2016

An Evaluation of exposures to respirable particulates, environmental PM2.5, PAHs and metal compounds in Western Australia

Desmond D. Menon

Edith Cowan University

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An Evaluation of Exposures to Respirable Particulates, Environmental PM$_{2.5}$, PAHs and Metal Compounds in Western Australia

Desmond Dev Menon

BSc (Hons – 1$^{st}$ Class)

This thesis is re-submitted for examination in fulfilment of the requirements for the award of the Degree of Doctor of Philosophy

School of Medical and Health Science
Edith Cowan University
2016
Declaration

I certify that this thesis does not, to the best of my knowledge and belief:

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Date……………..August 2016.........
Acknowledgements

I am grateful to Edith Cowan University for providing me with the opportunity to do this PhD study. I would also like to express my gratitude to a number of people without whom I would not have been able to complete my PhD thesis. First and foremost I would like to thank my supervisor, Associate Prof Jacques Oosthuizen, who not only showed his support, but also his enthusiasm throughout the study. I would also like to thank Prof. Wei Wang for his understanding and resourcefulness to which I am also indebted to. My educational and professional development in the field of air quality research from the context of public health has grown significantly under their guidance. I would also like to thank Dr Martyn Cross for taking the time to impart some of his practical experience in this area.

This work, of course, could not have been conducted without the help from the Shire of Collie and town of Dalyellup. I thank the councils for their support and our participating families who allowed us to gather the scientific data in this thesis. In particular, I am grateful to Leonie and James and their son, Fergus Scoffern for their enthusiasm and effort in helping to promote the study and recruit participants, and their friendship.

I would also like to thank Prof. Shao Bing, Director, Beijing Centre for Disease Prevention and Control Central Laboratory, and his diligent team, for analysing the samples professionally, and the Occupational Health Nurse, at the remote nickel and cobalt mine and refinery in Western Australia, for facilitating the participation of personnel, and the collection, storage and transport of urine samples for analysis.

Finally, I would like to offer my gratitude to my family and friends for their emotional support and helping me maintain my sanity through the obstacles in this experience. To my mum, Juliana Menon, I thank her for her constant belief and encouragement, and last but not least my wife, Takako Hosokawa, for her love and patience.
USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.
Abstract

It has been well established that air pollution is associated with health impacts. This study investigated the relationship between exposure to air pollutants and potential biomarkers of health effects. The research project was conducted in 2 separate study locations and cohorts.

Study 1: An Evaluation of Children’s Exposures to Respirable Particulates, Environmental PM$_{2.5}$, PAHs and Metal Compounds in The South West of Western Australia.

A cross sectional study to evaluate the exposures of children (n=18), and controls (n=15) to respirable particulates PAHs and metal compounds in the South West of Australia during 2011. Ambient particulate matter (PM$_{2.5}$) samples were found to be significantly higher in Collie as compared to Dalyellup. However, personal PM$_{2.5}$ concentrations between locations were not significantly different and both PAH and heavy metals were below the levels of detection. Urinary levels of 1-hydroxypyrene (1-OHpy) were below the level of detection. Copper, selenium and nickel were present in urine samples and these were not significantly different between locations, nor was there any correlation with residential areas within study locations. Urinary nickel concentrations were higher than expected for non-occupational cohorts and although statistically insignificant, mean values of urinary nickel were highest for homes using gas as a fuel source.

These data endorse current views that the reconstruction of PM$_{2.5}$ exposures and related respiratory health effects based simply on the mass of airborne particulate matter alone is not sufficient in providing an insight to the respiratory health of susceptible subgroups such as children. The presence of certain urinary heavy metals suggests possible accumulation in participants via alternative routes of entry, probably a dietary source. Studies that rely purely on data accrued from ambient PM$_{2.5}$ mass, and/or general health data might not detect or underestimate significant relationships between certain components of PM$_{2.5}$.

Study 2: Urinary levels of malondialdehyde and 8-deoxyguanosine as biomarkers of oxidative DNA damage induced by exposure to nickel and cobalt in metal refinery workers.

Metal mining and refinery workers in Australia have the potential to be occupationally exposed to quantities of heavy metals that may be associated with health impacts affecting major organ and immune systems. Current regulatory and internal company
policies and guidelines require regular monitoring of occupational exposures of employees through a combination of air borne sampling as well as biological monitoring for heavy metals.

Toxic levels of heavy metals accumulated in the body have been shown to elicit inflammatory responses linked to exacerbated health effects impacting the respiratory, cardiovascular and nervous systems. There are many studies that have established a significant link between heavy metal exposure and increased oxidative stress. In light of these observations, this study investigated urinary levels of nickel (Ni) and cobalt (Co) and Malondialdehyde (MDA) and 8-hydroxy-2’deoxyguanosine (8-OHdG) which are oxidative stress markers indicative of cellular and DNA damage.

A positive correlation between urinary Ni and Co exposure and oxidative stress markers among refinery workers was established. This finding has implications for occupational health management as individual responses to exposures can now be identified. In addition to implementing a global mean air borne exposure standard, individual variation and sensitivity can be accommodated through the use of urinary oxidative stress markers.
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<tr>
<td>µm</td>
<td>Micrometer (Micron)</td>
</tr>
<tr>
<td>1-OHp</td>
<td>1 – Hydroxypyrene</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>8-hydroxy-2’deoxyguanosine</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>Al</td>
<td>Aluminium</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of co-variance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APHEA-2</td>
<td>Air Pollution and Health: A European Approach – 2</td>
</tr>
<tr>
<td>As</td>
<td>Arsenic</td>
</tr>
<tr>
<td>BALF</td>
<td>Broncho-alveolar lavage fluid</td>
</tr>
<tr>
<td>BEI</td>
<td>Biological Exposure Indexes</td>
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<tr>
<td>BOEL</td>
<td>Biological Exposure Limit</td>
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<tr>
<td>CAA</td>
<td>Clean Air Act</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
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<tr>
<td>CO</td>
<td>Carbon monoxide</td>
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<tr>
<td>Co</td>
<td>Cobalt</td>
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<tr>
<td>COX2</td>
<td>Cyclooxygenase-2</td>
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<tr>
<td>Cr</td>
<td>Chromium</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>DEC</td>
<td>Department of Environment and Conservation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EPP</td>
<td>Environmental Protection Policy</td>
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<tr>
<td>Fe</td>
<td>Iron</td>
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<tr>
<td>FEV</td>
<td>Forced expiratory volume</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced volume capacity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>GC/MS</td>
<td>Gas Chromatography / Mass Spectrometry</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>HPLC-FD</td>
<td>High Performance Liquid Chromatography – Fluorescence Detection</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Inductively Coupled Plasma – Atomic Emission Spectrometry</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma – Mass Spectrometry</td>
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<tr>
<td>IL-1b</td>
<td>Interleukin-1beta</td>
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<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<td>MDA</td>
<td>Malondialdehyde</td>
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<td>Mn</td>
<td>Manganese</td>
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<td>MPO</td>
<td>Myeloperoxidase</td>
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<td>NAG</td>
<td>N-Acetyl glucosamine</td>
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<td>NATA</td>
<td>National Association of Testing Authorities</td>
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<tr>
<td>NEPC</td>
<td>(Australian) National Environmental Council</td>
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<tr>
<td>NEPM</td>
<td>National Environment Protection Measures</td>
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<tr>
<td>Ni</td>
<td>Nickel</td>
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<td>NO2</td>
<td>Nitrogen dioxide</td>
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<tr>
<td>O3</td>
<td>Ozone</td>
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<td>PAHs</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
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<td>Pb</td>
<td>Lead</td>
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<tr>
<td>PM</td>
<td>Particulate Matter</td>
</tr>
<tr>
<td>PM10</td>
<td>Particulate Matter – 10 microns in diameter or less</td>
</tr>
<tr>
<td>PM10nm</td>
<td>Particulate Matter – 10 nanometers in diameter or less</td>
</tr>
<tr>
<td>PM2.5</td>
<td>Particulate Matter – 2.5 microns in diameter or less</td>
</tr>
<tr>
<td>Ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
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<tr>
<td>Se</td>
<td>Selenium</td>
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<tr>
<td>Symbol</td>
<td>Term</td>
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<tr>
<td>Sn</td>
<td>Tin</td>
</tr>
<tr>
<td>SO2</td>
<td>Sulphur dioxide</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
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<tr>
<td>Th</td>
<td>Thallium</td>
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<tr>
<td>TNF-a</td>
<td>Tumour necrosis factor – alpha</td>
</tr>
<tr>
<td>US EPA</td>
<td>United States Environmental Protection Authority</td>
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<tr>
<td>V</td>
<td>Vanadium</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>Zn</td>
<td>Zinc</td>
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Study One:

1. An Evaluation of Children’s Exposures to Respirable Particulates, Environmental PM$_{2.5}$, PAHs and Metal Compounds in The South West of Western Australia

(Study 1 Submitted in 2014 and revised 2016)

1.1 Introduction

The South Western region of Western Australia (WA) is renowned for its viticulture and dairy farming and is a popular tourist destination. However, it also contains a number of industries including mining, fertilizer manufacturing, aluminium refining and coal fired power stations and these are concentrated in the Collie region. In recent years communities surrounded by the industries have been increasingly vocal about their concerns regarding air pollution and its effect on their health and there have been instances where industrial negligence have resulted in air pollution attributed adverse health problems (Community Newspaper Group, 2006, 2010).

More recently, increasing electricity demand has led to the re-commission of previously shut down power stations in the South West (Synergy, 2014). Power stations contribute significantly to particulate matter air pollution, which is linked to a myriad of adverse health effects (Department of Environment and Conversation (DEC), 2008) and this has fuelled existing concerns within the Collie community, related to air pollution and the respiratory health of local residents.

Particulate matter (PM) in respirable dust has been associated with increased respiratory and cardiovascular mortality and morbidity (Graff, et al., 2009; Suresh, et al., 2009; Karr, et al., 2009) and despite progressive decreases in PM exposure levels over the last few decades, no associated decreases in human health outcomes have been observed (World Health Organisation (WHO), 2013; Valberg, 2004). As such, the World Health Organization -Air Quality Group, have been unable to identify a threshold concentration below which ambient PM has no effect on health (WHO, 2003). According to the WHO, 2003, this issue needs to be resolved by obtaining a better understanding of PM and its constituents on the health of individuals, thus more specific studies of exposure, impact and health effects are required.
There are a number of factors that have contributed to the current lack of understanding in this area. In order to represent the populace in a study area, most epidemiological or pathological studies have resorted to utilising pre-existing data from fixed air pollutant monitoring sites, and/or pre-existing medical data of respiratory or cardiovascular, or all-cause morbidity and mortality as surrogates to represent population exposure to PM and health effects respectively (Zanobetti & Schwartz, 2009; Karr et al., 2007; Nieuwenhuijsen, 2003). While associations between population exposure and health effects can be observed, the lack of specificity in the data sets present a limited view of their relationship, and any conclusions that can be derived from them. In addition, the influences from confounding effects of other forms of environmental exposures as well as lifestyle factors such as smoking are difficult to quantify or isolate from the overall correlation.

Many of the initial studies used to determine suitable air quality guidelines were based on studies of healthy adults or other non-specific health data. As such, the effects on more susceptible sub-populations were not adequately defined, restricting the external validity of the study findings. Literature indicates that the majority of available data providing evidence of respiratory and cardiovascular morbidity from PM relates to the population in general and it is only in recent years that studies have been conducted to explore the vulnerability of populations that are deemed to be more susceptible (Peel, et al., 2007). Infants, children, the elderly, individuals with respiratory diseases such as asthma or bronchiolitis, cardiopulmonary disease, diabetes, mental impairment and hypertension, are among the individuals that are more susceptible to adverse health effects from air pollutants (Fang, et al., 2009; Chahine, et al., 2007; Committee on Environmental Health, 2004; Parker, et al., 2009; Suresh, et al., 2009; Hsu, et al., 2009; Loyo-Berrios, et al., 2007). In spite of this evidence much of the data providing evidence of cardiovascular or respiratory morbidity relates to the general population (Peel, et al., 2007).

There is evidence to support the hypothesis that exposure to a component of the particulates in air pollution known as PM$_{2.5}$ is associated with health impacts. These are very small particulates with a diameter equal to or $< 2.5$ microns ($\mu$m), and they readily reach the alveolar region of lungs where they have been shown to have a toxic effect (Feng, et al., 2016; Fang, et al., 2009; Goulaouic, et al., 2008). Numerous studies conducted on PM$_{2.5}$ have concentrated on spatial or meteorological parameters such as proximity to pollutant sources and these have consistently shown positive and significant associations with increased
emergency room visits and hospital admissions due to respiratory and cardiovascular symptoms, as well as increased mortality (Samoli, et al., 2015; Hoppo, et al., 2008; Goulaouic et al., 2008; Liu, et al., 2007; Chuang, et al., 2007; Rojas-Martinez, et al., 2007; Sarnat, et al., 2003; Sisovic, et al., 2002).

However, the evidence obtained from epidemiological and controlled exposure studies, as acknowledged by WHO (2013, 2003), suggests that the level of toxic effect is not simply related to just the quantity of PM in air pollution but that the actual composition of PM also contributes significantly to toxicity (Raaschou-Nielsen, et al., 2016; Hoppo, et al., 2008; Sharma, et al., 2007; Penn, et al., 2005; Graff, et al., 2009; Suresh, et al., 2009). Recent studies by Martinelli, et al., (2013) confirmed in earlier conclusions by the US EPA, (2004), that the PM and health effect relationship is more complex than a simple quantitative association with the overall PM concentration. Chemical composition and concentrations of substances in the PM are also critical. Health effects are therefore not simply related to the quantity of PM, but also its composition.

Polycyclic aromatic hydrocarbons (PAHs) and metals are examples of two such hazardous constituents found within PM. The toxicity of PAH and metal components carried in PM$_{2.5}$ in particular, have shown a significant association with respiratory health effects in individuals exposed to air pollution (Gao, et al., 2016; Gerlofs-Nijland, et al., 2009; Wallenborn, et al., 2009; Huang et al., 2009; Rouse, et al., 2008). However, to date, research focused specifically on understanding the magnitude of health impacts related to the composition of PM$_{2.5}$, particularly PAH and metal components, is still lacking.

