS

amongst other inflammatory responses (Buonanno et al., 2009, Héroux et al., 2010) and subsequent NO production. In a state of hypoxia stable end products of NO, NO_3^- and NO_2^- (measured in this chapter) are produced. Thus this kind of inflammation is called acute inflammation (Buonanno et al., 2009). The subjects in this study experienced acute inflammation, which was measured as increased NO_3^- and NO_2^- after the exposure in the Japanese restaurant. However if the production of the ROS is not contained by the antioxidant system, it then leads to excess production of ROS/RNS which leads to damage of DNA, proteins and other macromolecules thereby leading to oxidative stress which can be measured in the urine as 8-oxodG. That is, when the fractions bound to the UFP for example metals and organic compounds, either as redox active solid particles or as soluble compounds begins to interact with the modifications of DNA bases, enhance lipid peroxidation, and altered calcium and sulphhydryl homeostasis (Risom et al., 2005, White et al., 2012, Siversten et al., 2002, Pan et al., 2010). Then continued exposure to ROS/RNS now leads back to chronic inflammation (Buonanno et al., 2009). Thus this may be the reason why the 8-oxodG in this study did not significantly change (Table 5.4). Thus the exposure time at the Japanese restaurant may not be long enough to induce oxidative stress. However, the airways was challenged by these particles during exposure and in response, increased expression of nitrate and nitrites end products of NO, were generated at the site of the exposure (the lungs) to protect the lungs. This could be why the professional cooks assessed in the study by Luo et al. (2011) and Pan et al. (2008) that were constantly exposed to cooking fumes over length of time of cooking per day or length of time working as a cook had higher 8-oxodG measured as compared to the controls in their study. EBC- NO_3^- and NO_2^- are localised to the respiratory tract and is also referred to as respiratory tract oxidative stress whereas, 8-oxodG is systemic (Bogdan et al., 2000).

Rava et al. (2012) in their study consisting of 949 people suggested that certain environmental risk factors may modify nitrate and nitrite levels in plasma and EBC depending on the exposure route. However, in their study, dietary sources of nitrate and nitrite such as green leafy vegetables did not affect the levels of nitrate and nitrite in EBC but showed increased level in plasma. Nitrate and nitrite production in the plasma is more complex as compared to the EBC (Huang et al., 2016). That is, in EBC, nitrate and nitrite may be produced from NO after reaction with oxygen and or from activated immune cells present in the lining of the lungs during airway defence (Valko et al., 2005, Ghio, 1999). In plasma, nitrate and nitrite production comes from several sources, such as bacteria, enzymatic production and dietary sources (Kim et al., 2004). The subjects in this study were not restricted to a particular kind of diet consumption. Based on the Rava et al. (2012) epidemiological study, the change in levels of nitrate and nitrite more likely was attributed to the exposure to cooking fumes at the Japanese cooking technique “Teppanyaki” in the Japanese restaurant than to the type of meals eaten as only EBC concentrations were measured in this study.

There are however no legally set experimental exposure duration but various studies have used 2 hours as exposure time in controlled studies. The 2 hours exposure duration in the Japanese restaurant for this study might have been too short to produce measurable significant changes in the 8-oxodG concentration. Suggesting that for the exposure concentration to cause a systemic damage expressed as increase in 8-oxodG, the subject may have to be exposed for a longer period of time. Long-term continuous exposure studies have demonstrated adverse health effects associated with PM. In other studies, expression of significantly higher or increased 8-oxodG concentrations were measured in kitchen staff /cook as compared to the control and the predictors of these higher concentrations are

cooking duration per day, length of time as a kitchen staff, change of extraction system and state of health such as obesity (Vilcassim et al., 2014, Harper et al., 1983, Yang and Watts, 2005, Nel et al., 2006). Therefore in the case of professional cooks as compared to customers like in this study, continued exposure to ROS/RNS may have led to oxidative stress. While effects of short-term exposures can be difficult to quantify in people with varied background exposure and susceptibility factors (Allen et al., 2009). In a study with 10 subjects with metabolic syndrome after exposure for 2 hours to filtered air or DE (at 200 μ g/m³) on 2 different days, they did not observe any change in urinary 8-oxodG at 3 hours and then 22 hours after initiation of exposure (Allen et al., 2009). In a similar setting but with 18 healthy subjects and 3 hours exposure, Hemmingsen et al. (2015) did not observe any change in urinary 8-oxodG 3 hours and then 20 hours after exposure. In Figure 1.7, the expected physiological timeline for the appearance of 8-oxodG is within 24 hours and Moller et al. (2010) summarised that from their experience in their various studies, that oxidized DNA is observed within a lag period < 24 hr after the exposure to PM. This present study unfortunately did not have multiple 8-oxodG measurement at different time points after (for example at 3 hours, 6 hours, 9 hours after exposure) because sampling logistics could not accommodate these timelines. These multiple time points measurements would have been an advantage to this study because we would have been able to check if significant changes in 8-oxodG occurred between immediately after exposure and morning after exposure. However, in addition to the immediately after-exposure concentration, this study also measured morning after exposure for only 5 subjects. The morning after exposure did not significantly differ from the after exposure 8-oxodG concentrations (Table 5.4). Increased expression of localised oxidative stress (nitrate and nitrite) does not necessarily mean increased expression of systemic oxidative DNA damage expressed as urinary 8-oxodG. The "oxidative stress" occurred in the local area as seen in increased expression of nitrate and nitrite in the after-

exposure measurement showing acute response due to immune defence of the airways to “irritative effect” (suggesting a tissue (airways) specific response) that can be measured immediately.

It could appear that EBC reflected biomarkers of exposure before the urine. This could be because systemic oxidative stress biomarkers in this study measured as increased expression of 8-oxodG, may not be parallel to tissues (in this case the airways-lungs) specific changes; therefore, oxidative stress induced by exposure to particles (UFP, BC and PM_{2.5}) in the cooking fumes in the Japanese restaurant may not be represented in systemic analyses, or may be reflected in a time period or in other systemic biomarkers not shown in this present study.

It could be that 8-oxodG “dilute out” once expressed perhaps because no significant damage was done, therefore it could be difficult to see a significant fluctuation using a systemic biomarker of oxidative stress like urinary 8-oxodG level and 8-oxodG have a short half-life. For example, a study demonstrated that the disappearance of 8-oxodG from nuclear DNA (nDNA) in liver of mice *in vivo* is very rapid (half-life 11 mins) (Chao and Wong, 2002). They observed that 8-oxodG returned to the endogenous level within 45 mins. Another study, investigated the cellular repair of DNA base damage products in DNA of cultured human lymphoblast cells, which were exposed to oxidative stress by hydrogen peroxide and observed that the half-life for removal of 8-oxodG was 55.2 mins (Kefeni and Okonkwo, 2013). Even if there was an increase in between the after-exposure concentration and the morning after exposure concentration 8-oxodG measurement, because of the short half-life of 8-oxodG it may have diluted out by the time the morning after exposure 8-oxodG concentration were taken. However, Cooke et al. (2002), suggests that even in the absence of oxidative stress, a proportion of the radicals elude the antioxidant defence system, resulting in

a “background” level of lesions, as seen in the concentrations measured in the after exposure. These results in DNA repair processes that remove damaged DNA, although they only succeed in maintaining a minimum level, which, for the most part, appears to have no detrimental consequence (Cooke et al., 2002).

The EBC-pH in the before and after-exposure were quite similar with minor incremental changes for most subjects but showed no statistically significant difference (Table 5.3 and Table 5.4). Several studies have measured decreased EBC-pH in patients with inflammatory diseases. Thus suggesting that EBC-pH is a useful tool for measuring inflammatory responses due to air pollution exposure as supported in various studies. However results obtained from studies are a bit contradictory as some reported increased EBC-pH after exposure and some reported decreased EBC-pH after.

Decreased EBC-pH was observed in 60 subjects with mild to moderate asthma after traffic exposure on oxford road (McCreanor et al., 2007) bearing in mind that these subjects have asthma this present study measured EBC-pH in healthy subjects. Decreased EBC-pH was observed in adolescents exposed to ambient diesel exhaust particle exposures (Stevenson et al., 2011) the subjects in this study were exposed to cooking fumes. Decreased EBC-pH was observed in 75 grain workers and consistent effect of obesity, cumulative smoking, duration of work on the test day were associated with this decrease (Rank et al., 2001). Decreased EBC-pH was observed after an acute 5 hours of exposure of 9 healthy non-smoking volunteers in swine confinement farming (Long et al., 2000) length of exposure in this present study was shorter. Whereas the 15 swine confinement farmers assessed showed increased EBC-pH during the working week and decreased EBC-pH after a nonworking weekend (Long et al., 2000). This is because in the swine workers, signs of adaptations to chronic exposure were observed with a tolerance-like airway response followed by a

significant increase of EBC-pH during the working week in the swine confinement workers (Long et al., 2000). On the contrary, decreased EBC-pH was observed in 61 sawmill workers during the working week, especially in those with high mould concentration (Ćalušić et al., 2013). Increased EBC-pH was observed after smoking of 1 cigarette after overnight tobacco abstinence induced during the 2-hour observation period (Fonseca et al., 2016). While Kostikas et al. (2012) observed in 18 healthy never-smokers that EBC-pH decreased immediately after exposure to secondhand cigarette smoke and returned to baseline by 3 hours. Acute Effects of Smoking on EBC on 13 smokers did not show any significant difference between the before and after smoking while in the chronic effect, with a history of at least 10 pack-years compared to controls that showed decreased EBC-pH (Jia et al., 2005). Increased EBC-pH was observed in healthy welders exposed to welding fumes (Boyce et al., 2006, Lundberg and Weitzberg, 2010). Fireman et al. (2008) used 3 study groups totalling 67 people, they observed that the EBC-pH from the study Group 2 showed lower pH values than the study Group 1 and controls however all were alkaline (Lundberg and Weitzberg, 2010) as a result in different components of the welding fumes and duration of welding. Increased EBC-pH was observed following isocyanate exposure in 26 isocyanate-induced asthma positive and negative subjects (Wang et al., 2006). All the other studies show that exposure time (acute or chronic), health condition (inflammatory diseases, obesity), component of exposure (chemical: metals, non-metals and biological: mould) may affect the outcome of the hydrogen ion concentration in the airways. Therefore, possible interpretation of the lack of statistically significant changes in ECB-pH in this study could be: because these subjects are healthy, secondly the component of the Japanese cooking fumes and lastly, it could be that the twelve subjects that were accessed might be too small a sample size population to give enough statistical power when studying small changes in the biomarker concentrations.

5.5.4 Analysis of NO₃⁻/ NO₂⁻ ratio in measured in the before and after-exposure EBC

To measure the concentration difference of the nitrate and nitrite, the nitrate/nitrite ratio was calculated. This study demonstrated a small decrease with the presence of localised oxidative stress i.e. inflammation of the airways. This agrees with nitrate/nitrite and or nitrite/nitrate ratio in other studies as a parameter for measuring inflammation and oxidative stress damage. A study observed a gradual decrease in Saliva nitrate/nitrite ratios were higher in periodontitis and gingivitis groups with the presence of inflammation due to periodontal disease (Wang et al., 2011). Another study evaluated nitrite/nitrate ratio in urine of 73 patients with urinary tract infection (UTI) and 241 healthy subjects (Cohen et al., 2010). Chao et al. (2016) observed higher nitrite/nitrate ratio in the UTI patients as compared to the healthy subjects. They also observed a decrease in nitrite/nitrate ratio for 7 of the UTI patients who agreed to provide urine samples after a < 5 days antibiotics treatment. The nitrite and nitrate concentration in UTI patients especially nitrite is affected by the bacteria infect of the urinary tract and (Cohen et al., 2010, Pey et al., 2010) and also they inferred that urinary nitrite can be produced from locally produced NO due to inflammation of the urinary tract based on an in vitro study by Vermeer et al. (2004). Another study, observed the nitrite to nitrate ratio in plasma and wound fluid within 3 post wounding days in rat (Thorpe and Harrison, 2008). Žunić et al. (2009) observed on first day, wound fluid nitrite/nitrate molar ratio was significantly above corresponding plasma or wound fluid levels in the later time intervals. The higher level of nitrite/nitrate molar ratio in wound fluid as compared to plasma indicates that the production of the nitrite and nitrate was site specific or localised i.e. the wound site. While the significantly increased nitrite/nitrate ratio on the first day as compared to the others is because of less apoptosis cell and oxidative damage as Žunić et al. (2009)

observed that nitrite to nitrite molar ratio was inversely proportional both to the oxidative cell damages and their apoptosis.

5.5.5 Relationship between pollutants and biomarkers

One of the possible reasons of lack of statistically significant correlation between pollutants and biomarkers, inconsistent patterns and inverse correlation (Table 5.5) could be because of the size of study, 12 subjects. The lack of sufficient PM_{2.5} and BC data due to instrument malfunction, thus $\geq 20\%$ of the PM_{2.5} and BC were missing. However, a good positive significant correlation 0.71 ($P \leq 0.01$) (Table 5.5) was observed between after-exposure 8-oxodG and the UFP measured during the Japanese style cooking fumes exposure even though no significant change was observed between the before-exposure and after-exposure 8-oxodG. This suggests that UFP can induce oxidative stress as also confirmed by various researches, i.e. can initiate inflammation and oxidative stress through particle induced ROS/RNS formation. (Risom et al., 2005, Vinzents et al., 2005). With respect to the confinement of the results obtained in this study, a good positive correlation was observed between PM_{2.5} concentration and 8-oxodG and a strong correlation between particle size and nitrite but these were not significant. Also, positive correlations that were not significant were observed between UFP and nitrite, size and pH and BC and nitrite. These results suggest possibility of production of biomarkers induced by these pollutants again supporting the hypothesis but the sample size/population could be too small to achieve a satisfactory statistical power. Even with the small sample size, this work clearly show that following exposure to Japanese style cooking “Teppanyaki” fumes by healthy customers that visited the Japanese restaurant, increase in nitrite and nitrate were observed. This increase might be as a result of increase in NO expression due to production of free radicals, which suggests an indication of acute inflammatory response in the airways.

Table 5.5 Relationship between pollutant exposure concentration at the Japanese restaurant and the post exposure biomarkers concentrations

Pollutant	8-oxodG (ng/mg creatinine)	NO ₃ ⁻	NO ₂ ⁻	pH
UFP (#/cm ³)	0.71**	-0.19	0.37	-0.11
Size (nm)	-0.23	-0.79**	0.96	0.33
PM _{2.5}	0.63	-0.11	-0.11	-0.89
BC (ng/m ³)	-0.09	-0.78****	0.29	0.00

All values are Spearman r correlation values, **significant at $p < 0.01$

****significant at $p < 0.05$, 2-tailed probability test

5.6 Conclusion and implication

The results from this study show that during the 2 hours exposure to the cooking fumes in the Japanese restaurant, the subjects were exposed to high levels of the measured PM. Exposures to UFPs have been associated with deleterious health effects and concerns because of their characteristics such as their nano-size, large surface area and the chemical components such as metals and organic compounds adsorbed on these particles. Even though there were ventilation systems over the steel cooking plates, such high concentrations were measured. It may be an indication that the ventilation units are not efficient. Thus a more efficient system should be put in place. The exposure to these particulates induced inflammatory responses that was expressed as increase in nitrate and nitrite in the after exposure measurements, thus supporting our hypothesis that exposure to Japanese style cooking fumes can induce inflammatory responses. Other studies discussed in the chapter, have shown that health conditions (subjects in this study were healthy subjects without any cardiovascular diseases), smoking status, chemical and biological component of the particles and length of exposure time may be factors that affected the EBC-pH and 8oxodG outcome. Therefore, we did not confirm that exposure to PM in cooking fumes from Japanese style cooking causes systemic oxidative stress in healthy non-smoking adults within the confines of this experimental procedure, because EBC-pH and 8-oxodG did not significantly change in the after exposure

in comparison to the before exposure concentrations. However because of the acute response effects observed as increase in EBC-nitrate and nitrite during the short-term exposure, the health risk impact due to chronic exposure for the cooks and staff of the Japanese restaurant should be of great concern. Especially because previous studies of effects from cooking fumes have looked at diseases and chronic respiratory effects in cooks (Tsao et al., 2011, Brouwer et al., 2004). Also significant correlation observed between UFP and 8-oxodG supports the hypothesis of exposure to particles in this case particles in cooking fumes have the ability to initiate inflammation and oxidative stress through ROS/RNS production. The cohort-size of this study may have affected significant biological outcomes i.e. the measured biomarkers, especially in low concentration. Thus may have resulted in lack of sufficient statistical power to identify significant correlations between PE exposure/pollutant concentrations and biomarker outcomes. However, this study can be used as a preliminary study for assessing the cooks in the Japanese restaurant. Also subsequent 8-oxodG multiple monitoring time points should be added to subsequent studies in order to determine if any significant 8-oxodG changes will occur along the physiological timeline in Figure 1.7.

6 BIOMARKERS OF EXPOSURE TO SHISHA SMOKE

6.1 Introduction

Shisha (hookah, waterpipe, hubble bubble, narghile, goza, madâ'a, qalyân e.t.c as known in different languages (Bradshaw et al., 1998, Cozza et al., 2015)) smoking is now seen by public health officials as a global tobacco epidemic (Cozza et al., 2015). In the past decade, the prevalence of shisha smoking has increased exponentially particularly in young people and students (Gong et al., 2011) and the reason for this rapid growth has been explained in various literature (Hoffmeyer et al., 2015, Cozza et al., 2015, McCool et al., 1993).

Shisha smoking has been suggested to have similar health risk as cigarette (Hoffmeyer et al., 2016). The problem here is not just the smoker who is the first exposed person, but also the passive smokers. In fact a recent survey among college students in Britain showed that students consider shisha as an affordable and relaxing way to enjoy friend and family gatherings i.e. a place of social gathering (Ljubicic et al., 2014). Also within the shisha lounge, non-smoking and even smoking employees and other non-smokers are exposed continually to the shisha smoke. Therefore these non-smokers as well as the smokers may be affected by negative health outcomes due to exposure to shisha smoke.

UFP, particle diameter size, PM_{2.5} and other harmful substances have been measured in high concentrations indoors during shisha smoking, contaminating indoor air thereby can lead to poor indoor air quality (Raulf-Heimsoth et al., 2010), which may eventually result to acute or negative health effect depending on length, concentration and exposure dose.

6.1.1 Description of Shisha

Shisha smoking is a traditional method of smoking tobacco which is said to may be have originated from the Eastern Mediterranean area and have however spread around the world (Samet et al., 1993). In summary regardless of what it is called in various parts of the world as stated above, shisha smoking method involves heating of tobacco at a certain appropriate temperature i.e. about 450°C (Tomasiak-Lozowska et al., 2012), then passing the generated smoke through water before the smoker inhales the smoke (Samet et al., 1993, Bellocq et al., 1998). Figure 6.1 shows an illustration of the shisha device labelling the different components of the device.

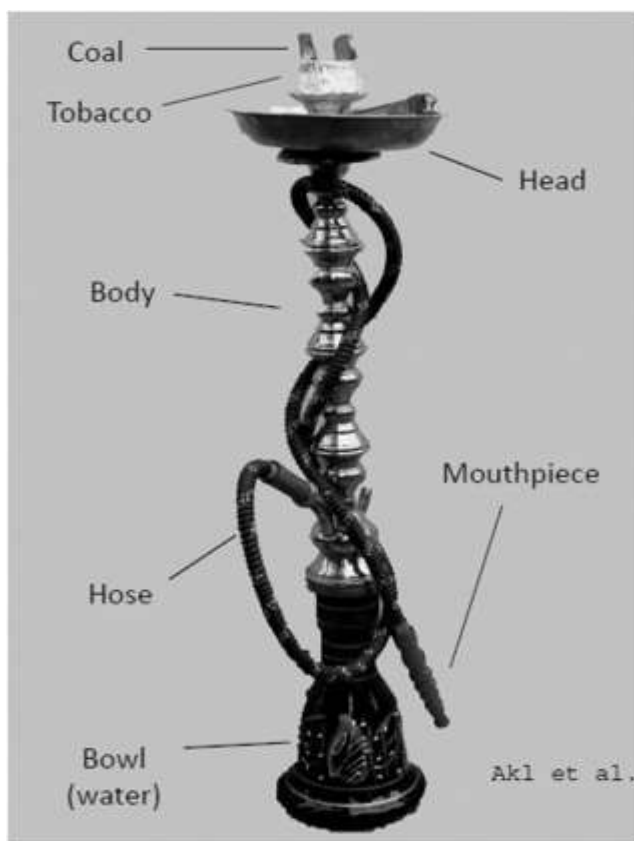


Figure 6.1. Shisha device

Adopted from Akl et al. (2010)

Tobacco is placed in the head and often covered with perforated aluminium foil; heated or burning charcoal is placed on top of the foil (Samet et al., 1993). Water half fills the bowl located at the bottom of the device, submerging a tube through which smoke enters, but not the hose connected tube through which smoke leaves (Samet et al., 1993). Thus, an inhalation at one end of the hose produces a vacuum in the air filled space of the water bowl, causing smoke to pass through the water (producing bubbles), into the hose-connected tube, and then to the smoker (Samet et al., 1993). The size of the hose may vary for different device or apparatus and to reduce the spread of communicable diseases such as tuberculosis the disposable mouthpieces can be used. Charcoal may be replenished or adjusted periodically during shisha smoking. A bowl of lit charcoal may be kept beside smoker.

6.1.2 Outcome of health studies/researches on exposure to shisha smoke by non-smokers

Many shisha smokers believe that shisha, is a safer alternative to cigarette smoking which is a misconception (Park et al., 2008), given the evidence of its main stream smoke constituents: carbon monoxide (CO), polycyclic aromatic hydrocarbons, formaldehyde, tar, nitrogen, nitric acid, nicotine, heavy metals such as arsenic, cobalt, chromium, and lead (Hieu and Lee, 2010, Das et al., 2008, Reuter et al., 2010, Kostikas et al., 2013) and various health effects. Various studies have associated smoking shisha to deleterious health effects such as development of acute and chronic respiratory diseases, cardiovascular disease, lung impairment, developing cancers (Samet et al., 1993, O'Donoghue et al., 2007), during pregnancy shisha smoking can lead to low fetal weight (Bradshaw et al., 1998) and spread of infectious diseases such as tuberculosis due to sharing of the shisha pipe (O'Donoghue et al., 2007).

Dependence/addiction to shisha smoking i.e. the nicotine/tobacco (Perwez Hussain and

Harris, 2007). Also nicotine has been found in the blood of shisha smokers and CO in their breath (De Flora et al., 1990).

It has been suggested that exposure of non-smokers (i.e. passive smokers) to shisha smoke can pose a serious risk (McCool et al., 1993, O'Donoghue et al., 2007) and this area of study is still emerging. WHO (2005) suggested based on Maziak et al. (2004) discussion on heavy metals in shisha main stream smoke from machine that the higher content of heavy metals in waterpipe smoke compared to cigarettes may also have adverse health effects on exposed non-smokers. A field based research conducted in Florida (USA) has shown that the amount of CO concentrations in personal breathing of non-smoker subjects exposed to shisha smoke in a shisha lounge was significantly higher (28.5 ppm) as compared to non-smokers visiting traditional bars (8.0 ppm) (Hemmingsen et al., 2015). In a review by Chaouachi (2009), it was summarised that exposed non-smokers to hookah smoke could retain in their respiratory tract 11–59% of the remaining exhaled main stream smoke particulate matter and 71–81% of nicotine.

Thus, based on the reviews of shisha related literature by Chaouachi (2009), Chaouachi (2009) concluded that most of shisha ETS is made up of exhaled main stream smoke which qualitatively differs from main stream smoke as observed in cigarette. Therefore suggesting that, more scientific ways should be employed in estimating non-smokers exposure to shisha smoke.

6.2 Aims and objectives

It has been mentioned severally that air pollutants may cause pulmonary and cardiovascular toxicity through several channel of action, of which includes local and systemic inflammation and oxidative stress (Gessner et al., 2003, Laumbach and Kipen, 2010). Therefore the aim of this chapter is to assess the acute effect of exposure to shisha smoke in exposed non-smokers by measuring change in biomarker concentrations. In order to achieve these listed below are the specific objectives;

- Measure concentrations of pollutants (UFP, size diameter, BC and PM_{2.5}) in the exposure shisha lounge
- Measure and compare biomarkers (EBC-nitrate, EBC-nitrite, EBC-pH and urinary 8-oxodG) in the before and after-exposure EBC and urine samples for exposed non-smokers and a control subject (non-smoker and not exposed)
- Determine relationship between after-exposure biomarkers concentrations and pollutants measured during exposure.

6.3 Materials and method

6.3.1 Study design

This study was done in collaboration with the health workers from the Birmingham city council, where they requested permission to visit various shisha lounges. Due to logistics constrain this study was able to collect EBC and urine sample for biomarker measurements for only 4 days out of the 12 days of sampling at the shisha lounge. The EBC and urine samples collected on day 3 was not analysed because only before-exposure samples were collected and no after-exposure samples were collected as we could not gain access into the lounge on that day. PSs used to measure the pollutants (UFP, particle size, BC and PM_{2.5}) were placed in a backpack and given to one of the non-smoking subject to be exposed. The control subject did not go into the shisha lounge. Measurement of the pollutants concentrations inside the shisha lounge was carried out as described in Table 6.1.

Table 6.1. Sampling itinerary

Sampling Day	Number of exposed non-smokers	Number of control non-exposed non-smoker	Pollutant sampling time
DAY 1	2	1	85 mins
DAY 2	3	1	60 mins
Day 4	2	1	45 mins

6.3.2 Sampling instruments

The PE concentrations of the pollutants were measured with the PSs. UFP concentrations and particle size diameters were measured using the DiSCmini, BC concentrations were measured using the MicroAethalometer and PM_{2.5} concentrations were measured using a RTI

MicroPEM. Method of operation of these instruments has been described in detail in chapter 2.

6.3.3 Biomarker measurements

The EBC and urine samples were collected before the subject was exposed to the shisha smoke and after exposure for the purpose of biomarker analysis. The method of collection, storage and analysis has been described in detail in chapter 2.

6.3.4 Data analysis

Data analysis was completed using IBM SPSS 22. Wilcoxon Tesst was used to test the difference of mean between pre and post-exposure biomarkers. Spearman correlation was use to test the relationship between measured pollutants and after exposure biomarkers.

6.4 Results and discussion

6.4.1 Pollutant concentrations

The mean concentrations measured at the Birmingham FS corresponding to the sampling time are listed in Table 6.2. Day 1 reported the lowest concentrations across the three FSs for BC and PM_{2.5} as compared to Day 2 and Day 4. This is reflective of the time of the day. That is, Day 1 sampling commenced at 9 pm at that time of the day traffic activities which is a major contributor to the ambient air measurement had drastically reduced. In Day 2 and Day 4 measurements were between 5 – 8 pm during rush hour. There were no data reported UFP because that data was not available from the AURN site. Table 6.3 show the concentrations of the pollutants measured in the shisha lounge on each sampling day. There is no Day 3 this has been explained in the methodology section. Day 1 showed the highest concentration for UFP, BC and PM_{2.5}, next to Day 2. There was no PM_{2.5} data for Day 4 because the instrument was not available, however Day 4 showed the smallest particle size diameter of 26.41 nm < Day 2 < Day 1. Table 6.3 shows that the concentrations measured at the shisha lounges are higher than those measured at the RFSs.

In an experimental study performed in a 57m³ room where four people smoked shisha for 40 mins each during a 4 hours period, a median UFP concentration of 28.9×10^{-4} Number/cm³ was measured (Raulf-Heimsoth et al., 2010) and in this study for the median measurement for the 3 days in the order of the sampling Day: 30.1×10^{-4} (85 mins), 6.8×10^{-4} (60 mins) and 2.6×10^{-4} (45 mins). In the same study Fromme et al. (2009) measured median PM_{2.5} as 393 µg/m³ and the highest measured median observed for this study is 225 µg/m³ there measurement appeared to be higher than the measurement in this study. In this study room size was not measured and ventilation in the rooms may be different. In a New York city study, the mean BC value observed after 1 – 2 hours measurement in 8 shisha lounges was

4.1 $\mu\text{g}/\text{m}^3$ (Khatri et al., 2013). While in this study, higher BC mean for the 3 days was observed 14.24 $\mu\text{g}/\text{m}^3$.

There are no concentrations guideline limits set for UFP but we know that the smaller the size, the increase in deposition of the particles in the air way also considering factors like the breathing pattern of the subject. A study reported that particles surface area and not the mass was related to biological effects, such as, inflammation, genotoxicity, and histology (Danielsen et al., 2008). Particles less than 4 μm (UFP < 100 nm, BC < 120 nm, $\text{PM}_{2.5}$ < 2.5 μm) have been said to have the ability to penetrate deep into the lungs causing health related effects (Gulliver and Briggs, 2004, Mutlu et al., 2001, de Nazelle et al., 2013). Another study observed particle size < 0.38 μm in the exhaled breath of a shisha smoker (Yue-dong, 1998), this is in the range of mean particle size measured in the shisha lounge in this study as shown in Table 6.3.

The United Kingdom Air Quality Standards Regulations (2010) set a $\text{PM}_{2.5}$ guide limit of 25 $\mu\text{g}/\text{m}^3$ per calendar year and a daily PM_{10} (Coarse particles (diameter between 2.5 and 10 micrometers)) limit of 50 $\mu\text{g}/\text{m}^3$ not be exceeded more than 35 times per calendar year. As shown in Table 6.3, the hourly $\text{PM}_{2.5}$ concentrations measured in the shisha premises are so high that they can pose health risk to the passive smokers in that environment. This is because the passive smokers may be in danger of exceeding the daily PM_{10} exposure guideline limit especially the workers. The levels of $\text{PM}_{2.5}$ concentrations measured in this study are in the hazardous zone of the USEPA Air Quality Guide to air pollution i.e. Hazardous ($\geq 211 \mu\text{g}/\text{m}^3$). This USEPA Air Quality Guide to air pollution or index grouped the ambient $\text{PM}_{2.5}$ from good to hazardous. In fact there are notable differences between the

measurement inside the shisha lounge and the ambient measurements at RFSs indicating high PE which have not been picked by the RFS measurement.

Following exposure to the pollutants generated in the shisha lounge, the non-smokers exposed to shisha smoke for a short period see Table 6.4, changes from the baseline in concentrations of biomarkers of exposure was identified in the healthy non-smoking exposed subject for this study. Therefore the non-smoking employees of such establishments who are exposed on a daily basis may be subject to chronic exposure health effects. Bearing in mind that chemical substance are bound to these particles therefore when they get into the airways are able to react to generate ROS/RNS leading to oxidative stress and inflammation.

Table 6.2. Mean concentrations at different fixed sites in Birmingham

	Birmingham Tyburn Background ($\mu\text{g}/\text{m}^3$)		Birmingham Tyburn Road side ($\mu\text{g}/\text{m}^3$)		Birmingham Acocks Green ($\mu\text{g}/\text{m}^3$)
Day	BC	PM _{2.5}	BC	PM _{2.5}	PM _{2.5}
Day 1	0.7	6.65	0.7	0.85	5.15
Day 2	1.5	20.16	4.90	21.83	20.90
Day 4	1.1	-	4.43	7.6	-

Table 6.3. Pollutant mean concentrations in the shisha lounge and fixed site pollutant concentrations

Day	UFP (Number/cm ³)	Size (nm)	BC ($\mu\text{g}/\text{m}^3$)	PM_{2.5} ($\mu\text{g}/\text{m}^3$)	Sampling time (mins)
Day 1	3.6×10^{-5}	39.40	31.5	358.31	85
Day 2	9.9×10^{-4}	31.96	7.77	348.76	60
Day 4	3.6×10^{-4}	26.41	3.44	-	45

6.4.2 Biomarker concentrations

6.4.2.1 Summary of biomarker concentrations

The mean of all the before-exposure biomarkers and after-exposure biomarkers for the three days was calculated and difference of mean was determined for the exposure and the control subject. The number of samples used to calculate the mean in the before and after EBC biomarkers for the expose was 7 and 3 for the control. For the urinary 8-oxodG, only 6 urine samples were used (3 exposed and 3 control samples were analysed because of fund). Table 6.4 showed EBC-nitrate and EBC-nitrite rose significantly from the baseline measured before exposure after a non-smoker was exposed to shisha smoke in the shisha lounge and the control subject did not show any significant change from the baseline concentrations.

An increase in EBC-nitrite and EBC-nitrate concentrations was observed in the after-exposure 65.61 μM and 21.48 μM respectively for the group to be exposed (Table 6.4). The nitrite concentration observed in this study was higher than the concentration of 20 healthy smokers measured in another study (Hunt et al., 2002). The increase in the nitrate was still within the range measured in other studies for healthy subjects using this method as this study (Corradi et al., 2003). The increase in EBC- NO_2^- and EBC- NO_3^- measured in this show to be statistically significant ($P < 0.05$) (Table 6.4). While there was no significant changes observed in the control subject.

In a study with 73 shisha smokers, the Fractional exhaled nitric oxide (FeNO) was measured and it was found to be lower as compared to their 73 non-smoker non-exposed control group (Sauvain et al., 2011). Furthermore another study also observed that exposure to passive and active smoking resulted in decrease in FeNO (Chuang et al., 2015). They suggested that the possible mechanisms include the high concentration of NO in tobacco smoke, which exerts

negative feedback on its synthesis down regulation of iNOS, and rapid conversion of NO to peroxynitrite by ROS/RNS (Sauvain et al., 2011, Chuang et al., 2015). Rava et al. (2012) suggests that since nitrite, nitrate and FeNO are both biological markers of the same pathway, their negative association with smoking may be explained by the same mechanism. However, another study observed increase in EBC-nitrate 30 mins after 15 healthy smokers, smoked 2 cigarettes (Balint et al., 2001). On the other hands some studies reported no effect of smoking on eNO levels (Maynard et al., 2006, Feng et al., 2015b). While an increase in eNO was observed at 1 and 10 mins in 24 healthy smokers after acute inhalation of cigarette (Iyengar, 1998). This is similar to the result in this study because, nitrite and nitrate are used to monitor eNO as they stable end products of eNO and are expressed by same mechanism. Therefore with an increase in eNO means increase in nitrate and nitrite in the EBC because NO converts to these stable end products. Cigarette smoke produces high concentrations of oxidizing radicals that challenges the human respiratory airways (Iyengar, 1998, Crinnion, 2010) and research has demonstrated that shisha smoke contains a wide variety of toxic compounds similar to those detected in cigarette smoke (Hieu and Lee, 2010, Samet et al., 1993). However studies have shown that exposure to shisha smoke may contains some higher concentrations of harmful substances and may even be more toxic than cigarette smoke (Hieu and Lee, 2010, Samet et al., 1993, Das et al., 2008, Yu et al., 2016, Hoet et al., 2012). Therefore when the airways is challenged by these potent oxidants bound to particulates (UFP, PM_{2.5} and BC) and or production of more ROS/RNS due to inflammatory immune responses provoked by the particulates, iNOS expression is activated in the presence of L-arginine thereby leading to the expression of NO. This NO is then further oxidised to more stable end products nitrate and nitrite. Therefore according to the results in this chapter nitrate and nitrite are produced during host defence of the airways because of exposure to the particulates just as seen in chapter 5.

In the EBC-pH and 8-oxodG, no significant changes were observed between the before and after-exposure of the non-smokers exposed to shisha smoke. Also no significant change was observed in the control subject. The result in this study suggests that exposure to shisha smoke may not be a predictor of 8-oxodG and EBC-pH in acute responses for healthy non-smokers. A study suggested that cigarette smoke radicals in cigarette PM in the gas phase can be deposited deep in the bronchopulmonary tree and then water-soluble materials can diffuse further into extrapulmonary fluids and into cells and produce the superoxide-hydrogen peroxide-hydroxyl radical chain of reactions and then can then lead to DNA damage, and perhaps lipid peroxidation (Crinnion, 2010). However, controversial results have been observed regarding cigarette smoke. Where some researchers have observed association between exposure to cigarette smoke and 8-oxodG both in human and animal studies, (Janssen et al., 2012, Eštoková et al., 2010, Howard et al., 1998, Thaiparambil et al., 2007) some have not seen any relationship (Arif et al., 2001). Thaiparambil et al. (2007) in their study with AJ mice observed that duration of a exposure to the cigarette smoke is a factor in the expression of 8-oxodG. They observed that, in their previous study, 2 day to 1 week exposure did not yield any significant changes in the concentration of 8-oxodG. However in their recent study, 6 hours daily cigarette smoke exposure for 5 days a week for a duration of 2, 4 and 6 weeks yielded an increase in 8-oxodG in the mice lungs. However, measurement time of the 8-oxodG was also important as measurements made at 1 hour after last exposure did not show any significant changes while at 3 hours significant changes were observed and decrease was observed after 6 hours. Arif et al. (2001) also exposed the rats for 6 hours, 7 days a week for 2 weeks and more but no increase in 8-oxodG was observed, perhaps because they measured for 8-oxodG after 15 hours of smoke exposure cessation. As previously explained in chapters 5, 8-oxodG have a short halflife. A study of 66 shisha smokers and 46 control, similar 8-OHdG between waterpipe smokers and non-smokers was

observed and they suggested that 8-OHdG is not a good biomarker for genotoxic effect of waterpipe smoking (Massey et al., 2013). Therefore based on the studies by Thaiparambil et al. (2007) and Arif et al. (2001) it may be possible that no significant changes were observed in this study because of length of exposure time as compared to their studies. Also because urine sample for 8-oxodG measurements were collected immediately after and no other post exposure samples were collected to check if concentrations change as further intervals after exposure. Also subjects in this study are healthy and the outcome may be different subjects with compromised health status e.g. cardiovascular disease, obesity and so on. This again relates to chapter 5 where post exposure 8-oxodG did not significantly change as seen in other studies where it change and the changes were associated to length of exposure and health status.

Studies have shown that airway acidification, expressed as decrease in EBC-pH is present in people with COPD and asthma (MacNee et al., 2011, Aarnio et al., 2005). Also decreased EBC-pH have been measured in healthy smokers as compared to non-smokers (Jia et al., 2005). Kostikas et al. (2012) observed decreased EBC-pH immediately after exposure in 18 healthy never smokers exposed to secondhand smoke. In the EBC of smokers collected after smoking, no significant increase in EBC-pH was observed but chronic cigarette smoking was associated with a lower EBC -pH in smokers without signs of airway disease, compared to non-smokers and the lowered pH depends on the duration of smoking, as it occurred only in subjects with a smoking history > 10 years, whereas smokers with ≤ 3 years showed no signs of decreased EBC-pH (Jia et al., 2005). In another study, 10 healthy smokers and 10 smokers with moderate persistent asthma, after 12 hours of abstinence smoked 2 cigarettes and no significant difference was observed in the EBC-pH of the healthy subjects, while EBC-pH decreased 30 min after in the subjects with moderate asthma (Papaioannou et al., 2010).

Another study showed that cigarette smoking acutely increased EBC-pH in 15 healthy smokers, after 10 hours abstinence smoked 1 cigarette.(Fonseca et al., 2016). Again factors like length of exposure and health status of the subject affected the EBC-pH measurements in some of these studies measured above.

Therefore it could be that in this study, that acute exposure of non-smokers to shisha smoke is more closely associated with localised oxidative stress expressed as increase in oxidised NO end product nitrate and nitrite.

Table 6.4. Mean and difference of mean for biomarker concentration in the before and after exposure for the sampling period

	EBC-Nitrite (μM)			EBC-Nitrate (μM)			EBC-pH			8-oxodG (ng/mg Creatinine)		
	Before-exposure	After-exposure	Wilcoxon	Before-exposure	After-exposure	Wilcoxon	Before-exposure	After-exposure	Wilcoxon	Before-exposure	After-exposure	Wilcoxon
Exposed	12.29	65.61	0.018	11.13	21.48	0.018	7.61	7.71	0.612	3.02	3.17	0.593
Control	0.91	1.02	0.109	7.14	9.95	0.109	7.45	7.47	0.655	1.53	1.25	0.180

Wilcoxon was used to test for difference of mean between the before exposure biomarker concentration and the after-exposure biomarker concentration.

6.4.2.2 Ratio between nitrate and nitrite in the before and in the after exposure

The $\text{NO}_3^-/\text{NO}_2^-$ ratio in EBC for the non-smokers exposed to shisha smoke was calculated for the before and after-exposure. The essence of calculating the ratio was to measure the changes in the NO_3^- and NO_2^- themselves during the before and after exposure. In the before-exposure the ratio observed was 10.19 and in the after-exposure 8.10. While for the control the before and after-exposure observed were 7.58 and 9.82 respectively. The ratio was higher in the before-exposure as compared to the after-exposure for the exposed non-smokers and for the control, the after-exposure was higher. Therefore in this study the decrease in ratio of EBC nitrate/nitrite observed in the measured concentrations of exposed non-smokers in comparison to the mean ratio in the before-exposure concentrations is associated with inflammation. This was also observed and explained in detail in chapter 5.

6.4.2.3 Relationship between the pollutants and biomarkers in the after-exposure measurements

In Table 6.5, 8-oxodG was excluded from the correlation matrix because more than 20 % of the data was missing. As previously explained because of funds some of the urine samples were not analysed for 8-oxodG. Only 1 urine sample per day was analysed for 1 non-smoker exposed subject and 1 non-smoker control subject. Table 6.5 shows the relationship between the pollutants and the biomarker. All the pollutants (UFP, size, BC and $\text{PM}_{2.5}$) showed significant correlation (spearman correlation < 0.90) not shown in this work, indicating that they have similar source in this study the shisha smoke from the main stream smoke and exhaled main stream smoke. EBC-nitrite showed good correlation with UFP, size, and $\text{PM}_{2.5}$ R^2 values 0.56, 0.56, 0.58 and 0.57 respectively but not at a significant level.

The result in Table 6.5 suggests that there is a chance that these exposures to the pollutants could induce inflammatory responses and oxidative stress. EBC-NO₂⁻ and EBC-NO₃⁻ correlated with EBC-pH weakly however not significantly. This could be as a result in the trend of increase of nitrate and nitrite in the exposure group. The cohort-size of this study is small. The size of this study may have affected significant biological outcomes i.e. the measured biomarkers, especially in low increment concentrations. Thus may have resulted in lack of sufficient statistical power to identify significant positive correlations between PE exposure and biomarker outcomes.

Table 6.5. Relationship between pollutants and biomarkers

	EBC-NO₃⁻	EBC-NO₂⁻	EBC-pH
UFP (Number/cm³)	-0.18	0.56	0.19
Size (nm)	-0.19	0.56	0.19
BC (µg/m³)	-0.18	0.58	0.18
PM_{2.5}	0.29	0.57	-0.28
EBC-NO₃⁻ (µM)	1	0.39	0.43
EBC-NO₂⁻ (µM)		1	0.61
EBC-pH			1

6.5 Conclusion

The commonly explored biomarkers for exposure to shisha smoke are exhaled breath for CO levels, blood for nicotine and cotinine and urine for systemic oxidative stress nitrosamine (Allen et al., 2009) mostly in the smokers. While in this study, non-smokers exposed to shisha smoke in a shisha lounge had their EBC assessed for EBC- NO_3^- , NO_2^- and pH and their urine for 8-oxodG. A significant increase observed in the EBC- NO_3^- and EBC- NO_3^- is an indication of inflammation which could be due to exposure to the shisha smoke. The ratio of $\text{NO}_3^-/\text{NO}_2^-$ measured in the after-exposure to the shisha smoke showed a decrease in the ratio value as compared to the before-exposure ratio measurement this is also indication of the presence of inflammation. Therefore, this study adds knowledge to the effects of exposure to shisha smoke as this is a novel area. This chapter was able to assess acute response effect of exposure to shisha smoke by measuring significant changes in nitrate and nitrite, thus supporting the hypothesis that exposure to shisha smoke can induce inflammation/oxidative stress in the airways.

7 MARKERS OF EXPOSURE ASSOCIATED WITH BUSY TRAFFIC SITE

7.1 Introduction

It is inevitable for people to be exposed to traffic related air pollutants (both in-vehicle and pedestrians) daily because substantial proportion of daily exposure to traffic related air pollutants occur during work/school and other commutes in rush-hour traffic (Mutlu et al., 2001, Montuschi, 2010). Studies have associated exposure to traffic-related air pollutants to increased respiratory and cardiovascular morbidity and mortality (Laumbach and Kipen, 2010). Toxicological and epidemiological studies have made available evidence that air pollutants may cause pulmonary and cardiovascular toxicity through several channel of action, of which includes local and systemic inflammation, as well as alterations in autonomic function (Gessner et al., 2003, Laumbach and Kipen, 2010) and decrease in lung function (Hambach et al., 2013).

The hypothesis of this chapter is, that exposure to air pollutants in a busy traffic site for 2 hours can causes health effects such as increment in inflammation (EBC-Nitrate, EBC-Nitrite, EBC-pH) in the airways and oxidative stress (measured in 8-oxodG), possible changes in the autonomic balance (blood pressure, pulse rate, V_E -HR) and decrease in lung function. Also exposure to PM can cause a change in the metal level concentration in the urine.

7.2 Aims and objectives

Giving that people can be exposed to near road-side pollutants that occur during activities such as walking, running, jogging, cycling, waiting for bus/commuting, therefore the aim of this chapter is to measure short term effects of traffic related air pollutants with healthy people on short-term in busy traffic sites and compare to the control site the park. Secondly investigate if urinary metals can be used as biomarkers of exposure to short term effect of traffic related particles

In order to achieve this, listed below are the specific objectives:

- Measure concentrations of air pollutants (UFP, particle size, BC, PM_{2.5}) in busy traffic site and control site
- Measure changes in biomarkers of exposure (EBC-Nitrate, EBC-Nitrite, EBC-pH, urinary 8-oxodG, systolic and diastolic blood pressure, pulse rate and lung function(FEV1,FEV6, FEV1/FEV6, FEF25-75%%)) in the before and after-exposure
- Estimate V_E-HR
- Estimate inhaled dose (load estimate) for PM_{2.5}, BC and UFP
- Relationship between air pollutant concentrations and biomarkers
- Measure and compare urinary metals in the before and after exposure for busy traffic site and for the park

7.3 Materials and methodology

7.3.1 Study design

Subjects walked for two hours (3pm – 5 pm) on Bristol road in a circle around the dark circled mark in Figure 7.1, where cars, buses, trucks, motorcycles all powered by either diesel or petrol engine. The Bristol road represents the busy traffic site. The same subject on another day walked in Calthrope Park which represents the control site for two hours at the same time as in the traffic road site (Figure 7.2). In the Park the subjects were about 230 m from a busy road called Pershore road.

Subjects were picked up from the University of Birmingham to a house about 2 mins drive from the sampling site. The subjects rested for 30 mins. Their before-exposure samples for biomarker measurements were taken: BP, pulse rate, lung function, EBC (for nitrate, nitrite and pH analysis), urine (urine collected was for 8-oxodG and urinary metal analysis) and they were given a heart-rate monitor to wear. The subjects were driven down to the sampling point and after 2 hours of exposure the subjects were driven back to the house, where after-exposure samples were collected.

The same process was repeated for the Park except that the subjects walked almost 1 min to the Park after the before-exposure samples were taken and they walked back after the 2 hours of exposure in the park.

The collection method, storage and analysis of the biological samples are as described in chapter 2.



Figure 7.1. Sampling route: Busy traffic site: Belgrave Middleway A38 Bristol road/street Birmingham (Picture from Google Earth)



Figure 7.2. Sampling route: Park: Calthrope Park Edward road Birmingham (Picture from Google Earth)

7.3.1.1 Sampling instrument

The PE concentrations of the pollutants were measured with the PSs. UFP concentrations and particle size diameter, were measured using the DiSCmini, BC concentrations were measured

using the MicroAethalometer and PM_{2.5} concentrations were measured using a RTI MicroPEM. The instruments were turned-on on arrival at the sampling site and turned-off at the end of two hours at the sampling site. Method of operation of these instruments has been described in detail in chapter 2.

7.3.2 Statistical analysis

7.3.2.1 Pollutants analysis

The mean, median, SD, minimum and maximum of all measured pollutants were determined to enable quantification of data.

7.3.2.2 Relationship between biomarkers

The Spearman correlation was applied to measure the relationship between the before and after-exposure biomarker measurements. Wilcoxon test was applied to measure difference of mean between the before and after-exposure (related samples) because the distribution was not a normal distribution. While Mann-Whitney U was used to test difference of mean between two independent samples i.e. difference of mean between after-exposure in the busy traffic site and after-exposure at the park. Coefficient of variation in percentage was used to measure variation of biomarkers between sampling days. $CV(\%) = SD/\text{mean}$ (7.1)

7.3.2.3 Relationship between the measured pollutants and the biomarkers

Spearman correlation was applied to measure the correlations between the pollutants (UFP, diameter size, BC and PM_{2.5}) and the biomarkers (EBC-Nitrate, EBC-Nitrite, EBC-pH, 8-oxodG, systolic and diastolic BP, pulse rate, lung function and minute ventilation (V_E) as ventilation-on-heart-rate (V_E-HR).

Statistical analysis was carried out using Microsoft Office excel and IBM SPSS Statistics 22.

7.4 Results and discussion

7.4.1 Concentration of measured pollutants

Table 7.1 show the descriptive summary of the pollutants measured in the busy traffic site and the control site the park. The concentration measured in the busy traffic site on the average was higher than the measurements of the park except for the $PM_{2.5}$. In fact the percentage differences between the sites are as follows UFP 78 %, particle size 1 % (slightly larger particle size), BC 85 % and for the $PM_{2.5}$ where the park had a higher concentration was -56%. The high PNC and BC percentage is not surprising as compared to the park because studies have shown that UFP are produced in large quantities by vehicular combustion (Mutlu et al., 2001) and BC is a surrogate of traffic related particles (Zhang et al., 2009). This higher concentration of UFP as compared to the control park site confirms Knibbs et al. (2011) that greater proximity vehicles is associated with increased UFP concentrations therefore a major source of ambient UFP. The mean particle diameter size in the busy traffic site was slightly larger than the park side but there was no significant difference between the two measurements. Some particle coagulation can occur very rapidly near the source of emission due to the rapid cooling of combustion exhaust like in the busy traffic site and the process of coagulation reduces rapidly as the particles move farther away from the source of emission (Hodan and Barnard, 2004). Therefore could explain the similarities between the particle size diameters measured at the busy traffic site and the control site the park. Participants had significantly higher exposure of UFP and BC in the busy road site. A busy traffic site with a mixture of vehicles running on both petrol and diesel engine can lead to increased localised air pollution thereby resulting in an increase in the concentration of UFP, decrease in particle size, increase in BC and $PM_{2.5}$. However, higher $PM_{2.5}$ concentration was observed in the park. During the park sampling, no visible strong

source or emission was identified. Therefore it is possible that the source of the higher PM_{2.5} in the park could be attributed to PM_{2.5} formation due to secondary aerosol reactions.

Precursor chemicals NO₂ and SO₂ measured at the RFS for the sampling days (park and Bristol road) were analysed and it was observed that there was no significant difference ($P > 0.05$) between the concentration on the park sampling days and Bristol Street sampling days. However, it is unlikely that the secondary aerosol reactions are responsible for the higher PM_{2.5} in the park because;

- 1) The PM_{2.5} concentration for the park and Bristol Street were not measured simultaneously. Therefore it is difficult to blame the secondary reactions for the higher PM_{2.5} park concentration. This is because other environmental factors such as precursor compounds, wind speed and direction, RH and temperature are involved in the process (Hodan and Barnard, 2004, Wang et al.), therefore they should be considered simultaneously.
- 2) PM_{2.5} formation due to secondary aerosol reaction takes time. Hodan et al.(2004) in their report suggested that the reaction time for sulphate precursor, show that sulphate oxidation happens at an average of 0.1 to 1% of sulphate per hour. They also suggested that because of the continuous change between the gas and condensed phase of nitrate in the atmosphere, it is impossible to quantify the reaction time of nitrates.

Table 7.1. Mean Concentrations of measured pollutants in the busy traffic site and park

	Busy traffic site				Park			
	UFP (#/cm ³)	Size (nm)	BC (µg/m ³)	PM _{2.5} (µg/m ³)	UFP (#/cm ³)	Size (nm)	BC (µg/m ³)	PM _{2.5} (µg/m ³)
Mean	12713	44.78	5.12	20.26	2741	44.28	0.76	31.64
SD	4409	3.53	2.04	6.33	1053	4.22	0.34	10.69
Minimum	6607	39.84	2.06	12.39	1425	40.04	0.34	13.98
Median	12427	45.43	4.98	19.07	2502	43.56	0.76	32.79
Maximum	19052	49.55	8.11	25.08	4239	51.02	1.32	42.49

7.4.2 Biomarkers of exposure

7.4.2.1 Exhaled breath condensate nitrate and nitrites

Table 7.2 shows concentration of the EBC NO_3^- and NO_2^- collected from the before and after exposure in a busy traffic site and the control site the park. Following the exposure, mean EBC NO_3^- and NO_2^- increased significantly ($P < 0.05$) at after-exposure compared to before-exposure concentration (34.20 μM vs 6.83 μM and 2.64 μM vs 2.44 μM) respectively for the busy traffic site. Like in this study, Laumbach et al. (2014) in their study of controlled exposure to traffic related air particles for 1.5 hours with 21 young healthy adults, observed mean EBC-nitrite was increased from baseline at immediately post-exposure comparing unfiltered to filtered rides. While in the park, the mean concentrations of the EBC- NO_3^- and NO_2^- decreased significantly ($P < 0.05$) at the after-exposure compared to the before-exposure (1.51 μM vs 7.65 μM and 0.67 μM vs 0.59 μM) respectively. Laumbach et al. (2014) observed a decrease in post-exposure EBC- NO_2^- as compared to pre-exposure. In another study, EBC- NO_3^- and NO_2^- were measured in 119 subjects from pre-to during-Olympics period and they observed a significant decrease in the EBC- NO_3^- and NO_2^- levels from the pre-to during-Olympics period (Bogdan et al., 2000). This is because the Chinese government made it compulsory that a temporary closure or relocations of industries and reduction in motor vehicles during the Olympics (Bogdan et al., 2000). Then they observed significant increase in the EBC- NO_3^- and NO_2^- in post Olympics because in the post Olympics, there was relaxation in pollution control and air pollutants increased in comparison to pre-Olympics (Bogdan et al., 2000).

The increase in the EBC-Nitrate and EBC-Nitrite may suggest a response to traffic related air pollutants that affect people exposed to it in the busy traffic areas and their decline in the

control site the park, demonstrates that when the air quality improves, production of particle induced EBC-NO₃⁻ and NO₂⁻ reduces. In other words the park exposure may confirm that the exposure to increased concentration to pollutants in the busy traffic caused the increase in EBC-NO₃⁻ and NO₂⁻.

Table 7.2. Summary of EBC nitrate and nitrite

	Busy traffic site			Park		
	Before (μM)	After (μM)	Wilcoxon	Before (μM)	After (μM)	Wilcoxon
NO₃⁻ Mean (SD)	6.83 (7.1)	34.20 (44)	0.01	7.65 (8.65)	1.51 (2.50)	0.03
NO₂⁻ Mean (SD)	2.44 (0.05)	2.64 (0.18)	0.01	0.67 (0.17)	0.59 (0.06)	0.05

Wilcoxon used to test difference of mean and significant at $P \leq 0.05$. 2-tailed probability test.

7.4.2.2 EBC-pH

Table 7.3 shows the concentrations of the EBC-pH that were evaluated in the before and after-exposure at the busy traffic site and at the park. At both sites the after-exposure EBC-pH appeared to be slightly higher than the before-exposure concentration however, not at a significant level ($P > 0.05$). A similar result has been found in a study with 16 subjects with mild asthma, where no clear pattern of change after exposures, and no statistically significant differences between exposure to diesel exhaust (DE) and clean-filtered Air (CA) at any point during the study i.e. EBC collected immediately post, 4 and 24 hours post DE and CA exposure for asthmatic subjects in a controlled exposure (Horváth et al., 2005). While other studies suggest that moderate asthmatic subject compared to mild asthma show significantly decreased EBC-pH when exposed to traffic related emissions and diesel exhaust as compared to the control site exposure (McCreanor et al., 2007, Malerba and Montuschi, 2012). Several studies have suggested that individuals with asthma are more sensitive to elevated PM exposure as compared to healthy subjects (Nordenhäll et al., 2001, Carpagnano et al., 2006). Therefore decreases in EBC-pH in patients with asthma suggests that acute asthma exacerbations may be accompanied by airway acidification that reflects inhibition of local epithelial proton pumps during airway inflammation (Wan et al., 2012). Whereas some other studies suggested that air pollution is associated with airway acidification in healthy subjects

(Bogdan et al., 2000, Stevenson et al., 2011) but the EBC-pH measurement in their study was not a comparison between the before and after exposure rather it was over a period of time and lag effects were also measured. In an occupational study the opposite was observed. It was found that EBC-pH was increased significantly following exposure to welding fumes (Boyce et al., 2006). This increase might be due to fractions such as metals in the welding fumes. Also Koskatis et al. (2012) measured a decrease in EBC-pH immediately after exposure to secondhand smoke in 18 never smoked subjects. The results obtained from different studies are a bit contradictory as some reported increased EBC-pH after exposure and some reported decreased EBC-pH after refer to Table 1.2 and chapter 5 and chapter 6.