A number of studies measuring respiratory health in relation to PM$_{2.5}$ exposure have utilised spirometry (Stanojevic, et al., 2008; Lagorio, et al., 2006). However, in recent years air quality and health related studies have tended to favour more robust measures of measuring health effects, stating that the use of spirometric measurements are prone to human error and variability resulting from a number of different sources, such as, physical differences among individuals, differences in technique and effort exerted, as well as the choice of calculations and reference populations utilised. Participant’s effort, cooperation and ability to comprehend and follow the stipulated instructions effectively are some of the factors that contribute to a lack of consistency and reproducibility in results, impacting on
both the validity and reliability of this method (Barr, et al., 2008, Roche, et. at., 2008, Stanojevic, et al., 2008, Salo, et al., 2001). Furthermore reference ranges for spirometric tools are either limited for children or other sub-groups within the population, or often do not take into consideration ethnic and age differences when diagnosing for their respective health effects (Martinez, et al., 2008, Stanojevic, et al., 2008, Innocenti, et al., 2007).

A current method of addressing this issue is to identify specific physicochemical properties of PM that are related to morbidity / mortality, and to utilise more objective measures of health effects and exposure such as biomarkers in blood or urine. This evidence may contribute to better understanding the risks associated with fine PM exposure.

In clinical studies, it was reported that respiratory effects induced by the inhalation of ultra-fine particles were also associated with oxidative stress and inflammation (Bell et al., 2009; Samet, et al., 2009; Suresh, et al., 2009; Mucha, et al., 2006; WHO, 2003). In particular it was found that exposure to PAHs result in the presence of 1-hydroxypyrene (1-OHp), a metabolite of PAH, which is subsequently excreted in the urine. Exposure to metals from the ambient environment also results in notable quantities of these metals in urine (Yuan, et al., 2013; Mukherjee, 2005; Schuhmacher, 2002). Accordingly, both components can serve as appropriate biomarkers of exposure, to compliment environmental monitoring when assessing exposures (Smolders, et al., 2009; Rainska, 2007; Mucha, et al., 2006).

Physiological responses associated with exposure to both PAHs and metals, are frequently expressed in the form of oxidative stress which appears as an inflammatory response (da Silva, 2016; Nieuwenhuijsen, 2003; Schuhmacher, 2002). The presence of malondialdehyde (MDA) and 8-hydroxy-2’ddeoxygenosine (8-OHdG) in urine are therefore indicative of increased oxidative stress which is associated with inflammation related to respiratory health effects. Both these biomarkers have demonstrated a positive association with oxidative stress induced by PAHs and metals (Aflanie, et al., 2015; Huang, et al., 2012; Kim, et al., 2009a; Kim, et al., 2009b; and Liu, et al., 2007). These physicochemical responses can also serve to validate spirometry.

This study therefore proposed to utilise these objective measures of physicochemical responses to exposure combined with an analysis of PM collected through both personal and environmental sampling to better quantify health effects associated with air pollution in the Collie area of Western Australia.
1.2 Study Design

The ability of an individual to deal with the inflammation and oxidative stress resulting from exposure to PM$_{2.5}$ is not only influenced by the chemical composition of the PM$_{2.5}$ but also the susceptibility of individuals to those contaminants, concentrations of PM$_{2.5}$ in the ambient air and the duration of exposure (Liu, et al., 2014; Kim et al., 2009a, Donaldson, et al., 2001). It has been shown that children are more susceptible to the effects of PM$_{2.5}$ than healthy adults (Mann, et al., 2010; Wheeler, et al., 2000). Therefore it is also expected that under circumstances where children are chronically exposed to PM$_{2.5}$ containing PAHs and metal components, any associated increase in respiratory symptoms should also be accompanied by biomarkers of exposure and inflammatory biomarkers indicative of oxidative stress (Bae, et al., 2010).

This cross sectional study investigated the relationship between exposure of children to heavy metals and polycyclic aromatic hydrocarbons (PAHs) in the PM$_{2.5}$ component of respirable dust and health effects measured through questionnaires and urinary biomarkers of air pollution exposure. A cohort of children that were perceived to be exposed to air pollution was recruited from Collie, which is located in the vicinity of a coal fired power plant and coal and bauxite mines. The control population was drawn from the residents of the relatively unpolluted seaside community of Dalyellup. Both communities share similar socio-economic status and age distribution status, as determined by the Australian Bureau of Statistics (2013), varying mainly only in their location to the integrated industry.

1.3 Purpose of the Study

The overall purpose of this study was to investigate the relationship between exposure to respirable PM$_{2.5}$ and its chemical composition, in terms of PAHs and metals, on the respiratory health of children living in close proximity to emission sources.

Specifically this study aimed to;

- Determine the environmental and personal levels of respirable dust, (PM$_{2.5}$), metals and PAH exposure of children living in close proximity to emission sources as compared to children in a control location with low levels of ambient air pollution.
• Analyse urine samples collected from the children living in close proximity to emission sources compared to children in a control location in order to determine if there are differences in concentrations of metals, PAHs, 8-OHdG and MDA.
• Investigate the relationship between levels of exposure to air pollutants and markers of exposure.
• Investigate the relationship between levels of exposure to air pollutants and measureable health effects.
• Assess the impact of other variables such as diet, indoor cooking, parental smoking, and heating facilities upon levels of exposure and biomarkers.

1.4 Hypotheses

• Children living in Collie are exposed to higher levels of respirable dust, environmental PM\(_{2.5}\) metals and PAH than children residing in Dalyellup where there are considered to be no point sources of air pollution.
• Children residing in Collie have a higher prevalence of self-reported respiratory symptoms than children residing in Dalyellup.
• Urinary levels of metals, PAHs, 8-OHdG and MDA among children living in Collie will be elevated when compared to the levels found in children residing in Dalyellup.
• Urinary 8-OHdG and MDA can be used as biomarkers of health effects subsequent to exposure to air pollutants.

1.5 Organisation of Thesis

Chapter 2 provides a critical review of existing literature covering in detail various studies that have investigated the association between air pollutants and respiratory health effects in exposed populations.

Chapter 3 describes the study design and methods and processes used to generate data for this study. In this chapter, the process of advertising the study to the public to recruit participants, and methods used to collect samples and lifestyle information are presented. The methods used for the analysis of the data are also elucidated. In this study, environmental ambient air respirable samples and personal exposure PM\(_{2.5}\) samples were collected on Teflon filters. Urine samples from participants in the study were also collected for urinalysis to determine individual exposure to ambient PM\(_{2.5}\) and associated health effects by analysing for PAHs, heavy metals and oxidative stress markers such as malondialdehyde and 8-
deoxyguanosine. Methods related to the collection of data related to micro-environmental variables, lifestyle factors and self-reported symptoms are also presented.

The results of the study are introduced in chapter 4. Micro-environment ambient air and personal PM$_{2.5}$ air samples collected in the Shire of Collie and Dalyellup are presented. All PM$_{2.5}$ samples were analysed for PAHs and heavy metals. Urine samples obtained from study participants were analysed for 1-OHpy and heavy metals. Study participants also completed a questionnaire to collect data related to micro-environmental and lifestyle variables as well as self-reported respiratory symptoms.

Chapter 5 presents the conclusion of the community based study and interprets the results with reference to published literature. This chapter also discusses the limitations and confounders associated with its application to a community cohort. The conclusion from this analysis, and recommendations for further investigation led to the development of a second study in an occupational cohort known to be exposed to Nickel and Cobalt.

Chapter 6 (study 2) investigates the relationship between heavy metal exposure in an occupational cohort working in a metal refinery, and oxidative stress markers, MDA and 8-OHdG in urine samples to evaluate the potential of both oxidative stress markers to provide a better correlation with individual exposure to toxic pollutants in ambient air.
2. Literature Review

2.1 Introduction

Ambient air consists of a mixture of varying amounts of gases, moisture and particulate matter, surrounding the earth’s atmosphere (Kim and Bernstein, 2009; Keeler, et al., 2005; Sorensen, et al., 2003) and air pollution has been recognised as a major public health threat. Numerous studies conducted over the last 80 years have established associations between air pollution and detrimental effects on environmental, animal and human health (Ghioa, et al., 2012; Kunzli, and Perez, 2009; Ozkaynak, et.al., 2009; Rudež, et.al., 2009; Seaton, et al., 1999).

Successive events from the 1930s in Meuse Valley, Belgium and Donora Valley, Pennsylvania, United States in 1948, and London in 1952, saw significant mortality and morbidity, resulting from ‘fog’ and industrial air pollution (Valavanidis, Vlachogianni, & Fiotakis, 2009). As this pattern of air pollution related health effects became more and more frequent, especially in developed and rapidly expanding cities, there was a rising concern for air quality and associated environmental problems. Scientist and governments were prompted to investigate the effects of air pollution on health and regulate emissions into the air (Valavanidis, Vlachogianni, & Fiotakis, 2009). In 1970, the amended Clean Air Act (CAA) was passed in the USA, and this initiated the movement to set standards for air quality (Fan, et al., 2008). Since then extensive efforts to assess and monitor air quality and the various components of air pollution have progressively resulted in determining permissible levels based on human health and/or environmental risk. Particulate matter arising from anthropogenic sources thus became the focus of many studies due to its direct correlation with morbidity and mortality rates. PM is currently recognised as one of the most widespread global health threats to date (WHO, 2013; WHO, 2003).


These are;

- The criteria pollutants, namely, nitrogen dioxide (NO₂), sulphur dioxide (SO₂), carbon monoxide (CO), ozone (O₃), lead (Pb) and particulate matter, including particulate matter with a diameter of less than 2.5 μm (PM₂.₅), these can be further divided into
a category of ultra-fine particulates that have a diameter less than 0.1 micrometres (μm) (PM₀.₁µm). These small particles are generally present in relatively high concentrations in polluted air and they arise from a number of different sources (Kunzli, and Perez, 2009; WHO, 2013).

- A diverse range of hazardous pollutants, including volatile and semi-volatile organic compounds and, heavy metals (USEPA, 2015; Sorensen, et al., 2003; NEPM, 2003). These pollutants are usually present in relatively low concentrations and they are toxic or persistent making them hazardous to human health (NEPM, 2003).

In the USA, a Clean Air Act Amendment, Title III, Hazardous Air Pollutants, was promulgated in 1990 (Clean Air Act Amendments of 1990, 104 Stat. 2468, P.L. 101-549). This resulted in emission control strategies of approximately 30 compounds presenting the greatest risk to public health (Leikauf, 2002). In 1997, the U.S. EPA revised the National Ambient Air Quality Standards (NAAQS) for airborne PM, which supplemented the previous standards. This focused on PM (Penn, et al., 2005). The following year in 1998, the U.S. EPA commenced the particulate matter supersite program to monitor and analyse air quality (Solomon, et al., 2008). Initially the focus of control measures were aimed at reducing levels of Nitrogen Oxides, Carbon Monoxide and Sulphur Oxides. However, epidemiological evidence indicated that these emission reductions did not achieve the anticipated reduction in associated health effects. Subsequent studies showed a significant portion of the observed mortality and morbidity among urban populations was associated with specific physicochemical properties within air pollution (Happo, et al., 2008). It has also been shown consistently that coarse particles (PM₁₀) and fine particles (PM₂.₅) in urban air have been equally potent in inducing respiratory-based hospitalisations and inflammatory responses resulting in disease exacerbation in susceptible groups (Diaz-Sanchez, et al., 2015; Pope and Dockery, 2006). These PM subsets arising from anthropogenic sources contain transition metals, and carbonaceous fractions in the form of PAHs, both of which are known to cause chronic respiratory and cardiovascular diseases and asthma (Uzoigwe, et al., 2013; Happo, et. al. 2008; Penn, et al., 2005).
2.2 Current situation

Although vast improvements in air quality have been achieved since the industrial revolution, much of the air pollution burdens in many parts of the world remain. These are predominantly caused by manmade synthetic by-products produced by industrialized nations (WHO, 2016; Kim and Bernstein, 2009).

Currently in the developing world air quality in many ways reflects the industrial revolution era experienced in the 1950’s in the USA, the UK and much of Europe. Despite strict air quality standards imposed by regulatory authorities, airborne pollutants in many countries appear to have increased, along with an associated increase in mortality and morbidity (Bose and Diette, 2016; Janke, Propper, and Henderson, 2009). Recent studies of industrial pollution indicate a significant association between the presence of systemic inflammatory markers and long term residential exposure to high levels of PM (Yan, et al., 2016; Hoffmann, et al., 2009; Kim, and Bernstein, 2009). Sources include residential heating, cigarette smoke and cooking fuels (Kim, and Bernstein, 2009; Ohura, el. al., 2004). In the United States, residential heating contributes approximately 16% of the potentially toxic carbonaceous fractions in PM, while mobile sources such as vehicle related pollutants, can contribute about 36%. Both of these have resulted in respiratory and/or allergic responses including asthma, wheezing and even mortality from cardiovascular infarction (Melen, et al., 2008; Ohura, el. al., 2004). Research by Pardo, et al., (2016), Yan, et al., (2016), Kim, and Bernstein, (2009), Kuusimaki, et al., (2003) and Ravindra, Mittal and Grieken, (2001), have similarly established an association between exhaust particles from ill-maintained vehicles on animal respiratory function as well as human cardiopulmonary health.

2.3 General health effects

Pollutants in ambient air are a major health concern and have been associated with numerous adverse health effects. These include minor effects such as headaches, tiredness, nausea, eyes, nose and throat irritation, and more significant respiratory and cardiovascular related illnesses which have contributed to morbidity and mortality (Valavanidis, Vlachogianni and Fiotakis, 2009).

In spite of the fact that air pollutant levels have decreased in most developed countries over the last forty years (WHO, 2013; Janke, Propper and Henderson, 2009), current levels still pose many challenges to human health outcomes (Chen, et al., 2015; Valberg, 2004,
Seaton, et al., 1999). As such, the World Health Organization - Air Quality Group, have similarly been unable to identify a threshold concentration below which ambient particulate matter has no effect on cardiovascular and respiratory health (WHO, 2013).