Table 7.3. EBC-pH

	Busy traffic site			Park		
	Before	After	Wilcoxon	Before	After	Wilcoxon
EBC-pH Mean (SD)	7.61 (0.14)	7.71 (0.19)	0.109	7.62 (0.19)	7.68 (0.13)	0.300

Wilcoxon used to test difference of mean and significant at $P \leq 0.05$. 2-tailed probability test.

7.4.2.3 8-oxodG (ng/mg creatinine)

Oxidative damage to DNA is most widely assessed with 8-oxodG as a biomarker (Barceloux and Barceloux, 1999). A slight not significant increase ($P \geq 0.495$) in the concentrations of the 8-oxodG was observed in the after-exposure as compared to the before exposure concentrations for both the busy traffic site and the park (Table 7.4). Literatures have reported links between PM increased urinary 8-oxodG. For example, increased urinary excretion of 8-oxodG was found in Copenhagen bus drivers from the central area as compared with drivers from the rural and suburban area (Montuschi et al., 2007) possibly because the drivers in the central area had higher concentration exposure. Another study showed 8-OHdG to be significantly elevated in 95 taxi drivers as compared to 75 community

men (control) (Chuang et al., 2003). However in their study no significant difference was observed between smoker-drivers and smoker-community men. Also no significant difference was observed between smoker-drivers and non-smoker drivers, suggesting that exposure to exhaust due to driving taxi may affect 8-OHdG concentrations and smoking also independently. The drivers in this study were exposed for about 12 hours daily and the community men about 1.9 hours per day. 8-oxodG was significantly elevated in study with 50 bus drivers and 50 controls (Rossner et al., 2008). Increased urinary 8-oxo-dG concentrations has also been observed in coke oven workers (Liu et al., 2006), and different types of workers exposed to vehicular/traffic emissions (Han et al., 2010, Lee et al., 2010). This suggests that 8-oxodG production is associated with length of exposure i.e. like in the cases mentioned in the studies above because of their occupation they are exposed to traffic related pollutants or work related exposures for a long time. Also cigarette and health status are predictors of 8-oxodG (see more explanation in chapter 5 and 6).

Table 7.4. Concentration of 8-oxodG (ng/mg creatinine)

	Busy traffic site			Park		
	Before	After	Wilcoxon	Before	After	Wilcoxon
8-oxodG (ng/mg creatinine) Mean (SD)	3.30 (1.39)	3.41 (1.42)	0.495	3.26 (1.50)	3.33 (1.46)	0.46

Wilcoxon used to test difference of mean and significant at $P \leq 0.05$. 2-tailed probability test.

7.4.3 Autonomic responses

7.4.3.1 Blood pressure and pulse rate

As shown in Table 7.5, the systolic and diastolic blood pressure and pulse rate averaged (SD) 117 (12), 78 (8) mmHg and 76 (8) Beats per minute (BPM) respectively for the before-exposure in busy road site and in the after-exposure measurements, the systolic and diastolic blood pressure and pulse rate averaged (SD), 116 (15), 79 (11) mmHg and 73 (11) BPM respectively. The before-exposure appeared to be very minutely higher than the after-exposure systolic measurement but no significant difference was found between both measurements. The after-exposure diastolic blood pressure measurement appeared to be very minutely higher than the before-exposure measurements. There was no significant difference in the diastolic before and after-exposure mean. The pulse rate decreased after exposure in the busy traffic site as compared to the before but no significant difference in their mean measurements.

As shown in Table 7.6, for the park, the systolic and diastolic blood pressure and pulse rate averaged (SD) 117 (13), 78 (9) mmHg and 77 (16) bpm respectively for the before-exposure and in the after-exposure measurements, the systolic and diastolic blood pressure and pulse rate averaged (SD), 117 (12), 82 (9) mmHg and 74 (16) bpm respectively. No significant difference was found between both measurements for the park. The after-exposure diastolic blood pressure measurement appeared to be higher than the before-exposure measurements. There was no significant difference in the diastolic before and after-exposure mean. The pulse rate was slightly higher in the before-exposure than the after-exposure in the park but no significant difference in their mean measurements.

In a study where ambient PM_{2.5} was concentrated to 150 µg/m³, healthy subjects were exposed for 2 hours on 3 different occasions, 2 to 4 weeks apart, they observed a significant increase in the diastolic pressure at the actual time of inhalation. Whereas in this present study the changes observed in the diastolic were no significant, probably because the PM_{2.5} concentrations measured (Table 7.1) were not as high the concentrations measured in their study (Peters et al., 2004). Brook et al. (2009) observed an increase in heart rate (pulse rate) with increase in diastolic BP and a decrease in heart rate variability. The pulse rate measurements in this study did not significantly change. That could be because the subject were not in an overly active mode (the leisurely walked or stood still at times) and the PM_{2.5} concentrations were not high enough to trigger a significant increase in diastolic BP and pulse rate. A study with 10 patients with lung function impairments showed that PNC 0.02–1 exposures at 1- to 3-hour moving averages were associated with the increased systolic BP, diastolic BP, and pulse rate (Lin et al., 2004a). Subjects in this present study are healthy without any cardiovascular diseases.

Table 7.5. Blood pressure, pulse rate and estimated ventilation for the busy traffic site

	Before-exposure				After-exposure			
	Systolic (mmHg)	Diastolic (mmHg)	Pulse rate (bpm)	V _E -HR (L)	Systolic (mmHg)	Diastolic (mmHg)	Pulse rate (bpm)	V _E -HR (L)
Mean (SD)	117 (12)	78 (8)	76 (8)	17 (3)	116 (15)	79 (11)	73 (11)	16 (4)

Table 7.6. Blood pressure, pulse rate and estimated ventilation for the park

	Before-exposure				After-exposure			
	Systolic (mmHg)	Diastolic (mmHg)	Pulse rate (bpm)	V _E -HR (L)	Systolic (mmHg)	Diastolic (mmHg)	Pulse rate (bpm)	V _E -HR (L)
Mean (SD)	117 (13)	78 (9)	77 (16)	18 (7)	117 (12)	82 (9)	74 (16)	17 (6)

7.4.3.2 Estimated ventilation as ventilation-on-heart- rate (V_E -HR)

The estimated ventilation for each subject was inferred from the pulse rate. The mean (range) estimated ventilation as ventilation-on-heart-rate (V_E -HR) in the before busy traffic site exposure was 17 (L) (14.07 – 25.48), in the after-exposure at the busy traffic site 16 (L) (10.57 – 23.86) (Table 7.5), in the before-exposure at the park 18 (L) (10.80 -34.67) and in the after park exposure 17 (L) (8 – 29.79) (Table 7.6).

7.4.4 Lung function

Lung function is an important measure of respiratory health and an early predictor of cardiovascular morbidity and mortality (Montuschi, 2007). Studies have associated exposure to air pollution with decrease in lung function (John, 2002, Bikov et al., 2010, Todor A, 2011, Montuschi, 2005). Therefore in order to evaluate the exposure influence on respiratory health, the associations between lung function and ambient air pollution in the busy traffic site and the park were measured.

The measurements included the following parameters: FEV_1 (L), which measures the amount of air exhaled during a forced breath in the first second of exhalation, FEV_6 (L) that is now used as a convenient alternative for forced vital capacity (FVC) (L), which is the total volume of expiratory air exhaled during the FEV_1 test. The use of FEV_6 in the place of FVC has been validated and it has been used in various other studies (Paschal et al., 1998, Montuschi and Barnes, 2002, Todor A, 2011). FEV_1 (L) measurement in Table 7.7 appeared in this order after-exposure > 2 hours into exposure > 1 hour into exposure > before-exposure i.e $2.37 > 2.32 > 2.24 > 2.01$. FEV_6 (L) followed the same pattern $2.70 > 2.48 > 2.47 > 2.00$. No exact pattern was found in the FEV_1/FEV_6 measurements: before-exposure > 2 hour into exposure = after-exposure > 1 hour into exposure i.e $0.98 > 0.97 = 0.97 > 0.95$. However, the outcome

of the FEV_1/FEV_6 suggests that there was no detection of airway obstruction because the corresponding FEV_1/FEV_6 was not < 0.73 which is the acceptable cut-off (Montuschi and Barnes, 2002). Also the corresponding FEV_1/FEV_6 was not < 0.70 proposed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guideline for FEV_1/FVC (Montuschi and Barnes, 2002, Koczulla et al., 2009b). Then the forced expiratory flow during the middle portion of a forced expiration ($FEF_{25-75\%}$) (L/s) showed the highest measurement in the after-exposure and lowest in the 1 hour into exposure. Meaning that the forced expiratory flow between 25% and 75% of the largest observed volume during the first 6 s of an FVC manoeuvre was observed at the after-exposure was the highest (4.07) and the lowest at the 1 hour into exposure (2.52).

Table 7.7. Average of lung monition measurements for before during and after exposure in busy traffic site

	Before exposure	1 hour into exposure	2 hour into exposure	After exposure
FEV_1 (L)	2.01	2.24	2.32	2.37
FEV_6 (L)	2.00	2.47	2.48	2.70
FEV_1/FEV_6	0.98	0.95	0.97	0.97
$FEF_{25-75\%}$ (L/s)	3.74	2.52	3.73	4.07

In Table 7.8 for the park measurements, FEV_1 (L) measurement appeared in this order after-exposure = 2hours into exposure > before-exposure > 1 hour into exposure (2.32 = 2.32 > 2.30 > 2.90). The FEV_6 (L) was in this order 1 hour into exposure > after-exposure > before-exposure > 2 hours into exposure (2.59 > 2.48 > 2.45 > 2.40). Like in the busy traffic site the corresponding FEV_1/FEV_6 did not show obstruction and the $FEF_{25-75\%}$ (L/s) showed that the before-exposure 4.43 is the highest and the after-exposure 4.07 is the lowest measurement, while 1 hour and 2 hours into exposure showed equal measurements of 4.14.

The FEV₁ measurement at 1 hour into exposure at the busy traffic site and at the park showed a strong significant correlation 0.91 ($P \leq 0.01$) but there was no significant difference between both averages. The FEV₁ at 2 hour into exposure at the busy traffic site and at the park showed a strong significant correlation 0.83 ($P \leq 0.01$) but there was no significant difference between both averages. The FEV₁ at the after-exposure showed significant correlation 0.69 ($P \leq 0.01$) but no significant difference in the average measurements of both sites. The ratio of FEV₁/FEV₆ for the before-exposure, 1 hour into exposure, 2 hours into exposure and after-exposure was calculated and the result ranged from 0.95 to 1.02. This result indicates that the measurement in the busy traffic site and park are quite similar. Thus, being that the park is the control it was expected that the lung function during the exposure at the park and after-exposure will be better than that obtained at the busy traffic site thereby noticeable significant difference was expected but this was not the case in this study. In a similar study, McCreanor et al. (2007) in their study with 60 mild to moderate asthma subjects observed a significantly more reduced lung function parameters after exposure in the busy Oxford street diesel exposure as compared to the control Hyde Park. The subjects in this study are healthy therefore may be the reason after exposure on the busy traffic site no significant change was observed and vice versa for the control park.

Table 7.8. Average of lung monition measurements for before during and after exposure in the park

	Before exposure	1 hour into exposure	2 hour into exposure	After exposure
FEV₁ (L)	2.30	2.29	2.32	2.32
FEV₆ (L)	2.45	2.59	2.40	2.48
FEV₁/FEV₆	0.97	0.96	0.97	0.96
FEF₂₅₋₇₅% (L/s)	4.43	4.14	4.14	4.07

7.4.5 Inhaled load estimate

Table 7.9 show the estimated inhaled dose that passes through the lungs (Eq. 1.2). Even though the park is the control site it showed that on the average that the subjects inhaled more PM_{2.5} particles than in the busy traffic site while more BC and UFP were inhaled in the busy traffic site. The reason why the park measured higher concentration of PM_{2.5} than the busy traffic site has been earlier discussed. UFP has a greater cytotoxicity and inflammatory capacity because of its unique characteristics. Table 7.1 showed the mean concentrations of the measured pollutants which was used to calculate the inhaled dose. Another factor that influenced the inhaled load estimate is the V_E-HR, however even though the park had a slightly higher after-exposure V_E-HR there was no significant difference between V_E-HR at the busy traffic site and the park control site. The Mann-Whitney U test was used to test the difference of mean between the estimated inhaled load of each pollutant for each site and it showed that they were significantly different.

Table 7.9. Mean UFP, PM_{2.5} and BC estimated inhaled load for 2 hours using the estimated ventilation

Busy traffic site			Park			UFP (particles/cm ³)	PM _{2.5} (µg)	BC (µg)
UFP (particles/cm ³)	PM _{2.5} (µg)	BC (µg)	UFP (particles/cm ³)	PM _{2.5} (µg)	BC (µg)	Mann-Whitney U	Mann-Whitney U	Mann-Whitney U
21027 (18560)	44.15 (18)	9.58 (3)	2775 (996)	62.01 (29)	1.63 (1)	0.000	0.05	0.000

7.4.6 Relationship between biomarkers

7.4.6.1 Relationship between biomarkers in the before

Table 7.10 shows that systolic against diastolic blood pressure, FEV₁ against FEV₆, FEV₁/FEV₆ against FEF₂₅₋₇₅% and V_E-HR against pulse-rate showed significant correlation. It is expected that the V_E-HR against pulse-rate significantly correlates because the V_E-HR is estimated from the pulse-rate. FEV₁ and FEV₆ should also correlate significantly, however if they don't agree it usually because of prolonged expiration time in some people. A study measured intraclass correlation coefficients for FEV₁, FVC, and FEV₆ and all were significantly correlated (De Prins et al., 2014). In a study of 140 systolic and diastolic subjects, they reported that at correlations > 0.5 (like observed in Table 7.10 and Table 7.11), that systolic and diastolic BP has a linear correlation in normotensive individuals (Møller and Loft, 2002) also like we have in our study. Some factors can affect these e.g. age, disease condition (Møller and Loft, 2002, Čalušić et al., 2011) or exposure to high concentrations of PM_{2.5} can induce prohypertensive response (Peters et al., 2004). The essence of checking association in the before-exposure is to compare to the after exposure to see if the same biomarkers correlated in the after-exposure and to see if more biomarkers correlated in the after-exposure.

Table 7.10. Relationship between biomarkers in the before busy traffic site exposure

			Correlations											
			EBC-Nitrate	EBC-Nitrite	EBC-pH	8-oxodG (ng/mg Creatinine)	Systolic	Diastolic	FEV1	FEV6	FEV1/FEV6	FEF25-75	Pulse-rate	VE-HR
Spearman's rho	EBC-Nitrate	Correlation Coefficient	1.000	.106	-.326	-.064	-.229	.224	-.418	-.314	.105	.146	.174	.174
		Sig. (2-tailed)	.	.707	.236	.820	.412	.422	.121	.297	.733	.635	.536	.536
		N	15	15	15	15	15	15	15	13	13	13	15	15
	EBC-Nitrite	Correlation Coefficient	.106	1.000	-.130	.028	-.004	.335	-.065	-.147	.230	-.292	-.077	-.077
		Sig. (2-tailed)	.707	.	.644	.922	.990	.222	.819	.631	.450	.333	.786	.786
		N	15	15	15	15	15	15	15	13	13	13	15	15
	EBC-pH	Correlation Coefficient	-.326	-.130	1.000	-.068	.399	-.187	.168	.118	.188	.157	.291	.291
		Sig. (2-tailed)	.236	.644	.	.810	.141	.504	.549	.700	.538	.609	.293	.293
		N	15	15	15	15	15	15	15	13	13	13	15	15
	8-oxodG(ng/mg Creatinine)	Correlation Coefficient	-.064	.028	-.068	1.000	-.045	-.167	.196	.275	-.384	-.148	-.436	-.436
		Sig. (2-tailed)	.820	.922	.810	.	.874	.553	.483	.364	.195	.629	.104	.104
		N	15	15	15	15	15	15	15	13	13	13	15	15
	Systolic	Correlation Coefficient	-.229	-.004	.399	-.045	1.000	.570*	.289	.194	.290	.484	.428	.428
		Sig. (2-tailed)	.412	.990	.141	.874	.	.027	.297	.526	.336	.094	.112	.112
		N	15	15	15	15	15	15	15	13	13	13	15	15
	Diastolic	Correlation Coefficient	.224	.335	-.187	-.167	.570*	1.000	.129	.099	.158	.270	.345	.345
		Sig. (2-tailed)	.422	.222	.504	.553	.027	.	.647	.747	.606	.372	.208	.208
		N	15	15	15	15	15	15	15	13	13	13	15	15
	FEV1	Correlation Coefficient	-.418	-.065	.168	.196	.289	.129	1.000	1.000**	.052	.104	-.145	-.145
		Sig. (2-tailed)	.121	.819	.549	.483	.297	.647	.	.	.865	.734	.606	.606
		N	15	15	15	15	15	15	15	13	13	13	15	15
	FEV6	Correlation Coefficient	-.314	-.147	.118	.275	.194	.099	1.000**	1.000	.052	.104	-.220	-.220
		Sig. (2-tailed)	.297	.631	.700	.364	.526	.747	.	.	.865	.734	.469	.469
		N	13	13	13	13	13	13	13	13	13	13	13	13
	FEV1/FEV6	Correlation Coefficient	.105	.230	.188	-.384	.290	.158	.052	.052	1.000	.629*	.434	.434
		Sig. (2-tailed)	.733	.450	.538	.195	.336	.606	.865	.865	.	.021	.139	.139
		N	13	13	13	13	13	13	13	13	13	13	13	13
	FEF25-75	Correlation Coefficient	.146	-.292	.157	-.148	.484	.270	.104	.104	.629*	1.000	.292	.292
		Sig. (2-tailed)	.635	.333	.609	.629	.094	.372	.734	.734	.021	.	.333	.333
		N	13	13	13	13	13	13	13	13	13	13	13	13
	Pulse-rate	Correlation Coefficient	.174	-.077	.291	-.436	.428	.345	-.145	-.220	.434	.292	1.000	1.000**
		Sig. (2-tailed)	.536	.786	.293	.104	.112	.208	.606	.469	.139	.333	.	.
		N	15	15	15	15	15	15	15	13	13	13	15	15
	VE-HR	Correlation Coefficient	.174	-.077	.291	-.436	.428	.345	-.145	-.220	.434	.292	1.000**	1.000
		Sig. (2-tailed)	.536	.786	.293	.104	.112	.208	.606	.469	.139	.333	.	.
		N	15	15	15	15	15	15	15	13	13	13	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

In Table 7.11 for the relationship between all biomarkers before the park exposure showed that EBC-NO₂⁻ against diastolic, systolic against diastolic, diastolic against pulse rate, FEV₁ against FEV₆, FEV₁ against FEV₁/FEV₆, FEV₆ against FEV₁/FEV₆, FEV₆ against pulse-rate, FEV₆ against V_E-HR, V_E-HR against diastolic and V_E-HR against pulse-rate were significant correlated.

There are some differences between Table 7.10 and Table 7.11. It would be expected that the same variables be related in both tables since they are both measuring before-exposure biomarker relationship for the same people but at different times. However the difference seen in Table 7.10 and Table 7.11 is because for each person, different marker measurements were observed before exposure (Table 7.1 to Table 7.8).

Table 7.11. Relationship between biomarkers in the before park exposure

			Correlations											
			EBC-Nitrate	EBC-Nitrite	EBC-pH	8-oxodG (ng/mg creatinine)	Systolic	Diastolic	FEV1	FEV6	FEV1/FEV6	FEF25-75	Pulse-rate	VE-HR
Spearman's rho	EBC-Nitrate	Correlation Coefficient	1.000	.048	-.142	-.133	.153	-.349	.190	.238	-.091	.271	-.307	-.307
		Sig. (2-tailed)	.	.889	.614	.636	.585	.202	.497	.434	.767	.371	.265	.265
		N	15	11	15	15	15	15	15	13	13	13	15	15
	EBC-Nitrite	Correlation Coefficient	.048	1.000	.163	.010	-.601	-.744**	.124	.150	-.280	.000	-.229	-.229
		Sig. (2-tailed)	.889	.	.632	.978	.051	.009	.717	.679	.433	1.000	.499	.499
		N	11	11	11	11	11	11	11	10	10	10	11	11
	EBC-pH	Correlation Coefficient	-.142	.163	1.000	-.050	.050	-.118	.181	.166	.142	.397	-.502	-.502
		Sig. (2-tailed)	.614	.632	.	.859	.859	.675	.519	.589	.645	.179	.057	.057
		N	15	11	15	15	15	15	15	13	13	13	15	15
	8-oxodG(ng/mg creatinine)	Correlation Coefficient	-.133	.010	-.050	1.000	-.286	.290	.318	.341	-.407	-.099	.329	.329
		Sig. (2-tailed)	.636	.978	.859	.	.301	.295	.248	.255	.167	.748	.231	.231
		N	15	11	15	15	15	15	15	13	13	13	15	15
	Systolic	Correlation Coefficient	.153	-.601	.050	-.286	1.000	.517*	-.161	-.229	.495	.504	.060	.060
		Sig. (2-tailed)	.585	.051	.859	.301	.	.048	.566	.452	.086	.079	.832	.832
		N	15	11	15	15	15	15	15	13	13	13	15	15
	Diastolic	Correlation Coefficient	-.349	-.744**	-.118	.290	.517*	1.000	-.200	-.264	.416	-.025	.517*	.517*
		Sig. (2-tailed)	.202	.009	.675	.295	.048	.	.474	.383	.158	.936	.048	.048
		N	15	11	15	15	15	15	15	13	13	13	15	15
	FEV1	Correlation Coefficient	.190	.124	.181	.318	-.161	-.200	1.000	.989**	.605*	.214	-.472	-.472
		Sig. (2-tailed)	.497	.717	.519	.248	.566	.474	.	.000	.029	.482	.075	.075
		N	15	11	15	15	15	15	15	13	13	13	15	15
	FEV6	Correlation Coefficient	.238	.150	.166	.341	-.229	-.264	.989**	1.000	-.699**	.110	.602*	.602*
		Sig. (2-tailed)	.434	.679	.589	.255	.452	.383	.000	.	.008	.721	.029	.029
		N	13	10	13	13	13	13	13	13	13	13	13	13
	FEV1/FEV6	Correlation Coefficient	-.091	-.280	.142	-.407	.495	.416	-.605*	-.699**	1.000	.460	.489	.489
		Sig. (2-tailed)	.767	.433	.645	.167	.086	.158	.029	.008	.	.113	.090	.090
		N	13	10	13	13	13	13	13	13	13	13	13	13
	FEF25-75	Correlation Coefficient	.271	.000	.397	-.099	.504	-.025	.214	.110	.460	1.000	-.099	-.099
		Sig. (2-tailed)	.371	1.000	.179	.748	.079	.936	.482	.721	.113	.	.748	.748
		N	13	10	13	13	13	13	13	13	13	13	13	13
	Pulse-rate	Correlation Coefficient	-.307	-.229	-.502	.329	.060	.517*	-.472	-.602*	.489	-.099	1.000	1.000
		Sig. (2-tailed)	.265	.499	.057	.231	.832	.048	.075	.029	.090	.748	.	.
		N	15	11	15	15	15	15	15	13	13	13	15	15
	VE-HR	Correlation Coefficient	-.307	-.229	-.502	.329	.060	.517*	-.472	-.602*	.489	-.099	1.000**	1.000
		Sig. (2-tailed)	.265	.499	.057	.231	.832	.048	.075	.029	.090	.748	.	.
		N	15	11	15	15	15	15	15	13	13	13	15	15

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

7.4.6.2 Relationship between pollutants and pollutants between after-exposure biomarkers

Table 7.12 shows that EBC- Nitrate was significantly correlated with UFP in the after-exposure for the busy street suggesting that increase in UFP concentration resulted in inflammation of the airways. EBC-Nitrite was significantly correlated with inhaled load estimated UFP thereby associating inflammation with the dose of UFP that passed through the lungs. Mermier et al. (2013) suggests that dose delivered to the target site in this chapter the airways, is a more biologically appropriate predictor of potential hazard to the lungs than the concentration in the air. The correlation observed with PM agrees with the hypothesis that PM can induce oxidative and nitrosative stress mediated by a particle-induced inflammation causing target cells like airway epithelial cells and the macrophages to release ROS/RNS, by transition metals, alongside organic hydrocarbons on the particle surface capable of generating ROS/RNS through the Fenton type reaction or by quinones in the particles that produce ROS through redox cycling (Li et al., 2003, Badmaev et al., 1999, Barceloux and Barceloux, 1999).

In table 7.12 and Table 7.13, EBC-Nitrate and EBC-Nitrite showed an inverse correlation with EBC-pH respectively in both the busy traffic site and park. Studies have shown that there is a relationship between airway pH and nitrite i.e. in asthmatic subjects, as the airway pH decrease, nitrite increases and it protonates converting to nitrous acid (HNO_2), which then goes on to form NO (Jaques and Kim, 2000). However, in this chapter, nitrate and nitrite increased or decreased significantly for the busy traffic site and the park respectively but no significant EBC-pH change. Therefore this not statistically significant inverse correlation may be associated with a trend for an increase of nitrate and nitrite (busy traffic site) and a reduction of nitrate and nitrite (park) in the study group. In this study, EBC-pH showed

inverse significant correlation with BC in the park in Table 7.13 and in the busy traffic site EBC-pH showed an inverse correlation with inhaled load estimate BC (BC that passed through the lungs) (Table 7.12). In the busy street as the higher BC concentration led to an increased inhaled dose (Table 7.1 and Table 7.9), EBC-pH did not significantly change (Table 7.3) and in the park as the BC concentration decreased (Table 7.1) the EBC-pH did not significantly change (Table 7.3) therefore causing the inverse correlation. However they both showed that exposure to BC could cause a change in the EBC-pH because of the observed relationship and the EBC-pH probably remained unchanged because the subjects in this study are healthy and are non-smokers. Koskatis et al. (2012) observed a decrease in EBC-pH immediately after exposure to secondhand cigarette smoke in 18 never smokers. It could be that the different constituents of the cigarette smoke as compared to traffic related air in this study were responsible for the decrease. Like Boyce et al. (2006) observed an increase in EBC-pH of 22 healthy subjects exposed to PM metal welding fume. Another study also observed increased in hydrogen ion concentration following exposure to metal particles in welding fumes for asymptomatic subjects (Lundberg and Weitzberg, 2010). Patel et al. (2013) observed among all subjects (i.e. asthmatic and non-asthmatic) that they sampled increases in 1- to 5-day averages of BC were associated with decreases in EBC-pH, and they attributed it to increased airway inflammation. Patel et al. (2013) sampled for 4 weeks for 6–7 h per weekday and they suggested that accumulated concentration of BC may have improved the assessment of BC exposure. While this study was just for 2 hours at the busy traffic site and 2 hours at the park on two separate days. Interestingly, they suggested that same-day and multiday average BC exposures were associated with EBC-pH whereas shorter averaging times (1- and 8-h) were not.

In the busy road side 8-oxodG showed a significant correlation with inhaled load estimate BC but not with the actual BC concentration that the subject was exposed to (Table 7.12), while in the park there was no correlation between 8-oxodG and any of the variables in the correlation matrix (Table 7.13). A study reported similar result stating that urinary 8-oxodG is not a predictor of PE to PM_{2.5} and black smoke but is a predictor in lymphocyte DNA (Barceloux and Barceloux, 1999). PM_{2.5} was not associated with 8-oxodG in a study with 19 wildland fire fighters, where 214 urine samples were collected over 10 burn days (Adetona et al., 2013). However this study shows that the dose that passed through the lungs could be a better indicator of oxidative stress which is then measured as urinary 8-oxodG.

In this study as shown in Table 7.12 and Table 7.13 in the busy traffic site and park respectively, systolic and diastolic BP did not show any significant correlation with the measured pollutants UFP, particle size, BC and PM_{2.5}. In another study 130 children's systolic BP was significantly associated with ambient increase in UFP concentration and particles < 100 nm in diameter measured in their school playground on the same morning, while larger particles, PM_{2.5} and PM₁₀ were not significantly associated (Gibson, 2012). However in their study, diastolic did not correlate with UFP or and PM. It is possible that the significant correlation they observed between the systolic and UFP (< 100 nm) could be because they are children and they have higher V_E, higher metabolic turnover developing lungs and other organs, therefore are more susceptible to air pollution effects (Sjaastad et al., 2010). Another study with 2607 subjects observed an increase in systolic BP associated with increase in air pollution, which could be related with change in cardiovascular autonomic control in subgroups with high plasma viscosity level and increased heart rate (Maître et al., 2003). Another study with 30 volunteers observed among all the health measurements that only blood pressure showed reasonably consistent unfavourable longitudinal associations with particulates, more with station or outdoor PM than with indoor or personal PM for subjects

with severe chronic obstructive pulmonary disease (Weichenthal et al., 2011). Subjects in this study were healthy with no cardiovascular or pulmonary health effects therefore may be the reason why there systolic and diastolic BP did not show effects i.e. did not correlate with the measured pollutants.

Even though the systolic and diastolic BP have different regulation pathway and respond to stimuli in different ways (Gibson, 2012) they were significantly correlated in both busy traffic site and park after exposure. The role of systolic BP is to force blood through the arteries during a heartbeat, which is responsive to the sympathetic nervous system and stress stimuli, the diastolic BP provides introduces blood into the peripheral tissues during heart relaxation (Gibson, 2012, Mermier et al., 1993). A significant inverse association between EBC-pH and systolic BP was observed (Table 7.13) cannot really be explained. However, a decrease in EBC-pH a marker of inflammation can lead to bronchoconstriction (Al-Obaidy and Al-Samarai, 2007) which can affect lung function and further BP. Previous studies have associated air pollution to BP and heart variability (Sarnat et al., 2014) and one of the mechanisms for this outcome is through inflammation and oxidative stress (Laumbach and Kipen, 2010).

FEV₁ and FEV₆ were significantly correlated with size and PM_{2.5} and FEF₂₅₋₇₅% was significantly correlated with UFP in the after park exposure both in Table 7.13 and FEV₆ against UFP. No other lung function parameter showed a significant correlation with the measured pollutants. A study, observed a decrease in lung function parameters and EBC-pH to be associated with UFP during most of their study and less consistently with EC. However BC and not EC was measured in this study. In a cohort, exposure to traffic-related BC, a component of PM, independently predicted decreased lung function in urban women, when adjusting for tobacco smoke, asthma diagnosis, and socioeconomic status (Zhang et al.,

2009). In a two-pollutant model developed in a study to check association between lung function and PM, they reported that it was consistent in the two-pollutant model that FEV₁ and FEV₆ were significantly (increase) associated with the PM₁₀ concentration for 37–60 hr preceding the lung function tests in children (Chuang et al., 2005). In this study before-exposure concentration of the pollutants at the busy traffic site and park were not measure because the aim was to measure biomarker relationship in short term exposure at the busy traffic. Therefore no Lag pollutants correlation with lung function measurements was produced. In this study there was no decrease in FEV₁ and FEV₆ in the busy traffic site all through the sampling and in the park no decrease in FEV₁ and FEV₆ after exposure as displayed in Table 7.7 and Table 7.8. Two studies, observed no association of lung function with any measure of PM in their study with subjects with respiratory diseases (de Hartog et al., 2009, Manney et al., 2012) and Manney et al. (2012) suggests that EBC NO_x may reflect an effect of PM exposure before such effects become marked enough to change airflow. In this study there were changes in nitrate and nitrite in EBC but no change in the lung function. It cannot be stressed enough that subject in this study are healthy therefore may be the reason why no significant change in lung function was observed.

There are various factors that affect pulse rate that influence pulse rate. They include; stress drugs, weather but the main factor that influences pulse rate is the consumption of oxygen, which is also related to ventilation. Therefore this explains the significant correlation found between pulse-rate against FEV₆ and pulse-rate against estimated inhaled PM_{2.5} for the busy traffic site as seen in Table 7.12 as they are all linked to oxygen in take. Therefore an obstruction due to exposure to PM_{2.5} can affect spirometric function. However, pulse-rate against estimated inhaled BC dose was not significantly correlated. In the busy traffic site and park (Table 7.12 and Table 7.13) respectively, pulse-rate significantly correlated with V_E-HR

which were both associated with intake of oxygen. In Table 7.12, for the busy traffic site pulse-rate was significantly associated with inhaled load estimate UFP and inhaled load estimate $PM_{2.5}$ respectively. Therefore, indicating a directly proportional relationship between pulse-rate, ventilation and dose of the pollutants that passed through the lungs.

In the park Table 7.13, BC was significantly correlated with UFP. In the park we can see that as the UFP decreased the BC decreased but $PM_{2.5}$ increased. BC and UFP are better correlated than BC and $PM_{2.5}$ because UFP and BC share provenance in vehicular emission (Ng and Moore, 1998).

Table 7.12. Relationship between pollutants and pollutants between after-exposure biomarkers in the busy traffic site

		Correlations																		Inhaled load estimated UFP	Inhaled load estimated PM2.5	Inhaled load estimated BC
		UFP	Size	BC	PM2.5	EBC-Nitrate	EBC-Nitrite	EBC-pH	8-oxodG (ng/mg creatinine)	Systolic	Diastolic	FEV1	FEV6	FEV1/FEV6	FEF25-75	Pulse-rate	VE-HR					
Spearman's rho	UFP	Correlation Coefficient	1.000	-.387	.229	-.174	.659	-.401	.456	.091	-.138	-.134	-.145	-.085	.171	-.014	-.140	-.140	.648	.096	-.241	
		Sig. (2-tailed)		.154	.411	.534	.008	.139	.088	.748	.625	.634	.607	.793	.594	.965	.620	.620	.009	.734	.387	
		N	15	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15	
Size	Correlation Coefficient	-.387	1.000	-.057	-.064	-.413	.057	-.202	.000	.214	.152	.195	.248	-.262	-.078	-.109	-.109	-.344	-.679	.002		
		Sig. (2-tailed)	.154		.840	.820	.126	.839	.470	1.000	.444	.588	.485	.437	.411	.810	.700	.700	.209	.005	.995	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
BC	Correlation Coefficient	.229	-.057	1.000	-.945	.058	.244	-.162	-.413	-.178	-.304	.272	.319	-.316	.050	-.408	-.408	.007	-.324	.614		
		Sig. (2-tailed)	.411	.840		.000	.838	.380	.564	.126	.527	.270	.328	.312	.317	.878	.131	.131	.980	.239	.015	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
PM2.5	Correlation Coefficient	-.174	-.064	-.945	1.000	-.123	-.340	.296	.322	.094	.243	-.290	-.291	.316	.035	.368	.368	-.047	.357	-.646		
		Sig. (2-tailed)	.534	.820	.000		.662	.216	.284	.242	.738	.383	.295	.359	.317	.913	.177	.177	.868	.192	.009	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
EBC-Nitrate	Correlation Coefficient	.659	-.413	.058	-.123	1.000	-.233	-.038	.011	-.216	-.027	-.354	-.378	.463	-.035	-.071	-.071	.457	.329	-.250		
		Sig. (2-tailed)	.008	.126	.838	.662		.402	.894	.970	.439	.924	.196	.226	.130	.914	.800	.800	.087	.232	.369	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
EBC-Nitrite	Correlation Coefficient	-.401	.057	.244	-.340	-.233	1.000	-.089	.109	.181	.058	.181	.414	-.450	.086	-.382	-.382	-.572	-.339	.136		
		Sig. (2-tailed)	.139	.839	.380	.216	.402		.754	.699	.519	.836	.519	.181	.142	.791	.159	.159	.026	.217	.630	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
EBC-pH	Correlation Coefficient	.456	-.202	-.162	.296	-.038	-.089	1.000	.216	-.058	-.280	.182	.354	-.048	.371	-.238	-.238	.027	-.232	-.547		
		Sig. (2-tailed)	.088	.470	.564	.284	.894	.754		.439	.837	.312	.516	.259	.882	.235	.393	.393	.924	.405	.035	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
8-oxodG(ng/mg creatinine)	Correlation Coefficient	.091	.000	-.413	.322	.011	.109	.216	1.000	-.064	-.116	.143	.224	-.292	.273	-.241	-.241	-.118	-.161	-.568		
		Sig. (2-tailed)	.748	1.000	.126	.242	.970	.699	.439		.820	.680	.612	.484	.357	.391	.386	.386	.676	.567	.027	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
Systolic	Correlation Coefficient	-.138	.214	-.178	.094	-.216	.181	-.058	-.064	1.000	.844	-.470	-.448	.114	-.644	.252	.252	.079	.064	.007		
		Sig. (2-tailed)	.625	.444	.527	.738	.439	.519	.837	.820		.000	.077	.144	.724	.024	.364	.364	.781	.820	.980	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
Diastolic	Correlation Coefficient	-.134	.152	-.304	.243	-.027	.058	-.280	-.116	.844	1.000	-.658	-.781	.481	-.641	.338	.338	.145	.297	-.055		
		Sig. (2-tailed)	.634	.588	.270	.383	.924	.836	.312	.680	.000		.008	.003	.113	.025	.218	.218	.607	.283	.845	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
FEV1	Correlation Coefficient	-.145	.195	.272	-.290	-.354	.181	.182	.143	-.470	-.658	1.000	.972	-.619	.685	-.275	-.275	-.196	-.457	.225		
		Sig. (2-tailed)	.607	.485	.328	.295	.196	.519	.516	.612	.077	.008		.000	.032	.014	.321	.321	.483	.087	.420	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
FEV6	Correlation Coefficient	-.085	.248	.319	-.291	-.378	.414	.354	.224	-.448	-.781	.972	1.000	-.740	.559	-.755	-.755	-.552	-.748	-.007		
		Sig. (2-tailed)	.793	.437	.312	.359	.226	.181	.259	.484	.144	.003	.000		.006	.059	.005	.005	.063	.005	.983	
		N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12		
FEV1/FEV6	Correlation Coefficient	.171	-.262	-.316	.316	.463	-.450	-.048	-.292	.114	.481	-.619	-.740	1.000	.064	.562	.562	.459	.569	-.178		
		Sig. (2-tailed)	.594	.411	.317	.317	.130	.142	.882	.357	.724	.113	.032	.006		.843	.057	.057	.133	.053	.580	
		N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12		
FEF25-75	Correlation Coefficient	-.014	-.078	.050	.035	-.035	.086	.371	.273	-.644	-.641	.685	.559	.064	1.000	-.483	-.483	-.392	-.329	-.322		
		Sig. (2-tailed)	.965	.810	.878	.913	.914	.791	.235	.391	.024	.025	.014	.059	.843		.112	.112	.208	.297	.308	
		N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12		
Pulse-rate	Correlation Coefficient	-.140	-.109	-.408	.368	-.071	-.382	-.238	-.241	.252	.338	-.275	-.755	.562	1.000	1.000	1.000	.577	.724	.347		
		Sig. (2-tailed)	.620	.700	.131	.177	.800	.159	.393	.386	.364	.218	.321	.005	.057	.112			.024	.002	.205	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
VE-HR	Correlation Coefficient	-.140	-.109	-.408	.368	-.071	-.382	-.238	-.241	.252	.338	-.275	-.755	.562	1.000	1.000	1.000	.577	.724	.347		
		Sig. (2-tailed)	.620	.700	.131	.177	.800	.159	.393	.386	.364	.218	.321	.005	.057	.112			.024	.002	.205	
		N	15	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15		
Inhaled load estimated UFP	Correlation Coefficient	.648	-.344	.007	-.047	.457	-.572	.027	-.118	.079	.145	-.196	-.552	.459	-.392	.577	.577	1.000	.026	.232		
		Sig. (2-tailed)	.009	.209	.980	.868	.087	.026	.924	.676	.781	.607	.483	.063	.133	.208	.024	.024		.026	.405	
		N	15	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15		
Inhaled load estimated PM2.5	Correlation Coefficient	.096	-.679	-.324	.357	.329	-.339	-.232	-.161	.064	.297	-.457	-.748	.569	-.329	.724	.724	.571	1.000	.157		
		Sig. (2-tailed)	.734	.005	.239	.192	.232	.217	.405	.567	.820	.283	.087	.005	.053	.297	.002	.002	.026		.576	
		N	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15	15		
Inhaled load estimated BC	Correlation Coefficient	-.241	.002	.614	-.646	-.250	.136	-.547	-.568	.007	-.055	.225	-.007	-.178	-.322	.347	.347	.232	.157	1.000		
		Sig. (2-tailed)	.387	.995	.015	.009	.369	.630	.035	.027	.980	.845	.420	.983	.580	.308	.205	.205	.405	.576		
		N	15	15	15	15	15	15	15	15	15	15	15	12	12	12	15	15	15	15		

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Table 7.13. Relationship between pollutants and pollutants between after-exposure biomarkers in the park

		Correlations																			
		UFP	Size	BC	PM2.5	EBC-Nitrate	EBC-Nitrite	EBC-pH	8-oxodG (ng/mg creatinine)	Systolic	Diastolic	FEV1	FEV6	FEV1/FEV6	FEF25-75	Pulse-rate	VE-HR	Inhaled load estimated UFP	Inhaled load estimated PM2.5	Inhaled load estimated BC	
Spearman's rho	UFP	Correlation Coefficient	1.000	.266	.560	.163	.346	.772	-.255	-.325	.209	.005	.222	.312	.005	.608	-.428	-.428	.447	.082	.283
		Sig. (2-tailed)	.	.338	.030	.561	.298	.005	.360	.237	.454	.985	.427	.278	.987	.021	.112	.112	.094	.773	.307
		N	15	15	15	15	11	15	15	15	15	15	15	15	14	14	15	15	15	15	15
Size		Correlation Coefficient	.266	1.000	.596	-.703	.207	.578	.010	-.021	.251	.169	-.700	.682	.523	.332	.080	.080	.187	-.567	.475
		Sig. (2-tailed)	.338	.	.019	.003	.542	.063	.972	.941	.367	.548	.004	.007	.055	.246	.777	.777	.505	.027	.074
		N	15	15	15	15	11	15	15	15	15	15	15	14	14	14	15	15	15	15	15
BC		Correlation Coefficient	.560	.596	1.000	-.431	.299	.694	-.584	-.041	.364	-.036	-.149	-.156	.076	.501	-.109	-.109	.408	-.498	.725
		Sig. (2-tailed)	.030	.019	.	.109	.371	.018	.022	.885	.182	.898	.595	.595	.796	.068	.700	.700	.131	.059	.002
		N	15	15	15	15	11	15	15	15	15	15	15	14	14	14	15	15	15	15	15
PM2.5		Correlation Coefficient	.163	-.703	-.431	1.000	-.114	-.403	-.026	.015	.120	.190	.553	.601	-.228	-.011	-.276	-.276	.129	.752	-.366
		Sig. (2-tailed)	.561	.003	.109	.	.739	.219	.926	.957	.670	.497	.032	.023	.432	.970	.320	.320	.648	.001	.180
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
EBC-Nitrate		Correlation Coefficient	.346	.207	.299	-.114	1.000	.301	-.532	-.287	.096	-.265	-.256	-.219	-.334	.351	-.057	-.057	.141	-.055	.036
		Sig. (2-tailed)	.298	.542	.371	.739	.	.368	.092	.392	.779	.431	.448	.518	.315	.290	.868	.868	.679	.873	.915
		N	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
EBC-Nitrite		Correlation Coefficient	.772	.578	.694	-.403	.301	1.000	-.153	.038	-.048	-.158	.191	.133	-.040	.439	-.287	-.287	.296	-.257	.277
		Sig. (2-tailed)	.005	.063	.018	.219	.368	.	.654	.911	.889	.643	.573	.696	.907	.177	.393	.393	.378	.445	.410
		N	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
EBC-pH		Correlation Coefficient	-.255	.010	-.584	-.026	-.532	-.153	1.000	.125	-.598	-.150	-.021	.044	.146	-.469	-.085	-.085	-.333	.014	-.403
		Sig. (2-tailed)	.360	.972	.022	.926	.092	.654	.	.657	.018	.593	.942	.881	.619	.091	.763	.763	.226	.960	.137
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
8-oxodG(ng/mg creatinine)		Correlation Coefficient	-.325	-.021	-.041	.015	-.287	.038	.125	1.000	-.148	-.044	.118	.130	-.176	-.226	.349	.349	.279	.073	.111
		Sig. (2-tailed)	.237	.941	.885	.957	.392	.911	.657	.	.598	.877	.675	.659	.547	.436	.202	.202	.314	.795	.694
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
Systolic		Correlation Coefficient	.209	.251	.364	.120	.096	-.048	-.598	-.148	1.000	.700	-.334	-.378	.373	.254	.064	.064	.296	.063	.286
		Sig. (2-tailed)	.454	.367	.182	.670	.779	.889	.018	.598	.	.004	.223	.183	.189	.381	.821	.821	.283	.024	.302
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
Diastolic		Correlation Coefficient	.005	.169	-.036	.190	-.265	-.158	-.150	-.044	.700	1.000	-.322	-.388	.609	-.121	.375	.375	.461	.389	.302
		Sig. (2-tailed)	.985	.548	.898	.497	.431	.643	.593	.877	.004	.	.241	.171	.021	.680	.168	.168	.084	.151	.274
		N	15	15	15	15	11	11	15	15	15	15	15	15	14	14	15	15	15	15	15
FEV1		Correlation Coefficient	.222	-.700	-.149	.553	-.256	.191	-.021	.118	-.334	-.322	1.000	.988	.554	-.057	-.399	-.399	-.030	.395	-.231
		Sig. (2-tailed)	.427	.004	.595	.032	.448	.573	.942	.675	.223	.241	.	.000	.040	.846	.140	.140	.914	.145	.408
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
FEV6		Correlation Coefficient	.312	-.682	-.156	.601	-.219	.133	.044	.130	-.378	-.388	.988	1.000	-.641	-.147	-.502	-.502	-.042	.367	-.284
		Sig. (2-tailed)	.278	.007	.595	.023	.518	.696	.881	.659	.183	.171	.000	.	.014	.615	.067	.067	.887	.196	.325
		N	14	14	14	14	11	11	14	14	14	14	14	14	14	14	14	14	14	14	14
FEV1/FEV6		Correlation Coefficient	.005	.523	.076	-.228	-.334	-.040	.146	-.176	.373	.609	-.554	-.641	1.000	.326	.191	.191	.092	-.139	.143
		Sig. (2-tailed)	.987	.055	.796	.432	.315	.907	.619	.547	.189	.021	.040	.014	.	.255	.514	.514	.755	.636	.625
		N	14	14	14	14	11	11	14	14	14	14	14	14	14	14	14	14	14	14	14
FEF25-75		Correlation Coefficient	.608	.332	.501	-.011	.351	.439	-.469	-.226	.254	-.121	-.057	-.147	.326	1.000	-.233	-.233	.191	-.235	.108
		Sig. (2-tailed)	.021	.246	.068	.970	.290	.177	.091	.436	.381	.680	.846	.615	.255	.	.422	.422	.512	.418	.714
		N	14	14	14	14	11	11	14	14	14	14	14	14	14	14	14	14	14	14	14
Pulse-rate		Correlation Coefficient	-.428	.080	-.109	-.276	-.057	-.287	-.085	.349	.064	.375	-.399	-.502	.191	-.233	1.000	1.000	.492	.224	.448
		Sig. (2-tailed)	.112	.777	.700	.320	.868	.393	.763	.202	.821	.168	.140	.067	.514	.422	.	.	.062	.423	.094
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
VE-HR		Correlation Coefficient	-.428	.080	-.109	-.276	-.057	-.287	-.085	.349	.064	.375	-.399	-.502	.191	-.233	1.000	1.000	.492	.224	.448
		Sig. (2-tailed)	.112	.777	.700	.320	.868	.393	.763	.202	.821	.168	.140	.067	.514	.422	.	.	.062	.423	.094
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
Inhaled load estimated UFP		Correlation Coefficient	.447	.187	.408	.129	.141	.296	-.333	.279	.296	.461	-.030	-.042	.092	.191	.492	.492	1.000	.406	.746
		Sig. (2-tailed)	.094	.505	.131	.648	.679	.378	.226	.314	.283	.084	.914	.887	.755	.512	.062	.062	.	.133	.001
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
Inhaled load estimated PM2.5		Correlation Coefficient	.082	-.567	-.498	.752	-.055	-.257	.014	.073	.063	.389	.395	.367	-.139	-.235	.224	.224	.406	1.000	-.091
		Sig. (2-tailed)	.773	.027	.059	.001	.873	.445	.960	.795	.824	.151	.145	.196	.418	.418	.423	.423	.133	.	.746
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15
Inhaled load estimated BC		Correlation Coefficient	.283	.475	.725	-.366	.036	.277	-.403	.111	.286	.302	-.231	-.284	.143	.108	.448	.448	.746	-.091	1.000
		Sig. (2-tailed)	.307	.074	.002	.180	.915	.410	.137	.694	.302	.274	.408	.325	.625	.714	.094	.094	.001	.	.
		N	15	15	15	15	11	11	15	15	15	15	15	14	14	14	15	15	15	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

** . Correlation is significant at the 0.01 level (2-tailed).

7.4.7 Urinary metals

Only 5 subjects (2 blacks, 2 whites and 1asian) were chosen from the 15 subjects sampled for the urinary metal analysis. Therefore the 5 subjects for the urinary metal comparison and analysis were the same for both the busy traffic site and the park. The mean urinary metals measured in the before and after exposure scenario (both busy traffic site and the park) were below the reference limits. Reference values were obtained from Hoet et al. (2013). The urinary metals analysed were Na, Mg, Ca, V, Cr, Mn, Fe, Ni, Cu, Sn, Sb and Pb.

7.4.7.1 Relationship between before and after exposures urinary metals in both the busy traffic site and the park

Table 7.14 and Table 7.15 show the comparison summary between the before and after urinary exposure in the busy traffic site and in the park respectively. For the busy traffic site, there was no significant increase between the urinary metals measured in the before-exposure as compared to the after-exposure ($P = 0.86$) (Table 7.14). For the park, there was a significant reduction between the urinary metals measured in the before-exposure and after-exposure ($P = 0.02$) (Table 7.15). Urine is the main route of excretion of metals, and urine concentrations of most metals show previous exposure within a few hours or days (Feng et al., 2015a). The reduction of urinary metals after the park exposure speculates that there may be a relationship between exposure concentrations and urinary metals, even though there was no significant change observed after the exposure at the busy road site. Some urinary metal concentrations may not reliably reflect environmental exposure, and may therefore lead to possible exposure misclassification (Feng et al., 2015a). However, several urinary metals have been associated with heart rate variability parameters due to metals in air particles (Feng et al., 2015a). Also the size of people assessed for this study, may be too small measure any significant changes in the busy traffic site. Although, concentrations of the urinary metals after exposure at the busy traffic site was then compared to the urinary metals measured after

the exposure at the park. It was observed that even though they were significantly correlated (0.96), the urinary metal after the busy traffic site exposure was significantly higher (0.009) than that measured after the park exposure. Also factors such as diet, ethnicity, life style/habit, medication and health conditions can affect the outcome of urinary metals (ChandrakalaAluganti et al., 2012, Jomova and Valko, 2011, Valko et al., 2007). A correlation between the pollutants and the urinary metals and biomarkers would have been an added advantage to this comparison to see if the pollutants and the urinary metals and the biomarkers significantly correlate but because of the very small size of 5 subjects whose urine were analysed for urinary metals the correlation may not give a good result. A study measured UCr of workers exposed to Cr during chrome-plating and control at different time intervals (Federico et al., 2007). Caglieri et al. (2006) sample collection time for their study: T_0 = before the work shift on Friday, T_1 = at the end of the Friday shift i.e. 8 hours of work and T_2 = before exposure of the following Monday. At all time intervals the exposed showed higher concentration of UCr as compared to the control. For the exposed, UCr measured for T_1 was significantly higher than T_0 and T_2 and then T_0 significantly higher than T_2 which is the start of a new week after the weekend off (Federico et al., 2007). In another study where urinary metals were measured in incinerator workers, the urinary metal concentrations were significantly higher at plant 1 as compared to plant 2 because of high concentrations of pollutants emanating from one old furnace (Patel et al., 2013).

As mentioned earlier most of the urinary metal assessment are in relation to exposure via inhalation are in the occupational scenarios. However, in this chapter based on the results show that after exposure at the park that is relatively less polluted than the busy site there was reduction in the concentration of urinary metals measured in 5 subjects. This suggests that there could be a connection between pollutant concentration exposure and urinary metals as

also seen in the occupational setting. However, it is recommended that this study be carried with a larger cohort size in order to get better understanding and enough statistical power for association between exposure and urinary metals. However the information is not enough to confirm urinary metals as a biomarker of short term exposure to air pollution.

Table 7.14. Comparison summary for the before and after urinary exposure in the busy traffic site

Metals	Units	Busy traffic site Before	Busy traffic site After	Wilcoxon
Na	mol/mol cr	8.81	12.90	0.86
Mg	mol/mol cr	0.22	0.23	
Ca	mol/mol cr	0.16	0.17	
V	μmol/mol cr	1.18	1.55	
Cr	μmol/mol cr	0.71	0.92	
Mn	μmol/mol cr	0.34	0.30	
Fe	μmol/mol cr	10.54	12.71	
Ni	μmol/mol cr	1.99	0.97	
Cu	μmol/mol cr	9.78	7.99	
Zn	μmol/mol cr	455.12	351.31	
Sn	μmol/mol cr	0.65	0.67	
Sb	μmol/mol cr	0.64	0.33	
Pb	μmol/mol cr	0.30	0.37	

Wilcoxon: testing difference of mean, Significant at ≤ 0.05

Table 7.15. Comparison summary for the before and after urinary exposure in the Park

Metals	Units	Park Before	Park After	Wilcoxon
Na	mol/mol cr	9.47	12.57	0.02
Mg	mol/mol cr	0.15	0.16	
Ca	mol/mol cr	0.08	0.11	
V	μmol/mol cr	2.87	1.37	
Cr	μmol/mol cr	3.27	0.91	
Mn	μmol/mol cr	0.23	0.09	
Fe	μmol/mol cr	41.69	9.16	
Ni	μmol/mol cr	1.61	1.22	
Cu	μmol/mol cr	6.35	5.66	
Zn	μmol/mol cr	332.36	293.69	
Sn	μmol/mol cr	0.56	0.50	
Sb	μmol/mol cr	0.11	0.06	
Pb	μmol/mol cr	0.28	0.17	

Wilcoxon: testing difference of mean, Significant at ≤ 0.05

7.5 Conclusion

Exposure to short-time traffic related air pollution has been associated with adverse health effect even in healthy individuals. Using markers of exposure to air pollution, this chapter sought to compare exposure in a busy traffic site against a control site the park.

This chapter demonstrates and supports with evidence the hypothesis that exposure to traffic related particles can induce inflammation. Significantly increased EBC-Nitrate and EBC-Nitrite were observed after exposure at the busy traffic site, while in the control site the park, significantly decrease EBC-Nitrate and EBC-Nitrite were observed. The significant increases in the busy road site after-exposure nitrate and nitrite concentrations, suggests increased pollutant (BC, UFP and decreased size) concentrations are associated with production of localized inflammatory or localised nitrosative/oxidative stress after short-term exposure. That is the innate immune system responds to the particles challenging the airways by releasing proinflammatory mediators like NO amongst others to protect the airways. Therefore with increased unstable NO, it oxidises to stable end product nitrate and nitrite. While in the park exposure where UFP and BC concentrations are lower the reverse happens suggesting that these particles play essential role in the production of particle induced inflammation.

There were no significant changes in the EBC-pH and 8-oxodG. Most studies as discussed in chapters 4 to chapter 7 suggests that significant changes in EBC-pH and 8-oxodG are associated with people with cardiovascular and other diseases, long term exposure due to occupational exposure, smoking and length of smoking years as predictors. However, without disregarding the fact that some studies including this have observed no significant changes

especially in healthy subjects. Some studies have also suggested incremental changes in EBC-pH could be due to the constituents of the particles like in the study of Boyce et al. (2006) and Fireman et al. (2008). 8-oxodG is a biomarker of DNA damage and repair, perhaps no immediate harm has been caused to the subject. However prolonged exposure could result in increased ROS/RNS that would cause modification to macromolecules thereby causing harm enough to measure fluctuations in the 8-oxodG concentrations. EBC-pH and 8-oxodG have shown contradictory results in various studies in health subjects. However, in subjects with compromised health status the end point may be different.

Pulmonary and autonomic function did not significantly change. This may be because the subjects in this study are healthy. However, one of the limitations of the study in this chapter is that more post exposure measurements of the biomarkers were not taken, in order to check if any significant alterations occurred e.g. 3hours or more after exposure.

Several biomarkers were significantly correlated to the pollutants, thus supporting particle induced inflammation and oxidative stress, pulmonary and autonomic function dysregulation.