Epidemiological and clinical studies established decades ago that toxic gases such as sulphur dioxide and inspired particulates from fossil fuel combustion were linked to detrimental respiratory and cardiovascular health effects. However, better and cleaner technology over the last 30 to 40 years have actively reduced the high sulphur content in air pollutants from traditional fossil fuels such as biomass, coal, wood, crude oil and diesel. Subsequently, air pollution studies have consistently indicated a similar significant association between respiratory health effects and particulate matter (including coarse [PM$_{10}$], fine [PM$_{2.5}$], and ultrafine [PM$_{0.1\mu m}$] particulates, arising from anthropogenic sources (Pardo, et al., 2016; Kim, & Bernstein, 2009; Ozkaynak, et al., 2009; Rudež, et al., 2009; Filho, et al., 2008). The lack of information about the components that constitute particulate matter has largely contributed to insufficient control over its emissions from industrial sources, which has resulted in fine and ultrafine particulate matter potentially gaining greater prominence as health effect causing pollutants (Argacha, et al., 2015; BBC, News, 2015; Valavanidis, Vlachogianni and Fiotakis, 2009).

More than a decade ago, an environmental health assessment report of the impact of air pollution on public health in a number of countries, including Austria, France and Switzerland; concluded that, more than 25,000 new cases of chronic bronchitis in adults, and 290,000 episodes of bronchitis in children, along with more than 0.5 million asthma attacks and 16 million person-days of restricted activity was attributed to air pollution (Kim, et.al., 2004). Analogous studies conducted in the USA also provided evidence that the complex mixture of ambient air pollutants at exposure levels permitted at that time exacerbated and facilitated the development of respiratory health effects such as asthma (Leikauf, 2002). More recent evidence from both experimental and epidemiological studies in South America also demonstrated a significant association between air pollution and adverse respiratory and cardiovascular health effects (Filho, et al., 2008; Schwarze, et al., 2006). Current research in North America and Europe still continue to show increases in respiratory symptoms, reduced lung function, and hospitalization and death from cardiac and respiratory diseases exacerbated by urban air pollution (Kim, Kabir and Kabir, 2015; Dales, Cakmak, and Vidal 2009; Ozkaynak, et.al., 2009).
2.4 Control measures

Since the 1970s a number of different methods have been utilised by many countries to monitor air quality. These methods range from fully automated numerical models, to manual data collection and analysis. Funded by the US EPA, five academic centres contributed a decade’s worth of data collected from source apportionment studies, personal exposure studies and epidemiological studies, that utilised all cause health effects data and air pollutant distribution data, to help address the uncertainties in air pollutant exposure based health effects (Fanning, et al., 2009). Similarly, large epidemiological studies conducted in Asia such as the “Public Health and Air Pollution in Asia” (PAPA) project have also investigated the association between air pollution and public health via multicity studies utilising similar strategies (Wong, et al., 2008). Smaller focused studies investigated direct associations between specific pollutants and health effects within a community through the use of actively measured micro-environmental air pollutant status and self-reported symptoms or parametric measurements of health impairments (Lee, et al., 2011; Parker, et al., 2009). Most recently studies have also targeted specific groups within communities to actively measure their interaction with their environment and the prevalence of a health effect to better understanding these correlations. (Chen, et al., 2015; Diaz-Sanchez, et al., 2015; Sarnat, et al., 2012).

It also became apparent, particularly to industrialized countries, that being able to compare air quality monitoring data was important not just to tackle trans-boundary pollution, but also from a research perspective when considering its effects on the environment and population health. The United States Environmental Protection Agency (EPA) published a series of air pollutant emission factors which are utilised as a monitoring tool for a number of industrial sources. Similarly Australia, Canada, China and many other countries in Asia as well as many parts of Europe, have published and utilise their own compilations, as well as regulations stipulated by the European Environment Agency. Some countries have also implemented other reduction efforts such as carbon or energy taxes to encourage industry to reduce their pollution emissions.

While the methods of monitoring and control of air quality may differ across countries depending on what resources are readily available, the underlying indicators for regulating air pollutants are inherently the same. Most methods focus on determining a number of variables about the air quality, including the potential toxicity of the constituents
in question with the aim of ensuring pollution levels remain below the threshold of health effects.

2.5 Situation in Australia

In Australia since 1998, the National Environmental Council (NEPC), under the National Environment Protection Agency (Ambient Air Quality) has measured, set and updated ambient air quality standards to protect human health and well-being (Kim, et.al., 2004). Australian researchers have also contributed to international air quality research in an attempt to determine if current permissible levels of particulate matter exposure are in fact appropriate in terms of the protection of the health of exposed populations (Hime, Cowie & Marks, 2015).

2.6 Particulate Matter

Ambient PM in air pollution generally comprises of a complex mixture of small particles and liquid droplets suspended in air, with organic chemicals, metals, pollen, soot and other components of smoke making up some of its constituents (Boullemant, 2011; Samet, et.al., 2009; Penn, et al., 2005; NEPM, 2003; WHO, 2003).

2.6.1 Particulate Matter and Health Effects

In the past decade, there have been numerous studies that have associated exposure to ambient PM with health outcomes providing epidemiological and clinical evidence of their effects, including systemic inflammatory markers of long term residential exposure to high levels of PM (Yan, et al., 2016; Chen, et al., 2015; WHO, 2013; Delfino, et al., 2011; de Hartog, et al., 2010; Karr et al., 2009; Chuang, et al., 2007; Penn, et al., 2005; Sorensen, et al., 2003; WHO, 2003).

In European countries, the Air Pollution and Health project (APHEA-2) measured short-term effects of air pollution on health, showing that all-cause daily mortality increased by 0.6% for each 10μg/m³ increase in particulate matter concentrations and this trend increased with the numbers of consecutive days with high PM concentrations (Valavanidis, Vlachogianni, and Fiotakis, 2009; Chow, et. al., 2006; Schwarze, 2006.

Particulate matter in air pollution has been linked to respiratory and cardiovascular diseases (Samet, et al., 2009; Simkhovich, Kleinman, and Kloner, 2009; Ostro et. al. 2009). The most commonly reported effects include impaired lung function, respiratory symptoms,
chronic bronchitis, increased respiratory and cardiovascular morbidity and mortality, headaches and impaired mental development (Uzoigwe, et al., 2013; Bartoli, et al., 2009; Bell, et al., 2009; Calderon-Garciduenas, et al., 2008; Dales, et al., 2009; de Hartog, et al., 2009; Gerlofs-Nijland, et al., 2009; Happo, et al., 2008; Ueda, et al., 2009; Valavanidis, Vlachogianni, and Fiotakis, 2009; Wong et al., 2008). Short-term PM exposure-peaks have been shown to aggravate bronchitis, asthma and other respiratory diseases as well as causing changes in heart rate in some people, while long-term exposure to high particle levels have been associated with increased risk of cancer, respiratory diseases and arteriosclerosis (Atkinson, et al., 2016; Kathrin, et al., 2015; Samoli, et al., 2015; Glinianaia, et al., 2004; Sorensen, et al., 2003).