Inhaled load estimate, showing that the load or dose of pollutant passing through the lungs could be a better indicator of potential health hazard associated with air pollution exposure rather than the concentrations of the pollutant. While in the park the decrease in the biomarkers significantly correlated with the concentrations of the measured pollutants.

Another limitation to the study in this chapter is the small cohort-size therefore findings may need replication with larger sampling cohort sizes.

Therefore Increases in markers of nitrosative/oxidative stress in EBC may represent early biological responses to exposures to traffic related air particles and air pollution. It also suggests that non-invasive markers like all the mediators used in this study can used to measure the mechanisms and kinetics of acute responses to traffic related air pollutants in a larger group or in a different controlled setting.

8 LUNG FUNCTION AND 8-OXOdG MEASUREMENTS OF 2 COMMUTERS

8.1 Introduction

Exposure to air pollution which includes traffic related air pollutant has been associated with both short-term and long-term health effects such as cardiovascular, pulmonary and respiratory health effects (Matter Aerosol, 2011, Valavanidis et al., 2011, Gessner et al., 2003, Huang et al., 2009, Morawska et al., 2008). Air pollution levels in various commuter modes have been compared in various studies. The resulting measured concentrations in the different commuter modes however vary and this is because of various factors such as time of sampling, type of fuel and route influence the sampling (Fierz et al., 2008). The focus of this chapter is not the concentrations measured but the short-term health related effects. Research work has been able to associate different types of commuter mode exposure to health effects however the information is still limited.

8.1.1 Toxicological and epidemiological information

In a comparison of PM dose and acute heart rate variability response in 32 cyclists, pedestrians, bus and train passengers, it was observed that elevated PM_{2.5} and PM₁₀ inhaled and lung deposited doses were significantly ($p < 0.05$) associated with decreased heart rate variability indices especially in cyclist and pedestrians in comparison to bus and train subjects (Guo et al., 2009). In a toxicological study where alveolar epithelium cell line was treated with PM_{2.5} from London Underground, inflammation and cytotoxic (% Lactate dehydrogenase release, plasmid DNA damage, and interleukin-8 release) effects were observed (Bourdon et al., 2012). A study observed that exposure to UFP and soot was weakly associated with nitric oxide which was indicative of airway inflammation and they also measured decrease in lung function after 6 hours after exposure in 12 cyclists (Matter Aerosol, 2011). A study review indentified increased myocardial infarction in susceptible

subjects after exposure to traffic related air pollutants regardless of whether they were in a car, public transport, cycling or even in a motorcycle (Liu et al., 2009). A study observed that Lung function was associated with truck traffic density but had a lesser association with automobile traffic density and exposure to particularly to DE particles may lead to reduced lung function in 1092 children from 13 schools living near major motorways (Abdullahi et al., 2013). Increased excretion of 8-oxodG in 30 bus drivers from central Copenhagen as compared with 27 drivers from rural/suburban greater Copenhagen were measured in study (Loft et al., 1999).

8.2 Aims and objectives

To measure the traffic related health risk is challenging due to factors such as exposures to complex mixtures with physical and chemical characteristics that change very quickly over short distances from their sources. However, studying these short-term effects or responses will help provide more evidence on health effects associated with commuter modes. Thus the aim of this chapter is to assess the effect of commuter mode exposure due to traffic related particles on oxidative DNA damage 8-oxodG and lung function. In order to achieve this, listed below are the specific objectives:

- Measure daily pollutant concentrations ($PM_{2.5}$, BC and EC) in different ME and commuter modes
- Measure biomarker 8-oxodG, a biomarker of oxidative damage
- Measure lung function in cycling and train journey
- Compare measured pollutant concentrations with biomarker and lung function parameters for cycling mode and train mode

8.3 Materials and method

8.3.1 Sampling method

Two subjects were sampled.

8.3.1.1 Subject one

In order to complete the commuter exposure the subject was asked to complete journeys in the following commuter modes: Cycling, Bus, Car and Walking. The cycling, Bus and car mode used the same journey route and during the same time slots but on different days. In addition to the planned route and time travel for the Car mode, the subject also used the car outside the planned route and time and these measurements were taken. The walking mode did not use the same pattern or route but exposure during anytime the subject walked was measured. Table 8.1 displays the Travel summary for the sampling period. The time slots were chosen so that the 8:15 – 9:15 and 16:30 – 17:30 represents the rush hour and the 13:00 – 14:00 represents the off-peak period. All sampling took place at Peterborough.

Table 8.1. Travel summary for the sampling period

Commuter mode	Day	Time of travel
Cycling	Day 1 – Day 5 (June 10 th -14 th)	8:15 – 9:15 13:00 – 14:00 16:30 – 17:30
Bus	Day 8 – Day 12 (June 17 th – 21 st)	8:15 – 9:15 13:00 – 14:00 16:30 – 17:30
Car	Day 15 – Day 19 (June 24 th – 28 th)	8:15 – 9:15 13:00 – 14:00 16:30 – 17:30
Car outside planned time and route	Day 1, Day 4, Day8 and Day 10	
Walking	On all days during the sampling except Day 10, Day 15 and Day 16.	

8.3.1.2 Subject two

Subject two was assessed for the train mode. Therefore Figure 8.1 shows the journey plan used for the 6 days of train travel. At Marylebone roadside sampling site the subject was there for 45 mins. The return journey followed the same route except that the subject waited at London Euston for about 2 hours before continuing back the Birmingham New Street station.

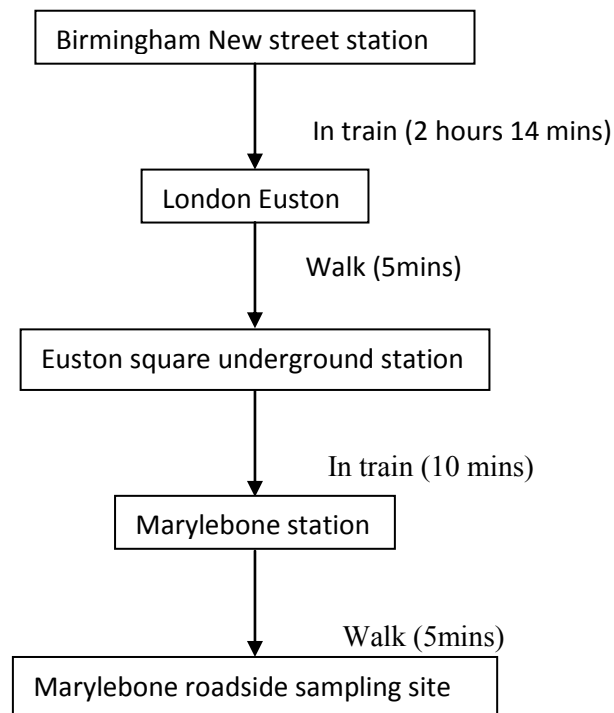


Figure 8.1. Travel route for the train commute

8.3.1.3 Personal sensors

Subject one used the following PSs to measure the pollutants in real time: the MicroAeth Model AE51 for the BC, RTI microPEM for the PM_{2.5} and the Matter-Aerosol DiSCmini for the UFP. The UFP measurements were not included in this study because when the data was downloaded only 2 days had valid data therefore it was decided not to include the UFP measurements in the analysis. All PSs have been discussed in detail in chapter 2.

Subject 2 used for the train commute a PM_{2.5} cyclone attached a pump to measure PM_{2.5} and BC. The measurement was not in real-time therefore the mass concentration of the PM_{2.5} collected on the Teflon filter was determined by gravimetric analysis and the soot collected on the quartz filter for the EC concentration was determined using the Sunset lab OC/EC analyser. Flow rate for the personal pumps was set at 2 LPM.

8.3.2 Collection of Urine samples

Morning after sampling early morning Urine sample was collected throughout the commuter exposure measurement for 8-oxodG analysis. Analysis is as described in chapter 2.

8.3.3 Lung function measurements

Vitalograph digital lung monitor was used to measure the lung function.

8.3.4 Statistical analysis

8.3.4.1 Pollutants analysis

The mean concentrations of the pollutants PM_{2.5}, BC and EC were determined to enable quantification of data.

8.3.4.2 Relationship between 8-oxodG and pollutants and lung function

Spearman correlation was applied to measure the correlations between the pollutants (BC and PM_{2.5}) and 8-oxodG and the lung function.

Coefficient of variation (CV) in percentage was used calculate variation between biomarker between sapling days.

$$CV = SD/\text{mean} \quad (7.1)$$

Statistical analysis was carried out using Microsoft Office excel and IBM SPSS Statistics 22.

8.4 Result and discussion

8.4.1 Subject 1

8.4.1.1 Concentrations of the pollutants in the different microenvironments and the commuter modes

Table 8.2, Table 8.3 and Table 8.4 summarises the measured concentrations of the pollutants BC and PM_{2.5}. Table 8.2 shows the daily exposure concentrations while Table 8.3 and Table 8.4 show the concentrations in the Home, others (any ME that is not home or transport mode) and the commuting modes (bus, car, cycling and walking). Days 6, 7, 13 and 14 are missing because the instruments were turned off to give a break before starting another commuter mode. The concentrations displayed were obtained after the data provided by the instruments. Some data were missing due to instrument error. Day 2 is missing from the BC data as shown in Table 8.2. In Table 8.3 in the Others environment, the measured concentration is missing from Day 10 and on Day 11, 1 hour 25 mins is missing from the bus commute. Others micro-environment represent every other environment that does not include the subjects home or when in transport mode.

Table 8.2. Daily measured BC and PM2.5 concentrations

Day	1	2	3	4	5	8	9	10	11	12	15	16	17	18	19
BC ($\mu\text{g}/\text{m}^3$)	1.46	-	0.72	1.37	1.99	1.17	2.31	4.01	2.22	2.52	1.43	1.71	1.88	1.72	1.71
PM _{2.5} ($\mu\text{g}/\text{m}^3$)	46.85	21.71	20.28	23.61	24.39	15.69	63.47	51.36	15.62	14.83	24.83	26.23	12.43	19.40	16.12

Table 8.3. Measured BC concentrations (ng/m³) in the different commuter modes

DAY	Microenvironment/BC concentration	DAY	Microenvironment/BC concentration
Day 1	Home – 1.33 x 10 ³ Car – 1.28 x 10 ³ Cycling – 2.17 x 10 ³ Walking – 0.57 x 10 ³ All commutes – 1.87 x 10 ³ Others – 1.25 x 10 ³	Day 11	Home – 1.51 x 10 ³ Bus – 6.63 x 10 ³ Walking – 1.38 x 10 ³ All commutes – 5.19 x 10 ³ Others – 1.66 x 10 ³
Day 3	Home – 0.48 x 10 ³ Cycling – 1.27 x 10 ³ Walking – 0.56 x 10 ³ All commutes – 1.13 x 10 ³ Others – 0.52 x 10 ³	Day 12	Home – 0.82 x 10 ³ Bus – 9.89 x 10 ³ Walking – 4.59 x 10 ³ All commutes – 8.29 x 10 ³ Others – 4.61 x 10 ³
Day 4	Home – 0.99 x 10 ³ Car – 1.91 x 10 ³ Cycling – 1.91 x 10 ³ Walking – 1.95 x 10 ³ All commutes – 1.92 x 10 ³ Others – 1.92 x 10 ³	Day 15	Home – 0.78 x 10 ³ Car – 3.86 x 10 ³ All commutes – 3.86 x 10 ³ Others – 1.43 x 10 ³
Day 5	Home – 2 x 10 ³ Cycling – 1.94 x 10 ³ Walking – 1.99 x 10 ³ All commutes – 1.96 x 10 ³ Others – 1.96 x 10 ³	Day 16	Home – 1.07 x 10 ³ Car – 6.42 x 10 ³ All commutes – 6.42 x 10 ³ Others – 2.07 x 10 ³
Day 8	Home – 1.11 x 10 ³ Car – 1.07 x 10 ³ Bus – 1.36 x 10 ³ Walking – 1.02 x 10 ³ All commutes – 1.33 x 10 ³ Others – 1.04 x 10 ³	Day 17	Home – 1.42 x 10 ³ Car – 7.04 x 10 ³ Walking – 1.46 x 10 ³ All commutes – 6.61 x 10 ³ Others – 1.71 x 10 ³
Day 9	Home – 1.80 x 10 ³ Bus – 5.03 x 10 ³ Walking – 9.03 x 10 ³ All commutes – 5.33 x 10 ³	Day 18	Home – 1.25 x 10 ³ Car – 5.20 x 10 ³ Walking – 1.24 x 10 ³ All commutes – 4.65 x 10 ³ Others – 1.73 x 10 ³
Day 10	Home – 3.26 x 10 ³ Car – 2.36 x 10 ³ Bus – 9.13 x 10 ³ All commutes – 7.63 x 10 ³ Others – 2.79 x 10 ³	Day 19	Home – 0.83 x 10 ³ Car – 6.70 x 10 ³ Walking – 0.84 x 10 ³ All commutes – 5.99 x 10 ³ Others – 1.27 x 10 ³

In this study because the focus is the commuter mode, the other MEs i.e. the Home and the Others will not be discussed. However they are measured to show that exposure to BC and $PM_{2.5}$ also occurred in the home and in the other ME and sometimes in magnitudes greater than the transport mode exposure. Different days showed different commuter modes with highest daily mean exposure concentrations (Table 8.3), i.e. the highest BC concentrations measured on the sampling days are as follows; Day 1 Cycling, Day 3 Cycling, Day 4 Walking, Day 5 Walking, Day 8 Bus, Day 9 Walking, Day 10 – Day 12 Bus and Day 15 – Day 19 Car. Also in Table 8.3 the highest $PM_{2.5}$ concentrations measured on the sampling days are as follows; Day 1 to Day 5 Cycling, Day 8 Bus, Day 9 – Day 10 Home, Day 11 – Day 12 Bus, Day 15 – Day 18 car and Day 19 Home. Comparison of air pollution concentrations in the different commuter modes observed in research studies have yielded different outcomes. In a study review, higher exposure to $PM_{2.5}$ in the bus and car commuter mode was observed than in the walking and in the cycling mode (Kamboures et al., 2013). Another with 2 cyclists and 2 drivers in 2 cars for 4 hours on 2 different days measured higher total dust concentration of about 2 to 4 times in the car mode compared to the cycling mode, therefore concluding that car drivers seem to be more exposed to airborne pollution than cyclists even with consideration of their (cycling) minute ventilation (Jeong et al., 2004). Another study using 468 samples shared equally among the 4 commuter modes collected over 18 months showed the $PM_{2.5}$ exposure concentration in this order bus > car > cycling > walking (Reis et al., 2013), while another study, whilst simultaneously walking and driving 48 routes in London observed higher exposure concentrations when walking than in a car by a factor of about 2.2 (Deville Cavellin et al., 2015).

For the BC exposure concentrations, a study examined personal exposure of 62 subjects and found highest exposure concentrations in car and bus mode when compared to cycling and

walking transport mode (Dons et al., 2012). Soot levels measured by Zuurbier et al. (2010) showed that highest median levels were observed in cars and buses, and lowest by bike along a low-traffic street.

The variation in the exposure for the different commuter modes for the different studies and this present study suggests that other factors such as proximity to the source, type of transport used in close proximity to source, meteorological conditions, time of travel, travelling time, urban area, traffic volume during time of travel, route of travel, type of transport ventilation system and filtration system, fuel type and more of which a summary can be found in Knibbs et al. (2011). Therefore making it quantitatively difficult to conclude that one commuter mode over the other is responsible for the highest exposure concentration.

Table 8.4. Measured PM_{2.5} concentrations (µg/m³) in the different commuter modes

DAY	Microenvironment/PM_{2.5} concentration	DAY	Microenvironment/PM_{2.5} concentration
DAY 1	Home – 48.64 Car – 30.24 Cycling – 56.76 Walking – 35.88 All commutes – 45.63 Others – 34.84	Day 11	Home – 16.94 Bus – 19.49 Walking – 10.14 All commutes – 15.01 Others – 11.16
Day 2	Home – 13.12 Car – 15.76 Cycling – 79.83 Walking – 20.29 All commutes – 68.68 Others – 12.65	Day 12	Home – 13.30 Bus – 24.05 Walking – 22 All commutes – 23.37 Others – 11.76
Day 3	Home – 10.81 Cycling – 61.83 Walking – 8.96 All commutes – 54.71 Others – 8.93	Day 15	Home – 17.24 Car – 36.20 All commutes – 36.20 Others – 24.40
Day 4	Home – 10.61 Car – 10.58 Cycling – 75.81 Walking – 10.27 All commutes – 51.15 Others – 8.45	Day 16	Home – 19.46 Car – 65.63 All commutes – 65.63 Others – 12.46
Day 5	Home – 10.20 Cycling – 87.47 Walking – 8.66 All commutes – 60.61 Others – 16.10	Day 17	Home – 9.48 Car – 31.49 Walking – 0.59 All commutes – 29.86 Others – 17.62
Day 8	Home – 12.95 Car – 0.85 Bus – 21.94 Walking – 9.12 All commutes – 20.97 Others – 10.84	Day 18	Home – 19.28 Car – 28.44 Walking – 13.66 All commutes – 26.39 Others – 15.53
Day 9	Home – 71.30 Bus – 26.87 Walking – 30.19 All commutes – 27.12	DAY 19	Home – 20.48 Car – 13.86 Walking – 13.15 All commutes – 13.79 Others – 12.76
Day 10	Home – 53.02 Car – 21.44 Bus – 45.78 All commutes – 44.90 Others – 34.84		

8.4.1.2 8-oxodG measurements

The summary of the 8-oxodG is displayed in Table 8.5. The concentrations that were measured are within the ranges that have been measured by other studies in healthy people. Various researches have measured 8-oxodG from 3 -50 (Hu et al., 2006, Shimoi et al., 2002, Lin et al., 2004b, Sjaastad and Svendsen, 2008) but no basal limit of 8-oxodG has been set (Hu et al., 2006). At the first day of each commuter mode, the 8-oxodG is high compared to the rest of the 8-oxodG in the rest of the days of the week of that commuter mode and then a steady decrease was observed (Table 8.5). The reason for this is not clear. However, the CV value for 8-oxodG, was 17 % indicating low variation between the 8-oxodG concentrations and also, the SD suggests that the variation between 8-oxodG concentration for the different days was not broad (Table 8.5) this suggests that there was no apparent damage. In a controlled random sequence study, 18 healthy subjects were exposed for 3 hours to DE at $276 \mu\text{g}/\text{m}^3$ from a passenger car or filtered air, with co-exposure to traffic noise at 48 or 75 dB(A) and no change in 8-oxodG was observed (Martin et al., 2015). Highest level of 8-oxodG was found in the exposed group 95 taxi drivers than in the 75 control subjects (Chuang et al., 2003). The 8-oxodG was measured in the urine across the 15 days of sampling for this subject.

Table 8.5. Summary of 8-oxodG (ng/mg creatinine)

Day	Commuter mode	8-oxodG
Day 1	Cycling	5.07
Day 2		6.41
Day 3		4.74
Day 4		4.24
Day 5		4.18
Day 8	Bus	6.46
Day 9		4.88
Day 10		4.03
Day 11		4.52
Day 12		4.05
Day 15	Car	4.70
Day 16		4.63
Day 17		4.61
Day 18		4.15
Day 19		3.80
Mean		4.70
CV % (SD)		17 % (0.79)
Minimum		3.80
Median		4.61
Maximum		6.46

Table 8.6 show the relationship between the daily pollutant concentrations measured and the corresponding morning after 8-oxodG for Subject 1. Table 8.6 showed that BC and PM_{2.5} concentrations measured showed a very poor correlation (0.037), indicating that the pollutants were not generated from the same source. That is, pollutants were generated in different MEs where the sources have their own unique “signature” i.e. composed of different chemical substances and maybe in different concentration and then the daily average of the pollutant was calculated. However, the 8-oxodG showed an inverse significant correlation with measured BC concentrations, while PM_{2.5} showed a weak agreement (not significant). Note that the daily concentration of the BC and PM_{2.5} includes all the ME the subject was exposed to.

Table 8.6. Relationship between daily concentration and 8-oxodG (ng/mg creatinine)

Correlations

			BC	PM2.5	8-oxodG (ng/mg Creatinine)
Spearman's rho	BC	Correlation Coefficient	1.000	.037	-.538*
		Sig. (2-tailed)	.	.899	.047
		N	14	14	14
	PM2.5	Correlation Coefficient	.037	1.000	.186
		Sig. (2-tailed)	.899	.	.508
		N	14	15	15
	8-oxodG(ng/mg Creatinine)	Correlation Coefficient	-.538*	.186	1.000
		Sig. (2-tailed)	.047	.508	.
		N	14	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

Table 8.7 show that an inverse significant correlation was observed between the BC measured during the 5 daily Bus commuter modes and the 8-oxodG.

Table 8.7. Relationship between 8-oxodG and BC measured in the different microenvironment

	Home	Bus	Car	Cycling	Walking	All commute	Others
N (number of days)	14	5	9	4	11	14	13
8-oxodG(ng/mg creatinine)	-0.28	-0.90****	-0.53	0.20	-0.24	-0.32	-0.82**

** Significant at $P \leq 0.01$ ****Significant at $P \leq 0.05$

In Table 8.8 no significant correlation was found between the measured $PM_{2.5}$ in the different commuter modes or any other ME and 8-oxodG.

Table 8.8. Relationship between 8-oxodG and $PM_{2.5}$ measured in the different microenvironment

	Home	Bus	Car	Cycling	Walking	All commute	Others
N (number of days)	15	5	10	5	12	15	13
8-oxodG(ng/mg creatinine)	-0.09	-0.50	-0.15	-0.40	0.06	0.31	-0.02

A study observed that none of the 8-oxodG measured correlated with the steel dust in New York subway particulates for the exposed 39 subway workers and the control 11 bus drivers and 25 suburban office workers (Grass et al., 2010). Multivariate logistic regression analysis identified $PM_{2.5}$ and PM_{10} levels, measured by stationary monitoring during a 3-day period before urine collection, as the only factors significantly affecting 8-oxodG levels in a group of 50 bus drivers in Prague (Rossner et al., 2008). This suggests lag effect of 8-oxodG measurements. In 50 students leaving in Denmark, no correlation was observed between urinary 8-oxodG and $PM_{2.5}$, rather significant correlation was observed with 8-oxodG in lymphocytes (Barceloux and Barceloux, 1999). In 76 students followed for 3 months, no association was observed between urinary 8-oxodG and $PM_{2.5}$ and PM_{10} rather association

between plasma 8-oxodG and sulphur dioxide and ozone (Chuang et al., 2007). This suggests that 8-oxodG in blood may show better correlation with pollutants than urine. The 8-oxodG measured in 36 schoolchildren during 5 time periods before and during the Olympic Games in Beijing (June 2007-September 2008), showed that 8-oxodG were significantly associated with concentrations of BC and PM_{2.5} (Lin et al., 2015b).

In Table 8.6 to Table 8.8, it is not clear whether the small cohort-size of the study in this chapter led to the inconsistency, weak agreements, lack of statistical power and or significant negative agreements observed between pollutants and 8-oxodG. Perhaps because of the small size of the study it was difficult to find a significant correlation between the low changes in 8-oxodG and measured pollutants. Therefore the result should not be considered a standard and may not be the same for people with compromised health, the elderly and children.

8.4.1.3 Lung function measurement during cycling exposure

The subject's lung capacity was measured during the cycling commute which is the first 5 days of the sampling period 10/06/2013 -14/06/2013. Three sets of 1 hour sampling was performed by the subject for each of the five days [8:15 – 9:15, 13:00 – 14:00, 16:30 – 17:30] associated with the daily rush hour. In each four sets of lung function measurements were made i.e. before each ride commences and the last at the end of the last ride at 17:30. The mean of the four sets of lung function measurement was calculated for each day and the outcome is displayed in Figure 8.2.

Figure 8.2 show that the lung function parameters decreased with exposure to air pollutant in the cycling commuter mode as the cycling week progressed, considering that the first measurement was taken on a Monday after a weekend of no cycling commuter mode. A study observed a decreased lung function in 12 healthy subjects after 6 hours of the 1 hour

exposure to traffic related air pollutants during cycling (Matter Aerosol, 2011). A study with 31 mild asthma and 29 moderate asthma compared with the control exposure showed reduction in FEV₁ and FVC 5 hours after exposure (Malerba and Montuschi, 2012). A study observed similar results like in this present study. The study where 15 healthy non-smoking adults cycled on a high traffic route and the control low traffic route, no changes in the lung function was observed immediately after exposure and 4 hours after exposure (Jarjour et al., 2013). Even though the subject in this study was healthy, Figure 8.2 showed that with continuous cycling exposure there was decrease in the lung function parameter. The ratio of FEV₁/FEV₆ which is used to check airway obstruction was measured and a daily reduction was observed, however not < 0.73 which is the acceptable cut-off for the ratio of FEV₁/FEV₆ (Montuschi and Barnes, 2002) but to 0.75 (this value is interpreted in percentage). The result obtained in the FEV₁/FEV₆ is not is not surprising given that the subject in this study is healthy and free from cardiovascular diseases like asthma. Jarjour et al. (2013) did not observe any change in their FEV₁/FVC equivalent of FEV₁/FEV₆. Bear in mind that this is not the only parameter used to check lung obstruction but this is the most glaring one.

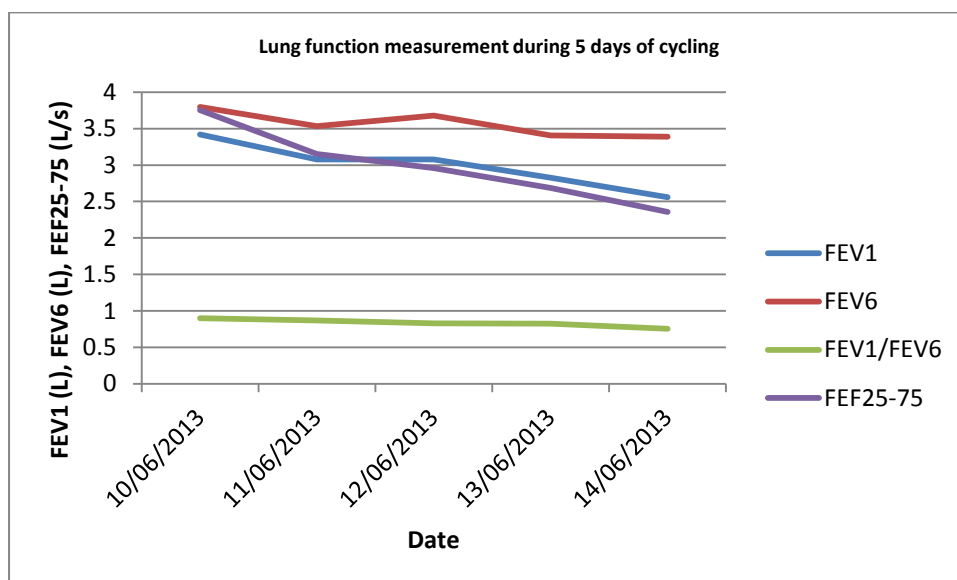


Figure 8.2. Lung function measurement during 5 days of cycling during the sampling period

Table 8.9 show the relationship between the measured pollutants and the lung function parameters and 8-oxodG. $PM_{2.5}$ respectively showed an inverse significant correlation with FEV_1 and FEV_6 respectively but no significant correlation between BC and FEV_1 and or FEV_6 . This supports the hypothesis that exposure to particulates during cycling commuter mode can cause a decrease in lung function because of exposure to increased concentration of the $PM_{2.5}$ and increased inhaled load estimates due to increased heart rate and V_E . FEV_1/FEV_6 and FEF25-75% showed a significant correlation with 8-oxodG respectively. Continuous exposure to increased $PM_{2.5}$ concentration can cause lung function impairment and also increase burden of 8-oxodG. This is in contrast with a study where 42 healthy subjects cycled for 1 hour in high and low traffic and indoors (Huang et al., 2012). They did not observe strong relationships between traffic related $PM_{2.5}$ and acute changes in respiratory outcome in urban cyclists in their study. However they observed an association between UFP and increase in FEF25-75% into 1 hour cycling but this study did not have any data for UFP, $PM_{2.5}$ was associated with a small increase in eNO 2 hr after cycling, BC did not significantly correlate with any respiratory outcomes and NO_2 levels were associated with significantly increased FEV_1 2 hours and 3 hours after the start of cycling.

An inverse relationship between FEV_1/FEV_6 ratio and 8-oxodG was expected i.e. a decrease in the FEV_1/FEV_6 ratio and an increase in the 8-oxodG, like in the study where the oxidative stress burden in the airway smooth muscles (ASM) of asthmatic subjects (mild to severe) was examined it was found to be related to the degree of airflow obstruction (Lin et al., 2015a). Sutcliffe et al. (2012) observed that when ASM in bronchial biopsies from asthmatic subject compared with 8-oxodG of healthy control subjects, a marked increase in the nuclear 8-oxodG staining was inversely correlated with FEV_1/FVC . Remember that FEV_1/FEV_6 is an accepted surrogate for FEV_1/FVC (Paschal et al., 1998, Montuschi and Barnes, 2002, Todor

A, 2011). Their result suggested that there is a link between the burden of oxidative stress and the features of disordered airway physiology (obstruction) that characterize asthma (Lin et al., 2015a). However in this present study, a positive significant correlation was observed opposite of the expected result. It could be because in the Sutcliffe et al. (2012) the cells used were from asthmatic subjects therefore more sensitivity to 8-oxodG nuclear staining or due to the small sample size used in this study measurement of small significant changes were not observed. Table 8.9 showed that there was no significant correlation between the BC and PM_{2.5} respectively with 8-oxodG. BC and UFP concentrations in the urban air are often more correlated than PM_{2.5} given that they both originate from vehicle emissions and that BC contribute to UFP chemical composition (Mutlu et al., 2001).

Table 8.9. Relationship between 8-oxodG, lung function parameters and mean pollutant concentration measured during the cycling mode

Correlations

			BC	PM2.5	FEV1	FEV6	FEV1/FEV6	FEF25-75	8-oxodG (ng/mg creatinine)
Spearman's rho	BC	Correlation Coefficient	1.000	-.200	.200	.200	.200	.200	.200
		Sig. (2-tailed)	.	.800	.800	.800	.800	.800	.800
		N	4	4	4	4	4	4	4
	PM2.5	Correlation Coefficient	-.200	1.000	-.900*	-.900*	-.700	-.700	-.400
		Sig. (2-tailed)	.800	.	.037	.037	.188	.188	.505
		N	4	5	5	5	5	5	5
	FEV1	Correlation Coefficient	.200	-.900*	1.000	1.000**	.900*	.900*	.700
		Sig. (2-tailed)	.800	.037	.	.	.037	.037	.188
		N	4	5	5	5	5	5	5
	FEV6	Correlation Coefficient	.200	-.900*	1.000**	1.000	.900*	.900*	.700
		Sig. (2-tailed)	.800	.037	.	.	.037	.037	.188
		N	4	5	5	5	5	5	5
	FEV1/FEV6	Correlation Coefficient	.200	-.700	.900*	.900*	1.000	1.000**	.900*
		Sig. (2-tailed)	.800	.188	.037	.037	.	.	.037
		N	4	5	5	5	5	5	5
	FEF25-75	Correlation Coefficient	.200	-.700	.900*	.900*	1.000**	1.000	.900*
		Sig. (2-tailed)	.800	.188	.037	.037	.	.	.037
		N	4	5	5	5	5	5	5
	8-oxodG(ng/mg creatinine)	Correlation Coefficient	.200	-.400	.700	.700	.900*	.900*	1.000
		Sig. (2-tailed)	.800	.505	.188	.188	.037	.037	.
		N	4	5	5	5	5	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

8.4.2 Subject 2

8.4.2.1 Exposure assessment for 6 daily commutes from Birmingham to London for one subject

The daily concentrations of the PM_{2.5} and EC measured for only during the journey taken by the subject is presented in Table 8.10. Day 2 showed the highest concentration of the PM_{2.5} (119.90 µg/m³) and the lowest PM_{2.5} concentration was measured on Day 1 (98.54 µg/m³). While for the EC, Day 1 presented with the highest concentration while Day 2 showed to have the lowest concentration. The remaining EC concentrations for day 4 to 6 were not analysed because of instrument breakdown. The PM_{2.5} and EC measurements are for the entire journey period from when the subject seats in the train at Birmingham New Street station until London Euston then about a 5 mins walk to Euston square station, underground to Marylebone station a 5 mins walk to the sampling site 30 to 45 mins at the sampling site and the same route back to Birmingham new street and the sampler is then turned off (Figure 8.1).

Exposure studies in different countries subway system, have reported concentrations of PM in subway systems to be usually several times higher than in the ambient environments (Chao and Cheng, 2002). In this present study, the PM_{2.5} concentrations measured during the other commuter modes (cycling, bus, car and walking) in Table 8.4 were significantly lower than the PM_{2.5} concentrations measured during the train commute (Table 8.10). The mean PM_{2.5} concentration measured for the 6 days of train commute as shown in Table 8.10 is 701.97 µg/m³. A study measured mean PM_{2.5} concentration in London Underground for 5 days between 20 – 24 May 1996 to be 892.54 µg/m³ and on another occasion for another 5 days 22 -26 January 1996 (the same period as this study (28 January – 3 February 2014 and at both times a cyclone head like in this study was used) and measured 708.60 µg/m³ (Lai et al.,

2010). The mean averages Sitzmann et al. (1999) measured are around the average observed in this study for the 6 day train commute. More PM_{2.5} concentrations measured in studies include; 247 µg/m³ in London underground was measured by 44 riders during the sampling (Adams et al., 2001), 157 µg/m³ was measured in London underground using 3 fixed samplers from 7 am – 5 pm for 3 days (Seaton et al., 2005) and 56 µg/m³ was measured in New York by 33 workers during 8 – 11 hours work shift for 1- 3 days (Grass et al., 2010). In this present study, the train journey does not only include underground train journey see Figure 8.1. The outbound of the train journey was during the peak period whereas as the return was during the off-peak. Furthermore, there are some evidences that the PM of subway air is substantially different from the above outdoor air or other transport air, in terms of physical and chemical properties (Chao and Cheng, 2002). For example, a study reported that iron-rich particles are the most abundant particles found in the London underground system (Lai et al., 2010) and we know that the most abundant element found in particles in ambient air is Fe. This Fe in the ambient air is usually in the range of 1 – 4 % of mass (Kamens et al., 1991) while another studies have reported that the Fe in the subway is about 40 % - 80% (w/w) of the PM mass fraction (White et al., 2012, Svendsen et al., 2002). The average EC concentration 4.01 µg/m³ (Table 8.10) measured for the train commute is similar to the 4.0 µg/m³ measured in a Helsinki subway station (Zhong et al., 1999). Only that the EC in Aarnio et al. (2005) was measured for 2 weeks, only on week days and only during the day while in this study the EC was measured for only 3 days (approximately 8.5 hours on each day). In another study of selected subway stations in Manhattan, BC concentrations ranged from 5 to 23 µg/m³, while real time PM_{2.5} levels ranged from 35 to 200 µg/m³ and mean EC levels ranged from 9 to 12.5 µg/m³ (Vilcassim et al., 2014). While at street level on the same days, the mean BC and PM_{2.5} concentrations were below 3 and 10 µg/m³ (Vilcassim et al., 2014). The EC concentrations on day 2 and day 3 in this present

study appeared to be very low this is because of power loss from the pump during some part of the journey. However day 1 is similar to the concentrations in Vilcassim et al. (2014).

Higher PM_{2.5} was measured in this study as compared to some of the studies mentioned above. Therefore particles in the Tube system can induce inflammatory and oxidative stress.

Table 8.10 also shows the biomarker urinary 8-oxodG measured in the morning after the journey (exposure). Highest urinary 8-oxodG measured during the commute was on Day 3 and lowest on Day 5. There was no 8-oxodG for Day 6 because the sample was contaminated. The studies on health outcome of exposure to train travel pollutants and exposure to underground or subway pollutants are few, thereby making the comparative discussion difficult. In a comparison study between the ability of particles from a subway station and a nearby very busy urban street, respectively, to damage DNA and to induce oxidative stress, it was observed that 8-oxodG was measured in the human lung cell DNA as a biomarker of oxidative stress due to exposure to the particles in the subway (White et al., 2012). Nilsson et al. (2005) suggested that subway particles are more genotoxic than urban street particles. However in another study after exposure of 20 healthy subjects for 2 hours to subway environment, no cellular response was found in the airways of the subjects (Cattaneo et al., 2010). While in this present study, the average urinary 8-oxodG observed in this study was 1.4 ng/mg of creatinine. This is low compared other studies (Hu et al., 2006, Lin et al., 2004b) but above the minimum concentration of 1.2 ng/mg of creatinine found in another studies for both smokers and non-smokers concentrations (Shimoi et al., 2002). There was a pattern of increase noticed within the daily 8-oxodG measurements. However CV between interday 8-oxodG concentrations was very low suggesting no apparent damage. The 8-oxodG measurement started on Tuesday and the 8-oxodG increased as the journey days progressed

until Friday then the trip stopped. The trip resumed again on Monday and 8-oxodG for Monday was measured as the lowest concentration for the train Journey.

Table 8.10. Summary of measurements following six daily commutes from Birmingham to London

	PM2.5 ($\mu\text{g}/\text{m}^3$)	EC ($\mu\text{g}/\text{m}^3$)	Sampling time (mins)	8-oxodG(ng/mg Creatinine)
Day 1	98.54	11.74	515	1.30
Day 2	1119.90	0.14	515	1.41
Day 3	408.33	0.15	540	1.56
Day 4	677.72	-	520	1.52
Day 5	1100.00	-	515	1.21
Day 6	807.28	-	515	-
Mean (SD)	701.96 (398.77)	4.01 (6.69)		1.42 (0.13)
CV%				9%

4 lung function measurements were made on each journey day by the subject. The first measurement was made when the subject was seated in the train at Birmingham New Street station just before the train departed and then the second measurement was made when the subject arrived London Euston train station and it was called Day 1-1. The same procedure was repeated for the return journey and was called Day 1-2. Figure 8.3 shows that for each pair of measurement showed that at the start of the journey, the FEV₁ and FEV₆ were decreased as compared to the start of the journey except for Day 3 and Day 4. This decrease continued as the journey days progressed. However at the start of Day 4 there was an increase and by the end of Day 4 the decrease started again. This increase at the beginning of Day could be associated with the break given to the subject over the weekend and then on Day 5 the journey commenced again. Nyström et al. (2010) in their study of exposure of 20 healthy subjects to metro environment for 2 hours did not notice any influence on the lung function.

For this train commute the FEV_1/FEV_6 ratio observed was in the range of 0.91 to 1 and did not decrease as compared to the cycling commute in Figure 8.1. It may be that the cyclist had higher inhalation dose associated with higher V_E and heart rate, as opposed to the inhalation dose of the subject in the train commute whose V_E was not as rapid as the cyclist. Therefore the cyclist may have inhaled more particles that could have affected lung function. However the V_E and inhaled dose parameters were not measured therefore this is just a speculation. A study with 34 volunteers observed that the V_E of cyclists, was twice the V_E of car and bus passengers, therefore the inhaled load estimate for cyclist were the highest (Fierz et al., 2008).

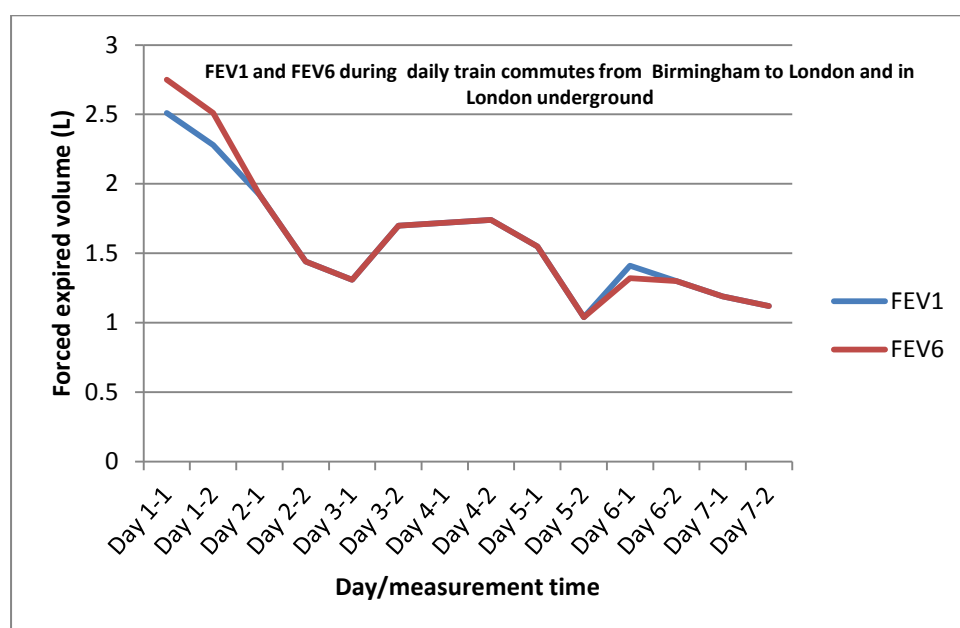


Figure 8.3. FEV1 and FEV6 during the 6 daily train commutes from Birmingham to London and in London underground

Table 8.11 show the relationship between $PM_{2.5}$ and 8-oxodG and the lung function parameters. The daily mean measurement of FEV_1 , FEV_6 , FEV_1/FEV_6 and $FEF_{25-75\%}$ (i.e.

average between the start journey and end of journey) were calculated and then used in the correlation matrix against the 8-oxodG and PM_{2.5} to find the relationship as displayed in Table 8.11. PM_{2.5} inversely associated with 8-oxodG and FEV₁. However the correlation was not significant. FEV₆ was inversely significantly correlated to FEV₁/FEV₆, the inverse correlation is due to the trend of reduction of the FEV₆ i.e. as the ratio of FEV₁/FEV₆ remained the same while FEV₆ decreased. A restrictive ratio of FEV₁/FEV₆ is proportional to a decrease in FEV₆. A slight prolonged time of expiration was observed on some days as shown in Figure 8.3 where FEV₁ and FEV₆ did not superimpose each other or the subject was starting to struggle to expand their lungs during deep breathing.

Table 8.11. Relationship between PM2.5 and biomarkers/outcome of exposure

Correlations

			PM2.5	FEV1	FEV6	FEV1/FEV6	FEF25-75	8-oxodG (ng/mg creatinine)
Spearman's rho	PM2.5	Correlation Coefficient	1.000	-.543	-.143	.169	-.029	-.200
		Sig. (2-tailed)	.	.266	.787	.749	.957	.747
		N	6	6	6	6	6	5
	FEV1	Correlation Coefficient	-.543	1.000	.371	-.676	.600	.100
		Sig. (2-tailed)	.266	.	.468	.140	.208	.873
		N	6	6	6	6	6	5
	FEV6	Correlation Coefficient	-.143	.371	1.000	-.845*	-.257	-.200
		Sig. (2-tailed)	.787	.468	.	.034	.623	.747
		N	6	6	6	6	6	5
	FEV1/FEV6	Correlation Coefficient	.169	-.676	-.845*	1.000	-.135	.335
		Sig. (2-tailed)	.749	.140	.034	.	.798	.581
		N	6	6	6	6	6	5
	FEF25-75	Correlation Coefficient	-.029	.600	-.257	-.135	1.000	.700
		Sig. (2-tailed)	.957	.208	.623	.798	.	.188
		N	6	6	6	6	6	5
	8-oxodG(ng/mg creatinine)	Correlation Coefficient	-.200	.100	-.200	.335	.700	1.000
		Sig. (2-tailed)	.747	.873	.747	.581	.188	.
		N	5	5	5	5	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

8.5 Conclusion

Exposure to particles in commuter mode has been associated with respiratory, inflammation, oxidative stress, pulmonary and cardiovascular health outcomes. Biomarker of exposure 8-oxodG, a biomarker of oxidative damage and lung function to measure the pulmonary function were used to measure the effects of particles exposure in commuter modes.

In the cycling commuter mode, a decrease in all the lung function parameters was observed. Secondly, the significant inverse association between the personal $PM_{2.5}$ and FEV_1 and FEV_6 respectively observed in the cycling mode, supports the hypothesis that negative health effect of exposure to ambient air pollution/ traffic related air pollution on pulmonary health. Also in the cycling mode, 8-oxodG was significantly associated with FEV_1/FEV_6 and $FEF_{25-75\%}$, indicating that as lung function decreases, airway obstruction starts to set-in. This obstruction due to decrease in lung function is due to exposure to air pollution which leads inflammation and oxidative stress in the airways, thereby leading to 8-oxodG a biomarker of oxidative damage. That is, due to the exposure, there is a potential increase in ROS production. The increased ROS production leads to infiltration of inflammatory cells into the airways and then leads to oxidative stress thereby affecting the ASM by mimicking the features of asthma (obstruction) leading to decrease in the FEV_1/FEV_6 ratio.

In the train commuter mode, a restrictive ratio was observed. In the train mode the 8-oxodG increased as the journey days increased and dropped right after a weekend where no train journey was made. It is possible that in the train travel exposure which includes underground as well as over ground journey, the fractions bound to the particles could be more genotoxic than those the cycling commuter was exposed. This agrees with Karlson et al. (2005) that subway particles are more genotoxic and can induce oxidative stress. Based on the trend

(increase) in 8-oxodG creatinine corrected measured during the train travel and the high concentrations of PM_{2.5} measured in the train travel this chapter also agrees with other studies that the PM of subway air is to a large extent, different from the above ambient air or other transport air, in terms of number, mass, size, concentration and chemical composition(Chao and Cheng, 2002).

The PM_{2.5} concentrations measured in the train commute system was observed to be higher than those of the cycling and even the other commuter mode. This agrees with literature, that PM_{2.5} concentrations in the subway systems, is higher than ambient environment and other commuter mode.

It is not clear whether the small cohort-size of the study in this chapter led to the inconsistency, weak agreements, lack of statistical power and or significant negative agreements observed between pollutants and 8-oxodG. Perhaps because of the small size of the study it was difficult to find a significant correlation between the low changes in 8-oxodG and measured pollutants. Therefore the result should not be considered a standard and may not be the same for people with compromised health, the elderly and children. Regardless, the results, agrees with the aim of this chapter that exposure to cycling and train commuter modes affects pulmonary health by altering the lung function of the exposed individuals. Also the association between PM_{2.5} and lung function suggests that traffic related particles are associated with the change in lung function.

9 CHARACTERISATION OF INDOOR PARTICLES AND PERSONAL EXPOSURE WITH BIOMARKERS OF EXPOSURE

9.1 Introduction

People are more aware that exposure to outdoor air pollution can impact human health negatively. However, most of them are not aware that exposure to indoor air pollution can also be detrimental to human health. According to the Environmental Protection Agency, indoor air can be 2 to 5 times, occasionally 100 times more polluted than outdoor air. Amongst these pollutants are UFPs and PM of various sizes. These UFP and PM constitutes of fractions that elicit various types and degrees of negative effects on the health (Cassee et al., 2013). Those fractions include different chemicals such as the metals (Cassee et al., 2013). Not very many researchers have conducted elemental compositions of indoor PM in homes. This chapter will focus on metallic compositions of the indoor home particles. The study in this chapter was the original design for the entire thesis. However, because of the problems and delays described in chapter 2, the cohort-size was reduced and the office particle size distribution measurement was removed (only samples for TEM analysis were collected in the offices).

9.2 Aims and objectives

Several campaigns have been done on the ambient air and there is an increase in the study of indoor air. However there is still a gap in characterisation of the indoor particles in terms of concentrations, mass-size and elemental distribution, bulk and surface chemistry of the particles, physical properties of the particles and health effects associated with the exposure to UFP and fine particles in the indoors especially those in the UFP range. It is general knowledge that people spend about 85 % of their time indoors, therefore the indoor air should be considered an important factor when assessing health effects due to exposure to UFPs and PM. The indoor and outdoor particulates differ in terms of toxicity and even mutagenicity because the outdoor differ in their physical and chemical properties particle in the various seasons (Happo et al., 2014, Monn and Becker, 1999). The aims of this study are

- 1) To measure and characterise indoor particles
- 2) To measure biomarkers of PE to these indoor particles and analyse health risk assessments.
- 3) To investigate if urinary metals can be used as biomarkers of exposure to indoor particles

In order to achieve the aims listed below are the main objectives:

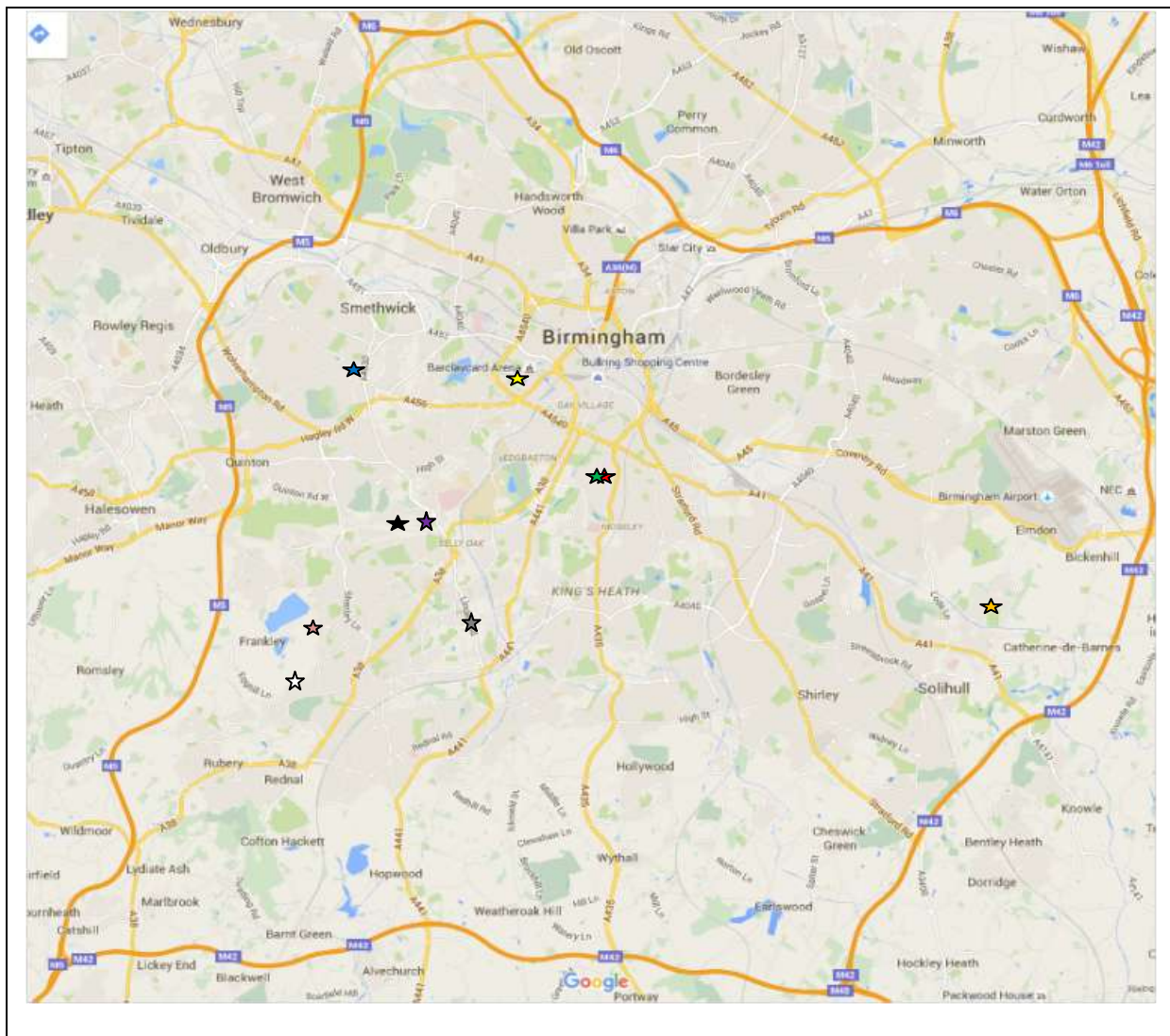
- To measure concentrations in different seasons
- To determine mass-size distribution of the PM
- Compare mass concentration between subjects based on location and season
- To measure elemental mass-size distribution
- Compare the elements among subjects based on location and season
- To characterise the indoor particles: size, morphology, chemistry
- Measure biomarkers: Nitrite, nitrate, pH and 8-oxoG in the subjects

- Compare PM concentration and elemental concentration with biomarkers
- To identify health risk associated with these particles
- To investigate the metal levels in urine and compare with other biomarkers of exposure and to compare with pollutants

9.3 Methodology

9.3.1 Home description

10 subjects and their homes were selected for sampling in the warm and cold season based on the criteria discussed in chapter 2 for this study. The intention was to compare between an equal number of urban and suburban homes. However after the selection criteria was applied to interested volunteers, only one urban and nine suburban homes were selected. Locations are as seen in Figure 9.1. Details of sampling are found in chapter 2. In this study the subject's homes may also be referred to as sites.



Only Home 1 is in the urban area all other homes are in the suburban

Figure 9.1. Map of Birmingham highlighting sampling sites in the urban and suburban areas. Adopted from Google Map

Table 9.1. Legend for the map

									
Home1	Home2	Home3	Home4	Home5	Home6	Home7	Home8	Home9	Home10

9.3.2 Sampling equipment and sampling method

9.3.2.1 SIOUTAS personal cascade impactor

This instrument has been described in chapter 2.

9.3.2.2 Experimental setup

The SIOUTAS impactor was placed in the living room of each subject for a maximum of 7 days and it sampled non-stop. Each subject was given a complete set of activity diary sheet to record their where about and activities, foods eaten and exposure to environmental tobacco smoke. Details of activity diary already discussed in chapter 2.

9.3.2.3 TEM Sample collection

UFPs were collected for analysis using the TEM and X-ray energy dispersive spectroscopy (EDS). The normal process of collecting TEM samples using nanoMOUDI i.e. by mounting the TEM grid on aluminium foil and placing in the nanoMOUDI was not used in this case. It was easier to mount the TEM grid (holey Carbon Films on nickel, Agar Scientific) on the PTFE filter by using tiny streak of adhesive at two opposite ends. Then placing them on the impactor stages and setting up the SIOUTAS impactor as normal. TEM samples were collected for the five stages representing the five size fractions. However, only the $< 0.25 \mu\text{m}$ size fractions were analysed for this study. This is because this study is more interested in the

particles in the UFP size range and these are in the respirable size. 5 sets of TEM samples representing homes and workplace were collected. The first TEM sample was collected for 4 hours at a subject's home during various indoor activities including cooking for 1 hour. The second TEM sample was collected for 1 hour in a home during various activities. The time was reduced from 4 hours to 1 hour because the TEM grid was very loaded (i.e. particles collecting on top of each other) when the particles were collected at 4 hours. Another 2 set of TEM samples were collected for 2 hours from two offices and the final TEM sample was collected for 2 hour from another subject's house during no particular indoor activity. It was found that 2 hours was ideal because the TEM grid was not too loaded or under loaded.

9.3.2.4 Biomarkers

Urine and EBC were collected from each subject on day 1 at the start of the sampling. The second set of urine and EBC was collected midway sampling and the last collected on the last day of the sampling as instrument is being stopped. For each subject, all urine and EBC samples were collected between 7:30 – 8:00 am in the morning. The urine was analysed for 8-oxodG and urinary metals and the EBC for pH, nitrate and nitrite. The methods of collection of the urine and the EBC samples have been described in chapter 2.

9.3.3 Analytical analysis

After collection of samples, mass concentrations were gravimetrically determined for all size ranges and all samples were analysed for (metals) elements: Ni, V, Cr, Mn, Sb, Pb, Sr, Cu, Zn, Mg, Sn, Na, Ca and Fe. Gravimetric analysis which include pre and post weighting have been discussed in details in chapter 2.

9.3.3.1 Acid digestion

The bulk chemistry analysis of the PTFE filters using the ICPMS (Inductively Couple Plasma Mass Spectrometry) and GFAA (Graphite Furnace Atomic Absorption spectroscopy) were

first carried out by acid digestion of the filters in reverse aqua regia. The reverse aqua regia is based on the technique described by Harper et al. (1983) and it has been used for previous studies by researches in the department (Environmental Health, UoB) (Reche et al., 2015). Aqua regia solution was prepared using pure grade (1:3) HNO_3 and HCl . 189 ml HCl and 66 ml HNO_3 were pipetted into a conical flask containing 18.2 M Ω deionised water (DDW), with further DDW then added until the total volume was made up to 1 litre.

The filters were put inside a 4 ml Nalgene polypropylene vials, 4 ml reverse aqua regia was added to the vials, the vials were screwed tightly, heated at 100 °C in a water bath for 30 mins and then sonicated at 50°C for 30 mins. The heating at 100 °C for 30 mins and sonicating at 50°C for 30 mins steps were repeated making a total of 2 hours of extraction. The resulting discoloured aqueous solutions were then transferred into 30 ml Nalgene polypropylene vials. The 4 ml extraction vials were rinsed with DDW three times to ensure the transfer of all of the aqueous solution. The total aqueous solution volume was then made up to 20 ml using DDW. Samples were stored in the refrigerator at 4 °C until they were analyzed.

All vials, pipette tips and glassware used for preparation of aqua regia and acid extraction were first washed using DDW and then soaked in a 2 % HNO_3 solution for a minimum of 24 hours, thoroughly rinsed using DDW, and placed in polypropylene containers and then placed in the oven to dry in order to eliminate contamination.

Blank filters were also subjected to the extraction method described above in order to determine limits of detection for the analysis.

9.3.3.2 Bulk chemistry analysis

The analysis of the acid extraction for the metals was carried out using the GFFA and other samples analysed using ICP-MS by Dr. Stephen Baker.

9.3.3.2.1 Inductively Couple Plasma Mass Spectrometry

An Agilent 7500ce ICP-MS with an Octopole Reaction System (ORS) operated in the helium collision mode was used for the analysis of V, Cr, Mn, Fe, Ni, Cu and Zn (to eliminate interference from polyatomic species) and in standard mode for Na, Mg, Ca, Sn, Sb and Pb. The ORS effectively removes all overlap due to molecular ions or polyatomic interferences and improve the overall sensitivity to allow analysis of ultra-trace or low level elements. The isotopes measured were ^{23}Na , ^{24}Mg , ^{43}Ca , ^{51}V , ^{52}Cr , ^{55}Mn , ^{56}Fe , ^{60}Ni , ^{63}Cu , ^{66}Zn , ^{118}Sn , ^{121}Sb and ^{208}Pb , using ^{45}Sc , ^{72}Ge , ^{115}In , ^{159}Tb and ^{209}Bi as internal standards.

The plasma operates with an RF power of 1500W. Samples were introduced using the Miramist nebuliser. The Calibration standards were prepared from VWR 1000 or 10000 mg/l stock solutions.

9.3.3.2.2 Graphite Furnace Atomic Absorption spectroscopy (GFAA)

The GFAA model: Perkin Elmer Aanalyst 600 with AS-800 autosampler was used in this study for the analysis of Cu, Sr and Cr. Very briefly the sample is injected into the graphite tube in the furnace. The sample is heated in a step by step manner in order to remove solvent i.e. drying the sample by evaporation and then atomise what is left of the sample. Light from the lamp is now passed through the tube which contains the atomised analyte. Then the light passes through a monochromator to isolate the radiation or specific wavelength to be measured and the detector then measures the amount of light that transmitted through the monochromator.

Only these elements were analysed using GFAA because of the availability of the lamp. The Sr was not analysed at all using the ICP-MS. Regression analysis between the measurements

made by the two methods i.e. ICPMS and GFFA showed very strong correlations for Cr and good correlation with Cu. For Cr R^2 value is 0.98 and the R^2 value for Cu is 0.84.

9.3.4 Characterisation of particles

The particles collected on the copper grids were analysed for Physico-chemical properties.

Shape and size characterisation using the Transmission electron microscope

Imaging of the samples was performed using the Tecnai F20 TEM, working on high tension up to 200kV using an extraction voltage of 4450Ev by Dr Paul Sanderson. For this study the analysis was done using the instrument in the STEM mode in connection with high angle annular dark field (HAADF) detector to allow for elemental identification and interpretation using the EDS.

9.3.4.1 Surface chemistry using the X-ray energy dispersive spectroscopy

The chemical analysis i.e. identification and interpretation of elements was performed using the EDS. The results generated by the EDS were managed by the INCA and AZTEC software. These software systems were designed by Oxford Instruments.

9.3.4.2 ImageJ

The images captured by the TEM were analysed using the ImageJ software. This software was used in determining the particle size and distribution.

9.3.5 Statistical analysis

9.3.5.1 Mass and elemental concentration and distribution analysis

The mass-size statistics were determined by calculating the mass-size distribution. Other statistical analysis like the central tendencies and quartiles were also applied to enable quantification of data.

Temporal variability of each site in the two different sampling seasons was determined by plotting the average concentrations of the different size cuts during the sampling season of each site and Spearman correlation coefficient (r_s) was applied in order to determine relationship between the same sample sites over different seasons.

9.3.5.2 Biomarkers and health risk assessment analysis

The relationship between the biomarkers (8-oxodG, EBC-pH, EBC NO_2^- and NO_3^-) and the particle size fractions were measured with the Spearman correlation. The relationship between the elements in the different size fractions and the biomarkers were analysed using the Spearman correlation. The difference of mean between the biomarkers in the summer and the winter measurements were determined using the Mann-Whitney U test. The relationship between the biomarkers was determined with the Spearman correlation. The Spearman and the Mann-Whitney U test were carried out using the IBM SPSS statistical 22 software. For the health risk assessment risk, the Excess cancer risk (ECRs) for the carcinogenic metals were calculated using equation 6.1 (Pope III and Dockery, 2006, Sørensen et al., 2003a):

$$\text{Excess cancer risk (inhalation)} = \text{concentration of pollutant } (\mu\text{g}/\text{m}^3) \times \text{unit risk } (\mu\text{g}/\text{m}^3)^{-1} \quad (6.1)$$

The information on the carcinogenic types and unit risks of the metals was obtained from the US EPA database for IRIS (Integrated Risk Information System) (available at <http://www.epa.gov/iris/>).

9.4 Results and Discussion

9.4.1 Particulate matter concentration

9.4.1.1 Particle mass concentration measurements

Size fractionated filter samples were collected from 10 homes in the summer and 9 homes in the winter. The winter was less one home because subject 9 moved out of Birmingham therefore that particular house could not be sampled. The home that was absent in the winter (subject 9) was then completely removed from the result analysis because it was not available in the winter and also in the summer it was sampled for only two and half days because the SIOUTAS developed a fault and subject was no longer available. Therefore instead of 10 homes in the summer and 10 homes in the winter, only 9 homes in the summer and 9 homes in the winter were analysed.

Generally with cascade impactors longer sampling times are required in order to collect enough mass on the filter for chemical analysis and in addition, indoor mass concentrations are usually lower than the outdoors therefore long sampling time is required. Also collection of lower mass particles like those in the ultrafine range requires a greater collection concentration, hence longer time. With the longer sampling time, volatiles can be lost but in this study the metals were sampled therefore volatiles were not a problem. However, volatility of particles is reduced in cascade impactors by decreasing the pressure drop over a reduced number of stages (Fonseca et al., 2016, Marple et al., 1991). One of the advantages of the use of the gravimetric method is that it allows for characterisation of particles in terms of size and shape as carried out in this chapter. However, the time integrated samplers like the real-time samplers can show temporal patterns. Therefore sampling for 7 days is ideal for this study for the mass-size distribution and elemental mass size distribution composition.

However, the sampling time can also depend on the study aim. In the case of size, shape and surface chemistry shorter time is needed so that the TEM grid is not overloaded.

The impactor has five stages which represent 5 size fractions. For this study the size fractions have been grouped as so: Outlet < 250 nm: Ultrafine particles, 250 - 500 nm: fine particle, 500 – 1000 nm: fine particle, 1000 – 2500 nm: fine particle and 2500nm – 5000 nm: Coarse particles). The entire fine particle size fractions are termed the accumulation mode particles and they range from 250 – 2500 nm. The lower tail of the 2500 nm size fraction is also part of the coarse mode but for the sake of categorising, this will be classified as the accumulation mode and Viana et al. (2005) also classified the 250 – 2500 nm range as the accumulation mode. Up to 250 nm in this study is classed as UFP because the impactor stage measuring the particles < 250 nm cannot separate particles larger particles from those < 100 nm and recent studies have included this size as part of the UFP (Salako et al., 2012) and quasi-ultrafine particles (Viana et al., 2014).

The average mass concentrations and mass-size distribution for each size fraction were used as one of the factors to characterise the indoor particles. One of the significance of PM is that they are carriers of many trace metals (Highwood and Kinnersley, 2006). Therefore the dividing of these metals into various size-range fractions, then measuring their concentration and assessing the link between both variables (size and concentration) is of great importance for the assessment of health effects. Table 9.2 and Table 9.3 show the mass concentration of each stage for each home and the mean mass concentration for each stage for all the homes for the summer and winter season respectively. Table 9.2 shows that in the summer Home 1 show the highest concentration for all the size fractions measured, especially in the < 0.25 μm and 0.25 – 0.5 μm size fractions. From the activity diary (not included in the thesis), Home 1 recorded that there was an incident where during grilling of steak for dinner the steak got

really burnt that it could not be eaten. Therefore may have influenced the concentration of PM in the different size fractions of Home 1 causing Home 1 to have highest concentrations for all the size fractions. This agrees with a study that showed that cooking; including oven cooking contributed primarily to PM with diameters between $< 0.25 \mu\text{m}$ and $0.25 - 0.5 \mu\text{m}$ (Riva et al., 2011). Another study suggested that grilling and roasting meat generate particles mean size of $0.024 \mu\text{m}$ and $0.038 \mu\text{m}$ respectively (Dennekamp et al., 2001a). Therefore based on this study particles generated from the charred steak will be around these sizes and therefore add to the mass of the $< 0.25 \mu\text{m}$ and $0.25 - 0.5 \mu\text{m}$. Home 6 show the lowest concentration for the $< 250 \text{ nm}$, $1000 - 2500 \text{ nm}$ and $2500 - 5000 \text{ nm}$ size fractions. Home 5 show the lowest concentration for $250 - 500 \text{ nm}$ size fraction and Home 3 show the lowest concentration $500 - 100 \text{ nm}$. In Table 9.3, Home 3 show the highest concentration for the all the size fractions except for $500 - 1000 \text{ nm}$ size fraction where Home 7 show the highest concentration. For the lowest concentration, Home 2 show the lowest concentration in the $< 250 \text{ nm}$, $500 - 100 \text{ nm}$ and $2500 - 5000 \text{ nm}$ Size fractions. Again like in the summer Home 6 show the lowest concentration $1000 - 2500 \text{ nm}$ and Home 10 in the $250 - 500 \text{ nm}$ size fraction.

Table 9.2 show that during the summer, the UFP size fraction $< 250 \text{ nm}$ show the highest concentration for each home except in Home 2 where the coarse fraction $2500 - 5000 \text{ nm}$ show the highest concentration. While in Table 9.3 the winter or cold season only in Home 10 by a very small margin was the coarse fraction $2500 - 5000 \text{ nm}$ with the highest concentration. Therefore in the exception of Home 2 in the summer and Home 10 in the winter the particle size fraction is driven by the indoor activity.

The mass-size concentrations indicated that the summer measured higher concentrations than in the winter (Table 9.2 and Table 9.3). Contrary to the findings in this chapter, a study where

PM₁₀ PM₅ PM_{2.5} and PM₁ were characterised in 5 homes, it was observed that the concentration of all the measured sizes was found to be highest in winter season and they attributed it to increase human activities and more space heating in indoors and due to low wind speed and high humidity in outdoors in comparison with other seasons (Sioutas et al., 2005). Their study is based on measurements in India while this Birmingham, UK. Therefore differences between their study and this present study could arise from different kind of indoor heating used in India. They also had increased bonfires to keep warm.

Remember that the major contributor of indoor pollutants is the indoor activities. In this present study all the homes used were non-smoking homes and in the Indian study, some of the subjects smoked. The possibility of increased smoking in the indoors during the winter period to keep warm could also account for the higher winter concentrations they measured. Another study found that the mean indoor PM_{2.5} concentrations in Tokyo in the winter and the summer were 69.9 and 34.6 $\mu\text{g m}^{-3}$, again making the winter concentration higher (Oberdörster, 2000). Again cigarette could be a contributing factor to their study as it was listed in their indoor source profile. It could be that the summer measurements were higher in this present study because, the corresponding outdoor concentrations found their way into the homes through open windows (higher ventilation) in the summer but it is difficult to assign the higher summer concentrations to the ventilation theory because the outdoor concentrations and the air exchange rate of the homes were not measured during the sampling. However, in this present study the difference measured between the summer and winter concentrations were not significant. This could be as a result of the sample size.

Table 9.2. Descriptive statistics for mass concentration measurements of each size fraction in summer

SUMMER					
Home	< 250 nm	250 - 500 nm	500 – 1000 nm	1000 - 2500 nm	2500 - 5000 nm
1	22.33	14.63	4.55	6.55	7.84
2	2.90	2.25	1.81	2.52	4.43
3	6.83	1.98	0.52	1.94	3.53
4	5.66	2.44	0.75	1.93	4.03
5	3.50	0.44	0.55	1.34	2.39
6	1.60	0.78	0.59	0.55	1.27
7	11.75	4.69	1.77	2.56	5.99
8	12.77	5.54	1.76	2.45	3.60
9	11.89	3.75	2.40	1.66	2.97
10	5.49	1.49	0.63	1.55	3.04
Mean	8.47	3.80	1.54	2.31	3.91
STDEV	6.28	4.14	1.26	1.61	1.86
Min	1.60	0.44	0.52	0.55	1.27
25%	4.00	1.61	0.60	1.58	2.99
Median	6.25	2.34	1.26	1.94	3.57
75%	11.86	4.46	1.80	2.50	4.33
Max	22.33	14.63	4.55	6.55	7.84

Table 9.3. Descriptive statistics for mass concentration measurements of each size fraction in winter

Winter					
Home	< 250 nm	250 - 500 nm	500 – 1000 nm	1000 - 2500 nm	2500 - 5000 nm
1	4.83	1.99	1.10	1.53	1.53
2	3.15	1.64	0.35	0.99	0.87
3	14.03	3.37	1.12	2.70	4.86
4	7.34	2.24	0.94	0.98	1.56
5	5.07	2.64	0.98	0.92	1.25
6	4.90	3.22	0.66	0.56	0.98
7	10.29	2.92	1.48	2.54	3.47
8	10.07	1.36	0.89	1.60	2.74
10	3.42	0.83	0.53	1.71	3.50
Mean	7.01	2.25	0.89	1.50	2.31
STDEV	3.71	0.87	0.34	0.74	1.40
Min	3.15	0.83	0.35	0.56	0.87
25%	4.83	1.64	0.66	0.98	1.25
Median	5.07	2.24	0.94	1.53	1.56
75%	10.07	2.92	1.10	1.71	3.47
Max	14.03	3.37	1.48	2.70	4.86

9.4.1.2 Mass-size distribution

The size ranges in the indoor environment are influenced by the activities or sources. The indoor sources such as combustion devices generate predominantly fine particles, cooking techniques generate fine and coarse particles and resuspension: dusting predominantly produce coarse particles (Bachmann et al., 1996). The size distributions of all the homes in Figure 9.2 for both summer and winter show a bimodal pattern except home 5 in the summer that has a unimodal distribution pattern. The distribution in the homes show a typical indoor particle distribution as suggested by Franck et al. (2003) in their study (Franck et al., 2003).

All the homes show that the mass-size distribution were all skewed to the left that is long tail at smaller sizes as opposed to the regular skewed to the right for most aerosol mass-size distribution (Hinds, 2012), but a study suggested that particles indoors are dominated by the particles in the UFP size fraction (LaRosa et al., 2002) this may be because UFP size fraction are driven by indoor activities as their primary source in addition to the outdoor sources of that size that infiltrated into the indoors. However in terms of mass the UFP size fraction may have a lower concentration in comparison to the other size fractions because their mass is smaller as compared to the larger particles. The distribution could also be skewed to the left because of the effect of the way that the distribution has been calculated for example all particles ≤ 250 nm are found in the skewed area of the distribution, there was no lower size cut.

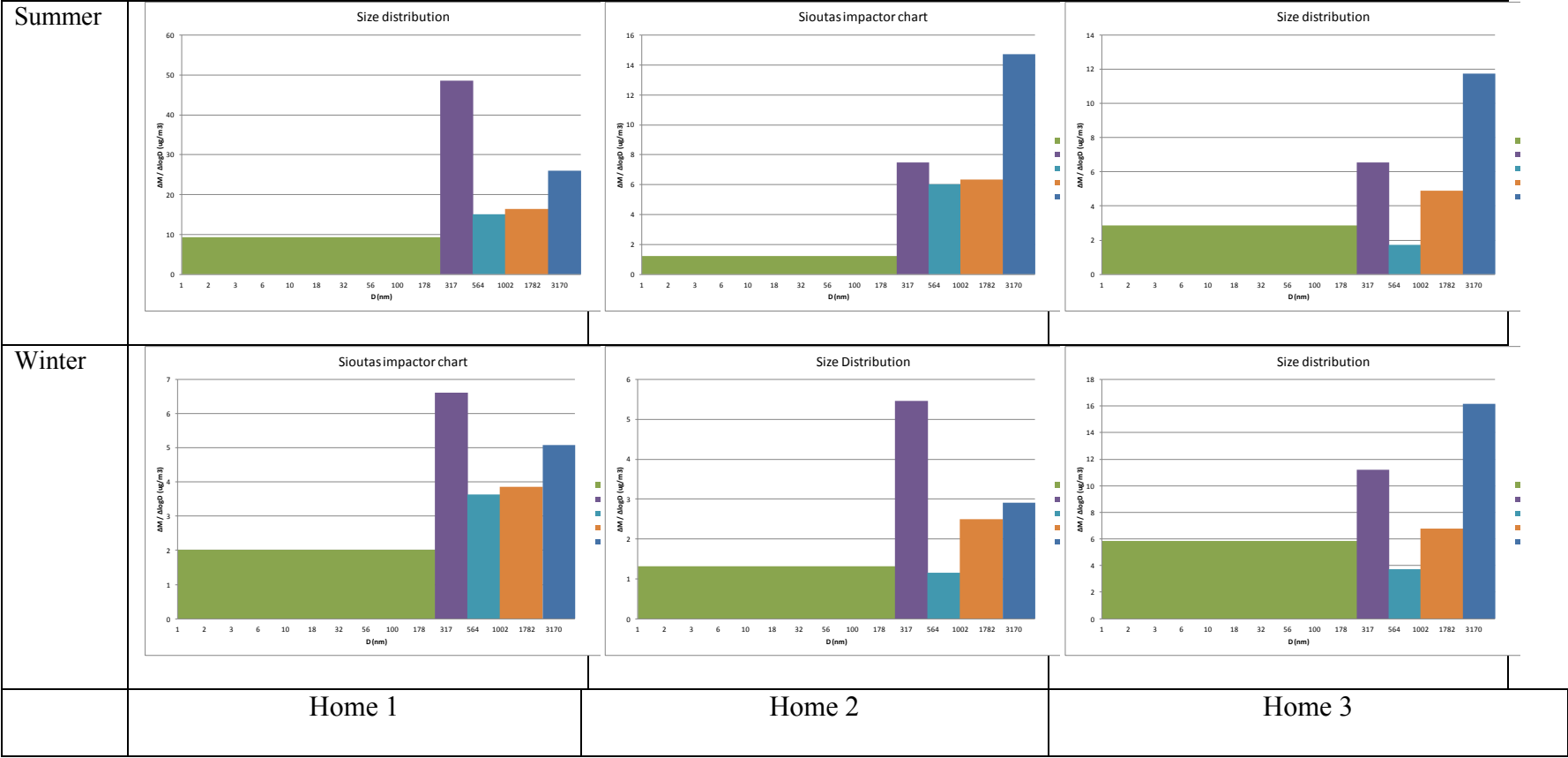
Figure 9.2 show that for both the summer and winter, that the UFP size fraction (< 250 nm) show a very large dominant mode (in regards to the size range) for all the homes. This could reflect on the artefacts of the way that the distribution was calculated.

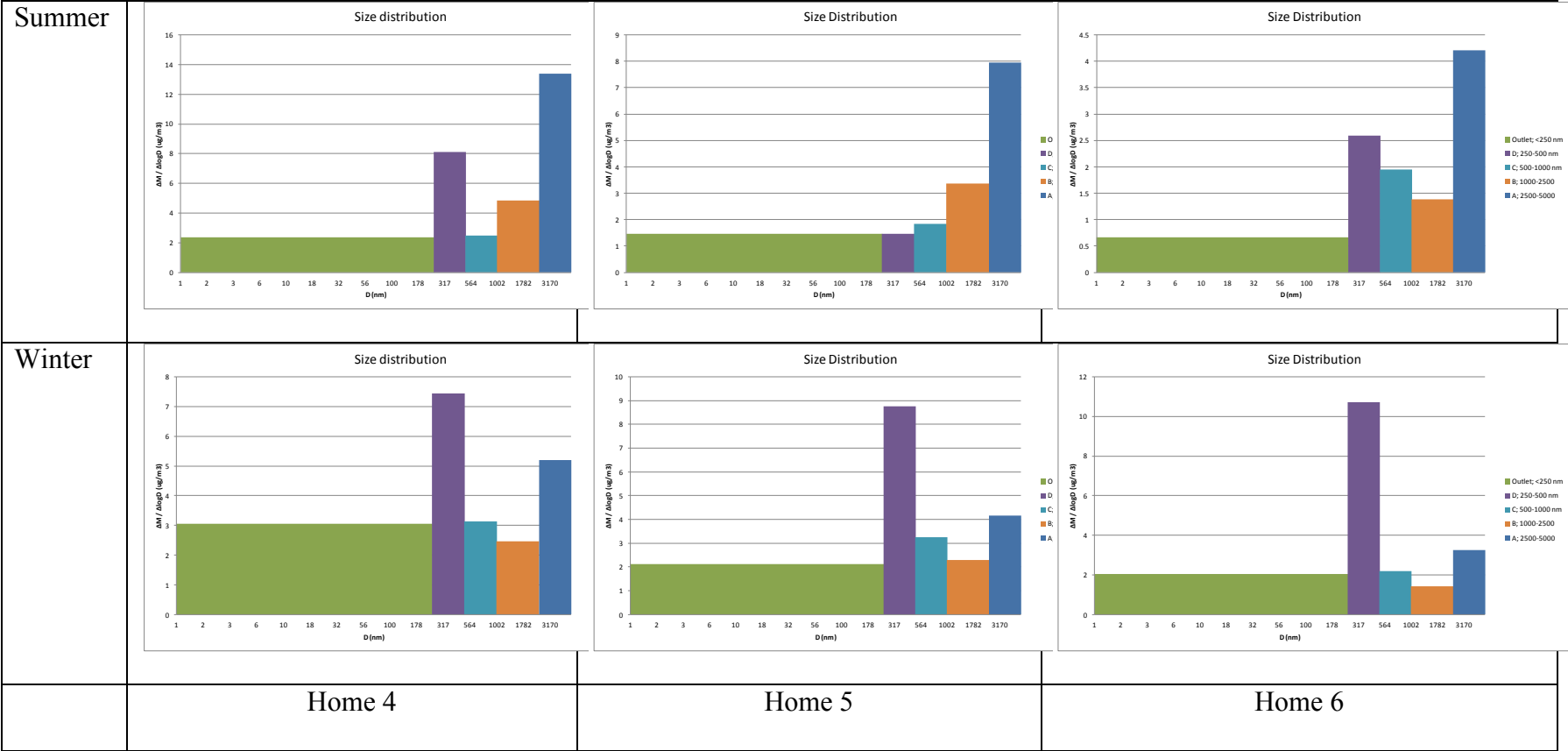
In Figure 9.2, the bimodal distribution observed showed two peaks (modes) one peak in the accumulation mode (250 – 500 nm) and the second peak in the coarse fraction (2500 – 5000

nm). The reason for this could be that the coarse size fractions which are characterised by a high deposition velocity as compared to the other sizes have short residence times. Therefore they have higher sedimentation rate. The particles in the accumulation mode remain in the indoor environment longer than the coarse and even the UFP (nucleation mode). The UFP (< 250 nm: the nucleation mode) are subject to Brownian motion thereby, higher rates of particle collision and coagulation which then increases the size of the individual particles and removes them from the < 250 nm nucleation mode.

From this study using a 5 stage impactor it can be concluded that the indoor mass-size distribution has a bimodal characteristics (in the 250 – 500 nm for the fine accumulation mode and > 2500 nm for the coarse mode). While another study on characterization of indoor particle sources using continuous mass and size Monitors observed that indoor sources predominantly emit particles in the UFP and coarse-mode regions (0.1 – 0.5 for UFP and > 2.5 for coarse) in their study (Brown et al., 2001). Their observation is quite similar to the observation in this present study.

Figure 9.2 below is spread across three pages because of size.





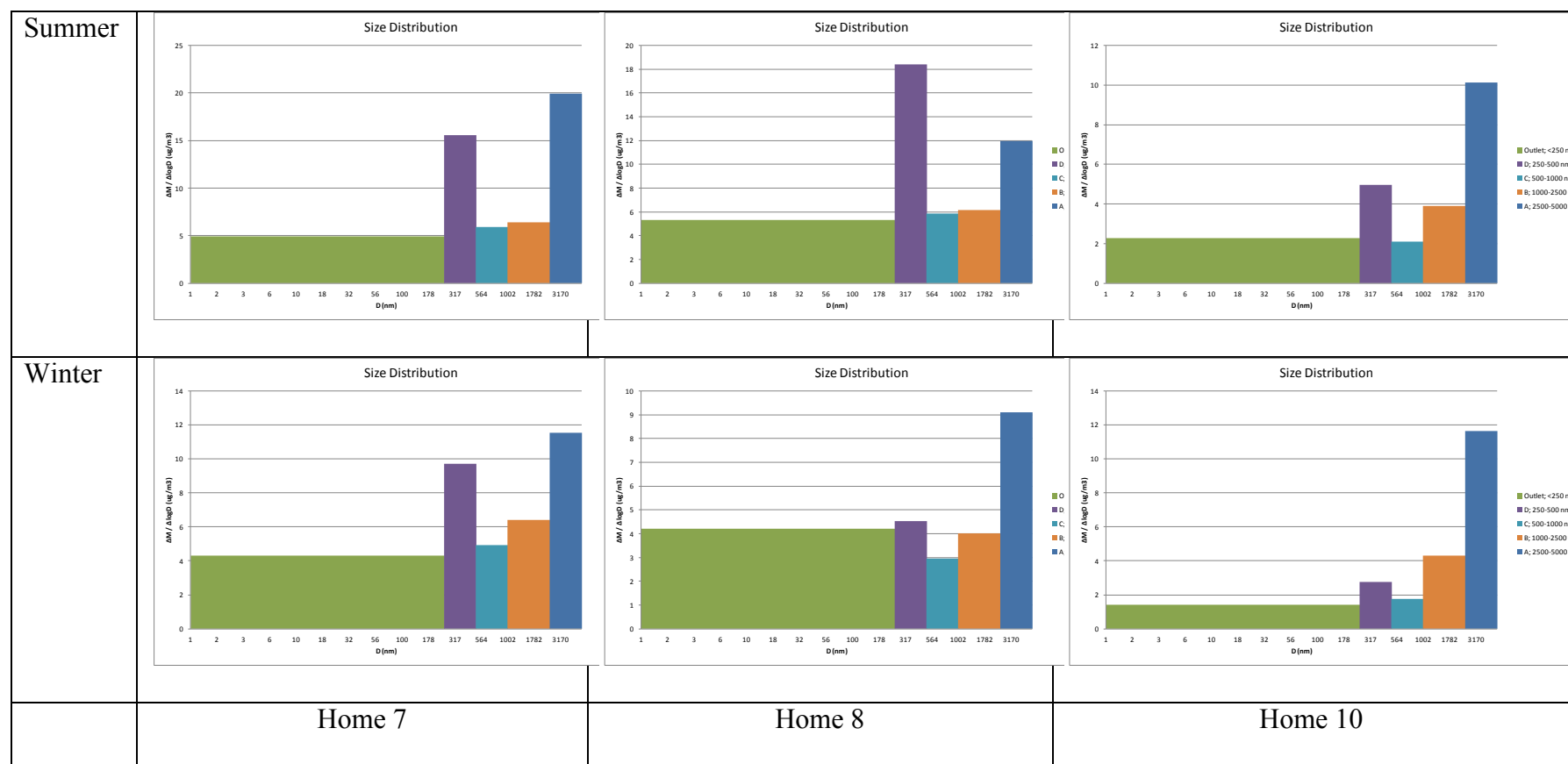


Figure 9.2. Particle size distribution of the different homes

9.4.2 Elements

9.4.2.1 Elemental mass concentration

The average concentrations of 14 elements in five different particle fraction collected from nine homes are shown in Table 9.4 for the summer and Table 9.5 for the winter. Table 9.4 show that $\text{Na} > \text{Ca} > \text{Fe} > \text{Mg}$ are the most abundant inorganic elements and crustal elements found in all the size fractions. Table 9.5 for the winter show that $\text{Na} > \text{Ca} > \text{Fe} > \text{Sn} > \text{Mg}$ are the most abundant inorganic elements found in all the size fractions. This outcome was expected for Na, Ca, Fe and Mg because in addition to anthropogenic sources they are crustal materials and make up a large percentage of the total suspended particles (Taner et al., 2013). The Na, Ca and Fe mean concentrations observed in this study are lower than expected. However a study on Hospital indoor $\text{PM}_{10}/\text{PM}_{2.5}$ and associated trace elements in Guangzhou, China showed measurements for mean Fe concentrations in the same range as the measurements in this present study (Svendsen et al., 2003) but their measurement was for $\text{PM}_{2.5}$. Another study on the elemental indoor concentration in Hong Kong showed that the range of Na concentration observed in their indoor study was 18.4 - 427.7, Ca 9.7 – 327.8 and Fe 44.9 – 387.7 all in ng/m^3 (Knibbs et al., 2011). Their higher concentration could be in addition to the crustal nature of these elements, their indoor activities could vary from the indoor activities in this study. It also could be due to difference in sampling time. This study sampling time was 7 days and theirs was for 4 days. The trace elements Ni, V, Cr, Pb, Cu, Zn and Mn for both seasons were found to be at a lower concentration when compared the crustal elements, bearing in mind that these trace metals are more hazardous to human health. These trace metals are said to have the ability to generate reactive oxidative specie and in the case where there is over production of ROS, oxidative stress is induced and some of them are said to be carcinogenic even in trace quantities (Polidori et al., 2009). The fine fraction

represents the $0.25 - 2.5 \mu\text{g}/\text{m}^3$ and the coarse fraction represents the $> 2.5 \mu\text{g}/\text{m}^3$. In this study as shown in Table 9.4 for the summer that the fine to coarse ratio were higher than 1 for all the elements except for Ca this suggest that the elements are predominantly in the fine fraction. The winter season as shown in Table 9.5 indicate that the fine to coarse ratio were higher than 1 for all the elements, again suggesting that the elements were predominantly in the fine fraction. Table 9.5, in the winter show that the average concentration for Sn in the fine was 11 times higher than the coarse fraction and that the < 0.25 (UFP) fraction for Sn was 17 times higher than the > 2.5 fraction (coarse).

In Table 9.4 in the UFP i.e. < 0.25 fraction, the abundance of the metals are in this order $\text{Na} > \text{Fe} > \text{Ca} > \text{Zn} > \text{Mg} > \text{Cu} > \text{Pb} > \text{Cr} > \text{Sn} > \text{Sr} > \text{Mn} > \text{Sb} > \text{Ni} > \text{V}$. For the winter season in Table 9.5, in the UFP fraction the abundance of the metals is in this order $\text{Na} > \text{Sn} > \text{Fe} > \text{Ca} > \text{Zn} > \text{Mg} > \text{Pb} > \text{Cu} > \text{Cr} > \text{Mn} > \text{Sb} > \text{Sr} > \text{Ni} > \text{V}$.

There is no guideline limit values for indoor air quality at homes, therefore the element concentrations measured in this study were compared to international and national guideline limit values and recommendations. The USEPA classified Pb as one of the criteria pollutants and has set its national ambient air quality standard of no exceedance of $0.15 \mu\text{g}/\text{m}^3$ in a 3 months rolling average. The highest concentration of lead found in this study is $4.01 \text{ ng}/\text{m}^3$ in the winter, in the $< 0.25 \mu\text{m}$ size fraction as show in Table 9.5 this indicates that the concentration found in this study is lower than the USEPA guideline level but this measurement is just for one week. WHO Air Quality Guideline for Pb is $500 \text{ ng}/\text{m}^3$. In the second edition of the WHO Air Quality Guidelines (2000), it was suggested that based on the available data from occupational studies, the lowest-observed-adverse-effect level of V can be assumed to be $20 \mu\text{g}/\text{m}^3$ based on chronic upper respiratory tract signs. Therefore, $20 \mu\text{g}/\text{m}^3$ was set as the guideline however the belief is that below $1 \mu\text{g}/\text{m}^3$ in an averaging time

of 24 hours environmental exposure to V may not likely cause any adverse effect on the health of humans.

Table 9.4 and Table 9.5 show that the metals Mg, Na, Ca and Fe are prevalent in the coarse (> 2.5 µm) size fraction more than the accumulation and UFP mode. This agrees with a study where the elemental composition of PM bound particles, was measured in Hong kong (Knibbs et al., 2011). However, Na deviated in the winter Table 9.5 showing prevalence in the accumulation (0.5 – 1 µm) mode.

Table 9.4. Average elemental concentration (ng/m³) for the summer in the different particle size fraction

SUMMER							
Elements	<0.25	0.25 - 0.5	0.5 - 1	1 - 2.5	> 2.5	Fine/Coarse	<0.25/>2.5
Ni	0.28	-	-	-	-	-	-
V	0.22	-	-	-	-	-	-
Cr	1.42	0.37	0.44	0.91	0.54	3.19	2.63
Mn	0.50	0.45	0.22	0.32	0.57	1.72	0.88
Sb	0.37	0.16	-		0.11	1.45	3.25
Pb	1.91	0.72	-	0.22	0.35	2.68	5.50
Sr	0.79	0.99	0.83	0.90	0.95	2.85	0.83
Cu	2.57	3.02	2.96	3.30	2.85	3.25	0.90
Zn	3.83	3.08	1.33	1.79	2.66	2.34	1.44
Mg	3.67	5.25	6.04	12.48	17.29	1.37	0.21
Sn	0.97	1.29	1.47	1.65	3.14	1.41	0.31
Na	8.69	16.70	20.96	59.64	64.12	1.52	0.14
Ca	7.19	12.38	13.36	34.62	78.70	0.77	0.09
Fe	8.04	6.73	6.84	18.80	30.89	1.05	0.26

-Below LOD

Table 9.5. Average elemental concentration (ng/m3) for the winter in the different particle size fraction

WINTER							
Elements	<0.25	0.25 - 0.5	0.5 - 1	1 - 2.5	> 2.5	Fine/Coarse	<0.25/>2.5
Ni	0.36	-	0.20	0.40	0.57	1.05	0.63
V	0.28	-	-	-	-	-	-
Cr	1.54	0.23	0.28	0.26	0.36	2.15	4.29
Mn	1.43	0.65	-	0.55	1.05	1.14	1.36
Sb	1.03	0.41	-	0.10	0.10	4.10	9.94
Pb	4.01	0.91	0.24	0.29	0.31	4.72	13.11
Sr	0.65	0.54	0.72	0.67	0.70	2.73	0.93
Cu	3.11	3.44	2.23	3.10	3.20	2.74	0.97
Zn	5.73	3.53	1.63	1.80	2.00	3.49	2.87
Mg	5.21	8.37	7.61	14.22	14.87	2.03	0.35
Sn	39.44	14.17	5.19	5.92	2.28	11.07	17.26
Na	41.91	47.74	155.82	115.44	64.11	4.98	0.65
Ca	16.31	18.64	23.79	38.28	69.69	1.16	0.23
Fe	8.01	4.34	4.77	10.56	16.42	1.20	0.49

-Below LOD

Table 9.6 show the difference of mean between mean concentrations of each individual element in the summer and winter. There was a significant difference between Mn, Sr and Sn in their summer as compared to their winter respectively. While the rest of the elements, did not show any significant difference between their summer and their winter measurements.

Table 9.6. Difference of mean between average elemental summer and winter measurements

Cr	Mn	Sb	Pb	Sr	Cu	Zn	Mg	Sn	Na	Ca	Fe
0.117	0.02	0.600	0.602	0.009	0.347	0.754	0.602	0.01	0.117	0.347	0.374

Two-tailed probability test
Significant at <0.05

9.4.2.2 Relationship between metals

Based on Spearman's correlation analysis the metals listed below show the elemental species that were significantly correlated across the houses during the sampling period.

9.4.2.2.1 Summer season inter-home metal correlation

The following were significantly correlated

- In the < 250 nm size range: Mn against Pb, Zn and Fe respectively, Sb against Zn, Pb against Zn, Fe against Zn and Mg against Ca (Table 9.7).
- In the 250 – 500 nm size range: Mn against Pb, Pb against Zn, Pb against Fe, Ca against Sr were positively correlated while Mn against Cu and Sr against Cu were negatively correlated (Table 9.8)
- In the 500 – 1000 nm size range: Na against Mg, Ca against Fe, Ca against Zn, Fe against Zn and Mg against Na (Table 9.9)
- In the 1000 – 2500 nm size range: Mn against Fe and Ca respectively, Mg against Fe and Na respectively, Ca against Fe and Fe against Zn (Table 9.10)
- In the > 2500 nm size range: Cr against Sr, Mn against Ca, Fe, Zn and Mg respectively, Pb against Ca, Pb against Zn, Na against Sn, Ca against Fe, Zn and Mg respectively, Fe against Zn, Fe against Mg and Zn against Mg (Table 9.11).

9.4.2.2.2 Winter season inter-home metal correlation

- In the < 250 nm size range: Sb against Pb, Sb against Zn, Pb against Zn and Ca against Mg (Table 9.12).
- In the 250 – 500 nm size range: Pb against Zn, Na against Mg, Ca against Fe were positively correlated while Cr against Na showed a negative correlation (Table 9.13).
- In the 500 -1000 nm size range: Na against Ca, Na against Mg and Ca against Mg (Table 9.14)

- In the 1000 – 2500 nm size range: Ca against Mg and Fe against Sn (Table 9.15).
- In the > 2500 nm size range: Cr against Ca, Cr against Zn, Na against Mg, Ca against Zn, Ca against Mg, Fe against Mg and Zn against Mg (Table 9.16).

In Table 9.7 to 9.16 some metals are missing in the matrix. That is because they were less than the limit of detection or at least 20 % of the data was missing due to being lower than the LOD.

Generally, for all the pairs with strong positive significant correlation between the metals suggests common sources. While the pairs with the negative significant correlations suggests that as one metal increases the other decreases, perhaps because the concentration increase in one metal may decrease the second or the factors that bring about a rapid release of the first metal may stabilize or affect the release of the second (Kefeni and Okonkwo, 2013).

Table 9.7. Relationship between metals in the < 250 nm size fraction for the homes during the summer

Correlations			Cr	Mn	Sb	Pb	Sr	Cu	Zn	Mg	Na	Ca	Fe
Spearman's rho	Cr	Correlation Coefficient	1.000	.234	-.179	-.067	-.393	.283	-.075	.000	.486	.134	.209
		Sig. (2-tailed)	.	.613	.702	.865	.383	.460	.847	1.000	.329	.731	.589
		N	9	7	7	9	7	9	9	9	6	9	9
	Mn	Correlation Coefficient	.234	1.000	.700	.883*	-.667	.288	.937*	.164	.400	.391	.918**
		Sig. (2-tailed)	.613	.	.188	.008	.219	.531	.002	.726	.600	.386	.004
		N	7	7	5	7	5	7	7	7	4	7	7
	Sb	Correlation Coefficient	-.179	.700	1.000	.571	.300	-.071	.757*	-.252	.200	.000	.536
		Sig. (2-tailed)	.702	.188	.	.180	.624	.879	.049	.585	.704	1.000	.215
		N	7	5	7	7	5	7	7	7	6	7	7
	Pb	Correlation Coefficient	-.067	.883**	.571	1.000	.000	.300	.787*	.427	.257	.276	.452
		Sig. (2-tailed)	.865	.008	.180	.	1.000	.433	.012	.252	.623	.472	.222
		N	9	7	7	9	7	9	9	9	6	9	9
	Sr	Correlation Coefficient	-.393	-.667	.300	.000	1.000	-.357	.198	.143	-.600	.360	-.324
		Sig. (2-tailed)	.383	.219	.624	1.000	.	.432	.670	.760	.400	.427	.478
		N	7	5	5	7	7	7	7	7	4	7	7
	Cu	Correlation Coefficient	.283	.288	-.071	.300	-.357	1.000	.285	.552	.543	.569	.075
		Sig. (2-tailed)	.460	.531	.879	.433	.432	.	.458	.123	.266	.110	.847
		N	9	7	7	9	7	9	9	9	6	9	9
	Zn	Correlation Coefficient	-.075	.937**	.757*	.787*	.198	.285	1.000	.147	.725	.487	.693*
		Sig. (2-tailed)	.847	.002	.049	.012	.670	.458	.	.706	.103	.183	.038
		N	9	7	7	9	7	9	9	9	6	9	9
	Mg	Correlation Coefficient	.000	.164	-.252	.427	.143	.552	.147	1.000	-.058	.697*	-.298
		Sig. (2-tailed)	1.000	.726	.585	.252	.760	.123	.706	.	.913	.037	.436
		N	9	7	7	9	7	9	9	9	6	9	9
	Na	Correlation Coefficient	.486	.400	.200	.257	-.600	.543	.725	-.058	1.000	.486	.771
		Sig. (2-tailed)	.329	.600	.704	.623	.400	.266	.103	.913	.	.329	.072
		N	6	4	6	6	4	6	6	6	6	6	6
	Ca	Correlation Coefficient	.134	.391	.000	.276	.360	.569	.487	.697*	.486	1.000	.105
		Sig. (2-tailed)	.731	.386	1.000	.472	.427	.110	.183	.037	.329	.	.788
		N	9	7	7	9	7	9	9	9	6	9	9
	Fe	Correlation Coefficient	.209	.918**	.536	.452	-.324	.075	.693*	-.298	.771	.105	1.000
		Sig. (2-tailed)	.589	.004	.215	.222	.478	.847	.038	.436	.072	.788	.
		N	9	7	7	9	7	9	9	9	6	9	9

** .Correlation is significant at the 0.01 level (2-tailed).

* .Correlation is significant at the 0.05 level (2-tailed).

Table 9.8. Relationship between metals in the 250 - 500 nm size fraction for the homes during the summer

			Correlations									
			Mn	Pb	Sr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Mn	Correlation Coefficient	1.000	.841	.400	.754	-.203	.638	-.928**	.754	.377	-.058
		Sig. (2-tailed)	.	.036	.505	.084	.700	.173	.008	.084	.461	.913
		N	6	6	5	6	6	6	6	6	6	6
	Pb	Correlation Coefficient	.841*	1.000	.357	.286	.262	.929**	-.595	.738*	-.167	.214
		Sig. (2-tailed)	.036	.	.432	.493	.531	.001	.120	.037	.693	.610
		N	6	8	7	8	8	8	8	8	8	8
	Sr	Correlation Coefficient	.400	.357	1.000	-.310	.810*	.333	-.786*	.405	-.095	.429
		Sig. (2-tailed)	.505	.432	.	.456	.015	.420	.021	.320	.823	.337
		N	5	7	8	8	8	8	8	8	8	7
	Na	Correlation Coefficient	.754	.286	-.310	1.000	-.217	.233	-.117	.067	.250	-.548
		Sig. (2-tailed)	.084	.493	.456	.	.576	.546	.765	.865	.516	.160
		N	6	8	8	9	9	9	9	9	9	8
	Ca	Correlation Coefficient	-.203	.262	.810*	-.217	1.000	.467	-.250	.267	-.067	.262
		Sig. (2-tailed)	.700	.531	.015	.576	.	.205	.516	.488	.865	.531
		N	6	8	8	9	9	9	9	9	9	8
	Fe	Correlation Coefficient	.638	.929**	.333	.233	.467	1.000	-.250	.567	-.050	.143
		Sig. (2-tailed)	.173	.001	.420	.546	.205	.	.516	.112	.898	.736
		N	6	8	8	9	9	9	9	9	9	8
	Cu	Correlation Coefficient	-.928**	-.595	-.786*	-.117	-.250	-.250	1.000	-.250	.250	-.452
		Sig. (2-tailed)	.008	.120	.021	.765	.516	.516	.	.516	.516	.260
		N	6	8	8	9	9	9	9	9	9	8
	Zn	Correlation Coefficient	.754	.738*	.405	.067	.267	.567	-.250	1.000	-.300	.429
		Sig. (2-tailed)	.084	.037	.320	.865	.488	.112	.516	.	.433	.289
		N	6	8	8	9	9	9	9	9	9	8
	Mg	Correlation Coefficient	.377	-.167	-.095	.250	-.067	-.050	.250	-.300	1.000	-.357
		Sig. (2-tailed)	.461	.693	.823	.516	.865	.898	.516	.433	.	.385
		N	6	8	8	9	9	9	9	9	9	8
	Sn	Correlation Coefficient	-.058	.214	.429	-.548	.262	.143	-.452	.429	-.357	1.000
		Sig. (2-tailed)	.913	.610	.337	.160	.531	.736	.260	.289	.385	.
		N	6	8	7	8	8	8	8	8	8	8

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9.9. Relationship between metals in the 500 – 1000 nm size fraction for the homes during the summer

Correlations

			Sr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Sr	Correlation Coefficient	1.000	.444	.117	.259	-.402	.159	.243	-.644
		Sig. (2-tailed)	.	.232	.764	.500	.284	.683	.529	.061
		N	9	9	9	9	9	9	9	9
	Na	Correlation Coefficient	.444	1.000	.133	.133	.050	.017	.717*	-.317
		Sig. (2-tailed)	.232	.	.732	.732	.898	.966	.030	.406
		N	9	9	9	9	9	9	9	9
	Ca	Correlation Coefficient	.117	.133	1.000	.700*	.417	.783*	.167	-.033
		Sig. (2-tailed)	.764	.732	.	.036	.265	.013	.668	.932
		N	9	9	9	9	9	9	9	9
	Fe	Correlation Coefficient	.259	.133	.700*	1.000	-.033	.733*	.400	-.217
		Sig. (2-tailed)	.500	.732	.036	.	.932	.025	.286	.576
		N	9	9	9	9	9	9	9	9
	Cu	Correlation Coefficient	-.402	.050	.417	-.033	1.000	.517	-.117	.417
		Sig. (2-tailed)	.284	.898	.265	.932	.	.154	.765	.265
		N	9	9	9	9	9	9	9	9
	Zn	Correlation Coefficient	.159	.017	.783*	.733*	.517	1.000	.050	.133
		Sig. (2-tailed)	.683	.966	.013	.025	.154	.	.898	.732
		N	9	9	9	9	9	9	9	9
	Mg	Correlation Coefficient	.243	.717*	.167	.400	-.117	.050	1.000	-.367
		Sig. (2-tailed)	.529	.030	.668	.286	.765	.898	.	.332
		N	9	9	9	9	9	9	9	9
	Sn	Correlation Coefficient	-.644	-.317	-.033	-.217	.417	.133	-.367	1.000
		Sig. (2-tailed)	.061	.406	.932	.576	.265	.732	.332	.
		N	9	9	9	9	9	9	9	9

*. Correlation is significant at the 0.05 level (2-tailed).

Table 9.10. Relationship between metals in the 1000 - 2500 nm size fraction for the homes during the summer

Correlations

			Mn	Sr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Mn	Correlation Coefficient	1.000	.657	.162	.775*	.991**	-.505	.450	.577	-.173
		Sig. (2-tailed)	.	.156	.728	.041	.000	.248	.310	.175	.711
		N	7	6	7	7	7	7	7	7	7
	Sr	Correlation Coefficient	.657	1.000	-.143	-.333	-.286	-.286	-.071	-.119	.190
		Sig. (2-tailed)	.156	.	.736	.420	.493	.493	.867	.779	.651
		N	6	8	8	8	8	8	8	8	8
	Na	Correlation Coefficient	.162	-.143	1.000	.083	.450	.633	.350	.783*	.142
		Sig. (2-tailed)	.728	.736	.	.831	.224	.067	.356	.013	.715
		N	7	8	9	9	9	9	9	9	9
	Ca	Correlation Coefficient	.775*	-.333	.083	1.000	.833**	-.483	.350	.500	-.209
		Sig. (2-tailed)	.041	.420	.831	.	.005	.187	.356	.170	.589
		N	7	8	9	9	9	9	9	9	9
	Fe	Correlation Coefficient	.991**	-.286	.450	.833**	1.000	-.017	.683*	.717*	.159
		Sig. (2-tailed)	.000	.493	.224	.005	.	.966	.042	.030	.683
		N	7	8	9	9	9	9	9	9	9
	Cu	Correlation Coefficient	-.505	-.286	.633	-.483	-.017	1.000	.167	.250	.226
		Sig. (2-tailed)	.248	.493	.067	.187	.966	.	.668	.516	.559
		N	7	8	9	9	9	9	9	9	9
	Zn	Correlation Coefficient	.450	-.071	.350	.350	.683*	.167	1.000	.283	.644
		Sig. (2-tailed)	.310	.867	.356	.356	.042	.668	.	.460	.061
		N	7	8	9	9	9	9	9	9	9
	Mg	Correlation Coefficient	.577	-.119	.783*	.500	.717*	.250	.283	1.000	-.025
		Sig. (2-tailed)	.175	.779	.013	.170	.030	.516	.460	.	.949
		N	7	8	9	9	9	9	9	9	9
	Sn	Correlation Coefficient	-.173	.190	.142	-.209	.159	.226	.644	-.025	1.000
		Sig. (2-tailed)	.711	.651	.715	.589	.683	.559	.061	.949	.
		N	7	8	9	9	9	9	9	9	9

*. Correlation is significant at the 0.05 level (2-tailed).

** . Correlation is significant at the 0.01 level (2-tailed).

Table 9.11. Relationship between metals in the >2500 nm size fraction for the homes during the summer

			Correlations										
			Cr	Mn	Pb	Sr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Cr	Correlation Coefficient	1.000	.286	-.143	.929 [*]	.536	-.107	.429	-.429	.214	.679	.321
		Sig. (2-tailed)	.	.535	.787	.003	.215	.819	.337	.337	.645	.094	.482
		N	7	7	6	7	7	7	7	7	7	7	7
	Mn	Correlation Coefficient	.286	1.000	.714	-.333	.167	.850 ^{**}	.983 ^{**}	.267	.867 ^{**}	.850 ^{**}	.333
		Sig. (2-tailed)	.535	.	.111	.381	.668	.004	.000	.488	.002	.004	.381
		N	7	9	6	9	9	9	9	9	9	9	9
	Pb	Correlation Coefficient	-.143	.714	1.000	-.314	-.486	.829 [*]	.486	.657	.829 [*]	.257	-.257
		Sig. (2-tailed)	.787	.111	.	.544	.329	.042	.329	.156	.042	.623	.623
		N	6	6	6	6	6	6	6	6	6	6	6
	Sr	Correlation Coefficient	.929 ^{**}	-.333	-.314	1.000	-.150	-.483	-.267	-.600	-.283	-.100	-.067
		Sig. (2-tailed)	.003	.381	.544	.	.700	.187	.488	.088	.460	.798	.865
		N	7	9	6	9	9	9	9	9	9	9	9
	Na	Correlation Coefficient	.536	.167	-.486	-.150	1.000	-.100	.233	.050	.217	.467	.667 [*]
		Sig. (2-tailed)	.215	.668	.329	.700	.	.798	.546	.898	.576	.205	.050
		N	7	9	6	9	9	9	9	9	9	9	9
	Ca	Correlation Coefficient	-.107	.850 ^{**}	.829 [*]	-.483	-.100	1.000	.800 ^{**}	.233	.733 [*]	.683 [*]	.117
		Sig. (2-tailed)	.819	.004	.042	.187	.798	.	.010	.546	.025	.042	.765
		N	7	9	6	9	9	9	9	9	9	9	9
	Fe	Correlation Coefficient	.429	.983 ^{**}	.486	-.267	.233	.800 ^{**}	1.000	.167	.833 ^{**}	.867 ^{**}	.317
		Sig. (2-tailed)	.337	.000	.329	.488	.546	.010	.	.668	.005	.002	.406
		N	7	9	6	9	9	9	9	9	9	9	9
	Cu	Correlation Coefficient	-.429	.267	.657	-.600	.050	.233	.167	1.000	.367	-.067	.167
		Sig. (2-tailed)	.337	.488	.156	.088	.898	.546	.668	.	.332	.865	.668
		N	7	9	6	9	9	9	9	9	9	9	9
	Zn	Correlation Coefficient	.214	.867 ^{**}	.829 [*]	-.283	.217	.733 [*]	.833 ^{**}	.367	1.000	.717 [*]	.367
		Sig. (2-tailed)	.645	.002	.042	.460	.576	.025	.005	.332	.	.030	.332
		N	7	9	6	9	9	9	9	9	9	9	9
	Mg	Correlation Coefficient	.679	.850 ^{**}	.257	-.100	.467	.683 [*]	.867 ^{**}	-.067	.717 [*]	1.000	.617
		Sig. (2-tailed)	.094	.004	.623	.798	.205	.042	.002	.865	.030	.	.077
		N	7	9	6	9	9	9	9	9	9	9	9
	Sn	Correlation Coefficient	.321	.333	-.257	-.067	.667 [*]	.117	.317	.167	.367	.617	1.000
		Sig. (2-tailed)	.482	.381	.623	.865	.050	.765	.406	.668	.332	.077	.
		N	7	9	6	9	9	9	9	9	9	9	9

^{**}. Correlation is significant at the 0.01 level (2-tailed).

^{*}. Correlation is significant at the 0.05 level (2-tailed).