Samet, et al., (2009), demonstrated that the physical attributes of PM, such as varying size fractions, play a role in the toxicity associated with exposure to PM. As a result, ambient respirable particulate matter is categorized according to size. The coarse fraction of particles, fall into the (PM$_{2.5 \mu m}$ – PM$_{10 \mu m}$) mean aerodynamic diameter range and these are typically referred to as coarse PM$_{10 \mu m}$ particles, typically derived from soil and dust. PM arising from anthropogenic sources is normally associated with abrasive mechanical processes, and exposures are particularly high in arid regions (Graff, et al., 2009; NEPM, 2003). The fine fraction, of mean aerodynamic diameter (PM$_{0.1 \mu m}$ – PM$_{2.5 \mu m}$) comprises particles generated through fossil fuel combustion and industrial activities such as power generation, or surface erosion from mines (Thurston, et al., 2015; Wang, et al., 2014; Belis, et al., 2013; Samet et al., 2009; Committee on Environmental Health, 2004; NEPM, 2003). Ultrafine fractions of PM have a mean aerodynamic diameter of (≤ PM$_{0.1 \mu m}$) and stem from heterogeneous particles derived from incomplete combustion of fossil fuels and industrial organics (Rouse, et al., 2008; Committee on Environmental Health, 2004; NEPM, 2003). Generally, these unstable particles aggregate from various sources, to produce larger particles, or they adhere to larger non-ultrafine particles, thereby compounding their toxicity (Chen, et al., 2016; Donaldson, et al., 2001).

Epidemiological evidence of the associations between inhalation of fine and ultrafine ambient PM [aerodynamic diameter ≤ 2.5 μm (PM$_{2.5}$)] and increases in cardiovascular and respiratory morbidity and mortality, prompted the U.S. EPA to revise the National Ambient Air Quality Standards (U.S. EPA 1997) for airborne PM, focusing on PM with aerodynamic diameters ≤ 2.5 μm (PM$_{2.5}$). Particles 0.1 μm and smaller represent the most chemically active part of the soil and thus have the potential to carry heavy metals, pesticides and spores
from microbes, as well as nutrients for plant growth (Chen, et al., 2016; Langley-Turnbaugh, Gordon, and Lambert, 2005).

There are a number of studies that have shown no significant health effects associated with PM and these inconsistencies become more apparent in studies that adjusted for PM$_{2.5}$ or other pollutants, or when climatic, regional and compositional variations in the coarse particulate matter were taken into consideration (Dales et al., 2009; Filho, et al., 2008). A significant rationale that these studies agree upon is the fact that PM$_{10\mu m}$ particles while inhalable essentially only collect in the upper respiratory tract. Fine PM$_{2.5 \mu m}$ and ultrafine PM$_{10nm}$ particles tend to embed into the deeper regions of the lung such as the alveoli (Kim, Kabir and Kabir, 2015; Samet, et al., 2009; Simkhovich, et al., 2009; Penn, et al., 2005; WHO, 2003). Some studies have shown that the contribution of the ultrafine particles to particulate matter toxicity though present, is negligible, as any statistical associations indicated were only significant when participants or animals in the studies were subjected to controlled exposures with unrealistic concentrations or particulate compositions (Samet et al., 2009; Penn, et al., 2005). Conversely; numerous epidemiological studies over the last decade have consistently shown positive and significant associations between increased hospital admissions or emergency room visits, respiratory and cardiovascular health effects and mortality with PM$_{2.5}$ exposure (Jones, et al., 2015; Boullemant, 2011; Linares, and Diaz, 2010; Zanobetti & Schwartz, 2009; Hanno, et al., 2008; Goulaouic et al., 2008; Liu, et al., 2007; Chuang, et al., 2007; Rojas-Martinez, et al., 2007; Sarnat, et al., 2003). In some studies, even small increases in levels of ambient PM$_{2.5}$ resulted in increases (> 1%) in cardiovascular and respiratory mortality (Penn, et al., 2005).

Causal studies have substantiated the association between the inhalation of fine PM with the increased incidence of respiratory symptoms, airway irritation, coughing or breathing difficulties, decreased lung function, aggravated asthma attacks, and chronic bronchitis even at low ambient concentrations (Jones, et al., 2015; WHO, 2013; Boldo, et al., 2011; Liu, et al., 2009; Hoffmann, et al., 2009; Kim, et al., 2009b, Hanno, et al., 2008; Melen, et al., 2008; Sharma, et al., 2007; Mucha, et al., 2006 and Donaldson, et al., 2001).

Similarly, a positive association with cardiovascular health effects was also noted by Fang, et al., (2009). A cohort of 25 people in a 36 day panel study showed positively correlated increases between ambient PM$_{2.5}$ levels and C-reactive protein (CRP), a biomarker for oxidative stress, which was in turn inversely associated with heart rate variability. This
associated impact and significant influence on the development of cardiovascular disease is not surprising. The American Heart Association (AHA) has acknowledged the presence of such mechanisms by potentially linking the effects of pulmonary exposure to air pollution, with increased risk of cardiovascular events (Chuang, et al., 2007). More current clinical investigations commonly present health effects initially localized in the bronchio-alveolar region of the respiratory tract, subsequently developing into cardiovascular disease indicated by cardiovascular dysfunction including, arrhythmia, non-fatal heart attacks or episodes and premature death in individuals with pre-existing disease (Simkhovich, et al., 2009; Rouse, et al., 2008).

Many studies attribute the significance of the association between exposure and health with PM$_{2.5}$ which readily reaches the alveolar region where PM has been shown to be toxic (Kim, et al., 2015; WHO, 2013; Bartoli, et al., 2009; Liu, et al., 2009; Hoffmann, et al., 2009; Kim, et al., 2009a; Fang, et al., 2009; Goulaouic, et al., 2008; Happo, et al., 2008; Melen, et al., 2008; Sharma, et al., 2007; Mucha, et al., 2006; Penn, et al., 2005; Donaldson, et al., 2001). The association between PM$_{2.5}$ exposure with both pulmonary and cardiac health effects is apparent in long-term as well as short-term epidemiological studies. (Atkinson, et al., 2016; Kathrin, et al., 2015; de Hartog, et al., 2009; Karr, et al., 2009; Fang, et al., 2009). Collated in- and out-patient clinical records and regional monitoring data of air pollutants showed an increased risk of respiratory symptoms such as bronchiolitis, or chronic coughing, correlated positively with lifetime average PM$_{2.5}$ exposure in infants in the Georgia air basin of British Columbia (WHO, 2013; Karr, et al., 2009).

Toxicological studies using animals or conducted in-vitro have also reinforced these findings. Bartoli, et al., (2009), reported that animals intratracheally instilled with PM$_{2.5}$ had increased variability in heart rate and arterial blood pressure. Lavaged lungs and bronchoalveolar lavage fluid (BALF) showed increased cellular damage on lung epithelial cells which correspond to the health effects observed in a study by Happo, et al., (2008). These associations were significant even without exposing either human or animal subjects to unrealistic concentrations and compositions, and they were consistent regardless of location, study design, regional and climatic differences, or when adjusted for other pollutants or confounders (Dales, et al., 2009; Sarnat, et al., 2003). It has been shown consistently that PM$_{2.5}$ is a good indicator of air quality and is associated with health impacts.
### 2.6.2 Particulate Matter Composition

The actual chemical composition of PM is dependent on the fossil fuel undergoing combustion or physical destruction, the conditions of combustion and of exhaust processing, non-combustive sources associated with vehicular transport, as well as the geological and meteorological conditions specific to the area (Samet, et al., 2009).