Table 9.12. Relationship between metals in the < 250 nm size fraction for the homes during the winter

Correlations

			Cr	Sb	Pb	Na	Ca	Fe	Cu	Zn	Mg
Spearman's rho	Cr	Correlation Coefficient	1.000	-.095	.262	-.036	.190	-.036	-.071	.048	.214
		Sig. (2-tailed)	.	.823	.531	.939	.651	.939	.867	.911	.610
		N	8	8	8	7	8	7	8	8	8
	Sb	Correlation Coefficient	-.095	1.000	.833*	-.464	.262	-.714	.119	.714*	.214
		Sig. (2-tailed)	.823	.	.010	.294	.531	.071	.779	.047	.610
		N	8	8	8	7	8	7	8	8	8
	Pb	Correlation Coefficient	.262	.833*	1.000	-.643	.552	-.287	.367	.900**	.433
		Sig. (2-tailed)	.531	.010	.	.086	.123	.490	.332	.001	.244
		N	8	8	9	8	9	8	9	9	9
	Na	Correlation Coefficient	-.036	-.464	-.643	1.000	.238	.455	.119	-.667	.119
		Sig. (2-tailed)	.939	.294	.086	.	.570	.257	.779	.071	.779
		N	7	7	8	8	8	8	8	8	8
	Ca	Correlation Coefficient	.190	.262	.552	.238	1.000	.395	.611	.594	.879*
		Sig. (2-tailed)	.651	.531	.123	.570	.	.333	.081	.092	.002
		N	8	8	9	8	9	8	9	9	9
	Fe	Correlation Coefficient	-.036	-.714	-.287	.455	.395	1.000	.515	-.048	.132
		Sig. (2-tailed)	.939	.071	.490	.257	.333	.	.192	.910	.756
		N	7	7	8	8	8	8	8	8	8
	Cu	Correlation Coefficient	-.071	.119	.367	.119	.611	.515	1.000	.417	.567
		Sig. (2-tailed)	.867	.779	.332	.779	.081	.192	.	.265	.112
		N	8	8	9	8	9	8	9	9	9
	Zn	Correlation Coefficient	.048	.714*	.900**	-.667	.594	-.048	.417	1.000	.450
		Sig. (2-tailed)	.911	.047	.001	.071	.092	.910	.265	.	.224
		N	8	8	9	8	9	8	9	9	9
	Mg	Correlation Coefficient	.214	.214	.433	.119	.879**	.132	.567	.450	1.000
		Sig. (2-tailed)	.610	.610	.244	.779	.002	.756	.112	.224	.
		N	8	8	9	8	9	8	9	9	9

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9.13. Relationship between metals in the 250 - 500 nm size fraction for the homes during the winter

Correlations

			Cr	Sb	Pb	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Cr	Correlation Coefficient	1.000	.738	.478	-.837*	-.717	-.717	-.120	.478	-.837*	-.359
		Sig. (2-tailed)	.	.155	.338	.038	.109	.109	.822	.338	.038	.485
		N	6	5	6	6	6	6	6	6	6	6
	Sb	Correlation Coefficient	.738	1.000	.486	-.257	-.257	-.600	-.657	.143	-.429	-.314
		Sig. (2-tailed)	.155	.	.329	.623	.623	.208	.156	.787	.397	.544
		N	5	6	6	6	6	6	6	6	6	6
	Pb	Correlation Coefficient	.478	.486	1.000	-.548	-.143	.048	-.071	.929**	-.690	.000
		Sig. (2-tailed)	.338	.329	.	.160	.736	.911	.867	.001	.058	1.000
		N	6	6	8	8	8	8	8	8	8	8
	Na	Correlation Coefficient	-.837*	-.257	-.548	1.000	.650	.467	.083	-.617	.883**	.600
		Sig. (2-tailed)	.038	.623	.160	.	.058	.205	.831	.077	.002	.088
		N	6	6	8	9	9	9	9	9	9	9
	Ca	Correlation Coefficient	-.717	-.257	-.143	.650	1.000	.817**	.083	-.217	.433	.083
		Sig. (2-tailed)	.109	.623	.736	.058	.	.007	.831	.576	.244	.831
		N	6	6	8	9	9	9	9	9	9	9
	Fe	Correlation Coefficient	-.717	-.600	.048	.467	.817**	1.000	.167	.133	.200	.150
		Sig. (2-tailed)	.109	.208	.911	.205	.007	.	.668	.732	.606	.700
		N	6	6	8	9	9	9	9	9	9	9
	Cu	Correlation Coefficient	-.120	-.657	-.071	.083	.083	.167	1.000	.183	.300	.383
		Sig. (2-tailed)	.822	.156	.867	.831	.831	.668	.	.637	.433	.308
		N	6	6	8	9	9	9	9	9	9	9
	Zn	Correlation Coefficient	.478	.143	.929**	-.617	-.217	.133	.183	1.000	-.633	-.133
		Sig. (2-tailed)	.338	.787	.001	.077	.576	.732	.637	.	.067	.732
		N	6	6	8	9	9	9	9	9	9	9
	Mg	Correlation Coefficient	-.837*	-.429	-.690	.883**	.433	.200	.300	-.633	1.000	.633
		Sig. (2-tailed)	.038	.397	.058	.002	.244	.606	.433	.067	.	.067
		N	6	6	8	9	9	9	9	9	9	9
	Sn	Correlation Coefficient	-.359	-.314	.000	.600	.083	.150	.383	-.133	.633	1.000
		Sig. (2-tailed)	.485	.544	1.000	.088	.831	.700	.308	.732	.067	.
		N	6	6	8	9	9	9	9	9	9	9

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9.14. Relationship between metals in the 500 - 1000 nm size fraction for the homes during the winter

Correlations

			Cr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Cr	Correlation Coefficient	1.000	.300	-.800	-.500	-.600	-.500	.100	.000
		Sig. (2-tailed)	.	.624	.104	.667	.285	.391	.873	1.000
		N	5	5	5	3	5	5	5	4
	Na	Correlation Coefficient	.300	1.000	.767*	-.543	-.429	-.200	.733*	-.476
		Sig. (2-tailed)	.624	.	.016	.266	.289	.606	.025	.233
		N	5	9	9	6	8	9	9	8
	Ca	Correlation Coefficient	-.800	.767*	1.000	-.486	.119	.100	.833**	-.405
		Sig. (2-tailed)	.104	.016	.	.329	.779	.798	.005	.320
		N	5	9	9	6	8	9	9	8
	Fe	Correlation Coefficient	-.500	-.543	-.486	1.000	.257	.600	-.543	-.314
		Sig. (2-tailed)	.667	.266	.329	.	.623	.208	.266	.544
		N	3	6	6	6	6	6	6	6
	Cu	Correlation Coefficient	-.600	-.429	.119	.257	1.000	.643	-.095	.714
		Sig. (2-tailed)	.285	.289	.779	.623	.	.086	.823	.071
		N	5	8	8	6	8	8	8	7
	Zn	Correlation Coefficient	-.500	-.200	.100	.600	.643	1.000	-.100	.167
		Sig. (2-tailed)	.391	.606	.798	.208	.086	.	.798	.693
		N	5	9	9	6	8	9	9	8
	Mg	Correlation Coefficient	.100	.733*	.833**	-.543	-.095	-.100	1.000	-.238
		Sig. (2-tailed)	.873	.025	.005	.266	.823	.798	.	.570
		N	5	9	9	6	8	9	9	8
	Sn	Correlation Coefficient	.000	-.476	-.405	-.314	.714	.167	-.238	1.000
		Sig. (2-tailed)	1.000	.233	.320	.544	.071	.693	.570	.
		N	4	8	8	6	7	8	8	8

*. Correlation is significant at the 0.05 level (2-tailed).

** . Correlation is significant at the 0.01 level (2-tailed).

Table 9.15. Relationship between metals in the 1000 - 2500 nm size fraction for the homes during the winter

Correlations

			Cr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Cr	Correlation Coefficient	1.000	.616	.359	.051	.400	-.564	.359	-.205
		Sig. (2-tailed)	.	.269	.553	.935	.600	.322	.553	.741
		N	5	5	5	5	4	5	5	5
	Na	Correlation Coefficient	.616	1.000	.317	.550	.405	-.133	.467	-.238
		Sig. (2-tailed)	.269	.	.406	.125	.320	.732	.205	.570
		N	5	9	9	9	8	9	9	8
	Ca	Correlation Coefficient	.359	.317	1.000	.550	.143	.450	.833**	.381
		Sig. (2-tailed)	.553	.406	.	.125	.736	.224	.005	.352
		N	5	9	9	9	8	9	9	8
	Fe	Correlation Coefficient	.051	.550	.550	1.000	.143	.517	.400	.714*
		Sig. (2-tailed)	.935	.125	.125	.	.736	.154	.286	.047
		N	5	9	9	9	8	9	9	8
	Cu	Correlation Coefficient	.400	.405	.143	.143	1.000	-.048	.143	-.107
		Sig. (2-tailed)	.600	.320	.736	.736	.	.911	.736	.819
		N	4	8	8	8	8	8	8	7
	Zn	Correlation Coefficient	-.564	-.133	.450	.517	-.048	1.000	.033	.333
		Sig. (2-tailed)	.322	.732	.224	.154	.911	.	.932	.420
		N	5	9	9	9	8	9	9	8
	Mg	Correlation Coefficient	.359	.467	.833**	.400	.143	.033	1.000	.286
		Sig. (2-tailed)	.553	.205	.005	.286	.736	.932	.	.493
		N	5	9	9	9	8	9	9	8
	Sn	Correlation Coefficient	-.205	-.238	.381	.714*	-.107	.333	.286	1.000
		Sig. (2-tailed)	.741	.570	.352	.047	.819	.420	.493	.
		N	5	8	8	8	7	8	8	8

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Table 9.16. Relationship between metals in the > 2500 nm size fraction for the homes during the winter

Correlations

			Cr	Na	Ca	Fe	Cu	Zn	Mg	Sn
Spearman's rho	Cr	Correlation Coefficient	1.000	.559	.893 ^{**}	.357	.000	.857 [*]	.714	-.071
		Sig. (2-tailed)	.	.192	.007	.432	1.000	.014	.071	.879
		N	7	7	7	7	7	7	7	7
	Na	Correlation Coefficient	.559	1.000	.661	.628	-.402	.444	.787 [*]	.351
		Sig. (2-tailed)	.192	.	.053	.070	.284	.232	.012	.354
		N	7	9	9	9	9	9	9	9
	Ca	Correlation Coefficient	.893 ^{**}	.661	1.000	.600	-.267	.900 ^{**}	.933 [*]	-.100
		Sig. (2-tailed)	.007	.053	.	.088	.488	.001	.000	.798
		N	7	9	9	9	9	9	9	9
	Fe	Correlation Coefficient	.357	.628	.600	1.000	-.100	.550	.783 [*]	.533
		Sig. (2-tailed)	.432	.070	.088	.	.798	.125	.013	.139
		N	7	9	9	9	9	9	9	9
	Cu	Correlation Coefficient	.000	-.402	-.267	-.100	1.000	-.383	-.167	.267
		Sig. (2-tailed)	1.000	.284	.488	.798	.	.308	.668	.488
		N	7	9	9	9	9	9	9	9
	Zn	Correlation Coefficient	.857 [*]	.444	.900 ^{**}	.550	-.383	1.000	.750 [*]	-.117
		Sig. (2-tailed)	.014	.232	.001	.125	.308	.	.020	.765
		N	7	9	9	9	9	9	9	9
	Mg	Correlation Coefficient	.714	.787 [*]	.933 ^{**}	.783 [*]	-.167	.750 [*]	1.000	.150
		Sig. (2-tailed)	.071	.012	.000	.013	.668	.020	.	.700
		N	7	9	9	9	9	9	9	9
	Sn	Correlation Coefficient	-.071	.351	-.100	.533	.267	-.117	.150	1.000
		Sig. (2-tailed)	.879	.354	.798	.139	.488	.765	.700	.
		N	7	9	9	9	9	9	9	9

^{**}. Correlation is significant at the 0.01 level (2-tailed).

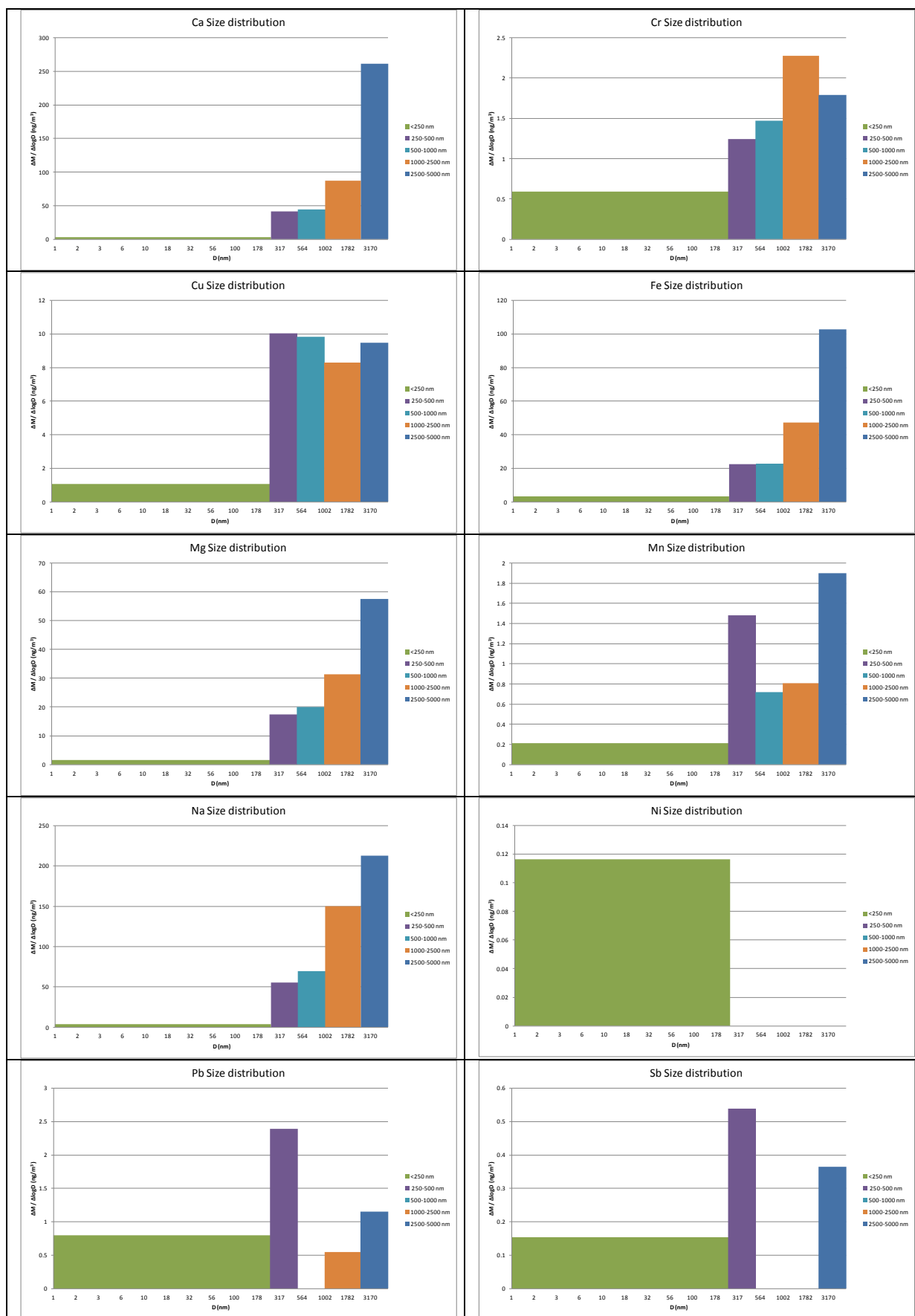
^{*}. Correlation is significant at the 0.05 level (2-tailed).

9.4.2.3 Elemental mass-size distribution

In Figure 9.2 the mass size distribution shows that the indoor particles show two modes mostly in the 250 – 500 nm to 2500 -5000 nm. The elemental distribution in Figure 9.3 for the summer and Figure 9.4 for the winter shows that these two modes are also the prevalent peaks. The elemental size distribution for Ca, Fe, Mg and Na (Figure 9.4) show one modal mass-size distribution prevalent in the coarse mode. Also in the winter (Figure 9.4), Ca, Fe, Ni showed a single mode prevalent in the coarse mode. While Na and Sn, showed a single mode that is prevalent in the fine mode (500 – 1000 nm and 250 – 500 nm respectively). It is typical for the crustal elements Ca, Fe, Mg and Na to show prevalence of peak in the coarse mode. This is because of the influence from the indoor sources.

Cr shows a single mode prevalent in the 1000 -2500 nm size range (Figure 9.3). While in the winter period Cr showed a bimodal distribution with the coarse fraction as the primary peak and the 500 100 nm size range as the secondary peak in (fine mode) (Figure 9.4). Viana et al. (2014) observed a bimodal distribution of Cr in their indoor size distribution of a school which is opposite of what is observed in this study. They observed a primary peak at the < 250 nm and secondary very low peak at the coarse size fraction. Cr in Figure 9.4 shows a unimodal distribution with prevalence in the fine, accumulation mode 1000 – 2500 nm mass-size fraction. This strongly suggests the concentration of Cr is mainly due to re-suspension of particles indoors and the high concentration of the coarse size fraction indicates that the outdoor is a main contributing factor for this metal indoors. Cr is a component of stainless steel and stainless materials are used in the homes (Taner et al., 2013). Cr from the outdoors is associated non-exhaust traffic sources as mentioned in a review study of metallic NPs (Sanderson et al., 2014).

Cr, Ni, Pb, Mn, Cu, Zn, Sb and V are said to be toxic metals (The Committee on Biological Markers of the National Research, 1987) and these metals were found indoor in trace amount in the homes that were sampled. Figure 9.3 also show that the mass-size distributions of Cu, Mn, Pb, Sb, Sr and Zn are bimodal. Cu, Pb, Sb, Sr, and Zn show two modes (fine-0.25 – 0.5 μm and coarse- 2.5 – 5 μm), with prevalence in the fine mode. Cu and has the fine – 0.25 – 0.5 μm size fraction as the prevalent mode indicating that an indoor sources (cooking fumes released from food and oils (Taner et al., 2013), in addition to the outdoors sources (transport associated (Knibbs et al., 2011). Mn show two modes (0.25 – 500 μm and coarse- 2.5 – 5 μm), with prevalence in the coarse mode. The two modes could an indication of at least one of the major contributing source is from the outdoors (Viana et al., 2014). Ni and V show size concentration only in the UFP mode (< 250 nm). Ni from the outdoors are mostly from vehicular traffic which infiltrated into the house (Viana et al., 2014).



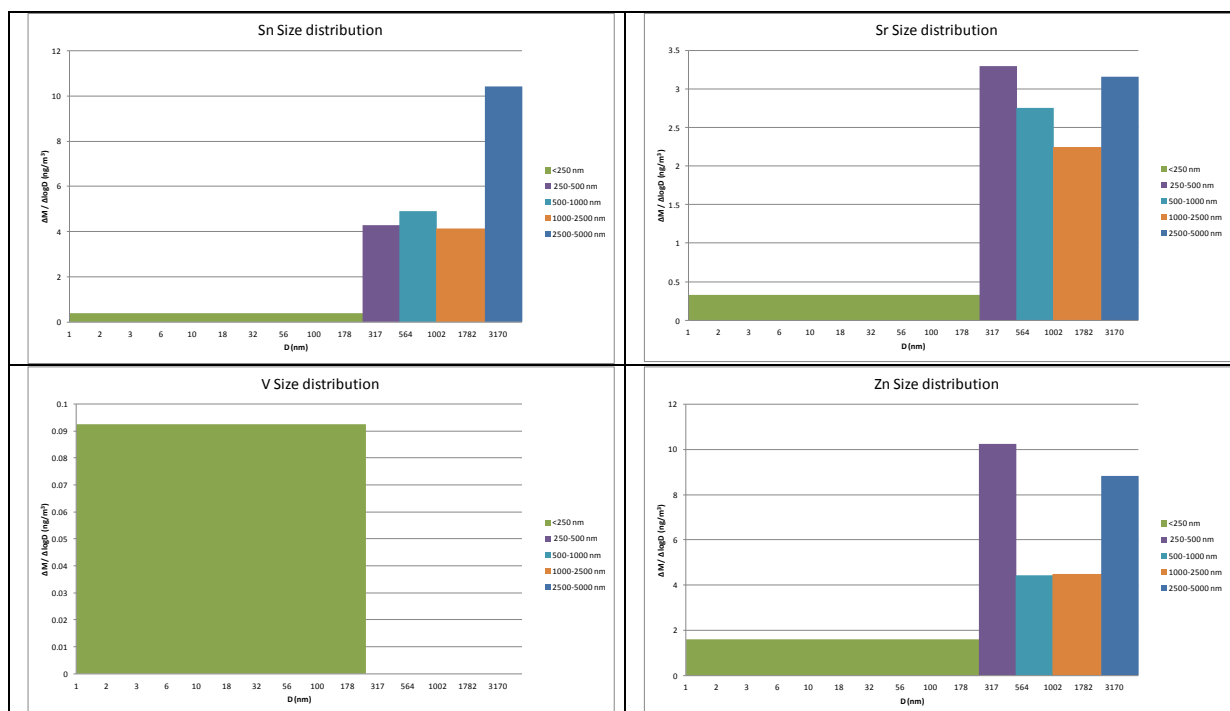
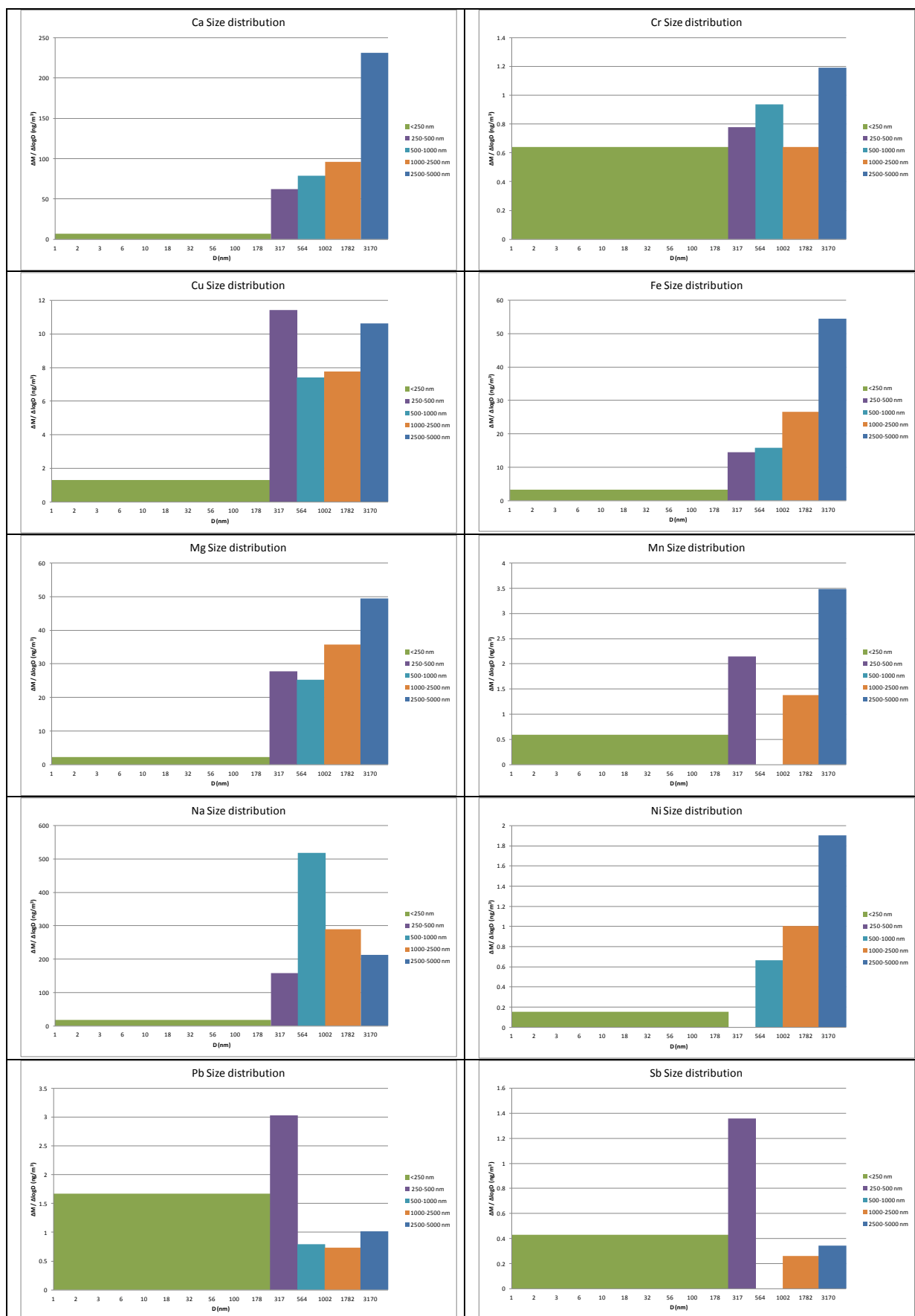


Figure 9.3. Average elemental size distribution for in the summer

In the winter (Figure 9.4), Cu, Mn, Pb, Sb, and Zn show a bimodal distribution just like during the summer season in Figure 9.3. While the Mg distribution in the summer, is different from the distribution in the winter. In the summer it was unimodal with the coarse fraction prevalent. While in the winter Mg showed a primary peak in the coarse size fraction and a secondary peak in the fine size fraction 250 – 500 nm (Figure 9.4). Sr show two modes; the primary mode 500 – 100 nm and secondary mode is the coarse mode $> 2.5 \mu\text{m}$.

Both in Figure 9.3 and Figure 9.4, where the mass-size distribution has a missing size range indicates that, the size range was below the detection limit. Also for both Figure 9.3 and Figure 9.4 due to inability to find suitable data for comparison, these two figures have been mainly described than discussed.



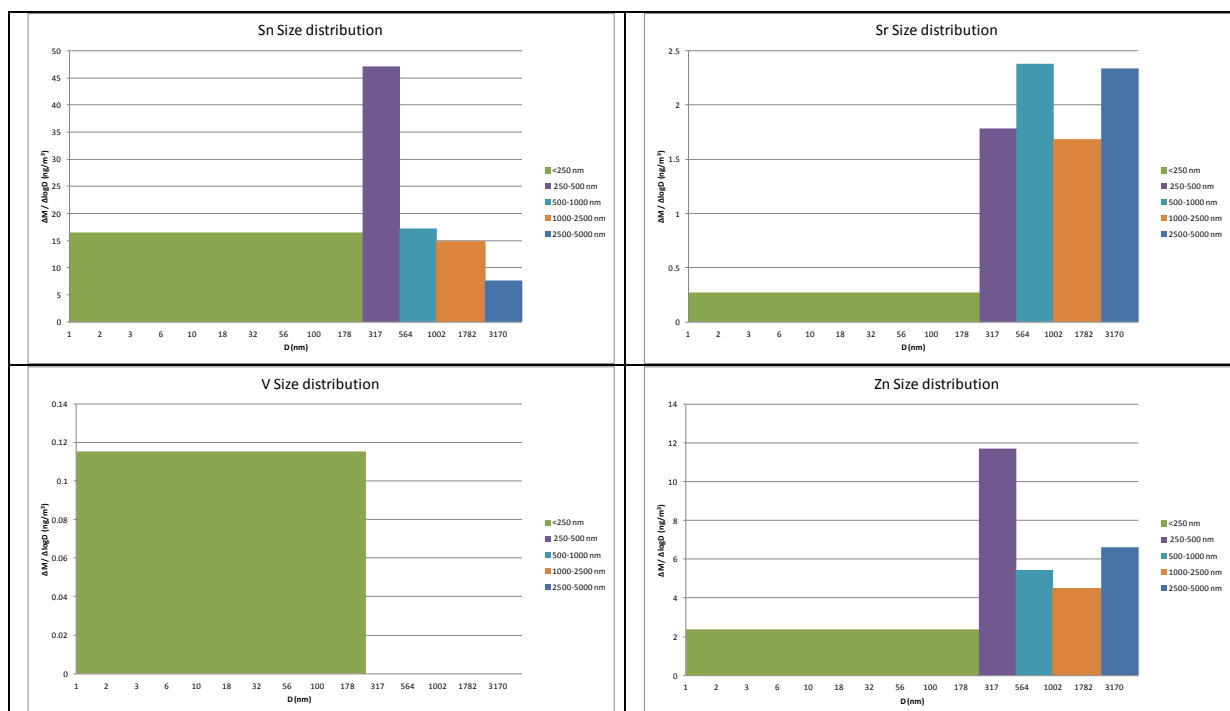


Figure 9.4. Average elemental size distribution for in the winter

Metals such as Cr, Fe, and Ni can also be leached from the stainless steel materials found inside the home or even during cooking when stainless steel utensils are subjected to high temperatures during cooking (See and Balasubramanian, 2008). Fe could also be from rust particles in the home due to breakdown of metals. Wall paints are sources of indoor metal metals bound to the PM fractions (Hulka, 1991). A study reported that Cr, Pb, Cu, Mn and Fe are from anthropogenic sources such as vehicle exhaust (Pope III and Dockery, 2006). Pb has been identified in road dust (Dreher et al., 1997). Sb, Zn, Fe, Sn and Mg are attributed to traffic/transport/ exhaust/ non-exhaust sources (Møller and Loft, 2010, Bonvallot et al., 2001). The salts Ca, Na and Mg are also of the crustal origin also based on the enrichment factor as shown in Figure 9.5 to Figure 9.9 and as described in literature (Bonvallot et al., 2001, Müller et al., 2010). V and Ni are from oil combustion in vehicle engines (Driscoll et al., 1997, Ghio et al., 2012).

9.4.3 Enrichment factor

The enrichment factors (EF) were calculated in order to speculate or determine if the indoor elemental PM originated from crustal or non-crustal sources. Fe was used as the reference element and the Upper continental crust (UCC) EF value used was obtained from the composition of continental crust (Wedepohl, 1995). Fe is one of the most common reference elements that is used in EF calculation and has been reported in literature (Delfino et al., 2011, Delfino et al., 2008). Fe was chosen as the reference because amongst the common reference elements used as reported in literature Al, Zr, Fe, Sc, and Ti (Delfino et al., 2011, Schmid et al., 2009), because the others were not among the elements measured in this study only Fe was measured therefore was used.

The EFs were calculated for each elemental size fraction using equation 2 below.

$$EF = (Conc_{element}/Conc_{Ref\ element})_{sample}/(Conc_{element}/Conc_{Ref\ element})_{ucc} \quad (6.2)$$

Crustal origin elements are mainly composed of rare and earth elements, natural mineral origin and marine origin from weathering, salt spray from the sea and soil remobilisation (Khodeir et al., 2012). While non-crustal are from anthropogenic sources such as industrial, vehicular and indoor activities/sources e.g cooking. EF of the elemental composition of particles is used in studies to determine sources of the particles. It helps suggests which elemental component is influenced by the anthropogenic activities or by crustal/marine origin. It is a preliminary source apportionment but the actual contribution of the source cannot be quantified by this method, a much more sophisticated method like the positive matrix factorization (PMF) and or chemical mass balance (CMB) may be applied for source apportionment.

Figure 9.5 to Figure 9.9 show the EF of the elements in the $< 0.25 \mu\text{m}$, $0.25 - 0.5 \mu\text{m}$, $0.5 - 1 \mu\text{m}$, $1 - 2.5 \mu\text{m}$ and $> 2.5 \mu\text{m}$ particles size fractions. The summer and winter for all the homes were pulled together.

Across the sampling period for both the summer and winter season as displayed in Figure 9.5 to Figure 9.9, it showed that these metals Na, Mg, Ca and Mn are of crustal origin and Na and Mg could also be from the marine origin. They have $EF < 10$ or close to 1 indicating that were mostly enriched by the soil. Also this is consistent with their size distribution in Figure 9.3 and Figure 9.4 where it showed that they were prevalent in the coarse mode ($> 2500 \text{ nm}$). Therefore parts of their indoor sources were contributed by their crustal/mineral/marine origin. The rest of the metals show EFs between 10 and 10000 indicating major influence of anthropogenic sources. Their concentrations is small in comparison to heavier concentration measured for Na, Ca and Mg this could mean that their anthropogenic sources are in the form of dust that made its way into the indoors via infiltration. Figure 9.5 to Figure 9.9 show that Cu, Sn and Sb were the most enriched elements. They are of vehicular non-exhaust emission origin such as the brake wear particles (Sjögren et al., 2009). Cu has also been said to be one of the most abundant metal found in cooking fumes and it may also be released from the food during cooking (See and Balasubramanian, 2008, Zhou et al., 2000). Zn, Pb, Ni, V, Cr, Sr are from anthropogenic sources such as vehicular and industrial sources. Most of these metals with moderate to high EF value are the trace metals that are said to have toxicological effects on humans

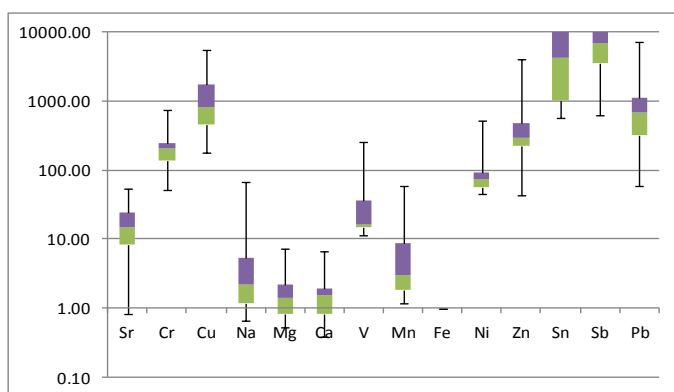


Figure 9.5. EF values of the elements in the $< 0.25 \mu\text{m}$ particles size fraction for all the homes

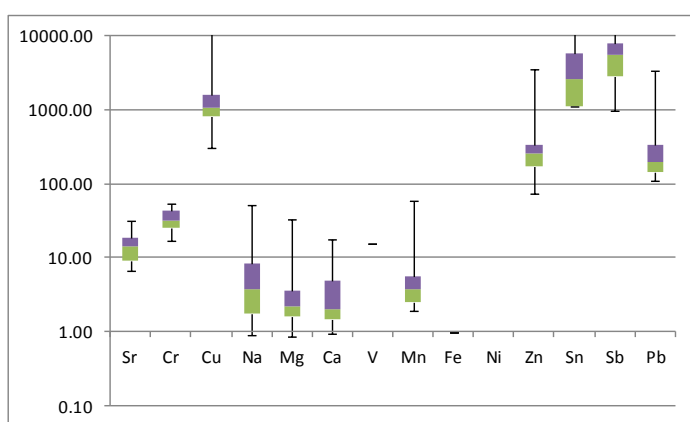


Figure 9.6. EF values of the elements in the $0.25 - 0.5 \mu\text{m}$ particles size fraction all the homes

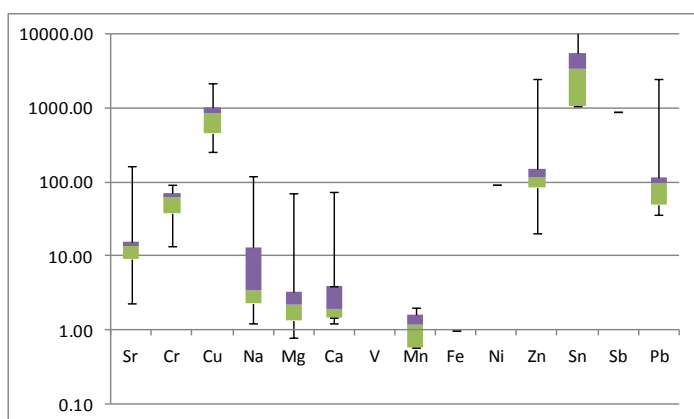


Figure 9.7. EF values of the elements in the $0.5 - 1 \mu\text{m}$ particles size fraction for all the homes

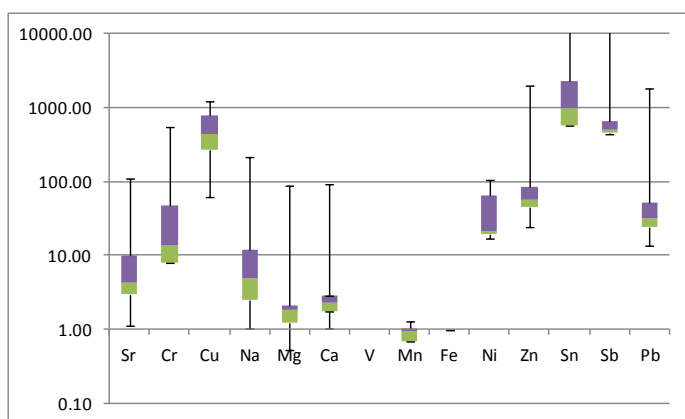


Figure 9.8. EF values of the elements in the 1 – 2.5 µm particles size fraction for all the homes

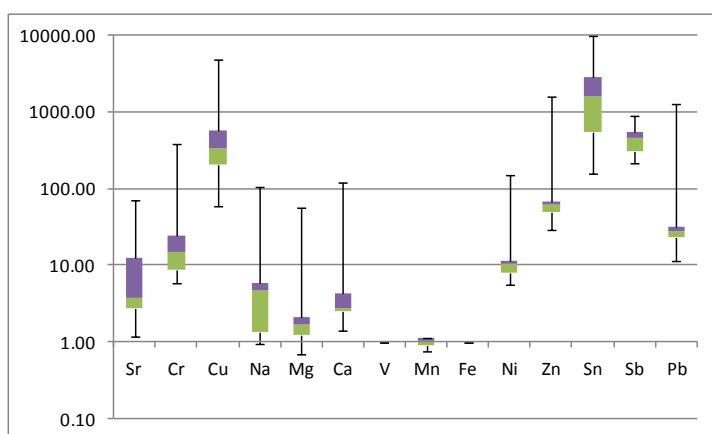


Figure 9.9. EF values of the elements in the > 2.5 µm particles size fraction for all the homes

There are various sources of these metals in the indoors and in addition, infiltration from the outdoor. They include materials from wear and tear from building materials, chemicals in paints, furniture, cooking, cooking utensils, cleaning, resuspension of dust due to human activities (See and Balasubramanian, 2008, Zhou et al., 2000, Taner et al., 2013, Hulka, 1991, Kulshrestha et al., 2014). Outdoor sources include vehicular sources (exhaust and non exhaust), road dust, crustal and marine sources such as Na, Mg, Si, and Ca are salts from crustal/minerals or marine origin. The different metals from vehicular sources (exhaust and

non exhaust) and road dust include these metals V, Cr, Ca, Mn, Fe, Ni, Cu, Zn, Sn, Sb and Pb and they originate from wear of brake lining, wear of tyres, combustion of lubrication oil, fuel additives (Bonvallot et al., 2001, Dreher et al., 1997).

Inhalation is a route for toxic metal exposure. Cr, Ni, Pb, Mn, Cu, Zn, Sb and V are said to be toxic (The Committee on Biological Markers of the National Research, 1987). Fe, Ni, V, Cr, Mn and Cu are redox catalysts, thus can catalyze oxidative stress reactions (Pan et al., 2010). Cu, Zn and Pb can deplete cellular antioxidant pool and interaction between GSH and metals can also produce or exacerbate cell injury (Ballatori, 1994) thus inducing oxidative stress reactions. There could be a possibility that they induce inflammation and oxidative stress through synergistic effects of more than two metals or the metals with other compounds. An in vitro study, observed synergistic effect of BC and iron oxide particles also induced oxidative stress (Seaton et al., 2005). Given that people spend most of their time indoors, it may be a potential health risk especially for it may be possible that susceptible people.

9.4.4 Size, morphology and surface chemistry

The TEM samples from three randomly selected homes and two offices will be discussed in details in this section. Morphology and elemental composition of particles provide information on their characteristics and sources (Kang et al., 2009). The size, morphology and surface chemistry for the particles collected on the $< 0.25 \mu\text{m}$ size fraction of the SIOUTAS in some of the homes and offices of some of the subjects were analysed using TEM/EDS. The particles observed in this TEM analysis were mostly in 5 shapes; 1) Irregular and aggregates of irregular shapes 2) Fibrous rod 3) Sphere and aggregates of spheres 4) Hexagon and 5) Rod. Table 9.17 show the number particles and frequency of shape observed per site. For all the sites, mostly irregular shapes were observed and this has been seen in

other studies in the outdoor (Adachi and Buseck, 2008). Relatively all particles on the TEM grid were viewed. Using the Image j software to analyse the viewed particles, background noises were removed and watershed image processing plugin in the software was used to show distinctive lines and segments on the particles. The particle diameters were measured and then counted. In order to get the particle count and frequency of size recorded per site (Table 9.17 and Figure 9.20), single particles were counted and recorded. However for agglomerates, the watershed image processing plugin in the Image J separates different single or primary particles (PPs) that make up agglomerate by defining lines and segments on the agglomerate particle, therefore showing different PPs that make up the agglomerates. The individual PPs were then counted and diameter measured. Where the software could not identify individual PPs and still viewed the agglomerate as a single particle, then the particle was counted as a single particle and diameter measured. Image J was used to measure particle diameter. Where the circularity generated by the software is less than 0.8 then manual measurements using the scale dimension on the image picture was used.

Based on the particles counted in the different sites (Table 9.17), the particles in Figure 9.10a to Figure 9.19a are representative of all the different sizes and shapes observed under the TEM. The particles in each site showed similar chemical compositions and a few variations. Therefore the similar pattern of composition and the variations in each site were selected and presented in Figure 9.10b to 9.19b. Thus the elemental maps in Figure 9.10b to 9.19b represents the compositions found in each site.

Table 9.17. Number of particles and frequency of shape observation per site

Shape/ location	Irregular and aggregates of irregular shapes (F)	Fibrous rod (F)	Sphere and aggregates of spheres (F)	Hexagon (F)	Rod (F)	Total particle numbers counted per site
Home 1	230 (66)	1 (0.28)	104 (29.8)	1 (0.28)	23 (6.6)	338
Home 2	21 (87)	-	1 (4.1)	2 (8.3)	-	24
Home 3	170 (90)	-	18 (10)	-	-	188
Office 1	61 (81.3)	-	11 (14.7)	-	3 (4)	75
Office 2	42 (87.7)	-	2 (4.1)	-	4 (8.2)	49

(F)= (Frequency of observation of shape per site (%)) , - = Shape not observed

Some particles per site (Figure 9.10 to Figure 9.19) will be displayed alongside elemental contribution to spectra (Table 9.18 to table 9.22) and size distribution of the particles viewed (Figure 9.20) will be displayed in this section.

9.4.4.1 Particles in the indoor: shape and surface chemistry

This study was done in an attempt to characterise the indoor (home and work place) particles in order to possibly understand their sources or their physico-chemical properties.

One of the most significant observations from the assessment of the various TEM particle images in Figure 9.10a, Figure 9.11a, Figure 9.12a, Figure 9.13 i, Figure 9.13 ii, Figure 9.13 v, Figure 9.14a, Figure 9.15a, Figure 9.17a, Figure 9.18a and Figure 9.19a showed that most of the particles existed as aggregates of primary particles (PPs), agglomerates of PPs or agglomerates of aggregates. Also the particles rarely existed as a single metallic particle. They existed as “shapeless” not exactly spherical all the time and mixed with carbon as was also observed by other studies (Mayeux, 2004, Adachi and Buseck, 2008).

The EDS spectra revealed that the dominant elements across the entire study were Si, S, K and Fe: Table 9.18 Si 26%, S 27%, K 6.09%, Table 9.19 Fe 44%, Si 40%, S 11.82%, K 0.51% , Table 9.20 Fe 63%, S 61%, Si 46%, K 59%, Table 9.21 Si 58%, S 16%, K 30% and Table 9.22 Fe 74%, Si 50%, S 29% and K 44% and rest of the relative contribution percentage of the elements of the other metals can be seen in Table 9.18 to Table 9.22.

Chithra et al. (2013) observed Si as one of the dominant elements in their indoor study (school in their case) and they attributed it to geological materials such a soil, road dust etc.

9.4.4.1.1 Home 1: shape and surface chemistry of some of the particles viewed

Figure 9.10a, show to be an agglomerate and Figure 9.10 b shows the elemental map surface chemistry constituents of the particle. The TEM grid is Ni, however it is obvious that this particle contains Ni because the Ni map is more emphasised than the area of the grid where there is no particle. The PPs in Figure 9.10a are more obvious as compared to the other particles in this chapter with an average diameter of 112 nm. Figure 9.10b shows that this particle in addition to Ni contain Cr, Fe and Ti possibly from the same source, this because they appear to be dispersed in exactly the same manner.

Figure 9.11b also contain in addition to Ni, Cr, Fe and Ti showing the same pattern of dispersion and Figure 9.11b show Cr, Fe and Ti displaying the same pattern. This is indicative of the same source, possibly stainless steel. Stainless steel is the preferred cooking utensils used in homes and also stainless steel is used for working surfaces and other building materials in the home. During collection of TEM sample in Home 1 there was cooking activity going on for one hour of the four hours of sample collection including other house chores. Cr is a component of stainless steel and stainless materials are used in the homes (Taner et al., 2013). Cr, Fe, and Ni can also be leached from the stainless steel wok and

spatula when subjected to high temperatures during cooking or are combustion by-products of the burner.

In addition to Cr, Fe, Ni, these particles contained other alloying elements added to enhance the structure and properties of stainless steel they include Ti, Cu and Mo.

Figure 10a and Figure 11a look like soot particles and also because they contain S could imply they are from a combustion source. It could also be that the S coagulated with the particles. Also they both have Si in the agglomerate. The Si could be from soil or road dust that coagulated with the particle.



Figure 9.10. a. Dark field of a particle image collected in Home 1 during indoor activities including cooking

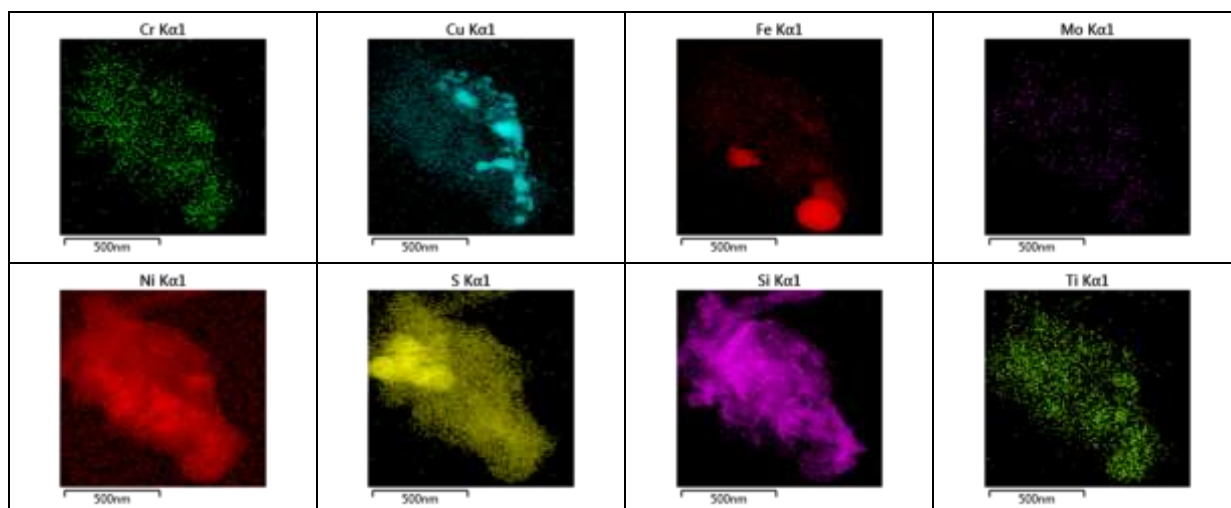


Figure 9.10. b. Elemental map: surface chemistry of the particle in Figure 9.10.a

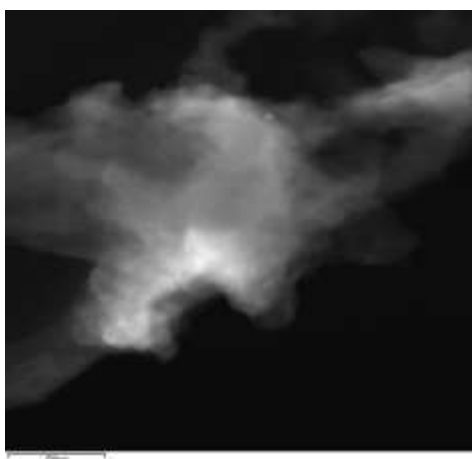


Figure 9.11. a. Dark field of a particle image collected in Home 1 during indoor activities including cooking

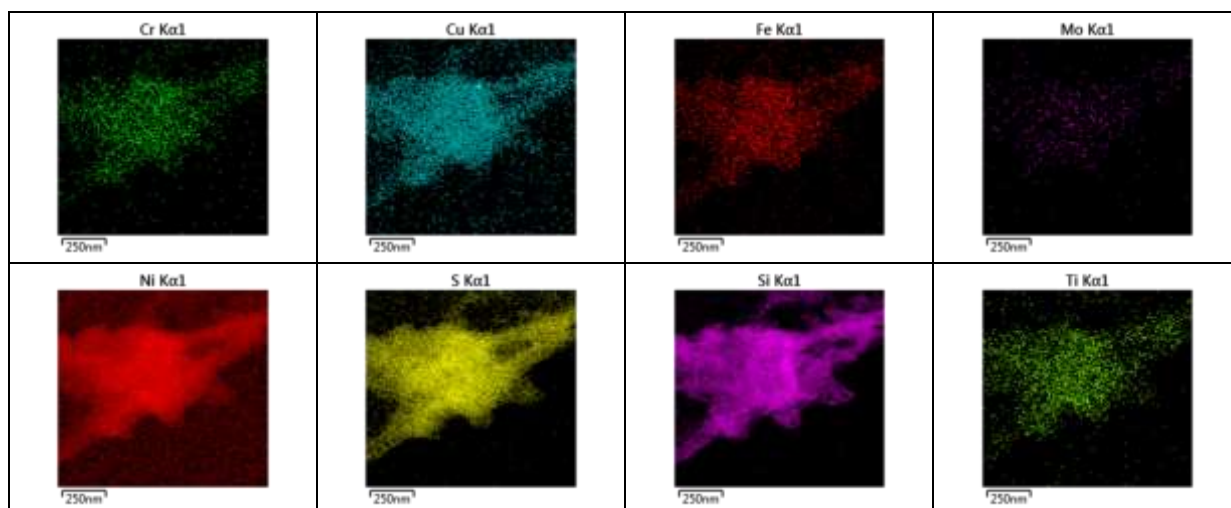


Figure 9.11. b. Elemental map: surface chemistry of the particle in Figure 9.11.a

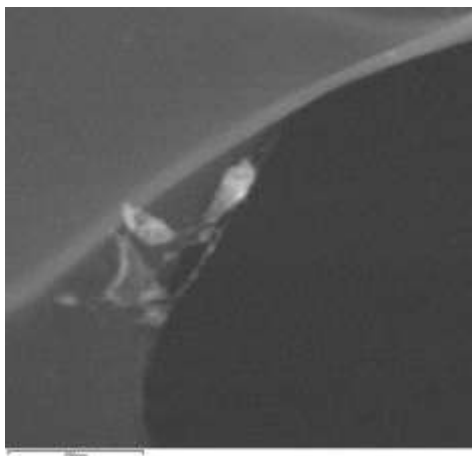


Figure 9.12. a. Dark field of a particle images collected in Home 1 during indoor activities including cooking

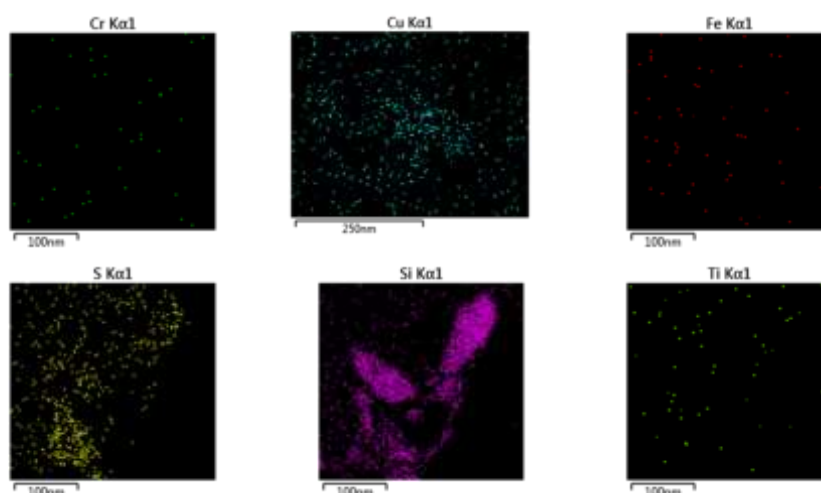


Figure 9.12. b. Elemental map: surface chemistry of the particle in Figure 9.12.a

Many studies on morphology and chemical composition of particles observed by electron microscopy (Toyokuni, 1999, Maskey et al., 2012, Bayr, 2005) have report different shapes of atmospheric particles such as spheres, aggregates, and irregular shapes etc., these different kinds of shapes have been observed indoors as shown in this chapter and Figure 9.13.

Research suggests that the morphology of particles are influenced by physical and chemical processes related to ambient conditions (Bayr, 2005).

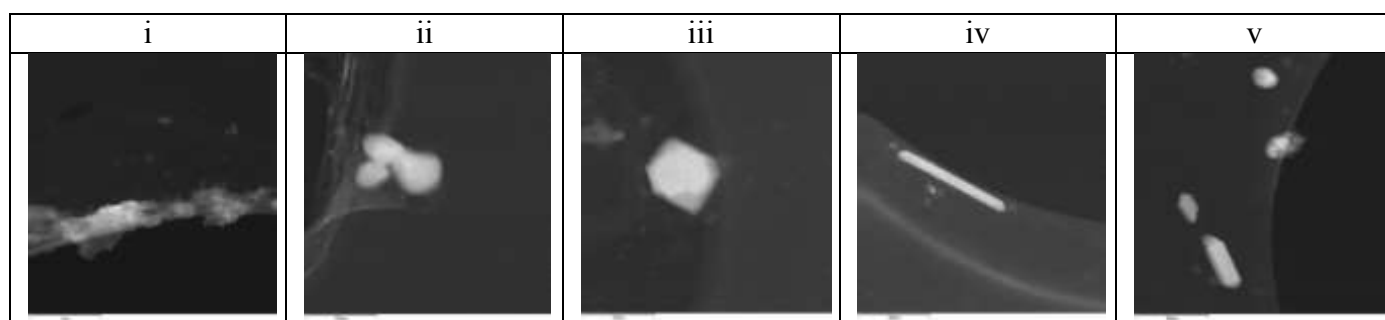
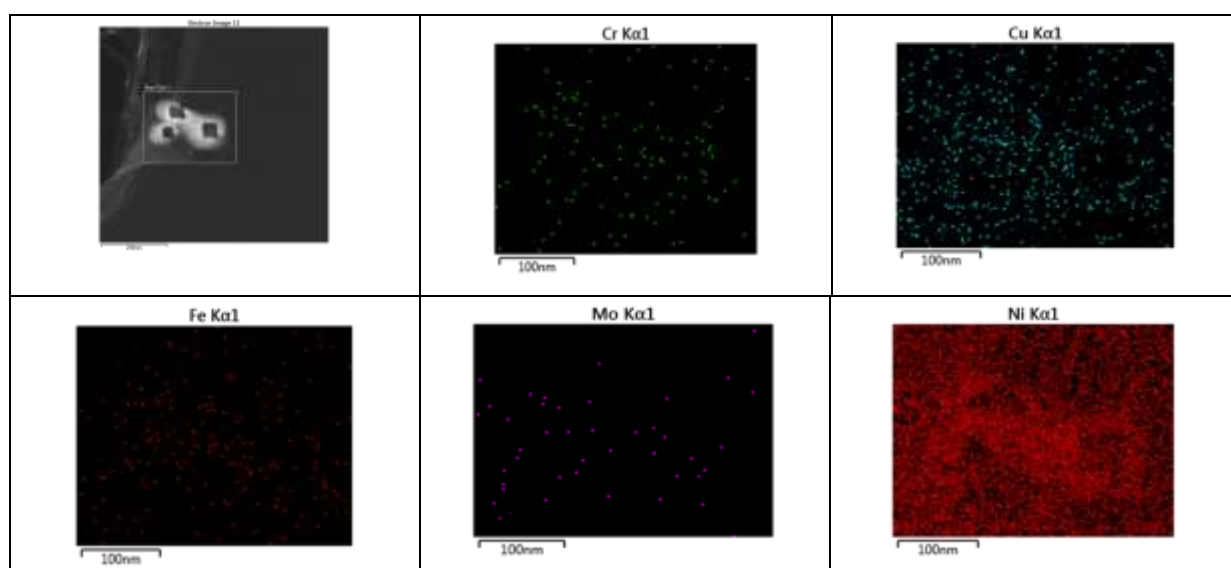


Figure 9.13. a. Overview of other different types of shapes found in Home 1

In Figure 9.13 b show the evaporation of volatile specie from Figure 9.13a. ii. This specie is sensitive to the electron beam. This evaporation takes place within few seconds under strong

electron beam. This particle is composed mainly of sulphur and other researchers have also identified particles containing sulphate that have such behaviour under strong beam under the TEM (Maskey et al., 2012, Toyokuni, 1999). Though the spectra is not shown here, the particle EDS elemental map show the evaporation of the sulphur from the particle in Figure 9.13b. Other particles that evaporated were found and all of them showed high average weight of S and Ca in them though they are not put into this chapter. Studies have suggested that these particles that contain S that decomposes under the electron beam are coated with organic materials and they suggested that S that forms the sulphates originate from petrol and diesel fuel (Adachi and Buseck, 2008). Another suggested that these particles that evaporate under the electron beam are regarded as mixtures of semi and/or less volatile organic species and ammonium sulfate/bisulphate (Maskey et al., 2012) and another suggests that ambient soot particles which have been processed for few hours are heavily internally mixed, primarily with ammonium sulphate (Geng et al., 2010). This suggests that the particles that contain these sulphates could be from outside sources infiltrating into the indoors.



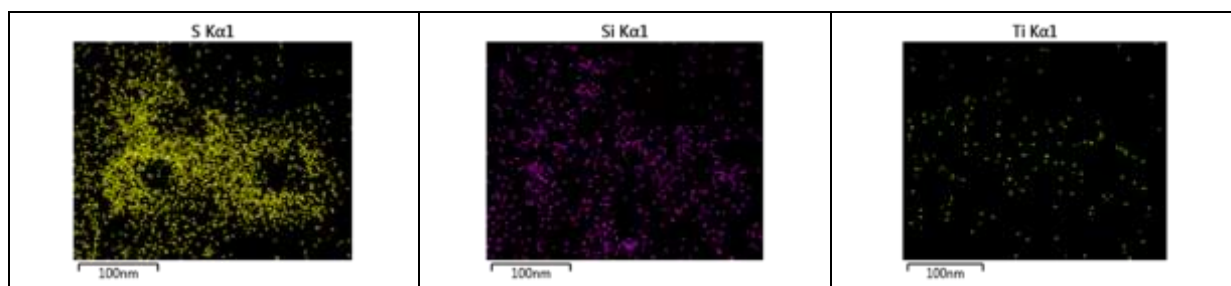


Figure 9.13 b. Dark field images showing evaporation of sulphur particles and surface chemistry of the agglomerate particle in Figure 9.13 a. ii.

Table 9.18 show the individual elemental contribution in terms of average weight (%) and the elemental relative percentage contribution of each element to all the particle images analysed from Home 1. Most of the particles collected were Ca and S and Si rich.

Table 9.18. Elemental relative contribution to spectra for the entire particles in Home 1

Elements	Average Weight (%)	SD	N	% Contribution
Si	26.34	31.83	43	18.31
S	27.22	22.24	43	18.92
K	6.09	-	1	4.23
Ca	58.90	24.67	20	40.94
Ti	0.79	2.85	22	0.55
Cr	0.74	3.30	24	0.52
Fe	4.83	17.58	41	3.36
Cu	7.91	18.47	35	5.50
Sn	5.17	-	1	3.59
Mo	5.87	1.95	24	4.08

SD=standard deviation

9.4.4.1.2 Home 2: shape and surface chemistry of some of the particles viewed

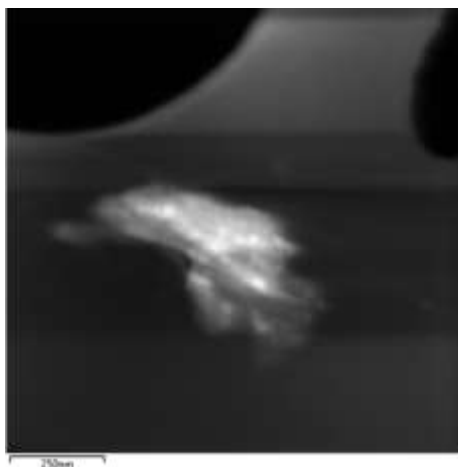


Figure 9.14. a. Dark field of a particle image collected in Home 2 during indoor activities

Figure 9.14b Cl is identified in the agglomerate mixture. This agglomerated particle contains Al, Ca, Si and all these are of soil and mineral origin. Therefore Since the Cl is mapped the same way as the Al and Ca it is of a clay mineral origin. However the main element found in the particles collected in this Home is Fe whereas the Cl makes up only 1.39% of the total elemental contribution (Table 9.19).

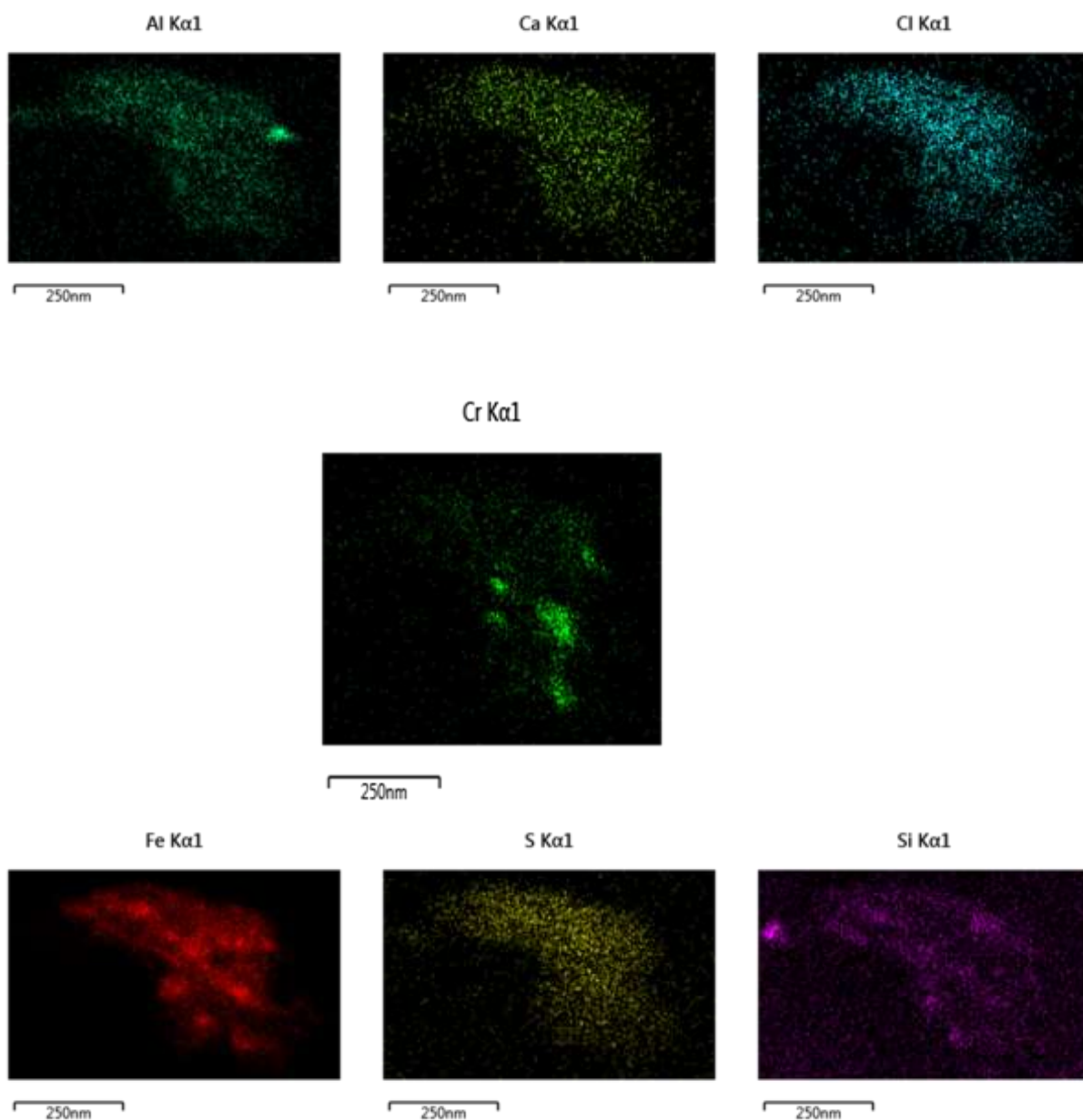


Figure 9.14. b. Elemental map: surface chemistry of the particle in Figure 9.14.a

Table 9.19 show the elemental contribution in terms of average weight (%) the elemental relative percentage contribution of each element of all the particle images analysed from Home 2. Fe then next to Si are the dominant elements found in this site during the sampling.

Table 9.19. Elemental relative contribution to spectra for the entire particles in Home 2

Elements	Average Weight (%)	SD	N	% Contribution
Na	16.39	21.31	2	12.53
Al	2.45	1.52	4	1.87
Si	40.24	43.67	8	30.76
P	0.85	0.29	3	0.65
S	11.82	14.86	6	9.04
Cl	1.82	1.36	3	1.39
k	0.51		1	0.39
Ca	4.01	5.76	4	3.06
Cr	7.08	7.86	4	5.41
Fe	44.01	27.75	4	33.64
Cu	1.65	0.26	2	1.26

SD=standard deviation

9.4.4.1.3 Home 3: shape and surface chemistry of some of the particles viewed

From the elemental map in Figure 9.15 b, it is obvious that the O follows the same pattern as the Fe and Cr indicating that the particle (Figure 9.15a) is an oxide of Fe or oxide of Cr. It could be a rust particle as rust exists most commonly as oxides of Fe.

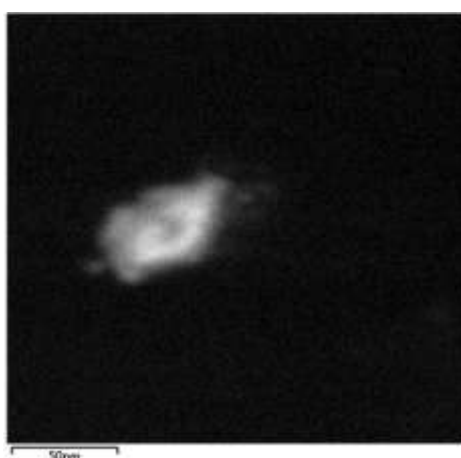


Figure 9.15. a. Dark field of a particle image collected in Home 3 with no indoor activities

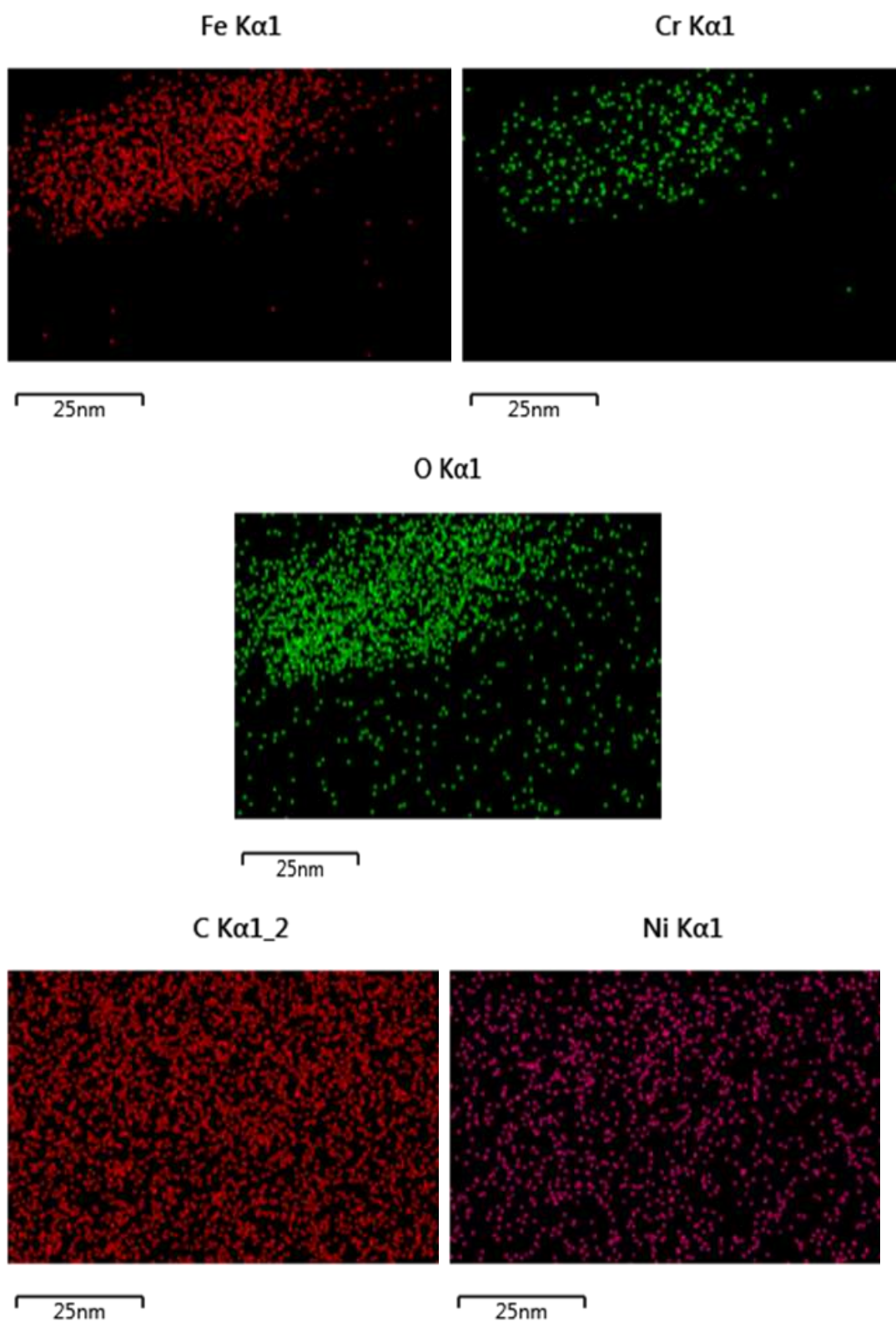


Figure 9.15. b. Elemental map: surface chemistry of the particle in Figure 9.15.a

Table 9.20 show the elemental contribution in terms of average weight (%) and elemental percentage contribution of each element to the particle images analysed from Home 3. Be showed the highest % contribution next to Fe in Home 3.

Table 9.20. Elemental relative contribution to spectra for the entire particles in Home 3

Elements	Average Weight (%)	SD	N	% Contribution
Be	100		1	27.37
Na	20.49		1	5.61
Si	45.78	37.96	7	12.53
S	61.56	36.31	6	16.85
k	59.37	29.05	4	16.25
Cr	15.43		1	4.22
Fe	62.80		1	17.19

SD=standard deviation

Figure 9.16 show the image of a single particle and spectrum of the particle showing that the particle is beryllium (Be). Be was unique to only Home 3 of the entire particles analysed with TEM/EDS images for this study. Also no indoor activity was going on during the 2 hours sampling. Therefore in the absence of no indoor activity, Be is found in electronic products but they are enclosed, it is most likely from an outdoor source such as the soil.

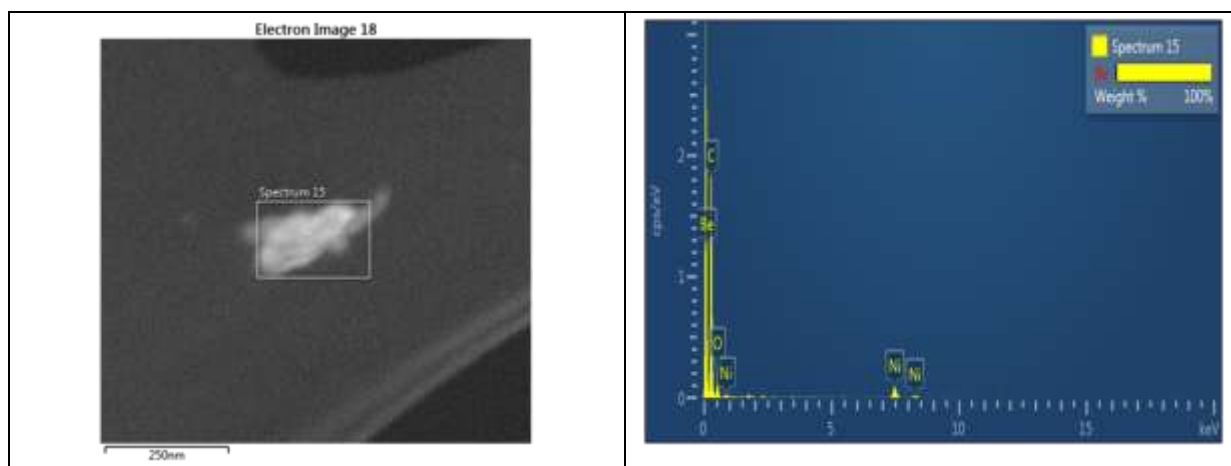


Figure 9.16. Dark field of a particle image and spectrum collected in Home 3 no indoor activities

9.4.4.1.4 Office 1: shape and surface chemistry of some of the particles viewed

Figure 9.17 a show an aggregate particle and Figure 9.17 b show the elemental map. The Elemental map in Figure 9.17 b indicates that this particle contains a mixture of S, Na, K, Si, C and O. It has the similarities of the doughnut shaped particle described by Maskey et al. (2012). This particle could be from soil origin because of the Na, K and Si.

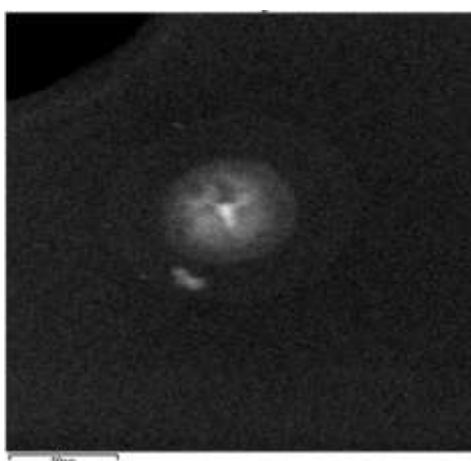


Figure 9.17. a. Dark field of a particle image collected in Office 1

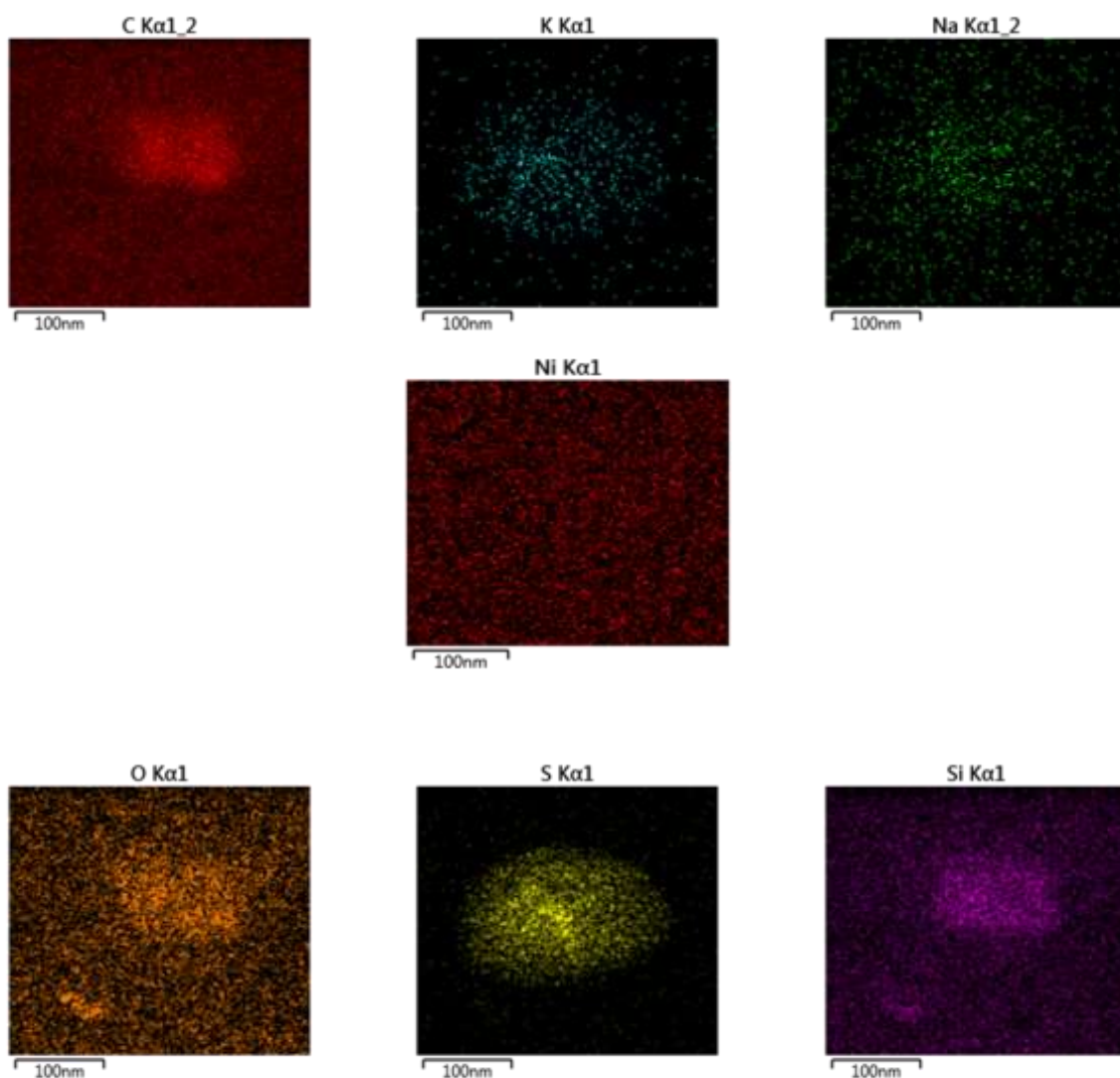


Figure 9.17 b. Elemental map: surface chemistry of the particle in Figure 9.17.a

The particle in Figure 9.18a is from a combustion source because it is rich in S, K, C and O. The Pb, Ni, Rb and Fe are from road dust (Møller and Loft, 2010) giving that the windows of the office was open and the window faced a background road in the University and a car park (that parks about 10 cars) with a grassy area and also a canal not too far away. The Cl and the Na could be from clay minerals or sea salt. While the Na is from crustal origin (Mayeux, 2004). This particle also contained F which is also from marine origin.



Figure 9.18. a. Dark field of a particle image collected in Office 1

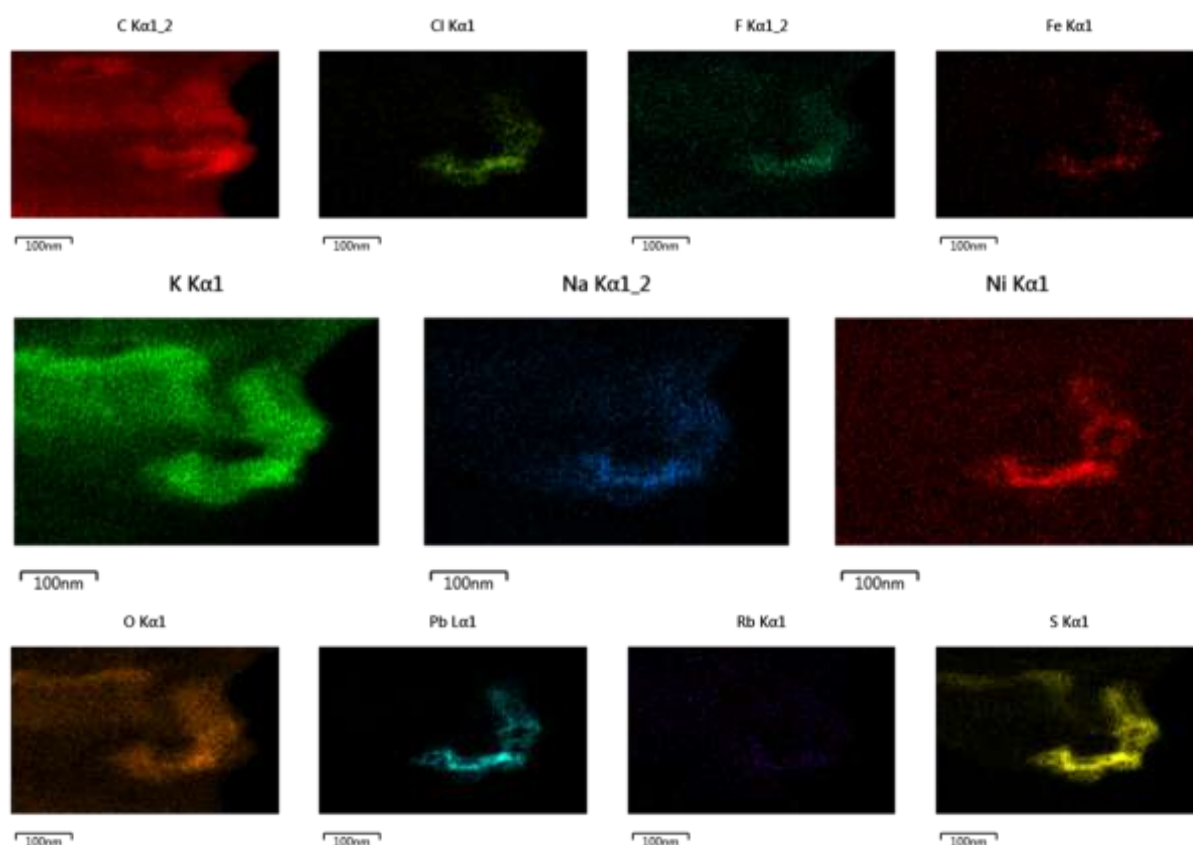


Figure 9.18.b. Elemental map: surface chemistry of the particle in Figure 9.18.a

Table 9.21 show the elemental contribution in terms of average weight (%) and elemental percentage of the total percentage contribution for the elements of all the particles images analysed from Office 1. The particles in this office are rich in Si, Pb and K.

Table 9.21. Elemental relative contribution to spectra for the entire particles in Office 1

Elements	Average Weight (%)	SD	N	% Contribution
F	2.70		1	1.82
Na	3.78	2.69	3	2.54
Si	57.89	36.56	10	38.93
S	15.64	15.71	8	10.52
Cl	1.30		1	0.87
k	30.08	23.73	5	20.23
Zn	0.78	0.28	2	0.52
Rb	2.99		1	2.01
Pb	33.52	9.33	3	22.55

SD=standard deviation

9.4.4.1.5 Office 2: shape and surface chemistry of some of the particles viewed

Figure 9.19 a show particle form Office. Office two is a Map room and photocopying activities takes place in it. Most of the primary particles in this particle have formed strong bonds by chemical forces, thereby making the primary particles less obvious. The particle is an aggregate of a mixture of different elements as seen in Figure 9.19 b. Sources of the elements found in this particle based on literature could include metals found in printer toners Ni, Mn, Fe and Si (Stocks and Hislop, 2002). Ca and Mg are constituents of paper (Stocks and Hislop, 2002). Ca, K, Mg, Si and Fe are from crustal origin. This particle also contains Co.

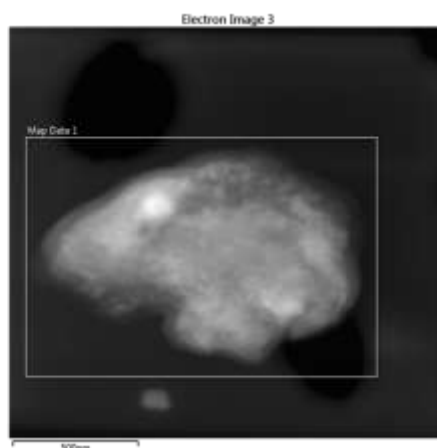


Figure 9.19. a. Dark field of a particle image collected in Office 2 (Map room and Photocopying/printing room)

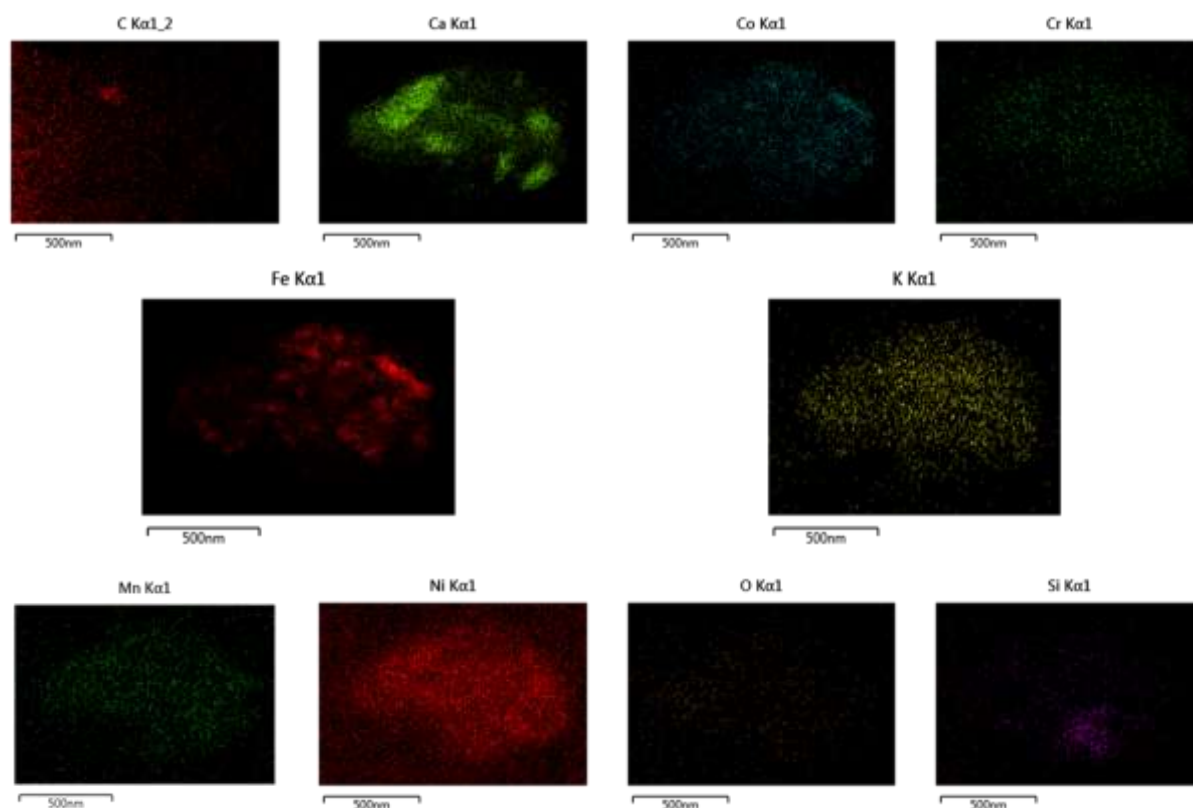


Figure 9.19.b. Elemental map: surface chemistry of the particle in Figure 9.19.a

Table 9.22 show the elemental contribution in terms of average weight and elemental percentage of the total percentage contribution for the elements of all the particles images analysed from Office 1. The particles in this office are rich in Si, S and K.

Table 9.22. Elemental relative contribution to spectra for the entire particles in Office 2

Elements	Average Weight (%)	SD	N	% Contribution
Na	23.44	5.70	4	8.37
Si	49.92	47.39	10	17.82
S	29.36	12.32	10	10.48
Cl	17.93	25.78	3	6.40
k	44.11	32.22	13	15.75
Ca	23.43	17.00	2	8.37
Cr	14.94		1	5.33
Mn	3.27		1	1.17
Fe	73.67	15.28	3	26.31
Co	0.22		1	0.08

SD=standard deviation

9.4.4.1.6 Summary of all the elements and compounds observed

Table 9.23 presents the summary of the elements observed and discussed for the TEM analysis in Figure 9.10 to Figure 9.19 and Table 9.18 to Table 9.22.

Table 9.23. Summary list of elements observed and their possible sources

Elements (Comments)	Possible sources	Location/activity
Agglomerates containing Ni, Cr, Fe, Ti, Cu, Mo (Several similar agglomerate mixture)	Stainless steel: from cooking utensils and coating of finishes in the house.	Home 1/ Indoor house chores and cooking
Sn (Observed only in Home 1)	Road dust re-suspension	Home 1/Indoor house chores and cooking
Al, Ca, Si, P, Na, Cl	Soil, clay mineral, marine origin (i.e. from water body spray)	Home /Indoor house chores: cleaning, sweeping, resuspension of particle due to the chores
Be (Observed only in Home 3)	Soil, vegetables	Home 3/No indoor activities just people moving around
Na, Mg, Ca, F, Na, Cl	Soil/crustal origin, marine origin i.e. from water body spray	Office 1/ Window open opposite a car park that parks about 12 cars, background road in the university, grassy land area beside window and a canal not far away
Pb, Rb (Observed only in Office 1)	Vehicular emissions	Office 1/ Window open opposite a car park that parks about 12 cars, background road in the university, grassy land area beside window and a canal not far away
Ni, Fe, Si, Co, Mn, Ca, Mg	Constituents of paper, printer toner	Office 2/Map room, photocopying and printing
Ni, Cr, Cu, Fe, S, Si, Na, Cl, Ca, K,	Vehicular emissions (exhaust and non-exhaust), road dust and wear and tear of building materials, Soil/crustal origin, marine origin i.e. from water body spray	Observed in all sampling locations

9.4.4.2 Particle size distribution of particles measured in each site

As shown in Figure 9.20 different particle sizes collected on the Ni grid mounted on the < 250 nm size fraction of the impactor were viewed. For each site, the mean particle size and numbers of particles viewed are: Home 1 is 39.54 nm based on the 338 particles counted on the grid, Home 2 is 92.58 nm and 24 particles were counted, Home 3 is 60.52 and 188 particles were counted, Office 1 is 34.63 nm and 75 particles were counted and finally Office

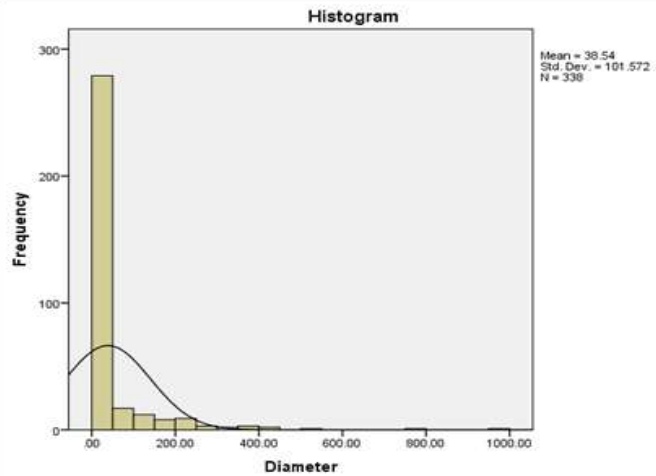
2 showed a mean of 102.64 and 49 particles were counted. These particles were collected on the < 250 nm size fraction therefore it would be expected that the sizes observed will be ≤ 250 nm but the size distribution (Figure 9.20) show that the size of some of the particles collected were about 1000 nm. This is most likely because as the single particles or primary particles (PP) or loosely attached particle (agglomerates) come in contact with each other during collection, they become tightly attached by metallic, ionic or covalent bonds forming aggregates. Some of these aggregates appear as a single particle with a diameter size as large as 1000 nm but was collected on a < 250 nm size fraction of the impactor and for example see Figure 6.19a. It could also be due to particle bounce during sampling. Studies have shown that due to the physical principle of particle collection associated with all impactors sampling artefacts like particle bounce from a larger size cutoff above into the next stage can occur (Wall et al., 1988, Fonseca et al., 2016). Fonseca et al. (2016) suggests that particle bounce particle bounce can only be expected when using the cascade impactor during dry collect or indoors like in the case of this study. Frequency at which the size ranges of the particles observed during the TEM analysis is shown in Figure 6.20.

The smallest mean was observed in Office 1 and then very closely Home 1 (Figure 9.20). This is not surprising because Office 1 was sampled in the summer and the office window was opened. The opened window faced a car park that parks 12 cars and a background road (inside the university). Therefore the small sizes observed maybe vehicular related pollution filtering into the office from the open window.

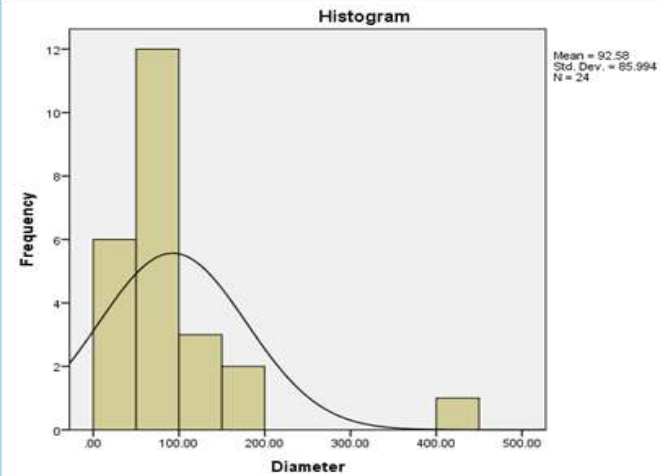
Home 1 had the highest amount of particles counted on the grid not only because it sampled for the longest amount of time but various activities indoor actives e.g. cooking, sweeping and other house chores were in progress during collection of sample. This cooking activity also accounts for the small mean size or diameter of particle collected. This is around what other researchers have observed. A study observed mode diameters of 40 nm, 50 nm, and 30

nm during scrambling eggs, frying chicken and cooking soups respectively (Hinds, 1999). Another study showed that cooking processes produced peak number of particles between 22 – 72 nm (Dennekamp et al., 2001a). Generation of particles in Home 1 and Home 2 resulted from cooking activities, cleaning and movement of people and Home 3 no indoor activity only movement of people. These activities have been suggested by researches to have significant impact on indoor particle concentration (Riva et al., 2011). The mean diameter measured demonstrates that indoor activities/sources, outdoor particle infiltration, factors affecting indoor particle concentrations and particle loss mechanism affects the particle size diameter (Wallace et al., 2013). Home 2 and Office 2 appeared to have a bimodal distribution but the primary mode was in the < 100 nm. While Home 1, Home 3 and Office 1 showed single mode with prevalent particle size also in the < 100 nm (Figure 9.20).

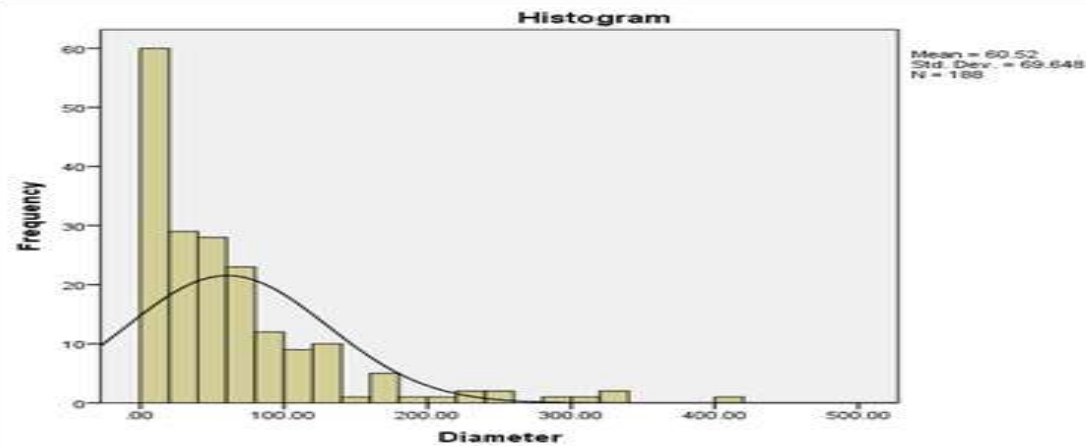
Home1



Home 2



Home 3



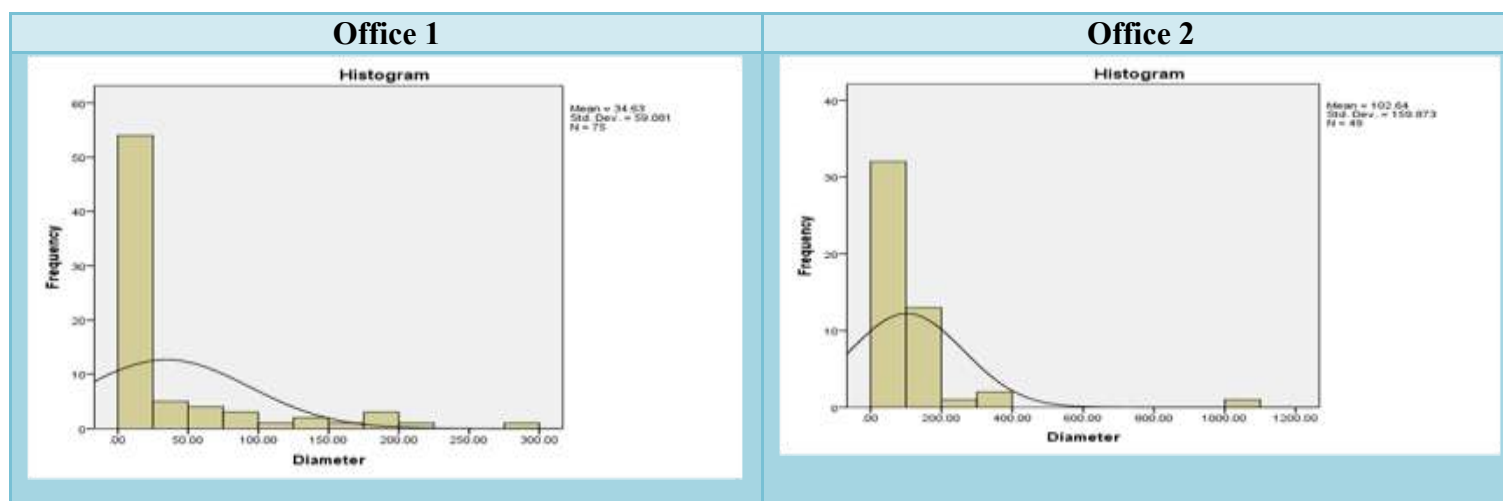


Figure 9.20. Size distribution of particles on the $< 250 \text{ nm}$ ($< 0.25 \mu\text{m}$) for all the sites

9.4.4.3 Consequences of the sizes, shapes and surface chemistry of the particles analysed with the TEM/EDS

Understanding human exposure to the particles in the indoors regardless of their size is important in order to understand how these particles affect humans and possible control measures. This is especially for particles in the ultrafine range because of their properties (properties already discussed in chapter 1). From the particles collected indoors (home and offices) different particle sizes and shapes with different/similar surface chemistry compositions were observed in TEM/EDS analysis (Figure 9.10 to Figure 9.19). Of these particles, size is one of the most important characteristics because smaller size particles have the ability to penetrate deeper into the respiratory tract. For example, a study by Renwick et al. (2004) showed that ultrafine BC and TiO₂ particles administered to rats, induced inflammation and epithelia damages to a greater extent than their bulk particle counterpart (Renwick et al., 2004). Of the particles viewed under the TEM, the particles with their sizes as < 100 nm appeared to have the highest frequency (Figure 9.20). When particles are inhaled, they are deposited throughout the entire respiratory tract and the smaller the particles, the deeper it penetrates into the lungs. The lung burden (rate of deposition and clearance) of these particles depends on the characteristics of the size, shape and chemical characteristics of the particle. Smaller particles as observed in this study have higher toxicity in comparison to larger particles because of their large surface area thus higher chemical reactivity. They also have longer resident time in the lungs since smaller particles can easily evade clearance system and phagocytosis. Different shapes were observed: irregular, spherical, non-spherical, rods, fibre, hexagon, agglomerates and aggregates (Figure 9.10 to Figure 9.19). Particles as observed in this study, exists more as aggregates and agglomerates as compared to single particles. Therefore some of them have sizes ≥ 100 nm. Thus when these aggregates and agglomerates are inhaled, they can be removed by the alveolar

macrophage through phagocytosis but their clearance depends on the shape of the particle. Non-spherical particles have higher surface area than the spherical particles of comparable aerodynamic diameter, thereby providing potentially more surface contact for interaction with the lungs (Carabali et al., 2012). Bound to these particles are trace metals, organic species and other compounds (Figure 9.10 to Figure 9.19). For example, insoluble compounds cause inflammation and oxidative stress by direct particle reaction with the airways as compared to soluble compounds that are dissolved and can pass into the circulatory system (White et al., 2012, Knaapen et al., 2002). The insoluble particle accumulate and if the macrophage clearance capacity is exceeded, then the lung defence mechanisms are overwhelmed, resulting in injury to the lung tissue (Buzea et al., 2007). Some of these chemical components have the ability to trigger inflammation, oxidative stress and other negative health effects. The size, shape and even the chemical components of these particles, determine the depth of inhalation into lungs, the deposition rate in the lungs and lung burden. Therefore further study on indoor particle sources and physico-chemical properties is recommended.

9.4.5 Biomarkers

Three early morning urine samples and EBC samples were collected from each subject during the sampling week. That is one set of both the urine and the EBC at the start of sampling, the second set of samples were collected midweek and the last set samples were collected on the last day of the sampling. The biomarkers 8-oxodG in the urine and nitrate, nitrite and pH in the EBC were then analysed.

One subject's urine and EBC sample for the summer was contaminated and therefore was excluded. For each subject the average of the 3 urinary 8-oxodG was average and used for the correlation analysis. Therefore there were 8 samples for the summer and 9 samples for the winter. The 9 urine samples representing the summer and the 9 urine samples for the winter were pulled together so that it became 17 samples (urinary 8-oxodG) so that the result could have statistical power. The same was done for the nitrate, nitrite and pH in the EBC.

9.4.6 Urine biomarker

8-oxodG was measured in the urine. All the 8-oxodG samples were corrected with creatinine.

9.4.6.1 8-oxodG ng/mg creatinine concentrations

The 8-oxodG concentrations in this chapter were within the range measured by other researches for healthy subject. References can be found in previous chapters. The descriptive summary of the 8-oxodG concentration is displayed in Table 9.24.

Table 9.24. Summary of biomarkers

Biomarker	Mean (SD)	Minimum	Median	Maximum
8-oxodG(ng/mg creatinine)	3.37 (1.76)	0.95	3.37	7.34
EBC-pH	7.50 (0.22)	6.84	7.54	7.77
EBC-Nitrate (μM)	24.18 (14.55)	4.83	23.45	44.78
EBC-Nitrite (μM)	1.08 (0.41)	0.62	1.27	1.98

9.4.7 Exhaled breath condensate

pH, nitrate and nitrite concentrations were measured in the EBC.

9.4.7.1 EBC-pH

As mentioned in the previous chapters, acidity of the airways measured as decrease in EBC-pH is a biomarker of airway inflammation. The EBC-pH concentrations in this chapter were within the range measured by other researches for healthy subject. References can be found in previous chapters. The summary concentration of the EBC-pH is displayed in Table 9.24.

9.4.7.2 EBC-Nitrate

The summary concentration of the EBC-Nitrate is displayed in Table 9.24. The mean concentration of nitrate is higher than the nitrite and the inter-nitrate concentration deviation is also higher in comparison to nitrite. Chladkova et al. (2006) in their study with 20 healthy and 20 controlled asthmatic patients as the control, they concluded that concentration of nitrate was found to be 2 to 3 times higher than that of nitrite and the variability between nitrates was also higher than the nitrite. In this study the nitrate concentrations measured were within the concentration in other studies (Chladkova et al., 2006, Corradi et al., 2003) and level of nitrate in this study is more than 3 times higher than the nitrite.

9.4.7.3 EBC-Nitrite

The EBC-Nitrite concentrations in this chapter were within the range measured by other researches for healthy subject. References can be found in previous chapters. The summary concentration of the EBC-Nitrite is displayed in Table 9.24.

9.4.8 Relationship between Particulates and biomarkers

The relationship between the PM concentrations of the different size fractions measured in the summer and winter were pulled together making it 18 homes. The home for the subject who had contaminated sample was removed as there was no biomarker for comparison. Therefore the comparison was based on 17 homes. The same was applied to the elemental and biomarker relationship.

9.4.8.1 Relationship between measured PM and biomarkers

Table 9.25 show the relationship between the measured PM in the different size fractions and the biomarkers. The < 250 nm size range significantly correlated with 8-oxodG, while the other size fractions didn't show significant correlations but positive associations were observed. Oxidative damage to DNA measured as 8-oxodG has been associated with exposure to polluted urban air (Loft et al., 1999). The significant correlation between the UFP size range (<250 nm) and 8-oxodG, agrees with the hypothesis that oxidative stress may be mediated by PM especially PM in the UFP range (Risom et al., 2005, Vinzents et al., 2005). In a study with 894 children, concentrations of PM₁₀ and PM_{2.5} measured by stationary monitors during a 7-day interval before sampling before sampling, were identified as independent factors significantly correlating with 8-oxodG (Svecova et al., 2009a). Studies have reported that associations between exposure to UFP and Fine particles with systemic oxidative stress (8-oxodG) respectively (Lin et al., 2015b, Schröder and Krutmann, 2005). Another study with two non-smoking security guards, were sampled for 29 days, the

pre-workshift urinary concentrations of 8-OHdG were associated with PM_{2.5} concentrations at the background site, while the post-workshift 8-OHdG concentrations were significantly and positively correlated with PM_{2.5} mass measured at the worksite (Wei et al., 2009). In a study where PNC and PM_{2.5} were measured at two locations within 9 km from the residence and workplace of 125 participating Beijing residents and UFPs and PM_{2.5} measured 1–7 days prior to biomarker measurements (lags) (Schröder and Krutmann, 2005). 8-oxodG was significantly correlated with PM_{2.5} at lag 1 and lag 2 while 8-oxodG was significantly correlated with UFP from lag 3 to lag 7 (Schröder and Krutmann, 2005). They suggested that the potential differences in timing of “actions” between UFPs and larger particles PM_{2.5} on the respiratory and the circulatory systems, is due to the differences between UFPs and PM_{2.5} in their airway interaction i.e. production of biomarkers are likely to reflect their differences in deposition, clearance, and translocation after inhalation (Schröder and Krutmann, 2005). Thus, the UFP compared to the PM_{2.5} appear to be cleared more slowly and retained longer within the lung after deposition (Schröder and Krutmann, 2005). In this present study, lag days were not measured. A one week measurement of the different size fractions were obtained, and three 8-oxodG samples (first day of sampling, mid sampling and last day of sampling) and then the mean of the 3 samples were used for each subject. It could that the concentration of the < 250 nm particles is more reflective of the exposure biomarker 8-oxodG because of their size thus longer retention in the airways to induce biological effects. Therefore the longer the interaction between the airways and the particle due to continued exposure, the possibility of increased production of ROS/RNS that overwhelms the defence system thereby leading to ROS/RNS interactions with macromolecules and DNA damage. However these studies mentioned above were carried out with the ambient air concentrations, where fractions and constituents of the PM may be different from those obtained indoor (home). Also, an in vitro study demonstrated that ambient PM have the

ability to produce 8-oxodG lesions in DNA (Knaapen et al., 2002). The indoors unlike the outdoors, provided prolonged periods of generally low-intensity exposure, with activities like cooking related to high exposure and constituents of the PM reflecting the indoor activities in addition to those of the outdoors that penetrated into the indoors. This present study shows that PM in the UFP range (< 250 nm) measured in the indoors are capable of inducing oxidative stress. Thus, suggesting that PE to the particles in < 250 nm size range in the indoor home environment is a predictors of 8-oxodG. These homes are non-smoking homes and the subjects are healthy non-smoking subjects. Therefore, the presence of tobacco smoke, which is a major source of fine and ultrafine particles and predictor of 8-oxodG, was highly unlikely in the homes of the subjects and unlikely to affect the 8-oxodG concentrations of the subjects.

EBC-pH did not significantly correlate with the different size fractions measured in this study (Table 9.25). However, all the insignificant decreases were negative except for in the 250 - 500 nm size range (Table 9.25). Studies have shown that airway acidity measured as decrease in EBC-pH is due to increase in airway inflammation associated with people with respiratory and inflammatory airway diseases (Aarnio et al., 2005, Rank et al., 2001, Hunt et al., 2000, Gessner et al., 2003, Ricciardolo et al., 2004a). Other studies have associated exposure to PM, with decrease in EBC-pH both in healthy smokers, healthy non/never smokers, and subjects with cardiovascular diseases. Koskatis et al. (2012) observed in 18 healthy never smokers that decreased immediately after exposure to secondhand smoke ($P < 0.001$) as compared to base line and returned to baseline by 180 min, but concentrations of pollutants were not measured. In two studies, increase in EBC-pH were observed after exposure to welding metal fumes in healthy welders than in control subjects (Boyce et al., 2006, Lundberg and Weitzberg, 2010). The increases in their study could be attributed to the metal components of the particulates. It could also be that the welders have developed survivors

immunity effect especially as Fireman et al. (2008) showed that welders who have worked longer had more alkaline EBC-pH as compared to those with shorter exposure working time and then the control. However correlation between welding particulates and EBC-pH were not measured. A study observed that 64 % of 25 non-smoking commercial cyclists, who drive for 8- 12 hours per day for 5 days per week, showed a decrease in EBC-pH (Brant et al., 2014). No significant correlation was observed between EBC-pH and NO₂ measured 14 days before assessment rather a significant correlation ($P < 0.036$) was observed between NO₂ and impaired mucociliary clearance (Brant et al., 2014). In a study with 87 non-smoking healthy male subjects working as street controllers or as office-workers, the street controllers showed significantly decreased EBC-pH as compared to the office-workers (Lima et al., 2013). PM₁₀ concentration was higher in the street controller work as compared to office work and no significant association was observed between PM₁₀ and EBC-pH (Lima et al., 2013). A study with 39 adults with prior myocardial infarction (>6 months), their PE measurements of PM₁₀, PM_{2.5}, and PM_{0.25} were taken over 24 hours and they observed a positive correlations between pH and all PM fractions (Folino et al., 2009). McCreanor et al. (2007), in their study with 60 mild to moderate asthma observed a decrease in EBC-pH after a two hours exposure to diesel traffic on busy Oxford street and decrease in EBC-pH was significantly associated ($P < 0.02$) with UFP but no association with PM_{2.5}. With similar group design as McCreanor et al. (2007), Zhang et al. (2009) also observed EBC-pH was significantly associated with UFP and NO₂ but no association with PM_{2.5} was observed. In a PE study with 32 asthmatic subjects followed for 2 years, with 6 EBC-pH measurements, PM was measured 24 hours before clinical assessment and no relationship was found between EBC-pH and PM_{2.5} and PM₁₀ (Maestrelli et al., 2011). In a study with 125 healthy volunteers biomarker of exposure including EBC-pH were measured alongside variety of air pollutants (PM_{2.5}, EC, OC, SO₂, O₃, NO₂) (Bogdan et al., 2000). Huang et al. (2012) observed an inverse significant

correlation with all the pollutants except O_3 that was a positive significant correlation. The negative association between $PM_{2.5}$ and EBC-pH were strongest at lags 0 and 1 days. While Patel et al. (2013) in their study, EBC-pH was collected twice a week from 18 asthmatics and 18 non-asthmatics over 4 weeks, they observed that associations of EBC-pH with $PM_{2.5}$, as a whole, were inconsistent; across the various lags days evaluated. This present study compared to the others referenced above, the PM with different size fractions were measured in the indoor (home). Therefore it could be that the low concentrations in the homes even though it is at a constant rate as compared to the high-traffic concentrations, traffic-related pollutants, cigarette smoke, and work exposure concentrations/ high metal components may exert greater effects on airway inflammation than pollutants in the indoor-home. Also EBC-pH is a biomarker expressed in response to challenge or irritation of the airways especially in short term exposures and or continuous high level of pollutant exposure due to occupational exposure. An increased risk of airway and lung inflammation, are likely to be more associated with the outdoors and occupational exposure. Therefore human activity pattern and environmental exposure could influence the outcome. This study also suffers from a small cohort-size that may influence the outcome of the small changes in EBC-pH which could in turn affect association with PM of different size fractions measured. Therefore further investigation with larger cohort-size is recommended, especially because there is no data on size fraction PM measurements with biomarkers in the indoor home environment.

As mentioned in previously discussed in the other chapters, nitrate and nitrites are relatively stable end products of NO, that have been measured in EBC (Chladkova et al., 2006, Laumbach and Kipen, 2010). They are implicated in the pathophysiology of respiratory diseases like asthma, COPD, and cystic fibrosis (Nadif et al., 2014) and used as a biomarker of oxidative stress in acute exposure to traffic related particle exposure (Laumbach et al.,

2014) and chapter 7 of this study, cigarette related exposure (Maynard et al., 2006, Balint et al., 2001), cooking fume exposure like in chapter 5 of this thesis, shisha smoke exposure chapter 6 of this thesis. EBC-nitrate and EBC-nitrite did not show any significant correlation with the size fraction except for EBC-nitrate and 2500 -5000 nm size fraction that showed a significant inverse correlation ($P < 0.01$) (Table 9.25). Unlike Manny et al. (2012) that observed a significant association between measured exposure to ambient coarse particles at central sites and EBC-NO_x (NO_x = nitrate + nitrite) and not with the indoor concentrations in their study of 133 volunteers with asthma or chronic obstructive pulmonary. However Manny et al. (2012) observed negative correlations like this study but not at a significant level. In another study involving 13 children with doctor diagnosed asthma, they observed that there was no significant association with EBC-nitrite with any of the fixed background and highway, and 7 mobile sites measured pollutants (PM₁₀, PNC, NO₂ and EC) (Ducret-Stich, 2014). The difference between this present study and Ducret-stich et al. (2014) study, is that they had child who suffered from asthma and asthmatic patients have higher nitrite levels and can be more sensitive to pollutant exposure (Hunt et al., 2000) while this study used healthy adults. EBC-NO_x were observed to be positively significant, and largest for PM_{2.5}, SO₄⁻², EC, OC, and SO₂ at lag 0, and CO and NO₂ at lag 1 and EBC-nitrite was positively associated with all pollutants, while EBC-nitrate varied more substantially in comparison to EBC-nitrite (Bogdan et al., 2000). In a controlled study on exposure to traffic related particles using 21 young healthy adults, PNC was significantly associated with EBC-nitrite and EBC-NO_x respectively (Laumbach et al., 2014). The correlation between EBC-nitrate could be per chance or the coarse particles have components that are not in the smaller size fractions. Patterns of EBC-nitrate and nitrite concentrations measured in addition to the small sample size, makes it unable to rule out undetected effects in cases where comparisons were not statistically significant.

Table 9.25. Relationship between PM and biomarkers

PM size fraction (nm)	8-oxodG (ng/mg creatinine)	EBC-pH	EBC-nitrate (µm)	EBC-nitrite (µm)
< 250	0.55****	-0.08	-0.19	-0.07
N	17	17	17	17
250 – 500	0.10	0.13	-0.29	-0.28
N	17	17	17	17
500 -1000	0.13	-0.61	-0.29	-0.39
N	17	17	17	17
1000 – 2500	0.414	-0.31	-0.48	-0.37
N	17	17	17	17
2500 – 5000	0.27	-0.18	-0.61**	-0.46
N	17	17	17	17

Correlation is significant at the ≤ 0.01 level (2-tailed), ** Correlation is significant at the ≤ 0.05 level (2-tailed), N=number of subjects

9.4.8.2 Relationship between elements and biomarkers

Studies have shown that PM have bound to them amongst other components metals (Pan et al., 2008, Lai et al., 2013). Table 9.26 show the relationship between elements in the < 250 nm size range and biomarkers. Na significantly correlated with EBC-nitrate and EBC-nitrite respectively, while Mn significantly correlated with 8-oxodG. A study in Dunkerque City, France showed that PM ≤ 250 nm which contained Na and Mn amongst other transition metals and components induced both 8-oxodG and NO in normal lung tissue (Svedahl et al., 2009) and relatively stable end product of NO is nitrate and nitrite (Chladkova et al., 2006, Laumbach and Kipen, 2010). Table 9.27: 8-oxodG against Cu, Mg against EBC-Nitrate and EBC-Nitrite respectively with elements bound to the 250 -500 nm. Table 9.28: Ca against EBC-Nitrate with elements bound to the 500 - 1000 nm. Table 9.29: Mn against EBC-Nitrite, Pb against EBC-Nitrite and EBC-Nitrate with elements bound to the 1000 - 2500 nm. Table 9.30: 8-oxodG against Ni, Ca and Mg with elements bound to the 2500 – 5000 nm and Cu showed a negative correlation with 8-oxodG. An *in vitro* study using alveolar macrophages suggested that indoor-generated fine particles was more bioactive than the ambient fine

particles (Zhang and Guttermann, 2007). Based on their suggestion another study concluded that PM in the indoor PE may contain fractions capable of inducing DNA damage that is not found in background fine particle concentration and that it is likely that PE could be more directly related to such biomarkers (Ghio et al., 2011). As we know, oxygen radicals are generated by environmental agents and by endogenous process. However, one of the proposed mechanisms of PM influenced oxidative stress induction is caused by transition metals contained by the PM (Khansari et al., 2009).