The complexity of PM lies not only in its mixture of particles with respect to size but also its chemical composition. Ambient PM is essentially a product of diverse chemical, physical and thermodynamic properties, subjected to numerous complex atmospheric processes in which source type, source strength, sinks, and meteorology interact continuously (Chung, et al., 2015; Keeler, et al., 2005). Classification by size and mass alone therefore is not appropriate. To gain a full understanding of the effect of particulates, their chemical composition also needs to be considered (Chung, et al., 2015; Simkhovich, et al., 2009; Nieuwenhuijsen, 2003). The historical reliance on mass and particle size alone to assess health impact has prevented proper assessments of health impacts associated with air pollution (Hime, Cowie & Marks, 2015; Gerlofs-Nijland, et al., 2009; Hanno, et. al. 2008; Penn, et al., 2005 & Valberg, 2004). Over the past ten years, the decrease in permissible ambient PM levels has not resulted in a decrease in associated human health effects (Valberg, 2004; WHO, 2003). Needless to say, current methods of estimating exposure to PM have not effectively addressed the impacts its constituents have on health outcomes (Gerlofs-Nijland, et al., 2009; Sharma, et al., 2007; WHO, 2003; Valberg, 2004).

According to Wallenborn, et al., (2009) and Valberg, (2004), the toxicity per unit of PM should ideally be related to both the size fraction as well as its constituents and the toxic effects of individual components within PM in respirable dust need to be evaluated (Boullemant, 2011; Grahame, 2009).

Studies such as the one conducted by Rogula-Kozłowska et al., (2013) and Stracquadanio, et al., (2007), displayed a significant correlation between the effects of PM$_{2.5}$ and its components such as PAHs. In studies by Kim, et al., (2013), Bartoli, et al., (2009), Huang, et al., (2009), Grahame, (2009), Hanno, et al., (2008), cellular damage as indicated by inflammation in lavaged lungs and bronchoalveolar lavage fluid, associated with PM$_{2.5}$ exposure, was observed to be positively associated with water-soluble and water-insoluble organic and inorganic constituents including PAHs as well as heavy metals in both animal and human alveolar macrophages.
PAHs, metal components and other organic compounds are examples of hazardous constituents within PM of respirable dust that contribute significantly to the potential toxicity of PM$_{2.5}$ and therefore contribute to respiratory and cardiovascular morbidity and mortality (WHO, 2013; Ostro, et al., 2009; Stracquadanio, et al., 2007). Through animal studies as well as recent epidemiological studies, both PAHs and metals, amongst other chemicals, bound to the surface of PM$_{2.5}$, are thought to induce many of the observed health effects through enzymatically catalysed reactions at target cells (Bae, et al., 2010; Bartoš, et al., 2009). Garnering a more comprehensive understanding of the effects of the constituents of PM requires further research to address the toxicity of the chemical constituents, and investigation of their relationship with relevant, specific health outcomes (Happo, et al., 2008; WHO, 2003).

### 2.7 Polycyclic Aromatic Hydrocarbons of PM$_{2.5}$ and Health Effects

Polycyclic Aromatic Hydrocarbons found in PM comprise a group of organic compounds consisting of three or more benzene rings that originate from incomplete combustion of organic matter and make up significant constituents of particulate matter (Suresh, et al., 2009; Gamboa, et al., 2008; Sisovic, et al., 2002). Coincidently, among the major types of PAHs; benzo(a)pyrene, benzo(b)fluoranthene and pyrene are quantitatively the most important as they are also mostly bound particularly to PM$_{2.5}$ (Suresh, et al., 2009; Goulaouic, et al., 2008). Major sources of PAHs from PM in urban areas are primarily from anthropogenic emissions such as fossil fuel combustion, biomass burning, and industrial activities (Cheng, et al., 2007). They have been shown to be important causative agents contributing to the increase in the prevalence of asthma, bronchitis, chronic obstructive pulmonary disease, respiratory tract infections as well as cardiovascular diseases (Kim, et al., 2013; WHO, 2013; Goulaouic, et al., 2008; Mucha, et al., 2006; Penn et al., 2005; Tsai, et al., 2001).

Occupational health assessment studies evaluating workers exposures to generated PAHs and their derivatives have presented well established links between many health impacts, and poorly controlled industrial emissions. The lack of emission control in manufacturing industries such as aluminium smelters, carbon black manufacturing and petrochemical industries can often lead to detrimental health effects. Studies such as those conducted by Kim, et al., (2013), Friesen, et al., (2009), Tsai, et al., (2001) and Kuljukka, et
al., (1996), are just some examples that show how personal inhalation exposure of PAHs contribute significantly to cases of cardiopulmonary morbidity and mortality.

The effects of PAHs on mortality and morbidity under different occupational settings is a great cause for concern both to industry and its employees, as in many instances it is apparent that occupational cohorts were exposed to legally permissible levels of air pollutants yet suffered ill health effects. Furthermore, workers exposed to PAHs on a daily basis also have significantly elevated concentrations of 1-hydroxypyrene or hydroxyphenanthrene, which are metabolites of PAHs in their urine (Liu, and Jia, 2016; Nelson, et al., 2010; Rossbach, et al., 2007; Sharma, et al., 2007; Kuusimaki, et al., 2003; Tsai, et al., 2001).

In-vivo and in-vitro studies have found a significant number of PAHs and their metabolites to be both carcinogenic and mutagenic, and these have in some instances been related to lung and skin cancer in humans, as well as toxicity in both humoral and cellular immune systems in animal models (Kim, et al., 2013; Gamboa, et al., 2008; Mucha, et al., 2006; Penn, et al., 2005; Vyskocil et al., 2000).

In view of this finding, epidemiological studies have also investigated the association between the effects of PAH exposure and health effects in non-occupational cohorts. Many of the super-fund and clean-air projects in the earlier part of the 21st century both in developing as well as developed countries were conducted to determine if residing close to such industries also resulted in residents becoming victims of PAH-triggered morbidities and mortalities by studying the relationship between PAH exposure and health effects. Many research projects have established strong associations between personal inhalation of industrial-based PAHs by local residents in close proximity to these sources and their persisting health effects (Boon, et al., 2001). However, there are also studies that report no link between industrial-based PAH pollution and community health outcomes, attributing the mismatch between personal concerns and the lack of significant health impacts in their studies to reporting bias (Ranzi, et al., 2011; Miller, and McGeehin, 1997). As such, it is often difficult to determine if actual toxicological events led to observed health effects, thereby leading to public concern, or that any public concern is largely attributed to reporting bias.