A study by Sorensen et al. (2005) indicated none of these transition metals (V, Cr, Ni, Pt, Cu, Fe) in their study was significantly associated with 8-oxodG in urine and in this study the Cu in the 250 -500 nm size range was significantly correlated with 8-oxodG. They suggested that this could be because Fe and Cu are tightly controlled in the intact organism (Khansari et al., 2009). It could be because they measured biomarker correlation with PM_{2.5} and in this study, Cu was in a smaller size range 250 -500 nm. Smaller size particles may have longer retention time and Cu is a redox active metal and a soluble metal (Siversten et al., 2002, Khansari et al., 2009). In another study, during 5 days of 20 workers exposure to residual oil fly ash and metal fumes during overhaul of oil fired boilers, they observed positive significant correlation between 8-OHdG and V, Mn, Ni and Pb (Kim et al., 2004). In comparison to Kim et al. (2004) this present study is an indoor study with lower metal concentrations.

The Mg bound to the 250 – 500 nm size range showed significant correlation with EBC-nitrate and EBC-Nitrite. Ca in the 500 – 1000 nm correlated with EBC-Nitrate. Mn in the 1000 – 2500 nm significantly correlated with EBC-Nitrite and Cu with EBC-pH. 8-oxodG showed significant correlation with Ni, Ca and Mg bound to the PM in the > 2500 nm size range. Various studies have shown that metals can solubilise and are taken up by the system

and also induce oxidative stress and inflammation. Due to their ability to participate in reduction/oxidation cycling, transition metals may cause acute lung injury by producing oxidative stress and inflammatory responses (Lai et al., 2013, Risom et al., 2005) leading to production of NO and further nitrate and nitrite.

As for Ni and pb, it has been suggested that the primary route for their toxicity is depletion of glutathione (leading to lipid peroxidation) and then binding to sulfhydryl groups of proteins (Ke et al., 2016, Siversten et al., 2002). Ni has been shown to inhibit DNA repair in a way that may play a role in its toxicity by increasing increases cellular ROS (Ke et al., 2016).

Studies have associated EBC-nitrate and EBC-nitrite with air pollution exposure (Bogdan et al., 2000). Again these metals catalyse ROS and RNS productions thereby inducing particle-induced NO formation: on exposure to particles, ROS and RNS generation is increased (Donaldson and Borm, 2006), because the lung cells are stimulated especially the alveolar macrophages, polymorphonuclear neutrophils and type II cells activating the production of ROS through the NADPH oxidase and the mitochondrial ETC and induce the release of the enzyme iNOS in the presence of L-arginine then reacts with oxygen in aqueous solution to form end product nitrate and nitrite. Zn was measured and was not significantly correlated with 8-oxodG. However, research suggests that Zn is a vital component of numerous proteins involved in the defence against oxidative stress and has been shown, that depletion of Zn may enhance DNA damage via impairments of DNA repair mechanisms (Siversten et al., 2002).

Studies have shown mechanisms by which metal fractions in PM can induce inflammation and oxidative stress using cell lines or *in vitro* studies (Knaapen et al., 2002, Ghio, 1999). They have shown that both soluble metals and insoluble metals can induce inflammation and oxidative stress through generating $\cdot\text{OH}$, by peroxidation of lipids and cytotoxicity (Ghio, 1999, Svedahl et al., 2009, Knaapen et al., 2002). Therefore, the result in Table 9.26 to Table

9.30 agrees with hypothesis that components of PM e.g. like the metals measured in this chapter can induce oxidative stress and inflammation. The results further show that indoor particles can cause such effect as the outdoor particles. Thus, agreeing with the hypothesis that indoor particles can be more genotoxic than outdoor air. Therefore this fulfils one of the objectives of this study that the indoor particle may be associated and firmly involved in oxidative stress and inflammation. However, it is worthy to note that the small sample size may have made it unable to rule out undetected effects in cases where comparisons were not statistically significant. This study was carried out with only 17 homes and corresponding subjects for the biomarkers and they are healthy and non-smoking subjects/homes therefore may not be representative of all homes. Therefore, a larger study group is recommended and in vitro study using particles from the homes will also add more credibility to the results.

Table 9.26. Relationship between elements in the < 250 nm size range and biomarkers

	Ni	Cr	Mn	Sb	Pb	Sr	Na	Ca	Fe	Cu	Zn	Mg
8-oxodG (ng/mg creatinine) N	0.26 10	0.09 16	0.67**** 9	0.09 16	0.18 17	-0.67**** 11	0.03 14	0.01 17	0.36 16	0.25 17	0.21 17	0.08 17
EBC-pH N	-0.09 10	0.94 16	-0.11 9	0.18 16	0.47 17	0.22 11	0.06 14	0.31 17	-0.16 16	0.46 17	0.23 17	0.41 17
EBC-nitrate (µm) N	-0.29 10	0.31 16	0.19 9	0.10 16	0.35 17	0.26 11	0.64**** 14	0.34 17	-0.16 16	0.29 17	0.16 17	0.38 17
EBC-nitrite (µm) N	-0.18 10	0.32 16	0.33 9	0.23 16	0.36 17	-0.29 11	0.64**** 14	0.004 17	-0.20 16	0.08 17	0.3 17	0.10 17

****correlation is significant at the 0.05 level (2-tailed) N =number of subject

Table 9.27. Relationship between elements in the 250 - 500 nm size range and biomarkers

	Pb	Na	Ca	Fe	Cu	Zn	Mg	Sn
8-oxodG (ng/mg creatinine)	0.24	0.27	0.39	0.34	0.49****	0.02	0.31	0.11
N	14	17	17	17	17	17	17	16
EBC-pH	0.05	0.12	-0.27	0.005	0.41	0.21	0.09	-0.34
N	14	17	17	17	17	17	17	16
EBC-nitrate (µm)	0.23	0.45	0.41	-0.24	0.47	-0.04	0.73**	0.22
N	14	17	17	17	17	17	17	16
EBC-nitrite (µm)	0.38	0.46	0.47	-0.25	0.29	-0.21	0.59****	-0.02
N	14	17	17	17	17	17	17	16

correlation is significant at the 0.01 level (2-tailed), **correlation is significant at the 0.05 level (2-tailed), N =number of subject

Table 9.28. Relationship between elements in the 500 - 1000 nm size range and biomarkers

	Sr	Na	Ca	Fe	Cu	Zn	Mg	Sn
8-oxodG (ng/mg creatinine)	0.20	0.24	0.25	0.11	-0.06	0.05	0.38	-0.17
N	10	17	17	17	16	17	17	16
EBC-pH	-0.45	0.14	-0.16	0.05	0.16	0.14	0.12	0.12
N	10	17	17	17	16	17	17	16
EBC-nitrate (μm)	-0.26	0.45	0.53****	-0.23	-0.13	0.05	0.39	0.21
N	10	17	17	17	16	17	17	16
EBC-nitrite (μm)	-0.01	0.35	0.39	-0.32	-0.33	-0.18	0.18	0.04
N	10	17	17	17	16	17	17	16

****correlation is significant at the 0.05 level (2-tailed), N =number of subject

Table 9.29. Relationship between elements in the 1000 - 2500 nm size range and biomarkers (EBC-Nitrate and nitrite are in μM)

	Mn	Pb	Na	Ca	Fe	Cu	Zn	Mg	Sn
8-oxodG (ng/mg creatinine)	0.44	-0.14	0.20	0.26	0.16	0.33	-0.08	0.29	0.03
N	9	10	17	17	17	17	17	17	17
EBC-pH	-0.17	0.77**	0.01	-0.16	-0.05	0.53****	0.28	-0.03	-0.09
N	9	10	17	17	17	17	17	17	17
EBC-nitrate (μM)	0.44	0.69****	-0.14	-0.46	-0.70**	0.23	0.14	-0.16	0.07
N	9	10	17	17	17	17	17	17	17
EBC-nitrite (μM)	0.79****	0.64****	-0.16	-0.40	-0.65**	-0.12	0.16	-0.29	-0.12
N	9	10	17	17	17	17	17	17	17

correlation is significant at the 0.01 level (2-tailed), **correlation is significant at the 0.05 level (2-tailed), N=number of subject

Table 9.30. Relationship between elements in the 2500 - 5000 nm size range and biomarkers (EBC-Nitrate and nitrite are in μM)

	Ni	Cr	Mn	Pb	Sr	Na	Ca	Fe	Cu	Zn	Mg	Sn
8-oxodG (ng/mg creatinine) N	0.80**** 8	0.04 14	0.55 10	0.35 9	-0.31 11	0.33 17	0.59**** 17	0.33 17	-0.52**** 17	0.24 17	0.59**** 17	-0.23 17
EBC-Ph N	0.09 8	-0.04 14	-0.29 10	-0.39 9	-0.11 11	0.15 17	-0.38 17	-0.18 17	0.35 17	-0.24 17	-0.15 17	0.17 17
EBC-nitrate (μM) N	0.42 8	-0.46 14	-0.42 10	-0.27 9	-0.14 11	-0.05 17	-0.26 17	-0.48 17	0.08 17	-0.44 17	-0.15 17	-0.28 17
EBC-nitrite (μM) N	0.58 8	0.18 14	0.52 10	-0.23 9	-0.38 11	-0.11 17	-0.05 17	-0.31 17	0.06 17	-0.15 17	0.02 17	-0.39 17

****correlation is significant at the 0.05 level (2-tailed), N =number of subject

9.4.9 Metals measured in the urine samples

18 samples of urine collected but only were measured for metals in urine. The concentrations of metals measured in this study are shown in Table 9.32. The mean concentrations of the metals where the reference values limits were available are also included in the table (Table 9.32). All metals in the urine were corrected with creatinine. The metals measured in urine are Na, Mg, Ca, V, Cr, Mn, Fe, Ni, Cu, Zn, Sn, Sb and Pb and these metals were selected because of their source contribution in air, especially ambient air (Bonvallot et al., 2001).

Table 9.31. Sociodemographic table for the subjects used for urinary metal analysis

Sociodemographic and lifestyle	Number and comments
Ethnicity/race	
Black	3
White	2
Asian	2
Hispanic	1
Life style	Non-smokers in non-smoking houses
Education	All educated above masters degree level
Age	31 – 37 years (age range)
Sex	
Female	5
Male	3

Summer and winter data were combined, thus each subject had 2 sets of urine therefore making it 16 subjects

Various factors such as diet, water, lifestyle, environmental exposure, (Chandrakala Aluganti et al., 2012, Jomova and Valko, 2011, Valko et al., 2007) are said to influence concentrations of metals in urine. However, ethnicity/race may be considered as one the major factors that influence urinary metal concentrations because of ethnic influence on diet; people of the same ethnic group tend to eat similar kind of food thus may be exposed to uptake of the same kind of metals in the food. That is influence of regional or ethnic diet. They may drink the same kind of water if they are getting their water from the same source. People of the same

ethnic group or race may have the same kind of life style for example cooking technique/style. Certain ethnic groups may have low income jobs and poor education that may affect the quality of food they eat and the knowledge of nutrients in the foods that they consume. Thus affect the levels of metals in food that they are exposed to. Table 9.31 show the sociodemographic and lifestyle of the subjects that their urine were analysed for urinary metal in this study. The small size and almost equal number of ethnic group representation and the other characteristics used for urinary metal analysis may make it impossible for detection of difference in urinary metal concentrations due to ethnicity. Also all the subjects have lived in Birmingham UK for at least 3 years. Therefore, we did not think that the ethnicity of the subjects may have any significant effect on this study. However, the aim of measuring urinary metals is to investigate if they can be used as biomarkers of exposure to indoor particles.

Table 9.32. Concentration of measured metals in the urine

	Na	Mg	Ca	V	Cr	Mn	Fe	Ni	Cu	Zn	Sn	Sb	Pb
	mol/mol creatinine	mol/mol creatinine	mol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine	μmol/mol creatinine
Mean	9.32	0.23	0.12	0.69	0.41	0.20	26.67	1.86	12.11	417.13	0.56	0.09	0.64
Mean (μg/g creatinine)	-	-	-	0.31	0.19	0.10	-	0.96	6.80	241.09	0.58	0.10	1.17
SD	2.28	0.06	0.08	0.28	0.15	0.22	81.05	0.88	4.11	230.61	0.65	0.08	0.73
Minimum	5.61	0.13	0.03	0.32	0.23	0.04	1.17	0.57	6.51	176.80	0.08	0.03	0.17
Maximum	14.04	0.34	0.28	1.35	0.67	0.91	330.00	3.32	22.39	1006.83	2.28	0.36	3.06
Reference values (μg/g creatinine) (Hoet et al., 2013)	-	-	-	2	0.55	1	-	5	14	770	2.5	0.25	3

9.4.9.1 Relationship between biomarkers and metals in urine and relationship between urinary metals

Table 9.33 show the relationship between the biomarkers of exposure and metals measured in the urine. Oxidative stress and inflammation may be important mechanisms in response to exposure to metals bound to PM. However with urine, it may be difficult to predict internal dose of metal due to exposure because other factors such as diet, medication can influence the urinary metal concentration (ChandrakalaAluganti et al., 2012).

Only EBC-pH and UMn showed a positive significant correlation (Table 9.33). 8-oxodG and UMn showed an inverse significant correlation as well as EBC-nitrate and EBC-nitrite showed inverse significant correlation with USn respectively (Table 9.33). Fe, Ni, V, Cr, Mn and Cu are redox catalysts, thus can catalyze oxidative stress reactions (Pan et al., 2010). CU, Zn and Pb can deplete cellular antioxidant pool and interaction between GSH and metals can also produce or exacerbate cell injury (Ballatori, 1994) thus inducing oxidative stress reactions. A study observed increase in concentration of Ba, Mn, Sn and Ni in spot urine samples from 1000 children (15 different urinary metals) exposed to ETS, which was then associated with increased urinary 8-oxodG and 8-Isoprostane (Jamaludin, 2014). Caglieri et al. (2016) in their study observed that UCr and EBC-Cr were significantly correlated but UCr showed weak to no correlation with biomarkers of free radical production (Federico et al., 2007). Caglieri et al. (2016) also observed good correlation between Cr levels and airway free radical production. This suggests that it is possible to measure metals in EBC but difficult measure the metals in urine because of the perhaps complex breakdown process when they are internalised and the mechanisms of some metals are unknown (Huang et al., 2016). A study reported that in their study of exposure to Cd in 71 solderers, no statistically significant correlation was found between 8-OHdG (tautomer of 8-oxodG) and Cd in blood and urine, even though slightly higher 8-OHdG was measured in their exposed group as compared to a control group (Fitzpatrick et al., 2009). However, another study showed that in 66 exposed welders, UAl, UCr, UMn, UFe, and UNi were all significantly correlated with the biomarker of welding exposure neutrophil gelatinase-associated lipocalin (Chuang et al., 2015). In 2460 community-living Chinese adults, multiple urinary metals are associated with altered pulmonary function, restrictive lung disease and obstructive lung disease risks (Feng et al., 2015b).

A study suggests that asthma prevalence in the Chinese adults was positively correlated with urinary Cr, Cu, Se, Mo, Cd, and U, and negatively correlated with urinary Mn, Fe, Ni, Zn, Rb, Ba and Pb (Huang et al., 2016) and the biomarkers EBC-pH, EBC-nitrate and EBC-nitrite measured in this study have been implicated in the pathophysiology of asthma.

Most of the studies where urinary metals have been measured and compared with biomarkers of exposure are in occupational studies; where exposure levels of metals were high or where length of daily exposure was long. In this study no subject was exposed in a place of relatively high exposure in comparison to occupational exposure. All subjects in this study are non-smokers in a non-smoking house.

Table 9.33. Relationship between biomarkers (8-oxodG (ng/mg creatinine) and metals measured in the urine (UNa, UMg and UCa measured in mol/mol and the other urinary metals are in $\mu\text{mol/mol}$) and relationship between urinary metals

	UNa	UMg	UCa	UV	UCr	UMn	UFe	UNi	UCu	UZn	USn	USb	UPb
8-oxodG	-0.42	0.07	-0.33	-0.15	-0.11	-0.53****	-0.32	0.05	-0.09	-0.03	0.12	0.32	-0.04
N	16	16	16	16	16	16	16	16	16	16	16	16	16
EBC-Ph	-0.13	-0.08	-0.12	0.10	-0.08	0.79**	0.18	-0.29	0.24	0.19	-0.28	0.17	0.32
N	16	16	16	16	16	16	16	16	16	16	18	16	16
EBC-nitrate	-0.37	-0.16	-0.01	0.09	-0.36	0.15	0.30	-0.02	-0.13	0.22	-0.58****	0.25	-0.08
(μM)	16	16	16	16	16	16	16	16	16	16	16	16	16
N													
EBC-nitrite	-0.45	-0.18	-0.13	-0.17	-0.37	0.08	0.44	0.007	-0.11	0.42	-0.73**	0.11	0.24
(μM)	16	16	16	16	16	16	16	16	16	16	16	16	16
N													

Using Spearman's correlation the relationship between the metals bound to the PM and trace metals measured in the urine for the different size fractions were measured. Only the significant relationships are listed below:

- In the < 250 nm: Sr against UCa, Na against UFe, Fe against USn and Fe against UCr were positively significantly correlated. Mn against UMg showed a significant inverse correlation.
- In the 250 – 500 nm: Cu against UPb and Cu against UMg were positively significantly correlated, while Na against UMg, Sn against UMg and Sn against USn showed an inverse significant correlation.
- In the 500 – 1000 nm: Zn against USn showed a positive significant correlation while Na against UMg was an inverse significant correlation.
- In the 1000 – 2500 nm: Sr against UMn, Na against UCa, Cu against UCu, Cu against USn and Zn against USb were positively significantly correlated. Ca against UMg, Fe against UFe, Cu against UFe and Zn against USb showed have inverse significantly correlation.
- In the 2500 - 5000 nm: Sr against UCa, Cu against UMn and Cu against UFe showed a positive significant correlation. Pb against UZn, Sr against UNi, Na against UCa and Ca against UMn showed a significant negative correlation.

There was no significant correlation between the metal bound to PM and their corresponding urinary counterpart except for the 1000 – 2500 nm Cu bound PM and UCu that showed to be significantly correlated. The results obtained above makes it very difficult to find a relationship between metals bound to PM and urinary metals. A study suggests that while many trace metals are excreted in the urine, all potentially useful biomarkers of vehicular abrasion processes are predominately excreted through faecal route (Iyengar, 1998,

Jamaludin, 2014) and another suggests that Urinary concentrations of metals could reflect long- and/or short-term exposure to metals from all sources besides air pollution (Crinnion, 2010) which may limit their usefulness of the result in this present study. Some of the metals in the urine may not reflect air particulate exposure. A study with 83 participants, observed elevated UCr was significantly associated with increased Cr concentrations in personal PM_{2.5} but after adjustment for false discovery, the significant association was lost and no other significant correlation was observed between PM metal and their corresponding urinary counterpart (Yu et al., 2016). However such relationship may be found in high exposure scenario like occupational exposure. A study with 24 chrome-plating workers, found Cr ambient Cr levels sampled during the afternoon to be moderately correlated with UCr ($r = 0.48$, $p < 0.05$) (Federico et al., 2007). However, UMn has been widely investigated as a biomarker of occupational exposure to Mn and were found to be inconsistently related to Mn in the air (Hoet et al., 2012). Therefore this result should be interpreted with caution to avoid misclassification. This study does not have enough information to ascertain that urinary metals can be used as biomarkers of exposure to indoor particles.

9.4.10 Health risk assessment

As displayed in Table 9.34 the ECR for the PM bound in different size fractions (< 0.25 , $0.25 - 0.5$, $0.5 - 1$, $1 - 2.5$, > 2.5) to the carcinogenic metals were calculated. The highest ECR is the Cr in the $< 0.25\mu\text{m}$ size fraction (UFP mode). While the lowest is the Pb in the $> 2.5\mu\text{m}$ size fraction for the winter season. Another study identified Cr as having the highest ECR in their indoor and outdoor study however they measured only the PM_{2.5} bound metals (Karlsson et al., 2005). Therefore suggesting that human exposure to Cr in the indoor can easily get cancer in comparison to the outdoor location (Karlsson et al., 2005). Bearing in mind that this study is for Birmingham, UK and Massey et al. (2013) is India. The total

values for the carcinogenic risk which is the ECR for the winter were calculated as 2.7×10^{-6} , 4.1×10^{-7} , 5.3×10^{-7} , 5.4×10^{-7} and 7.5×10^{-7} for the $PM_{<0.25}$, $PM_{0.25-0.5}$, $PM_{0.5-1}$, $PM_{1-2.5}$, $PM_{>2.5}$ respectively. This suggests that the subjects were more exposed to the UFP particles i.e. the $< 0.25\mu m$ size fraction that are emitted indoors and could be linked to adverse health effects and that the ECR for the $PM_{<0.25}$ size fraction suggest that 2 to 3 people in a million may get cancer from exposure to toxic metals in Birmingham UK in a life time, in relation to the concentration of the metals measured during the sampling in this study. Also these mean total ECR values 2.6×10^{-6} for the $PM_{<0.25}$ exceed the acceptable limit USEPA value of 1.0×10^{-6} (EPA, 1991).

Table 9.34. Excess cancer risk of carcinogenic elements in the different size fractions

Element	Size fraction	Concentration ($\mu g/m^3$)	Inhalation unit risk ($\mu g/m^3$) ⁻¹	Excess cancer risk (ECR)
Cr (VI) ($\Sigma Cr/7$)	$< 0.25 \mu m$	2.0×10^{-4}	1.2×10^{-2a}	2.6×10^{-6}
	$0.25 - 0.5$	4.0×10^{-5}	1.2×10^{-2a}	4.0×10^{-7}
	$0.5 - 1$	6.0×10^{-6}	1.2×10^{-2a}	5.0×10^{-7}
	$1 - 2.5$	7.0×10^{-5}	1.2×10^{-2a}	4.0×10^{-7}
	> 2.5	3.6×10^{-4}	1.2×10^{-2a}	6.0×10^{-7}
Ni	$< 0.25 \mu m$	3.6×10^{-4}	2.4×10^{-4a}	8.6×10^{-8}
	$0.25 - 0.5$	0	2.4×10^{-4a}	0
	$0.5 - 1$	2.0×10^{-4}	2.4×10^{-4a}	4.8×10^{-8}
	$1 - 2.5$	4.0×10^{-4}	2.4×10^{-4a}	9.6×10^{-8}
	> 2.5	5.7×10^{-4}	2.4×10^{-4a}	1.4×10^{-8}
Pb	$< 0.25 \mu m$	4.0×10^{-3}	1.2×10^{-5b}	4.8×10^{-8}
	$0.25 - 0.5$	0.91×10^{-4}	1.2×10^{-5b}	1.1×10^{-8}
	$0.5 - 1$	0.24×10^{-4}	1.2×10^{-5b}	3.0×10^{-9}
	$1 - 2.5$	0.29×10^{-4}	1.2×10^{-5b}	3.0×10^{-9}
	> 2.5	0.31×10^{-4}	1.2×10^{-5b}	4.0×10^{-9}

^a Values taken from IRIS (Integrated Risk Information System). ^b Values taken from Cal EPA (California Environmental Protection Agency).

9.5 Conclusion

This chapter aimed at characterising indoor particles by describing their mass and elemental components concentrations, mass-size distribution patterns of the PM and also for the metals bound to the particles in this size range $< 0.25\ \mu\text{m}$, UFP, $0.25 - 0.5\ \mu\text{m}$, $0.5 - 1\ \mu\text{m}$, $1 - 2.5\ \mu\text{m}$ Fine particles and the coarse mode $> 2.5\ \mu\text{m}$. Particles were found to exist in two modes in the homes the 250 -500 nm and the $> 2500\ \text{nm}$ mode. Particles such as Na, Mg, Fe, Mn and Ca existed indoors in the coarse size fraction in a single mode. The EF of these metals also suggests that they are of crustal and or marine origin. Toxic metals such as Ni, V, Mn, Sb, Zn, Cr and Cu were measured in the homes and existed more in < 2.5 size ranges, suggesting that toxic metals in the homes measured are in the respirable range. Therefore can be inhaled deeper into the lungs where they can induce inflammation and oxidative stress. Also their EF suggests that due to high enrichment they are of anthropogenic sources and made their way into the homes via infiltration and are also enriched by indoor sources.

Physicochemical (shape, size and surface chemistry) properties of the particles found in the indoors were analysed using the TEM/EDS. The data available in this work showed the presence of metals and other non metal components bound to the particles analysed using the TEM/EDS. In the indoors, the prevalent components were Si, S and Fe. A prevalent combination (showing the same pattern) in the indoor (home) was between Cr, Fe and Ni also mixed with Ti, Mo and Cu. This demonstrates that it is likely stainless steel and stainless steel is the preferred cooking utensil, work surfaces and building materials in homes because it is less likely to rust as compared to Fe. The type of microenvironment also affected the kind of particles that were generated. For example in one of the offices where loads of paper and photocopying was used, the particle was suggestive of an aged soot aggregated particle containing Ni, Mn, Fe, Si, Ca and Mg found in printer toners and papers and the printer was

the source of heat for the combustion. However, Be was found in one of the homes that was sampled and the likely source is the outdoor soil sources.

Biomarkers of exposure to indoor PM and PM components were also measured alongside health risk indicators. The aim was to find a relationship between exposure biomarkers and the PM and PM metal components of different size fractions. The relationship showed the PM in the < 250 nm size range (UFP) significantly correlated with 8-oxodG. Also, some of the metals in the different size ranges also showed significant correlation with biomarkers of oxidative stress and localised oxidative/nitrosative stress. Thus, supporting the hypothesis that exposure to particles can induce oxidative stress. Even though, UMn showed a significant correlation with EBC-pH and an inverse significant correlation with 8-oxodG and UCu significantly correlated with Cu bound to PM, the results obtained were not enough to conclude if urinary metals can be used as biomarkers of exposure to indoor particles.

ECR was calculated for the carcinogenic PM bound metals in the different size fractions. The ECR for $PM_{<2.5 \mu m}$ exceeded the acceptable limit indicating that 2 to 3 persons in a million in a life time may get cancer from indoor home exposure in Birmingham UK based on the concentrations in this study. However, this should be interpreted with caution because only a small size of homes were used and may not be representative of all the homes. Thus ECR should be carried out on a larger scale.

10 CONCLUSION AND FUTURE RESEARCH

10.1 Conclusion

For over a decade now, the trending air pollution issue is that particles are important health indicators of adverse effect. Therefore particles were chosen as the pollutants to measure in acute/short term exposure and in the indoor exposure. In my opinion, particles are very important because they modulate the effects of the particle bound chemicals in human exposure via inhalation. The main particles measured in this study are UFP, PM_{2.5} and BC. Only in the indoor size fraction studies were particles between 2.5 – 5 µm are measured. BC is a primary component of PM. BC is a harmful component of PM and UFP (Ng and Moore, 1998) which is said to be an indicator of adverse health effect (Janssen et al., 2011). BC is the light absorbing component of PM (Janssen et al., 2011). BC particles are generated from incomplete combustion and are dispersed in air. While the UFP and PM describes the different particle sizes. UFP are mainly particles ≤ 100 nm (in the acute exposure PNC were used as the surrogate for UFP) however, these days, the size cut-off has expanded a bit and in this study we have considered UFP to be particle ≤ 250 nm for the indoor size fraction studies. PM describes a wider size range and for this study the PM ≤ 2500 nm were our concern. UFP/PM are made up of different chemical elements/compounds (as shown in chapter 9) and in this study, we were concerned with the metal components in the different size fraction measurements and other non metal elements/compounds (P, S, Cl and F) were also observed from the surface chemistry in the TEM analysis (chapter 9). BC is made up of carbon that can be measured by light absorption (McNabola et al., 2008). BC is a better indicator of harmful particulate substances from combustion sources (especially traffic) than undifferentiated PM mass (Janssen et al., 2011, Janssen et al., 2012). However, studies suggest that both BC and PM can induce inflammation and oxidative stress. Also, synergistic

effect of BC and PM components can induce oxidative stress, for example: BC and iron oxide particles induced oxidative stress in an *in vitro* study (Seaton et al., 2005) but, there are not enough clinical or toxicological studies to allow an evaluation of the qualitative differences between the health effects of exposure to BC or to PM mass (in different health outcomes). However, in terms of UFP (measured as PNC), because they are smaller, they can travel deeper into the lungs. In this research, we have observed that in some of the studies BC, UFP/PM have shown significant association with biomarkers of inflammation and oxidative stress.

One of the aims of this work was to characterise PEs and indoor concentrations of NPs/UFP, PM and BC in different indoor and personal exposure scenarios and to study their associations with biological markers of inflammation and oxidative stress.

In order to characterise PE, elimination of inconsistencies associated with distance between the sampler and the sampled subject, as in the case of RFS sampling, the use of PSs were employed. The performances of 4 PSs (3- UFP-DiSCMini, 4- BC-Micro-aethalometer AE51, 4- PM_{2.5}-MicroPEM and 6- NO₂-Aeroqual sensor) were validated before use in PE sampling, both in the laboratory and in the field (ambient air). During the validation study, the O-ring of the PM_{2.5} PS- the MicroPEM was not placed properly; therefore the sampling was not sealed and because of the leak the results obtained from this sensor was affected. However, this was rectified and another validation of the instrument was carried. The report of the second validation is not reported in this study but can be found in ““USE OF REAL-TIME SENSORS TO ASSESS MISCLASSIFICATION AND TO IDENTIFY MAIN SOURCES CONTRIBUTING TO PEAK AND CHRONIC EXPOSURES: 5-MONTH YEAR 2 PROGRESS REPORT by Dr Juana Maria Delgado-Saborit”. However, the validation

assessment concluded that all the PSs were capable of delivering meaningful results that can be comparable to the reference methods samplers except the NO₂-Aeroqual PS. Therefore the NO₂-Aeroqual PS was not used in the PE studies.

The values obtained from the UFP and PM_{2.5} PSs were used as absolute values. While the correction factors were applied to the BC data. Validated/peer reviewed correction factors for BC MicroAethalometer and Aethalometers can be found in literatures/other studies (Apte et al., 2011, Hagler et al., 2011, Weingartner et al., 2003). It could be argued that this work should have corrected the absolute values for the PM_{2.5} and UFP data with the correction factors obtained in the report by “USE OF REAL-TIME SENSORS TO ASSESS MISCLASSIFICATION AND TO IDENTIFY MAIN SOURCES CONTRIBUTING TO PEAK AND CHRONIC EXPOSURES: 5-MONTH YEAR 2 PROGRESS REPORT.

Author: Dr Juana Maria Delgado-Saborit, University of Birmingham, September 2014.

However, deriving of correction factors were not part of this work, and the correction factors in the report by Dr Juana Maria Delgado-Saborit, had not yet been published, neither had they been peer-reviewed by the time that I had done the analysis of the data in this thesis. Therefore, I was unable to use correction factors and had to use the absolute values instead and I have used the same sensors for all the data collection in all the studies in this thesis. One of the objectives of this study is mainly looking at correlations between the BC, PM, UFP concentrations and the biomarkers, the fact that the data in this study was not corrected and only absolute values have been used, does not interfere with the results obtained in the pollutant-biomarker correlations.

The validated PSs were used in PE measurements alongside activity diaries and GPS in comparison to measurements with reference fixed sensors at the Tyburn FS. With the aid of the PSs, temporal and spatial variability between PE and FS measurements were observed. This study showed that for PE and biomarker comparison, the PS measured concentration for the different activities/MEs appeared to be better predictors of biomarkers expression, rather than the entire daily measurement and/or FS measurement. Also, the BC FS concentration for the daily measurement also appeared to be a predictor of EBC-nitrite.

Within the confines of this study where chapter 5, chapter 6 and chapter 7 were grouped as the elevated exposure scenario, EBC and urine samples were collected for biomarker analysis. In the EBC, nitrate, nitrite and EBC-pH were measured and in the urine 8-oxodG was measured in the before and after exposure scenario. Exposure to particles in these scenarios resulted in “irritative effects” in which the response appeared to be specific to only the tissue (airways-lungs) that was involved. Therefore biomarker of localised oxidative stress were expressed and measured in EBC as increase in nitrate and nitrite immediately after, 2 hours of exposure in each of the different scenarios in the chapters mentioned above. However, a non-significant increase was observed in the EBC-pH after exposure. While the 8-oxodG that represents systemic oxidative stress due to DNA damage and repair did not show any significant change.

Still, during the elevated exposure in the busy traffic site and the control site- the park (chapter 7), no significant changes were observed in the pulmonary and autonomic functions both for the exposure and the control. In chapter 8 during the cycling and train commuter exposure, a steady decrease in the lung function was observed suggesting that exposure to transport related air pollutants may have altered the pulmonary function parameters (FEV₁,

FEV₆, FEV₁/FEV₆ and FEF₂₅₋₇₅). However this data should be interpreted with caution as only 2 subjects (1 cyclists and 1 train commuter) were used. Therefore comparison with other individuals should be carried out in a larger study.

The second aim was to characterise the PM concentrations and metal composition in nine Birmingham UK homes, study their associations with biological markers of inflammation and oxidative stress and quantify their toxicity evaluation of carcinogenic trace metals Cr, Ni and Pb.

PM exposure that occurs indoors is a significant fraction in relation to an individual's overall exposure to particles because people spend most time indoors. As seen even in chapter 4 the subject spent 93% of time in the indoors. Different size fractions of the indoor (home) PM were measured and characterised: mass-size distribution, elemental chemical composition with special regard to metal content, particles morphology/shape, size and surface chemistry.

- Indoor particles (size range: < 250, < 250 -500, 500 -1000, 1000 – 2500, > 2500 - 5000 nm) were characterised and it showed that the mass-size distribution in the indoor (homes) for this study were mainly in 2 modes; the 250 – 500 nm (accumulation mode) and the 2500 - 5000 nm (coarse mode).
- Toxic metals such as Ni, V, Mn, Sb, Zn, Cr and Cu were in quantifiable concentrations inside the homes.
- Particles of irregular shapes and agglomerates were the most observed shapes of the particles viewed under the TEM analysis and also various sizes were observed.
- Elements constituting the PM such as Fe, Si and S were the most prevalent and other elements such as Al, K, Ca, Cl, Mg, Cr, Ni, Cu, Mn, Mo, Ti, Pb, Na, P, Sn, Rb and

Be were observed under the TEM from indoor particles. Some of the particles originated from outdoor infiltration while others reflected the indoor activity. For example, the components of the particles observed in Office 2 (chapter 9) showed that the prevalent activity in that office is printing and photocopying.

- The results demonstrate the complexity of indoor PM nature.
- Some of these metals are redox active and can induce inflammation and oxidative stress following exposure. As seen in chapter 9, some of the PM in different size fractions and metal content of PM of different size fractions were significantly correlated with biomarkers of exposure (EBC-nitrate, EBC-nitrite, EBC-pH and 8-oxodG).
- Among the PM size fractions measured, chapter 9 suggests that the subjects were more exposed to the UFP particles i.e. the $< 0.25\mu\text{m}$ size fraction that were emitted indoors and could be linked to adverse health effects and that the ECR for the $\text{PM}_{<0.25}$ size fraction suggest that 2 to 3 people in a million for a life time may get cancer from exposure to Cr in Birmingham UK, in relation to the concentration of the metals measured during the sampling in this study.

10.1.1 Biomarkers of exposure

Biomarker assessments have been employed in various studies to assess the impact of air pollution to exposure. Quantification of the effect of the exposure then relies on association between health outcomes and the concentration inhaled by the subject or the concentration the subject is exposed to in an environment. Following exposure to particles, the airways/lungs is challenged, resulting in responses that can be measured within hours to days after exposure. This study is interested in acute responses due to the influence of short-term air particulate exposure by measuring changes in biomarker levels in healthy non-smoking

adults. Therefore the results obtained in this study in comparison with available literature on biomarkers and exposure to particles will help provide new insights on short-term related acute changes in inflammatory biomarkers.

10.1.1.1 EBC-nitrate and EBC-nitrite

Nitrate and nitrite are relatively stable end products of NO that were measured in the EBC in this study. In this study during the elevated exposures they were detected immediately after a 2 hours exposure. Acute increase in nitrate and nitrite following the exposure suggests that they are site/tissue specific biomarkers. That is, as the airways are challenged, the immune system generates response to protect the airways and in the process nitrate and nitrite expression increases.

The question is can exposure to traffic related particles, cooking fumes from Japanese style cooking “Teppanyaki” and secondhand shisha smoke via inhalation cause acute effect responses in healthy non-smoking adults? This study shows that:

- Exposure to traffic related particles induced localised nitrosative stress marker nitrate and nitrite
- The non existence of biomarker effect after exposure in the park (control exposure) and the positive effect after exposure in traffic roadside (previous bullet point), suggests traffic aerosol, measured in this study as BC and UFP, as mediating the localised nitrosative stress marker nitrate and nitrite
- Exposure to cooking fumes particles induced localised nitrosative stress marker nitrate and nitrite
- Exposure to secondhand shisha smoke induced localised nitrosative stress marker nitrate and nitrite

The significant changes in EBC-nitrate and nitrite were within this study expected physiological timeline of appearance (Figure 1.7) and within that measured by some other studies (Table 1.3). EBC-nitrate and nitrite are biomarkers of acute response and their expression following acute exposure may not be affected by factor such as smoking status, health status, length of exposure (using 2 hours) and type of occupation. However subjects who are biased by any of these factors mentioned may have a higher concentration of nitrate and nitrite as they have shown in multiple cross sectional studies to be increased in people with asthma, COPD, and cystic fibrosis and in smokers.

This is the only (as far as I know) study that has measured acute effect of exposure to cooking fumes (Japanese style cooking) in customers and not the restaurant workers. Therefore, the health risk impact due to chronic exposure for the cooks and staff of the Japanese restaurant should be of great concern. Very few studies have measured EBC-nitrate and nitrite as biomarkers of acute effect of exposure to traffic related particles in healthy adults (Knaapen et al., 2002, Laumbach and Kipen, 2010). In their study, after exposure, they observed increased EBC-nitrite and EBC-nitrate + nitrite and a decrease in their control where they used filtered air. This study used the park as the control site and also got the same reduction in nitrate and nitrite. The use of the park as the control site is applicable to real life scenarios, for example pedestrians, avoiding the use of busy traffic road and instead use background or back roads will help them to reduce their exposure to traffic related particles.

10.1.1.2 EBC-pH

Decrease in EBC-pH is a biomarker for measuring airway acidity. The summary obtained from most researchers discussed through this study and the results obtained in this study suggests that for a significant decrease in EBC-pH after exposure to occur, the subject will be affected by one or more of these predictors; that the subjects already suffer from

cardiovascular diseases, long term exposure to pollutants due to their occupation or living in a highly polluted area, smoking and length of years smoked. Some studies have also suggested that incremental changes in EBC-pH could be due to the constituents of the particles -like in the study of Boyce et al. (2006) and Fireman et al. (2008) where metal welding fumes was suggested to have caused increase in EBC-pH . Cigarette exposures have also shown inconsistent outcomes for EBC-pH following exposure to cigarette smoke or smoking. EBC-pH results in different studies have been contradicting because of these predictor factors mentioned above. Therefore using EBC-pH for short term studies especially in subjects who are healthy (i.e. without cardiovascular or pulmonary diseases), non-smoking/never smoked and do not work in occupation where they are constantly exposed high particle concentrations may not be recommended. However, it is good non invasive technique for measuring pH of the lungs.

This study did not observe any significant changes in EBC-pH after exposure, within this study expected physiological timeline of appearance (Figure 1.7) thus EBC-pH was not ideal for measuring acute effect changes based on the confines of this study.

10.1.1.3 8-oxodG

8-oxodG is a biomarker of DNA damage and repair representing the entire system. Across this study, no significant changes to 8-oxodG were observed in the elevated exposure and CV for all the interday 8-oxodG concentration in chapter 4 and chapter 9 were very low suggesting no apparent damage between sampling days. 8-oxodG formation is more complex than formation of nitrate and nitrite. There has to be damage; i.e. when the ROS/RNS produced overwhelm the antioxidant system and starts to interfere/modify with DNA, proteins and other macromolecules enough to cause oxidative stress that can be measured as significant fluctuations in the 8-oxodG concentrations. Based on the results observed in the

various studies in this research, and other studies by other researches, exposure duration of 2 hours might be too short to produce measurable significant changes in the 8-oxodG concentration. In order to cause a systemic damage expressed as increase in 8-oxodG, the subject may have to be exposed for a longer time or continuous exposure (Wall et al., 1988) like in occupations such as cooks (Vilcassim et al., 2014, Harper et al., 1983, Yang and Watts, 2005, Nel et al., 2006), drivers (Montuschi et al., 2007, Chuang et al., 2003), compromised state of health in addition to exposure can affect 8-oxodG concentration. Smoking status in addition to exposure and time between exposure and 8-oxodG measurement (Thaiparambil et al., 2007) can affect 8-oxodG expression. However in subjects with compromised health status, in children and in the elderly the end point may be different.

Similar result as obtained in this study has been observed in other studies (Martin et al., 2015, Allen et al., 2009). EBC-pH and 8-oxodG have shown contradictory results in various studies in healthy subjects. Urinary 8-oxodG did not significantly change within this study expected physiological timeline of appearance (Figure 1.7) and within that measured by other studies (Table 1.3). Therefore expression of 8-oxodG may not be suitable for acute response effects especially in non-smoking healthy subjects like in this study. It would have been good to have more monitoring after exposure time points of 8-oxodG in order to check if at any point along the physiological time points in Figure 1.7 there was a change in the expression of 8-oxodG because the effects of short-term exposures can be difficult to quantify in people with different background exposure and susceptibility factors

10.1.1.4 Other parameters measured

Pulmonary and autonomic function parameters were measured in the busy traffic site exposure and in the control the park. No significant changes were observed between the

before and after exposures for both the busy traffic site and the park. McCreanor et al. (2007) observed decrease in the pulmonary function parameters while this study did not. This may be because the subjects in this study were healthy subjects and theirs had asthma.

10.1.1.5 Urinary metals

Thirdly the aim of this study is to investigate if urinary metals can be used as biomarkers of exposure to PE to short term effects.

Urinary metals were significantly reduced after exposure in the park- the control site in chapter 7 suggesting that the effect of traffic aerosol, measured as BC and UFP in this study, could be mediating the observed effect. While in chapter 9, only UMn and EBC-pH were significantly correlated no other urinary metals with other biomarkers. Also only UCu significantly correlated with its corresponding counterpart PM bound Cu in the 1000 – 2500 nm size fraction. However these results should be interpreted with caution as a study suggests that while many trace metals are excreted in the urine, all potentially useful biomarkers of vehicular abrasion processes are predominately excreted through faecal route (Iyengar, 1998, Jamaludin, 2014) and another suggests that Urinary concentrations of metals could reflect long- and/or short-term exposure to metals from all sources besides air pollution (Crinnion, 2010) which may limit their usefulness of the result in this present study. Therefore further studies on urinary metals and exposure in non-occupational scenarios and metabolomic studies should be investigated. However, at this time, this study does not have enough evidence to suggest that urinary metals can be used as biomarker of exposure to short term effects.

10.1.1.6 Correlations between biomarkers and pollutants

While this study was limited by small cohort-size that may have affected the statistic power of the correlation between exposure concentration of the pollutants and biomarkers measured, significant correlations were observed between the particles and biomarkers across the different studies. Suggesting that exposure to UFP, PM_{2.5}, BC and PM bound metals can induce inflammation and oxidative stress. Also, inhaled load estimate (dose) i.e. the amount of the particles that passed through the lungs also showed correlations with the biomarkers. For example, after busy road exposure, inhaled load estimate showed a significant correlation with EBC-pH in chapter 7. This suggests that lung dose is important indicator of inflammatory responses.

10.1.1.7 Strengths

This study was able to use various non-invasive markers to analyse end health points. This study carried out personal monitoring by using personal exposure to measure concentrations in the subjects breathing zone and not relying on fixed site. However fixed sites also have their importance. In the personal monitoring, we had control over the microenvironments by selecting exposure scenarios that are commensurate with natural and real life exposures. For example people walk on busy traffic streets and others seat in the park. Therefore, using these two environments will help add knowledge to the kind of responses that can be observed in the cardiopulmonary health of individuals. People travel by train, car, buses and cycling, therefore information on health effects in these microenvironments will help policy makers decided on friendlier travelling alternatives. Various elevated exposure studies were carried out and EBC-nitrate and nitrite consistently showed that they are biomarkers of “irritative effect” or acute response effect they appear to defend/protect the airways.

10.1.1.8 Limitations

One of the major limitations faced by this research was with the instrumentation (nanoMOUDI) that was to be used in the initial project design. As discussed in chapter 2, when the instrument could not be made indoor friendly (as a result of higher than background noise, smell from running the oil pump, high temperature e.t.c), then the smaller projects were created. Thus a lot of time was lost trying to modify the nanoMOUDI and trying different scenarios for the nanoMOUDI to be placed outside and tubing inside the homes. Which again did not work because of drop in pressure and the instrument could not bring in enough air through the inlet for sampling. Also while we were waiting for funds approval for the personal impactor SIOUTAS, the smaller individual projects commenced. Generally time was lost and cohort sampling size reduced when the SIOUTAS arrived.

The different cohort-sizes used for the different studies that make up this entire research study were small. The cohort-size of this study may have affected significant biological outcomes i.e. the measured biomarkers, especially in low concentration. Thus may have resulted in lack of sufficient statistical power to identify significant correlations between PE exposure/pollutant concentrations and biomarker outcomes. Especially for some of the studies like chapter 4 and chapter 8 where single individuals were used, larger cohort size observation should be carried out so that result can be more representative of a larger population. However results should not be considered as gold stand and therefore may not be the same for people with compromised health, the elderly and children. More after exposure urine and EBC samples at different intervals rather than only immediately after exposure urine and EBC samples would have helped buttressed the 8-oxodG and EBC-pH results because there could have been significant changes along the timeline that was not measured in this study.

With the small size limitations, this work cannot be used as an epidemiological study observation, but the methods used and the results obtained can be expanded upon and applied to larger cohort size studies that will benefit epidemiological findings.

All the urine samples from all the individual studies were sent as a single batch for the 8-oxodG analysis. It would have been better to do the 8-oxodG analysis after each individual study. That way the protocol for urine sample collection for 8-oxodG analysis may have been changed. That is, subsequent 8-oxodG multiple monitoring time points may have been added to the other studies in order to determine if any significant 8-oxodG changes will occur along the physiological timeline in Figure 1.7.

10.1.1.9 Significance

Exposure to particles clearly leads to localised oxidative stress, in non-smoking healthy and young adults. Association between particles and inflammation and oxidative stress respectively were observed in this study. The natural microenvironments used in this experiment validated the short term effects measured and the common pathophysiologic pathways in air pollution health studies. The reversibility of biomarkers observed in the elevated and control study calls for intervention measures to improve air quality. There is enough information on adverse effects of ambient air therefore people are aware of the health effects associated with exposure to outdoor air. This study points out that the indoors are also contributors to pollutants that can cause adverse effects and it is detrimental especially to the susceptible population that spends a lot of time indoors. In order to completely study exposure effects of PM on human health, the indoors must be included and not just the ambient exposure. As seen in this study that indoor PM can induce inflammatory responses

and studies have suggested that PM is one of the most important indoor air pollutants involved in a number of adverse health effects (Eštoková et al., 2010).

10.2 Future research

I intend to further study toxicity of particles focusing more on NPs. I analysed indoor particle morphology and chemistry in this present study. These appear to be useful. As a further extension to these findings, I would like to conduct acute *in vitro* exposures to cell lines using replica particles of the particles observed in this study or other NPs in order to investigate health end points such as oxidative stress and cell genotoxicity due to particles generated in the indoor.

In the future it will be good to expand on the cycling exposure. Using a larger cohort-size group, I would like to monitor lung function, minute ventilation and inhaled dose both in and out of the laboratory. Inputting the data obtained into a spatial model to predict effects of high and or low pollutant concentration to cyclists' lung function, especially those with cardiovascular and pulmonary health diseases. This will help advice on alternate routes during high concentration episodes.

Future work regarding indoor particles characterisation needs to include Urban, suburban and rural homes. More home numbers as compared to this study should be included. This will enable source apportionment of the PM components and better identification on health risk analysis in relation to excess cancer risks. The results also stresses the need for further research to a more complete understanding of the chemical nature of indoor PM in regards to their surface reactivity. Due to the negative biological influence of PMs and their specific properties resulting in synergistic effect with other pollutants in the indoor air, it is necessary to investigate ways to reduce occurrence and exposure to indoor PM.

From the results obtained in chapter 4 a time activity model has already been created, therefore, I would like to test this out on about a 100 people in order to validate the model and possible expand it for epidemiological studies.

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12 APPENDICES

12.1 Appendix A

The selected central fixed monitoring site description (adopted from validation study proposal).

Birmingham Tyburn Background Site (Tyburn), an urban background site located within the car park of Birmingham Council owned offices. The nearest road (other than car park access road) is approximately 60 metres north of the station, the A38 Tyburn Road, which has an average annual daily flow (AADF) of traffic of 20,000 vehicles. The site is located approximately 600 metres to the north of the M6 motorway (AADF = 28,000 vehicles).

London Marylebone Road (Marylebone), a kerbside station located in Marylebone Road opposite Madame Tussauds. The nearest road, the 6 laned A501 is approximately 1 metre from the station. The road is frequently congested. The surrounding area forms a street canyon and comprises of education buildings, tourist attractions, shops and housing (AADF > 20,000 vehicles).

London North Kensington (North Kensington), an urban background site located the grounds of Sion Manning School. The nearest road, St. Charles Square, is a quiet residential road approximately 5 metres from the station. The surrounding area is mainly residential.

Harwell Observatory Site (Harwell), a rural site where the UK Department of Environment Food and Rural Affairs (DEFRA) currently has a rural air monitoring site located ca. 800m away from the A4185 (AADF= 8,000).

Reference sensors

The European Committee for Standardization (**CEN**, French: Comité Européen de Normalisation) CEN reference method equipment as per the 2008/50/EC Directive requirements for gases and reference method equivalent equipment for particulates were used in this validation monitoring sites to compare against the results measured by the personal sensors.

The reference instrument used for real time PM_{2.5} was the Filter Dynamic Measurement System tapered element oscillating microbalance monitor (FDMS- TEOM). The unit of measurement for this instrument is $\mu\text{g}/\text{m}^3$ including both volatile and non-volatile PM components. The sampler constantly samples the ambient air by the means of a switch valve, which changes the path of the air main flow every 6 minutes. Air drawn in passes through a Nafion dryer bundle to remove water which allows the TEOM filter element to be maintained at 30 °C (Schwab et al., 2006, Heal et al., 2012). At this 30 °C temperature loss of volatiles are reduced (Heal et al., 2012). For the first 6 minute time block, the air goes through a purge filter and all PM from the sample stream is removed and then passed over the TEOM filter where it is collected and weighed (Heal et al., 2012). The average concentration over the 6 minutes is known as the *base* measurement. Then the

switch valve switches diverting the path of the airstream. The air streams through a filter and then goes into a 4°C temperature chamber. The air then passes through a filter which collects the PM in the airstream but allows the gases to pass through.

The clean air is then passed over the TEOM filter where it is measured to give the baseline measurement known as the *reference* measurement (TEOM FDMS equipment manual). The total PM measurement generated by the sensor is obtained by computing the difference between the *base* and the *reference* measurement. That is this mass change is then added to the mass recorded during the previous “base” cycle to give the total mass of PM (Heal et al., 2012). Many detailed description of the FDMS- TEOM instrumentation is available in literature (Schwab et al., 2006) and in the TEOM FDMS equipment manual.

The reference instrument used for real time UFP was the Condensation Particle Counter TSI 3022A (CPC) and Scanning mobility particle sizer (SMPS). Particles in the CPC are detected by light scattering. When air is drawn into the CPC through the inlet, the aerosol sample passes through a heated saturator, in which n-butanol is vaporised and diffused into the sample stream. Then the aerosol sample becomes saturated with the vapour from the n-butanol and then flows into a cooled condenser. Inside the condenser, the vapour becomes supersaturated. When sufficient supersaturation is achieved, the vapour begins to condense on the particles thereby making particles to grow into larger liquid droplets. The enlarged particles are detected and counted by light scattering using the optical detector. Light scattered by an individual particle is recorded as a voltage pulse. The SMPS includes a differential mobility analyser (DMA) and a CPC. The particles entering the system are neutralised by being introduced to a bipolar charger radioactive source and then these charged particles are passed to the DMA. The DMA classifies particles

using the electrical mobility technique and the classified particles are then counted by the CPC (Ristimäki et al., 2002).

The reference instrument used for the measurement of BC is the Magee Scientific, model AE 22 Aethalometer. The Aethalometers quantify i.e. determines mass concentration of Black Carbon on a quartz filter based on the transmission of light through a sample. A two-wavelength Aethalometer (Model AE-22) was employed: with the 880-nm wavelength in the IR range was used to estimate BC concentrations and the 370-nm wavelength probe which measures the UV component of the aerosol which was not used (Wallace et al., 2011). The light transmits through a pristine portion of the filter i.e. the reference, and also transmits through the sample spot i.e. sample on the filter at time t (Arnott et al., 2005). The system evaluates changes in two optical sensors (sample and reference), with the light source both on and off, such that independent measurements of the change in attenuation of the sample are produced for averaging periods then the absorption coefficient for material added during the period, is calculated from the attenuation change, the filter area, and volume of the sample which is then converted to a Black Carbon concentration for the period (Butterfield et al., 2010). The same correction factor model applied to the personal BC sensor AE-51 was also applied to the reference sensor.

The reference instrument used for the measurement of NO_2 is the Chemiluminescence API M200E i.e. M200E Photolytic Analyser of Teledyne-API Inc.. The M200E Photolytic Analyser measures NO and NO_x ($\text{NO} + \text{NO}_2$) and derives NO_2 from it (Sluis et al., 2010). The instrument: Calculates the amount of NO present by measuring the amount of chemiluminescence given off when the sample gas is exposed to ozone (O_3)

(Teledyne API – M200E NO_x Analyzer Operation Manual). To measure NO₂, a catalytic-reactive converter converts any NO₂ in the air sample gas to NO, which is then mixed with O₃, chemiluminescence occurs, and the detected light response is proportional to the concentration of all nitrogen oxide species NO_x (Teledyne API – M200E NO_x Analyzer Operation Manual). Then NO₂ can also be calculated as the difference between NO_x and NO (API Model 200E Instrument Manual 10.0.0).

12.2 Appendix B

Mode of operation of the Philips Aerasense NanoTracer: Personal sensor used in chapter 4

Airflow, ϕ , passes via a fan located at the top of the sensor. Then the airborne particles are electrically charged in the charging section through diffusion theory by corona discharge from a needle-tip electrode set at a voltage, V_{cor} (Marra et al., 2010). This V_{cor} is set at a high voltage high enough to ionize the air then inducing diffusion charging of airborne particles inside the conduit (Marra et al., 2010). The needle-tip electrode is surrounded by a screen electrode, wherein the screen voltage, V_{scr} , much lesser than the electrode (V_{cor}) is imposed. The charged particles enter the precipitation section where voltage pulse varying between $V_{PI}=0$ and $V_{PI} = V_1$ are imposed between two parallel electrode plates separated by a distance d_{PI} (Marra et al., 2010, Buonanno et al., 2014a). At the initial voltage pulse $V_{PI}=0$ charged particles pass through the precipitation section freely and then are captured in a filter inside a faraday cage that is connected via a current meter to a reference potential. This current meter records the total particle charge that deposits per unit time inside the Faraday cage as an electrical current $I_{sensor} = I_1$. At $V_{PI} = V_1$, some of the charged particles precipitate inside the plate due to the field strength between the electrode plates. Then all the remaining particles are subsequently captured inside the Faraday cage hence a second current signal I_2 (Buonanno et al., 2014a). The average particle diameter $d_{p,av}$, can be inferred from a relationship between L/N where L = particle length concentration and N = Particle number concentration and L and N can be gotten as a function of I_1 i.e. $I_1/(I_1 - I_2)$ (Marra et al., 2010).

12.3 Appendix C

Appendix Table 1 to Appendix Table 7 and Appendix Figure 1 to Appendix Figure 2 represent the time series for the PE measured with PSs and RFS for the individual sampling days. All the time series were presented in tables and only 2 were chosen at random and presented in Figures (graphs). In the tables, Home-Sleeping, Home-Bedroom-Sleeping and Sleeping are interchanged they all mean sleeping at home. Home-Bedroom and Home-Bedroom not sleeping are also interchanged.

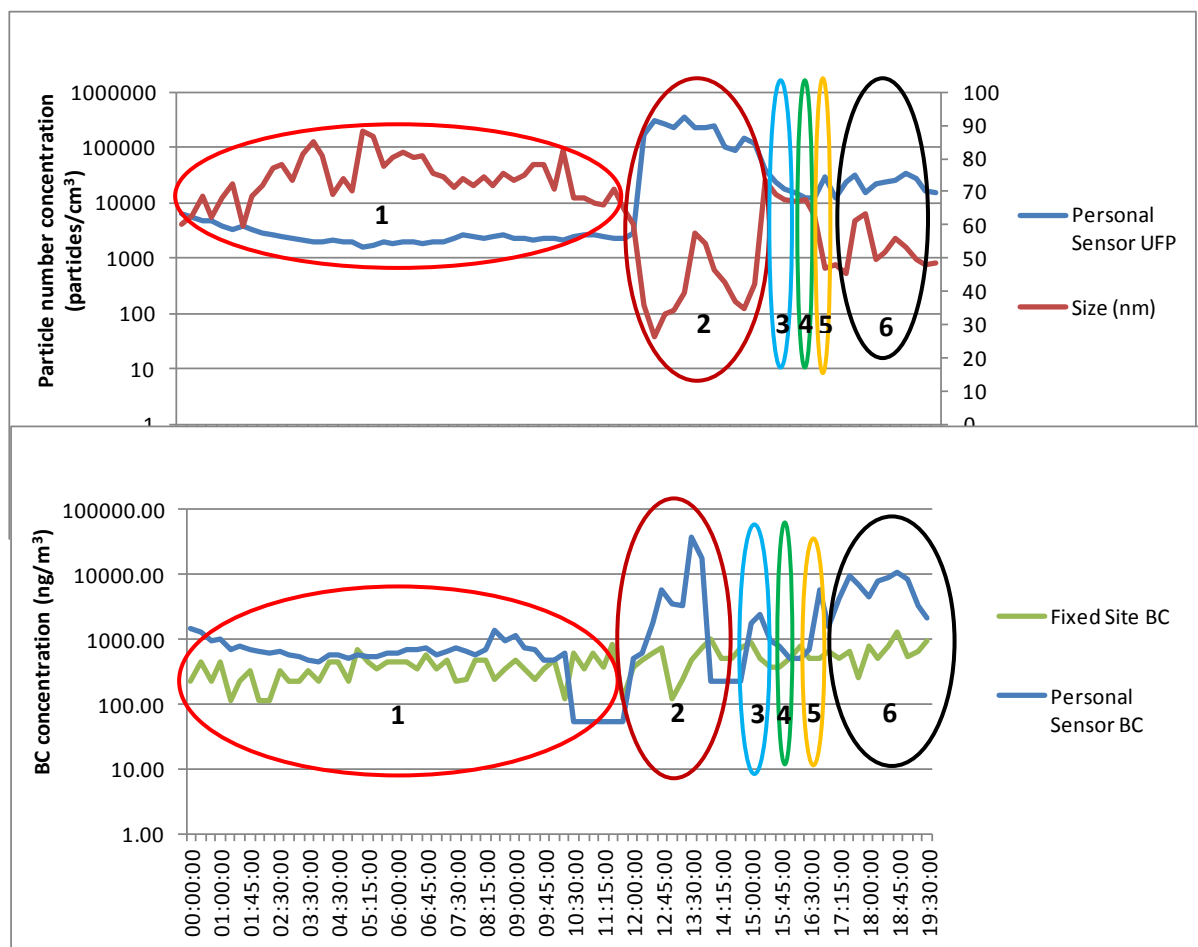
12.3.1 Appendix Table 1: Time-series Table for Day 1 PE in the different activities/ME

Time	Personal Sensor UFP (particle/cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
11:00:00	3719.54	65	1128.92	488.95	Home-Bedroom
11:15:00	3620.11	63	795.04	373.82	Home-Bedroom
11:30:00	3382.59	64	913.34	366.55	Home-Bedroom
11:45:00	19954.91	40	1150.57	339.87	Home-Bedroom
12:00:00	41702.87	43	809.57	383.71	Kitchen-Food-grilling
12:15:00	130256.67	44	1280.97	592.51	Kitchen-Food-grilling
12:30:00	84273.49	47	702.33	678.22	Home-Bedroom
12:45:00	50927.31	55	823.62	757.72	Home-Bedroom
13:00:00	33792.02	51	1064.40	733.06	Car-Driving
13:15:00	9545.46	64	832.85	2892.70	Car-Driving
13:30:00	9720.63	61	1076.33	2010.30	Car-Driving
13:45:00	9315.89	59	962.49	1806.46	Car-Driving
14:00:00	13109.63	59	1089.30	2221.08	Car-Driving
14:15:00	13091.35	53	1218.64	346.96	Car-Driving
14:30:00	11385.98	58	858.16	1031.06	Home-Bedroom
14:45:00	9876.57	61	1357.76	1206.29	Home-Bedroom
15:00:00	9328.98	61	1242.76	1183.18	Home-Bedroom
15:15:00	8839.37	61	1377.54	1173.56	Home-Bedroom
15:30:00	8532.00	61	1261.92	1161.87	Home-Bedroom
15:45:00	8358.07	62	1142.49	1264.53	Home-Bedroom
16:00:00	7953.13	64	1278.06	1183.63	Kitchen-Food-Microwaving,boiling water
16:15:00	8165.28	65	1287.83	4205.25	Kitchen-Food-Microwaving,boiling water
16:30:00	8724.25	65	1296.57	1215.20	Housework-Washingup, heating on
16:45:00	9464.93	60	1305.35	1335.44	Housework-Washingup, heating on
17:00:00	8850.50	60	1314.18	1124.71	Home-Bedroom, heating off
17:15:00	7595.07	62	1190.75	971.27	Home-Bedroom, heating off
17:30:00	6792.42	63	930.83	1128.90	Home-Bedroom, heating off
17:45:00	6586.63	60	1070.07	1069.75	Home-Bedroom, heating off
18:00:00	5950.26	63	806.61	1155.36	Home-Bedroom, heating off
18:15:00	5621.35	64	944.99	1153.26	Home-Bedroom, heating off
18:30:00	5312.43	67	948.96	1227.52	Home-Bedroom, heating off
18:45:00	5509.69	65	680.67	1118.59	Home-Bedroom, heating off
19:00:00	5621.38	65	819.54	1240.81	Home-Bedroom, heating off
19:15:00	5750.15	63	548.65	1346.66	Home-Bedroom, heating off
19:30:00	5814.20	64	687.53	1301.14	Home-Bedroom, heating off
19:45:00	5821.46	62	551.87	1373.38	Home-Bedroom, heating off
20:00:00	6027.56	62	553.25	1314.04	sleeping
20:15:00	6244.82	60	555.10	1363.83	sleeping
20:30:00	6085.26	63	695.61	1417.96	sleeping
20:45:00	6020.98	63	278.94	1402.78	sleeping
21:00:00	6127.80	62	699.67	1249.19	sleeping
21:15:00	5694.31	63	702.00	1269.38	sleeping
21:30:00	5698.31	64	986.08	1259.73	sleeping
21:45:00	6227.81	58	565.35	1157.79	sleeping
22:00:00	7572.96	57	425.07	1092.33	sleeping
22:15:00	7179.26	57	568.17	1178.98	sleeping
22:30:00	6429.30	60	569.59	911.82	sleeping
22:45:00	5973.29	63	428.25	1021.69	sleeping
23:00:00	5758.85	63	429.32	913.06	sleeping
23:15:00	5624.20	60	430.03	927.38	sleeping
23:30:00	5251.84	63	574.80	904.79	sleeping

12.3.2 Appendix Table 2: Time series Table for Day 2 PE in the different activities/ME

Time	Personal Sensor UFP (particle/ cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
04:00:00	4051.19	55.38	296	478.50	Sleeping
04:15:00	3495.96	70.29	296	480.73	Sleeping
04:30:00	3438.11	70.72	296	456.04	Sleeping
04:45:00	3014.25	80.80	297	458.03	Sleeping
05:00:00	3248.09	72.94	297	441.74	Sleeping
05:15:00	3230.45	71.82	298	490.09	Sleeping
05:30:00	3094.43	75.69	298	482.07	Sleeping
05:45:00	2898.31	79.93	298	385.26	Sleeping
06:00:00	3177.89	76.65	299	463.04	Home-Bedroom
06:15:00	3029.00	75.04	299	351.73	Home-Bedroom
06:30:00	2770.63	77.35	299	361.78	Home-Bedroom
06:45:00	3101.33	70.46	300	412.95	Home-Bedroom
07:00:00	3076.95	67.05	150	356.83	Home-Bedroom
07:15:00	2991.06	72.54	150	316.93	Home-Bedroom
07:30:00	3104.42	72.82	150	326.56	Home-Bedroom
07:45:00	3080.17	76.87	150	300.60	Home-Bedroom
08:00:00	3494.13	67.52	151	405.77	Housework-Tidying,Washingup, Heating on
08:15:00	2649.76	79.17	151	413.15	Housework-Tidying,Washingup, Heating on
08:30:00	3422.93	71.37	302	1077.28	Kitchen-Food-Toasting,Microwaving
08:45:00	125318.31	34.58	302	632.89	Kitchen-Food-Toasting,Microwaving
09:00:00	69578.83	30.81	302	128.05	Sleeping
09:15:00	39631.09	33.98	454	128.04	Sleeping
09:30:00	25957.63	35.93	152	128.05	Kitchen-Housework-Washingup
09:45:00	17507.60	38.44	304	128.07	Kitchen-Housework-Washingup
10:00:00	12491.35	40.54	304	920.23	Home-Bathroom,Home-Bedroom
10:15:00	8553.61	45.83	305	795.02	Home-Bathroom,Home-Bedroom
10:30:00	6286.07	50.13	458	749.22	Home-Bathroom,Home-Bedroom
10:45:00	18672.40	54.78	459	563.64	Home-Bathroom,Home-Bedroom
11:00:00	36918.85	74.87	460	1032.13	Case @ Home-Volunteer not there, Heating off
11:15:00	21034.98	79.46	461	1611.10	Case @ Home-Volunteer not there, Heating off
11:30:00	13859.16	81.87	615	828.21	Case @ Home-Volunteer not there, Heating off
11:45:00	11613.06	76.67	926	705.69	Case @ Home-Volunteer not there, Heating off
12:00:00	10096.42	73.67	619	612.31	Case @ Home-Volunteer not there, Heating off
12:15:00	9764.00	67.30	621	608.71	Case @ Home-Volunteer not there, Heating off
12:30:00	8787.42	66.42	467	573.67	Case @ Home-Volunteer not there, Heating off
12:45:00	7951.46	64.80	468	564.97	Case @ Home-Volunteer not there, Heating off
13:00:00	8206.96	60.61	625	567.56	Case @ Home-Volunteer not there, Heating off
13:15:00	7164.75	63.80	313	629.48	Case @ Home-Volunteer not there, Heating off
13:30:00	6976.70	61.39	471	637.18	Case @ Home-Volunteer not there, Heating off
13:45:00	6744.55	60.96	472	674.04	Case @ Home-Volunteer not there, Heating off
14:00:00	6642.02	61.81	631	703.20	Case @ Home-Volunteer not there, Heating off
14:15:00	6544.11	58.71	790	792.66	Case @ Home-Volunteer not there, Heating off
14:30:00	5916.06	58.09	951	1022.97	Home-Bedroom-Sleeping
14:45:00	5859.09	57.83	795	985.96	Home-Bedroom-Sleeping
15:00:00	5663.40	58.24	802	751.79	Housework-Laundry
15:15:00	5594.49	55.40	402	1078.18	Housework-Laundry
15:30:00	4949.57	58.57	201	1499.26	Housework-Laundry
15:45:00	4340.72	66.72	303	1639.56	Housework-Laundry
16:00:00	4460.13	59.45	405	1550.19	Housework-Laundry
16:15:00	4451.94	58.57	406	1618.29	Housework-Laundry
16:30:00	4337.89	61.62	407	3744.47	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
16:45:00	3831.50	65.50	409	6204.19	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
17:00:00	3862.36	63.65	307	12031.56	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
17:15:00	6973.74	51.15	514	6476.57	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
17:30:00	6877.67	50.57	515	4032.11	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
17:45:00	6483.58	56.73	517	3768.11	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
18:00:00	5905.63	53.48	311	3284.36	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
18:15:00	6265.25	53.04	312	2477.18	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
18:30:00	9587.74	52.69	521	3029.61	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
18:45:00	11900.78	52.27	627	4424.94	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
19:00:00	10769.87	54.09	420	11470.49	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
19:15:00	14414.48	51.96	421	18067.32	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
19:30:00	15159.27	55.27	634	31796.81	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
19:45:00	25050.56	61.76	212	20198.63	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
20:00:00	22836.48	60.70	425	12038.29	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
20:15:00	19375.37	62.33	532	9899.02	Housework-Laundry, Food-Microwaving,BBQ infiltration windows closed
20:30:00	18374.80	62.20	213	8511.25	Sleeping
20:45:00	17279.07	63.31	321	7225.19	Sleeping
21:00:00	15444.93	66.04	536	7468.58	Sleeping
21:15:00	14360.31	65.35	215	8789.81	Sleeping
21:30:00	13376.65	64.84	108	13272.25	Sleeping
21:45:00	14888.24	63.46	432	14818.30	Sleeping
22:00:00	14848.09	59.33	324	8444.72	Sleeping
22:15:00	12354.76	61.67	108	6165.22	Sleeping
22:30:00	10964.39	63.24	325	4774.34	Sleeping
22:45:00	9972.52	63.54	326	3715.80	Sleeping
23:15:00	8190.20	62.73	327	2411.58	Sleeping
23:30:00	7266.93	64.65	327	2128.20	Sleeping

12.3.3 Appendix Figure 1: Time series Chart for Day 3 PE in the different activities/ME



1 = Sleeping

2 = Kitchen-Food-Cooking

3 = Housework-Cleaning-Washing-up

4 = Home-Bedroom

5 = Car-Taxi

6 = Car-Driving

12.3.4 Appendix Table 3: Time series Table for Day 4 PE in the different activities/ME

Time	Personal Sensor UFP (particle/cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
00:00:00	4883.52	83	1702.70	1853.23	Home-Bedroom-Sleeping
00:15:00	4571.48	83	1395.55	1880.78	Home-Bedroom-Sleeping
00:30:00	4358.25	82	1517.36	1921.39	Home-Bedroom-Sleeping
00:45:00	4366.78	84	1421.28	2241.95	Home-Bedroom-Sleeping
01:00:00	4593.80	80	1434.92	2151.53	Home-Bedroom-Sleeping
01:15:00	4951.09	78	1336.08	2943.07	Home-Bedroom-Sleeping
01:30:00	5093.02	81	1234.32	2776.31	Home-Bedroom-Sleeping
01:45:00	5435.89	81	1130.88	3294.62	Home-Bedroom-Sleeping
02:00:00	6250.43	83	1138.72	3068.96	Home-Bedroom-Sleeping
02:15:00	6385.59	81	1146.61	3305.20	Home-Bedroom-Sleeping
02:30:00	6039.07	84	1154.54	2978.55	Home-Bedroom-Sleeping
02:45:00	5891.48	82	1045.37	2995.16	Home-Bedroom-Sleeping
03:00:00	5633.56	87	1051.68	2836.54	Home-Bedroom-Sleeping
03:15:00	5604.20	85	1058.03	1871.39	Home-Bedroom-Sleeping
03:30:00	5749.39	82	946.14	1982.37	Home-Bedroom-Sleeping
03:45:00	5569.07	84	1070.81	2380.88	Home-Bedroom-Sleeping
04:00:00	5614.29	83	1197.97	1848.04	Home-Bedroom-Sleeping
04:15:00	5345.19	85	1206.20	1753.38	Home-Bedroom-Sleeping
04:30:00	5069.02	88	971.58	1620.51	Home-Bedroom-Sleeping
04:45:00	4989.35	89	977.41	1663.54	Home-Bedroom-Sleeping
05:00:00	5027.65	87	1106.17	1619.43	Home-Bedroom-Sleeping
05:15:00	5267.85	83	1112.79	1593.27	Home-Bedroom-Sleeping
05:30:00	5415.09	83	995.06	1728.24	Home-Bedroom-Sleeping
05:45:00	5174.22	88	1127.08	2021.96	Home-Bedroom-Sleeping
06:00:00	6434.26	85	1260.85	1770.09	Home-Bedroom
06:15:00	6861.94	85	1268.35	3578.63	Home-Bedroom
06:30:00	6672.96	86	1021.58	2128.07	Home-Bedroom
06:45:00	5992.94	89	899.20	2067.40	Home-Bedroom
07:00:00	5607.60	84	1161.99	1373.61	Case at home-but volunteer not there
07:15:00	5323.22	84	648.28	951.92	Case at home-but volunteer not there
07:30:00	4912.55	84	911.43	1023.92	Case at home-but volunteer not there
07:45:00	4316.76	91	653.78	893.07	Case at home-but volunteer not there
08:00:00	4074.63	92	787.18	608.05	Case at home-but volunteer not there
08:15:00	3901.95	91	394.92	924.08	Case at home-but volunteer not there
08:30:00	4207.22	82	659.86	1137.84	Case at home-but volunteer not there
08:45:00	3711.35	89	662.64	472.88	Case at home-but volunteer not there
09:00:00	3512.41	88	531.45	475.45	Home-Bedroom-but not sleeping
09:15:00	3189.59	93	666.55	491.20	Home-Bedroom-but not sleeping
09:30:00	3175.78	89	400.94	458.46	Home-Bedroom-but not sleeping
09:45:00	3141.91	86	804.58	326.88	Home-Bedroom-but not sleeping
10:00:00	2932.33	88	673.30	326.92	Home-Bedroom-but not sleeping
10:15:00	2505.47	100	945.79	454.36	Home-Bedroom-but not sleeping
10:30:00	2881.56	85	678.96	463.64	Kitchen-Food-Microwaving
10:45:00	2721.33	91	1090.90	931.13	Kitchen-Food-Microwaving
11:00:00	2833.80	86	547.28	662.67	Home-Indoor activity
11:15:00	2762.61	85	961.74	559.79	Home-Indoor activity
11:30:00	2712.60	85	551.87	459.26	Home-Bedroom-Sleeping
11:45:00	2648.24	88	830.56	436.04	Home-Bedroom-Sleeping
12:00:00	2354.36	94	973.85	474.08	Kitchen-Food-Microwaving
12:15:00	2572.30	86	697.93	707.82	Kitchen-Food-Microwaving
12:30:00	2745.52	84	840.31	491.17	Home-Bedroom-Sleeping
12:45:00	2631.43	85	562.07	491.06	Home-Bedroom-Sleeping
13:00:00	2599.93	84	987.72	618.93	Home-Bedroom-Sleeping
13:15:00	2735.48	87	850.14	874.45	Home-Bedroom-Sleeping
13:30:00	2501.35	89	853.67	478.83	Home-Bedroom-Sleeping
13:45:00	2531.37	88	857.22	531.70	Home-Bedroom-Sleeping
14:00:00	2743.61	82	572.90	782.49	Home-Bedroom-Sleeping
14:15:00	2847.78	81	862.92	800.49	Home-Bedroom-Sleeping
14:30:00	3012.91	81	866.49	772.90	Home-Bedroom-Sleeping
14:45:00	2928.38	89	579.57	611.30	Home-Bedroom-Sleeping
15:00:00	3203.09	83	1017.61	889.74	Home
15:15:00	3057.20	85	583.41	866.97	Home
15:30:00	3609.43	85	1171.65	1874.09	Home
15:45:00	3774.13	79	735.91	2439.29	Home
16:00:00	3845.80	80	738.34	401.44	Home
16:15:00	3472.40	82	1186.21	401.51	Home
16:45:00	2824.52	91	1105.82	542.74	Home
17:00:00	2790.58	88	809.20	693.46	Home
17:15:00	2903.00	88	813.47	749.48	Home
17:30:00	3677.22	87	818.49	1384.29	Home
17:45:00	3799.39	86	719.95	820.24	Home
18:00:00	4153.50	88	931.35	931.18	Home
18:15:00	3970.72	85	624.17	1156.17	Home
18:30:00	3732.67	87	942.00	1222.34	Kichen-Boiling,Kitchen-Cleaning
18:45:00	4067.24	90	736.52	1444.59	Kichen-Boiling,Kitchen-Cleaning
19:00:00	4484.20	87	1059.55	879.69	Home-Bedroom-but not sleeping
19:15:00	4075.82	90	852.09	1000.00	Home-Bedroom-but not sleeping
19:30:00	3689.69	95	642.42	1189.22	Home-Bedroom-but not sleeping
19:45:00	3640.18	91	1077.25	1171.81	Home-Bedroom-but not sleeping
20:00:00	3172.37	101	649.73	987.19	Home-Bedroom-but not sleeping
20:15:00	2927.33	103	979.70	853.77	Home-Bedroom-but not sleeping
20:30:00	2646.47	109	546.65	797.97	Home-Bedroom-but not sleeping
20:45:00	2576.42	104	990.82	781.53	Home-Bedroom-but not sleeping
21:00:00	2675.98	98	996.00	797.05	Home-Bedroom-but not sleeping
21:15:00	2467.56	101	667.46	681.27	Home-Bedroom-but not sleeping
21:30:00	2330.35	101	670.94	709.08	Home-Bedroom-but not sleeping
21:45:00	2299.56	100	898.46	720.54	Home-Bedroom-but not sleeping
22:00:00	2162.52	102	790.25	706.92	Sleeping
22:15:00	1886.06	111	680.87	657.34	Sleeping
22:30:00	1874.85	108	569.36	650.52	Sleeping
22:45:00	1942.06	99	801.24	733.46	Sleeping
23:00:00	1810.06	106	574.29	760.69	Sleeping
23:15:00	1821.02	109	923.63	784.54	Sleeping
23:30:00	1563.61	117	579.76	854.89	Sleeping

12.3.5 Appendix Table 4: Time series Table for Day 5 PE in the different activities/ME

Time	Personal Sensor UFP (particle/cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
00:00:00	1808.93	92	584.27	562.74	Home-Bedroom-Sleeping
00:15:00	1431.05	110	586.28	562.97	Home-Bedroom-Sleeping
00:30:00	1402.09	113	588.30	571.66	Home-Bedroom-Sleeping
00:45:00	1505.76	106	471.85	595.26	Home-Bedroom-Sleeping
01:00:00	1385.25	110	118.27	590.14	Home-Bedroom-Sleeping
01:15:00	1512.93	105	473.88	574.47	Home-Bedroom-Sleeping
01:30:00	1414.31	107	118.67	462.92	Home-Bedroom-Sleeping
01:45:00	1361.76	107	475.51	463.03	Home-Bedroom-Sleeping
02:00:00	1468.15	92	477.14	442.71	Home-Bedroom-Sleeping
02:15:00	1354.07	94	478.78	402.08	Home-Bedroom-Sleeping
02:45:00	1400.80	92	480.01	391.64	Home-Bedroom-Sleeping
03:00:00	1315.95	92	360.63	375.72	Home-Bedroom-Sleeping
03:15:00	1174.17	104	120.41	375.86	Home-Bedroom-Sleeping
03:30:00	1233.31	92	482.89	265.02	Home-Bedroom-Sleeping
03:45:00	1224.47	87	726.20	248.00	Home-Bedroom-Sleeping
04:00:00	1109.81	92	242.69	248.02	Home-Bedroom-Sleeping
04:15:00	1081.62	99	608.28	184.07	Home-Bedroom-Sleeping
04:30:00	951.57	107	854.50	184.09	Home-Bedroom-Sleeping
04:45:00	963.87	100	859.62	184.11	Home-Bedroom-Sleeping
05:00:00	1037.37	88	1358.92	184.12	Home-Bedroom-Sleeping
05:15:00	977.48	90	869.93	184.12	Home-Bedroom-Sleeping
05:30:00	848.13	97	999.30	184.15	Home-Bedroom-Sleeping
05:45:00	1006.13	89	1129.95	1210.49	Home-Bedroom-Sleeping
06:00:00	1088.19	89	1011.25	3880.22	Kitchen-Food-Microwaving, Home-Bedroom
06:15:00	1132.24	91	1655.86	111.15	Kitchen-Food-Microwaving, Home-Bedroom
06:30:00	993.75	95	1928.48	126.40	Home-Bedroom-but not Sleeping
06:45:00	876.06	101	1557.19	329.75	Home-Bedroom-but not Sleeping
07:00:00	991.20	101	1964.64	378.71	Home-Bedroom-but not Sleeping
07:15:00	855.28	112	1718.53	351.95	Home-Bedroom-but not Sleeping
07:30:00	1238.24	82	1867.92	435.47	Home-Bedroom-but not Sleeping
07:45:00	1523.31	76	2294.98	223.52	Home-Bedroom-but not Sleeping
08:00:00	1500.69	85	1771.23	100.77	Home-Indoor activity Kitchen-Food-Microwaving
08:15:00	1364.22	95	1787.58	100.77	Home-Indoor activity Kitchen-Food-Microwaving
08:30:00	1042.67	95	968.99	179.42	Home-Bedroom-but not Sleeping
08:45:00	1078.02	97	1392.37	362.57	Home-Bedroom-but not Sleeping
09:00:00	1683.63	86	1401.68	1777.72	Kitchen-Housework-Washing up
09:15:00	1931.29	85	1696.05	492.17	Kitchen-Housework-Washing up
09:30:00	2506.39	75	995.13	820.18	Home-Bathroom, Home-Bedroom
09:45:00	3260.17	68	1286.89	301.18	Home-Bathroom, Home-Bedroom
10:00:00	4196.71	67	1295.44	184.63	Home-Bathroom, Home-Bedroom
10:15:00	5959.64	58	1447.74	97.29	Home-Bathroom, Home-Bedroom
10:30:00	29735.50	50	1603.06	5536.56	Walking in the street, Bus-Waiting at Bus stop, Bus-Travelling on Bus, Walking in the street
10:45:00	28273.72	43	1465.77	9483.84	Walking in the street, Bus-Waiting at Bus stop, Bus-Travelling on Bus, Walking in the street
11:00:00	2734.47	62	1476.67	483.70	Walking in the street/uni, Uni-Office
11:15:00	1904.91	79	1636.40	68.72	Walking in the street/uni, Uni-Office
11:30:00	2188.51	73	1197.95	68.72	Uni-Office
11:45:00	2272.81	71	1356.56	68.72	Uni-Office
12:00:00	2782.31	68	1211.77	152.10	Uni-Office
12:15:00	2732.57	71	1216.74	158.55	Uni-Office
12:30:00	2603.83	71	917.04	320.34	Uni-Office
12:45:00	2747.55	70	1693.63	378.50	Uni-Office
13:00:00	2785.56	70	3267.69	414.96	Uni-Office
13:15:00	2497.06	72	3634.53	409.00	Uni-Office
13:30:00	2765.31	69	2233.92	472.60	Uni-Office
13:45:00	2752.95	70	1506.61	438.83	Uni-Office
14:00:00	2672.56	72	1621.24	452.51	Uni-Office
14:15:00	2372.17	79	1436.12	463.71	Uni-Office
14:30:00	2850.54	67	1556.33	510.84	Uni-Office
14:45:00	3073.33	67	1888.96	500.43	Uni-Office
15:00:00	2938.67	72	1592.10	547.59	Uni-Office
15:15:00	2955.54	70	1285.96	521.35	Uni-Office
15:30:00	3050.51	69	2499.33	584.70	Walking on the street/uni,Bus-waiting @ Bus stop, Travelling on bus, Walking on the street
15:45:00	4499.81	63	2540.91	687.09	Walking on the street/uni,Bus-waiting @ Bus stop, Travelling on bus, Walking on the street
16:00:00	13678.19	42	2470.77	3504.42	Walking on the street/uni,Bus-waiting @ Bus stop, Travelling on bus, Walking on the street
17:00:00	7608.13	58	4551.46	1780.99	Home
17:15:00	7559.72	62	4412.96	3346.65	Home
17:30:00	7513.13	60	3342.70	3097.87	Home
17:45:00	6917.09	64	2743.54	3050.81	Home
18:00:00	7200.41	60	4665.87	3039.65	Home
18:15:00	6693.49	62	4227.23	2925.86	Home
18:30:00	6321.70	64	5173.27	3036.31	Home
18:45:00	5918.56	66	3414.69	3065.49	Home
19:00:00	6040.85	65	2451.00	2899.90	Home
19:15:00	5752.00	65	2333.66	2615.10	Home
19:30:00	5768.46	64	2060.54	2223.68	Home
19:45:00	5352.56	65	2831.16	2248.65	Home
20:00:00	5338.63	64	3635.31	2161.49	Home
20:15:00	4977.09	68	3849.15	2149.71	Home
20:30:00	5281.80	65	4065.49	2463.16	Home
20:45:00	5329.44	67	4138.65	2918.88	Home
21:00:00	5018.64	70	5504.63	2850.33	Home
21:15:00	5212.98	68	3031.82	2868.96	Home
21:30:00	5405.64	68	3296.99	3198.30	Home
21:45:00	5239.49	70	2733.27	3063.32	Home
22:00:00	4952.30	72	2783.82	2684.08	Home
22:15:00	4944.48	70	2726.12	2349.94	Home
22:30:00	5002.07	67	2436.77	2404.11	Home
22:45:00	5155.22	66	2362.55	2459.47	Home
23:00:00	4979.18	68	3204.96	2157.86	Home
23:15:00	4638.83	72	2916.32	2304.56	Sleeping
23:30:00	4577.17	65	2966.84	2325.19	Sleeping

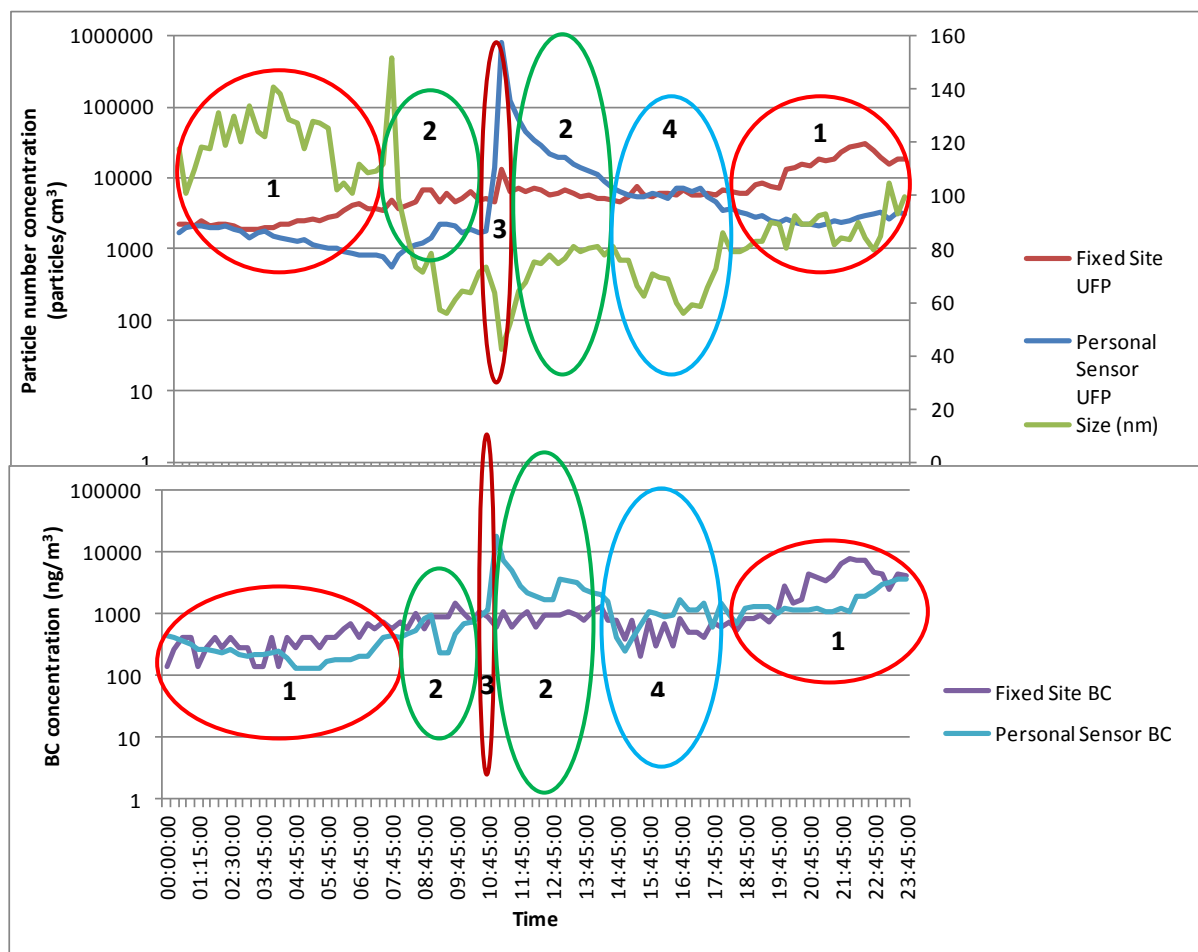
12.3.6 Appendix Table 5: Time series Table for Day 6 PE in the different activities/ME

Time	Fixed Site UFP (particle/cm ³)	Personal Sensor UFP (particle/cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
00:00:00		3362.93	75	2954.82	2024.63	Home-Bedroom-Sleeping
00:15:00		3121.11	77	2496.12	2087.19	Home-Bedroom-Sleeping
00:30:00		3210.69	73	1892.87	2030.88	Home-Bedroom-Sleeping
00:45:00		2959.45	78	2428.32	1937.69	Home-Bedroom-Sleeping
01:00:00		2955.35	75	1419.01	2002.47	Home-Bedroom-Sleeping
01:15:00		2858.94	80	1428.63	1990.49	Home-Bedroom-Sleeping
01:30:00		2893.96	78	1967.95	1822.05	Home-Bedroom-Sleeping
01:45:00		2817.78	82	1056.67	1908.99	Home-Bedroom-Sleeping
02:00:00		2744.62	82	1195.77	1823.02	Home-Bedroom-Sleeping
02:15:00		2883.71	76	801.21	1827.86	Home-Bedroom-Sleeping
02:30:00		2994.74	71	1073.67	1797.42	Home-Bedroom-Sleeping
02:45:00		2682.72	78	808.64	1496.90	Home-Bedroom-Sleeping
03:00:00		2593.61	79	812.71	1463.13	Home-Bedroom-Sleeping
03:15:00		2522.22	76	1225.21	1318.00	Home-Bedroom-Sleeping
03:30:00		2327.07	84	1370.48	1040.23	Home-Bedroom-Sleeping
03:45:00		2163.63	83	1103.73	1092.82	Home-Bedroom-Sleeping
04:00:00		2023.67	87	1527.79	940.58	Home-Bedroom-Sleeping
04:15:00		1913.00	85	2384.83	935.76	Home-Bedroom-Sleeping
04:30:00		2037.57	80	1557.30	793.90	Home-Bedroom-Sleeping
04:45:00		2102.82	77	1711.60	812.59	Home-Bedroom-Sleeping
05:00:00		1778.52	92	1437.00	719.12	Home-Bedroom-Sleeping
05:15:00		1761.20	85	1592.51	685.71	Home-Bedroom-Sleeping
05:30:00		1652.67	89	726.86	632.17	Home-Bedroom-Sleeping
05:45:00		1749.06	85	1609.69	640.00	Home-Bedroom-Sleeping
06:00:00		1912.11	82	1029.42	671.46	Home-bedroom
06:15:00		1591.02	96	1481.54	680.14	Home-bedroom
06:30:00		1510.44	101	1791.04	573.15	Home-bedroom
06:45:00		1417.78	111	1351.02	577.31	Home-bedroom
07:00:00		1658.44	100	1663.46	809.43	Home-bedroom
07:15:00		1714.85	89	1828.09	1226.88	Home-bedroom
07:30:00		1586.80	94	919.29	844.02	Kitchen-Food-Boiling Home-Indoor activity
07:45:00		4960.33	80	1385.69	2758.89	Kitchen-Food-Boiling Home-Indoor activity
08:00:00		61243.67	36	1394.75	1123.50	Home-bedroom
08:15:00		37083.00	32	1401.58	480.85	Home-bedroom
08:30:00		22336.81	35	3313.11	317.69	Home-bedroom
08:45:00		14540.80	38	634.66	818.46	Home-bedroom
09:00:00		8743.83	42	1274.46	824.14	Home-bedroom
09:15:00		6003.82	47	960.49	283.31	Home-bedroom
09:45:00		9600.22	64	1004.41	910.90	Home-bedroom
10:00:00		13756.69	76	1215.93	1898.43	Home-bedroom
10:15:00		9390.81	77	1327.71	3431.26	Home-bedroom
10:30:00		18338.61	67	1030.31	5072.27	Walking on the street,Bus-waiting @ Bus stop, Travelling on bus, Walking on the street
10:45:00		20849.33	55	1246.16	10280.39	Walking on the street,Bus-waiting @ Bus stop, Travelling on bus, Walking on the street
11:00:00		3724.70	71	1361.86	1775.20	Uni-Office
11:15:00		3607.96	75	1376.22	364.01	Uni-Office
11:30:00		3632.54	75	1172.65	762.83	Uni-Office
11:45:00		4003.51	71	1075.37	863.84	Uni-Office
12:00:00		3677.61	81	1408.98	863.27	Uni-Office
12:15:00		3777.07	79	1313.09	862.96	Uni-Office
12:30:00		3310.71	86	1545.30	754.55	Uni-Office
12:45:00		3163.93	84	889.18	842.64	Uni-Office
13:00:00		2988.02	87	1457.48	847.86	Uni-Office
13:15:00		3702.33	72	2269.58	842.40	Uni-Office
13:30:00		3186.24	83	1722.88	897.04	Uni-Office
13:45:00		3423.04	74	1507.38	863.00	Uni-Office
14:00:00		3508.67	72	1286.49	881.59	Uni-Office
14:15:00		3442.15	73	1413.13	941.63	Uni-Office
14:30:00		3611.00	73	1068.06	1026.07	Uni-Office
14:45:00		3670.85	73	1315.52	1024.39	Uni-Office
15:00:00		4061.41	72	1933.23	1012.68	Uni-Office
15:15:00		3952.55	74	1462.36	983.92	Uni-Office
15:30:00		4396.78	67	1350.83	984.68	Uni-Office
15:45:00		4083.20	66	1484.98	1013.50	Uni-Office
16:00:00		3978.72	69	1248.06	1062.63	Uni-Office
16:15:00		3596.43	73	1381.05	1015.18	Uni-Office
16:30:00		3091.41	81	1520.73	978.39	Uni-Office
16:45:00		3390.81	74	1404.68	960.65	Uni-Office
17:00:00		3237.16	74	1415.42	988.38	Uni-Office
18:15:00	5529.981	3300.50	72	660.42	1585.82	home-Bedroom
18:30:00	4861.523	2830.20	77	795.84	1619.87	kitchen-microwaving,boiling hot water
18:45:00	4389.911	2946.20	82	665.43	1333.05	kitchen-microwaving,boiling hot water
19:00:00	4523.581	2853.37	78	800.54	840.78	Home-Bedroom
19:15:00	4892.842	2396.35	89	535.49	652.84	Home-Bedroom
19:30:00	3675.184	2388.83	84	537.29	687.16	Home-Bedroom
19:45:00	3987.188	2361.42	81	808.64	633.23	Home-Bedroom
20:00:00	3867.499	2421.87	90	540.90	722.61	Home-Bedroom
20:15:00	4110.624	2192.59	94	542.72	758.46	Home-Bedroom
20:30:00	4654.452	2253.49	94	816.81	754.71	Home-Sleeping
20:45:00	3826.279	1974.35	105	682.95	621.94	Home-Sleeping
21:00:00	3623.299	2059.13	94	410.80	500.67	Home-Sleeping
21:15:00	4351.596	2153.17	97	1100.05	528.81	Home-Sleeping
21:30:00	3182.898	2182.69	98	275.70	652.54	Home-Sleeping
21:45:00	2882.409	1793.56	105	691.56	496.24	Home-Sleeping
22:00:00	3652.788	1784.96	102	415.63	453.20	Home-Sleeping
22:15:00	4279.595	1726.83	108	695.61	434.62	Home-Sleeping
22:30:00	4049.601	1475.78	110	418.06	410.93	Home-Sleeping
22:45:00	3857.383	1575.50	107	559.27	364.50	Home-Sleeping
23:00:00	2375.711	1443.81	111	420.15	363.36	Home-Sleeping
23:15:00	2535.319	1344.02	109	280.80	362.05	Home-Sleeping
23:30:00	1897.120	1187.96	121	421.90	376.16	Home-Sleeping

12.3.7 Appendix Table 6: Time series Table for Day 7 PE in the different activities/ME

Time	Fixed Site UFP (particle/cm ³)	Personal Sensor UFP (particle/cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
00:00:00	2597	1215.69	103	140.99	337.09	Home-Bedroom-Sleeping
00:15:00	1953	1383.75	95	423.66	267.28	Home-Bedroom-Sleeping
00:45:00	3431	1320.37	85	212.36	257.44	Home-Bedroom-Sleeping
01:00:00	2893	1220.91	91	212.36	254.99	Home-Bedroom-Sleeping
01:15:00	2038	1056.87	106	283.61	246.82	Home-Bedroom-Sleeping
01:30:00	1339	1195.57	105	283.85	243.88	Home-Bedroom-Sleeping
02:00:00	3096	1055.19	106	284.32	208.33	Home-Bedroom-Sleeping
02:15:00	3086	1103.15	99	284.56	208.38	Home-Bedroom-Sleeping
02:30:00	1496	948.28	106	213.77	164.36	Home-Bedroom-Sleeping
02:45:00	1421	1034.09	97	213.77	125.83	Home-Bedroom-Sleeping
03:00:00	1613	932.36	96	142.75	125.84	Home-Bedroom-Sleeping
03:15:00	1408	799.13	111	142.75	125.86	Home-Bedroom-Sleeping
03:30:00	2093	823.65	105	285.98	119.73	Home-Bedroom-Sleeping
03:45:00	1972	819.30	109	143.11	112.74	Home-Bedroom-Sleeping
04:00:00	1780	778.02	101	430.03	112.75	Home-Bedroom-Sleeping
04:15:00	1853	725.78	106	143.46	112.77	Home-Bedroom-Sleeping
04:30:00	1889	719.44	107	287.40	88.35	Home-Bedroom-Sleeping
04:45:00	2401	814.42	88	287.64	88.35	Home-Bedroom-Sleeping
05:00:00	2577	703.85	106	432.53	88.36	Home-Bedroom-Sleeping
05:15:00	2967	773.33	85	288.59	88.36	Home-Bedroom-Sleeping
05:30:00	3013	717.49	91	723.87	88.38	Home-Bedroom-Sleeping
05:45:00	3616	701.95	102	435.40	88.38	Home-Bedroom-Sleeping
06:00:00	4134	581.17	112	1019.29	88.40	Home-Bedroom
06:15:00	4199	520.80	111	731.07	126.78	Home-Bedroom
06:30:00	4601	609.02	89	1027.73	152.38	Home-Bedroom
06:45:00	3977	687.43	82	885.27	152.41	Home-Bedroom
07:00:00	4710	821.07	97	888.19	198.36	Home-Bedroom
07:15:00	4828	746.50	85	1041.35	267.34	Home-Bedroom
07:30:00	5239	816.96	85	896.26	267.41	Home-Bedroom
07:45:00	5293	919.89	76	1351.02	201.53	Home-Bedroom
08:00:00	6210	1518.70	61	1511.00	201.57	Kitchen-Microwaving
08:15:00	6511	1629.56	64	1520.92	201.59	Kitchen-Microwaving
08:30:00	7205	1826.44	59	1840.07	315.51	Home-Bedroom
08:45:00	7503	2179.11	64	1390.22	740.17	Home-Bedroom
09:00:00	7401	4733.57	46	1710.26	756.55	Kitchen-Washingup, Home-Bathroom
09:15:00	7590	7506.69	44	1566.19	512.73	Kitchen-Washingup, Home-Bathroom
09:30:00	7212	3832.94	51	1576.39	992.29	Home-Bedroom
09:45:00	7759	4298.94	59	1746.72	1575.06	Home-Bedroom
10:00:00	7000	3833.28	62	1759.48	3811.73	Car-Taxi
10:15:00	7249	8520.64	64	1772.31	472.99	Uni-Office
10:45:00	9772	4370.57	57	1719.54	470.09	Uni-Office
11:00:00	9153	4439.35	57	1328.87	801.83	Uni-Office
11:15:00	8293	4922.87	57	1756.13	1222.26	Uni-Office
11:30:00	10118	4975.94	55	1777.79	1161.64	Uni-Office
11:45:00	12916	5172.11	62	1586.56	1575.79	Uni-Office
12:00:00	9243	2174.69	76	1389.49	155.08	Uni-Office
12:15:00	8761	2670.36	75	1292.70	155.09	Uni-Office
12:30:00	8967	6015.87	42	1632.83	189.57	Uni-Office
12:45:00	9332	5743.96	42	1429.95	212.58	Uni-Office
13:00:00	7760	4779.45	46	1330.30	228.74	Uni-Office
13:15:00	7050	3817.70	51	1454.96	247.19	Uni-Office
13:30:00	7164	3359.02	50	1467.60	266.42	Uni-Office
13:45:00	6343	3034.15	59	1481.61	299.74	Uni-Office
14:00:00	6264	2760.60	59	1494.45	359.93	Uni-Office
14:15:00	7326	2482.39	66	1508.68	346.38	Uni-Office
14:30:00	5972	2242.00	70	1404.65	333.97	Uni-Office
14:45:00	6316	2035.40	74	1415.55	401.30	Uni-Office
15:00:00	6623	2222.25	71	1787.75	368.16	Uni-Office
15:15:00	5199	2082.20	70	1201.06	390.08	Uni-Office
15:30:00	5037	1988.59	75	1452.40	422.17	Uni-Office
15:45:00	5417	2211.91	69	1219.68	404.40	Uni-Office
16:00:00	4888	2098.25	68	1104.29	176.24	Uni-Office
17:00:00	4456	4985.53	81	1133.80	2037.16	Home bedroom
17:15:00	4347	3876.81	97	1013.82	2192.44	Home bedroom
17:30:00	4307	4537.16	94	1019.86	1648.25	Home bedroom
17:45:00	4407	3680.15	106	768.14	1443.26	Home bedroom
18:00:00	4081	3786.89	93	900.72	885.56	Home bedroom
18:15:00	4856	3079.42	103	775.32	1071.95	Home bedroom
18:30:00	5089	3571.31	95	908.36	786.82	Home bedroom
18:45:00	5315	3318.35	98	651.57	763.77	Home bedroom
19:00:00	4923	3109.91	98	916.06	698.95	Home bedroom
19:15:00	3992	2757.22	103	393.59	652.35	Home bedroom
19:30:00	3990	2950.26	94	658.20	715.88	Home bedroom
19:45:00	4663	2459.57	105	660.42	597.62	Home bedroom
20:00:00	4988	2289.51	103	662.64	574.19	Home bedroom
20:15:00	4601	2172.65	103	664.32	696.97	Home bedroom
20:30:00	4775	1770.73	116	533.24	449.97	Home-Sleeping
20:45:00	3703	1688.85	118	400.94	536.14	Home-Sleeping
21:00:00	3296	1875.07	109	401.95	535.77	Home-Sleeping
21:15:00	3560	1768.70	110	536.84	477.28	Home-Sleeping
21:30:00	3290	1629.02	111	538.19	444.27	Home-Sleeping
21:45:00	3138	1704.22	106	539.54	420.06	Home-Sleeping
22:00:00	3090	1578.29	115	405.34	445.33	Home-Sleeping
22:15:00	2990	1703.70	115	541.81	465.39	Home-Sleeping
22:30:00	3109	2030.04	108	407.38	391.84	Home-Sleeping
22:45:00	3269	2118.35	102	408.40	429.97	Home-Sleeping
23:00:00	2836	2063.37	104	409.43	471.96	Home-Sleeping
23:15:00	2726	1904.69	107	409.77	418.75	Home-Sleeping
23:30:00	2595	2103.36	93	410.46	296.94	Home-Sleeping

12.3.8 Appendix Figure 1: Time series Chart for Day 8 PE in the different activities/ME



1 = Sleeping

2 = Home-Bedroom

3 = Kitchen-Food-Frying

4 = Housework

12.3.9 Appendix Table 7: Time series Table for Day 9 PE in the different activities/ME

Time	Fixed Site UFP (particle/cm ³)	Personal Sensor UFP (particle/cm ³)	Personal Sensor Size (nm)	Fixed Site BC (ng/m ³)	Personal Sensor BC (ng/m ³)	Activity/Microenvironment
00:00:00	19592	3907.51	94	4490.31	4082.42	Home-Bedroom-Sleeping
00:15:00	17786	4324.57	88	3590.74	4734.25	Home-Bedroom-Sleeping
00:30:00	19613	4285.52	93	4487.21	5138.13	Home-Bedroom-Sleeping
00:45:00	20166	4303.47	96	4980.02	5995.07	Home-Bedroom-Sleeping
01:00:00	20294	5312.19	84	5000.68	6485.99	Home-Bedroom-Sleeping
01:15:00	20474	5374.62	90	6041.79	6771.80	Home-Bedroom-Sleeping
01:30:00	19831	5344.67	89	5454.78	7130.94	Home-Bedroom-Sleeping
01:45:00	17862	5097.60	95	4932.50	7202.10	Home-Bedroom-Sleeping
02:00:00	18540	5530.43	91	6035.14	6973.20	Home-Bedroom-Sleeping
02:15:00	18139	5465.67	91	5498.44	6594.96	Home-Bedroom-Sleeping
02:30:00	19534	5473.22	91	5945.54	6692.69	Home-Bedroom-Sleeping
02:45:00	18249	5382.15	94	5657.67	7150.92	Home-Bedroom-Sleeping
03:00:00	19397	5278.06	95	5023.14	7072.48	Home-Bedroom-Sleeping
03:15:00	15787	5213.67	95	3874.32	6954.71	Home-Bedroom-Sleeping
03:30:00	18784	5364.98	96	4882.86	6562.52	Home-Bedroom-Sleeping
03:45:00	17043	5575.57	91	4056.66	6674.26	Home-Bedroom-Sleeping
04:00:00	19239	5099.80	96	4585.26	6167.77	Home-Bedroom-Sleeping
04:15:00	16597	5109.17	96	3847.81	6133.91	Home-Bedroom-Sleeping
04:30:00	17385	4878.91	100	4059.27	6037.90	Home-Bedroom-Sleeping
04:45:00	18847	4913.83	100	4289.90	6179.77	Home-Bedroom-Sleeping
05:00:00	24402	5053.37	95	4990.23	5830.77	Home-Bedroom-Sleeping
05:15:00	18638	4756.33	100	4294.28	6069.76	Home-Bedroom-Sleeping
05:30:00	16905	4621.93	102	4157.53	5501.94	Home-Bedroom-Sleeping
05:45:00	17425	4748.68	97	4508.31	5510.16	Home-Bedroom-Sleeping
06:00:00				4492.19	5233.06	Home-Bedroom
06:15:00				4333.14	5096.84	Home-Bedroom
06:30:00				3215.62	4752.66	Home-Bedroom
06:45:00				2031.78	4992.20	Home-Bedroom
07:00:00				1778.65	3299.08	Home-Bedroom
07:15:00				825.04	3459.10	Home-Bedroom
07:30:00				690.41	3043.39	Home-Bedroom
07:45:00	4478	5671.17	66	553.71	3853.33	Home-Bedroom
08:00:00	3860	4215.53	96	277.32	3250.38	Home-Bedroom
08:15:00	4869	3370.89	105	416.67	3537.94	Home-Bedroom
08:30:00	6386	2843.31	107	139.01	3398.96	Home-Indoor activity
08:45:00	9100	2492.13	104	278.47	1988.39	Home-Indoor activity
09:00:00	12921	1741.52	123	697.93	1453.25	Home-Indoor activity
09:15:00	13070	3780.00	106	559.74	4797.14	Home-Indoor activity
09:30:00	14939	25571.48	36	420.85	528.37	Kitchen-Food-Toasting, Microwaving,Boiling
09:45:00	10607	25847.53	34	844.51	528.33	Kitchen-Food-Toasting, Microwaving,Boiling
10:00:00	9142	19513.11	39	423.66	1204.03	Kitchen-Housework-Washing up, Cleaning Home-Bathroom
10:15:00	8354	16668.06	42	709.04	350.95	Kitchen-Housework-Washing up, Cleaning Home-Bathroom
10:30:00	7757	9757.28	51	568.17	350.85	Home-Bedroom
10:45:00	8882	8453.00	67	712.57	350.91	Home-Bedroom
11:00:00	8654	6067.74	84	571.48	1641.85	Home-Bedroom
11:15:00	9794	4630.02	83	429.32	1294.04	Home-Bedroom
11:30:00	13820	3718.06	84	0.00	1161.55	Home-Bedroom
11:45:00	9020	3386.35	83	575.28	685.69	Home-Bedroom
12:00:00	8421	3653.65	76	575.75	991.91	Home-Bedroom
12:15:00	8022	3338.52	80	721.48	705.96	Home-Bedroom
12:30:00	7784	3082.02	82	289.31	844.75	Home-Bedroom
12:45:00	8511	3049.20	79	434.32	624.69	Home-Bedroom
13:00:00	8220	2837.65	86	145.01	671.72	Home-Bedroom
13:15:00	7289	3009.02	80	290.75	692.61	Home-Bedroom
13:30:00	7103	3301.24	76	728.67	566.49	Kitchen-Food-Microwaving,Home-Bedroom
13:45:00	7667	3314.19	70	730.47	1080.99	Kitchen-Food-Microwaving,Home-Bedroom
14:00:00	7754	3455.19	68	585.82	862.70	Home-Bedroom
14:15:00	7601	3251.48	69	587.27	791.28	Home-Bedroom
14:30:00	7422	3160.04	69	441.18	980.25	Kitchen-Food-Microwaving,Home-Bedroom
14:45:00	6904	2985.02	79	147.42	3075.31	Kitchen-Food-Microwaving,Home-Bedroom
15:00:00	6939	2623.87	88			Home-Bedroom
15:15:00	10189	2833.36	78			Home-Bedroom
15:30:00	10442	2718.00	86			Home-Bedroom
15:45:00	7537	3701.33	79			Home-Bedroom
16:00:00	7924	4942.53	79			Home-Bedroom
16:15:00	7600	4854.52	78			Home-Bedroom
16:30:00	7821	4722.70	76			Home-Bedroom
16:45:00	7424	4383.85	77			Home-Bedroom
17:00:00	7033	3839.67	84			Home-Bedroom
17:15:00	8287	3838.49	80			Home-Bedroom
17:30:00	7400	4569.72	88			Home-Bedroom
17:45:00	7236	17364.80	102			Home-Bedroom
18:00:00	6581	12279.89	111			Home-Bedroom
18:15:00	5582	9609.96	122			Home-Bedroom
18:30:00	10026	8046.00	132			Home-Bedroom
18:45:00	15653	5730.35	148			Home-Bedroom
19:00:00	16039	6485.91	130			Home-Bedroom
19:15:00	13649	6191.07	133			Home-Bedroom
19:30:00	18723	5784.19	137			Home-Bedroom
19:45:00	17921	5591.31	136			Home-Bedroom
20:00:00	16804	5348.50	137			Home-Living room
20:15:00	15965	5857.02	134			Home-Living room
20:30:00	15642	4728.39	145			Home-Bedroom-Sleeping
20:45:00	14460	4478.67	150			Home-Bedroom-Sleeping
21:00:00	15144	4633.33	138			Home-Bedroom-Sleeping
21:15:00	15781	4537.07	144			Home-Bedroom-Sleeping
21:30:00	17861	4643.24	133			Home-Bedroom-Sleeping
21:45:00	16691	4626.31	129			Home-Bedroom-Sleeping
22:00:00	17392	4440.38	126			Home-Bedroom-Sleeping
22:15:00	18740	3991.67	138			Home-Bedroom-Sleeping
22:30:00	22561	3961.49	132			Home-Bedroom-Sleeping
22:45:00	22244	3627.96	140			Home-Bedroom-Sleeping
23:00:00	19406	3731.80	135			Home-Bedroom-Sleeping
23:15:00	19522	4380.80	119			Home-Bedroom-Sleeping
23:30:00	21256	4394.87	120			Home-Bedroom-Sleeping
23:45:00	22050	4194.05	123			Home-Bedroom-Sleeping