The progressively increasing numbers of studies being conducted in different settings are helping to cut through some of this initial confusion. It is apparent from these studies that individuals with lower or pre-clinical indicators of susceptibility experience more severe PM-
associated health effects. Infants, children, the elderly or individuals with underlying impairments in vascular health and increased systemic inflammation are among the most susceptible to adverse health effects from PAH in air pollution (Chen, et al., 2014; Parker, et al., 2009; Filho, et al., 2008; Chahine, et al., 2007; Committee on Environmental Health, 2004). Cross-sectional studies carried out by Liu, (2013), Mucha, et al., (2006), Mielzynska, et al., (2006), and Vyskocil, et al., (2000), investigated the effects of proximity to urban sources of PAH exposure from ambient particulate matter on children. These studies indicate that even at low environmental concentrations, PAHs accumulate within the body and result in children having measurable concentrations of 1-hydroxypyrene in their urine, an indicator of PAH exposure (Sánchez-Guerra, et al., 2012; Mucha, et al., 2006; Vyskocil, et al., 2000). What is also apparent from these studies is that living near a local industrial source such as a steel mill and coking facility resulted in higher levels of exposure to PAHs than residing in an area of high urban traffic (Sánchez-Guerra, et al., 2012; Goulaouic, et al., 2008; Vyskocil, et al., 2000). In case-control studies conducted by Suresh, et al., (2009), and Gamboa, et al., (2008), PAH exposure from ambient particulate matter resulted in genotoxicity following the presence of increased oxidative stress markers in venous blood. Children living at different proximities to industrial sources of PAH in the state of Tabasco, Mexico, showed a positive association between PAH exposure and DNA damage in blood lymphocytes (Gamboa, et al., 2008). In the study by Suresh, et al., (2009), phenanthrene concentrations in blood indicative of PAH exposure, were associated with lower blood glutathione (GSH) levels and elevated malondialdehyde (MDA) levels which occur to counter airway oxidative stress in bronchial asthma sufferers. Cellular inflammatory mediators such as tumour necrosis factor – alpha (TNF-α), a cytokine involved in systemic inflammation, were also shown to be involved in activating a pro-apoptotic protein, Bax, resulting in lung tissue apoptosis in rats exposed to the PAH surrogate, β-naphthoflavone (Ghanem, et al., 2006). As such, while the association between PAH exposure and mortality is not clear, the association between such air pollutant exposures and the cardiopulmonary morbidities experienced in the more susceptible individuals is apparent.

2.8 Metal Components of PM$_{2.5}$ and Health Effects

It is not uncommon for PM$_{2.5}$ and PM$_{10}$ fractions in respirable dust of air pollution from anthropogenic point sources such as industry as well as communities close to mines, mine waste, power plants, incinerators and smelters to contain heavy metals which contribute
significantly to the overall mass of PM (Zota, et al., 2009; Shah, et al., 2007; Sharma, et al., 2007; Figueroa, et al., 2006). As such, atmospheric dispersion of particles from industrial sources is potentially an important source of human exposure to metals in communities in close proximity to these activities (Wallenborn, et al., 2009; Zota, et al., 2009).

According to the WHO (2003), a significant portion of inflammatory responses associated with PM$_{2.5}$ have also been shown to be potentiated by transition metals in particulate matter. Examples of these include metals such as arsenic (As), vanadium (V), zinc (Zn), iron (Fe), and nickel (Ni), manganese (Mn), cadmium (Cd) and nickel (Ni), all of which have been linked with exacerbating health effects by encouraging various inflammatory responses in the respiratory system, and even impacting the cardiovascular and nervous systems when present at toxic levels (Ściskalska, et al., 2014; Jomova, & Valko, 2011; Nelson, et al., 2010; Zota, et al., 2009). From an occupational health perspective, worker exposures to these contaminants have been investigated extensively Nelson, et al., (2010) and when metals are present in significant quantities, they have been associated with health effects of the lungs, kidney, liver, heart and nervous system and they can evoke an immune response in exposed individuals (McChoumwo, et al., 2012; Rouse, et al., 2008; Lynes, et al., 2007). Coal is known to contain high concentrations of trace elements such as Cd, cobalt (Co), copper (Cu), Ni, tin (Sb), and Zn, all of which have a high potential to be mobile and are toxic to biological systems in sufficient quantities (Fernandez-Turiel, et al., 1994).

In a retrospective cohort study, it was shown that workers exposed to inorganic lead from a smelting plant had significant associations with adverse health effects such as lung symptoms as compared to workers belonging to the control group (Stoleski, et al., 2008). Metals therefore play an important part in the toxic effects observed from exposure to particulate matter (Huang, et al., 2009). In addition, water soluble metals found in PM$_{2.5}$ in particular, were readily bioavailable, and therefore may have a more direct link to the adverse health effects observed in individuals chronically and/or acutely exposed to them (Rivara, et al., 1997). It was reported in studies collated by Karthikeyan, et al., (2006), that an increase in water-soluble metal content found in PM$_{2.5}$, which was collected in Singapore from February to March 2005 as a result of bushfires, was associated with increased daily respiratory uptake of several metals including Zn, Cu and Fe.

Both essential common transition metals such as Cu, Zn, Ni and Fe, and other non-essential ones such as Mn, Cd, Pb, mercury (Hg) and V found in ambient particulate matter
are known to disturb normal biological functions, evoking cellular responses, and in some cases are even transported directly to the brain via olfactory pathways when present in excess (Bell, et al., 2009; Wallenborn, et al., 2009; Lynes, M.A., et al., 2007; Tjalve, et al., 1999). Experimental evidence as well as studies analysing heavy metals emitted from coal fired power stations suggests that the metal constituents such as As, Cu, Cd, Se, Hg, Pb, V, Zn, Fe and Ni often present in particulate matter, undergo speciation to form reactive oxygen species when present in excess (Shah, et al., 2008). These are toxic and are known to elicit inflammatory responses which in turn account for a significant amount of the respiratory and cardiovascular health effects associated with particulate matter exposure and are therefore part of Australia’s National Pollution Inventory (NPI) Air Toxic program (Huang, et al., 2009; Shah, et al., 2008; WHO, 2000).

Recent studies on animals were used to garner a deeper understanding of the relationship between heavy metal exposure and tissue inflammation (Tchounwou, et al., 2012; Lynes, et al., 2007). Gerlofs-Nijland, et al., (2009) utilising rats, reinforced the correlation between exposure to PM$_{2.5}$ containing high levels of metals and an increase in oxidative stress and tissue inflammation. In the study, rats were exposed to PM$_{2.5}$ samples containing elevated levels of Al, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, As, Fe and Zn. In response to accumulated metals within the organism, there were increased numbers of total cells, neutrophils, lymphocytes, eosinophils and monocytes in BALF samples. There were also increases in proteins, N-Acetylglucosamine (NAG), Myeloperoxidase (MPO), a peroxidase enzyme, present in the neutrophil granulocytes, C-reactive protein (CRP) and TNF-α in the blood, all of which were indicative of oxidative stress (Gerlofs-Nijland, et al., 2009). Other signs included enhanced inflammation parameters, such as cellular toxicity symptoms comprising, higher concentrations of lactate dehydrogenase (LDH) enzymes and albumin in BALF samples. Binding and inactivating these metals subsequently resulted in a decrease in the inflammatory markers, suggesting that the metals contributed to the significantly different levels of systemic effects observed (Gerlofs-Nijland, et al., 2009).

In Chile, elevated levels of airborne arsenic pollution from a copper smelter were shown to have significant associations with high lung and larynx cancer rates. High arsenic content was also found in hair and urine samples of individuals working and residing in the surrounding communities (Rivara, et al., 1997). Concurrently, many studies began to investigate the effects of exposure to metals within fine particulate matter in non-occupational settings. It was noted that there was a positive relationship between the quantity
of metal content in air pollution and particulate matter as there was a propensity for metal agglutination onto PM$_{2.5}$ and PM$_{10}$. Consequently metal pollutant movement tended to emulate particulate matter movement (Hawas, et al., 2005). Research conducted in Puerto Rico supported this finding, showing that air pollution samples from the industrialised Guaynabo district contained significantly higher concentrations of heavy metals such as Ni, V, Fe, Cu, Pb, Zn and As in PM$_{2.5}$ when compared to reference sites like Fajardo with less PM$_{2.5}$ pollution (Figueroa, et al., 2006). PM$_{10}$ because of its larger size and weight, travels only relatively short distances from its sources, PM$_{2.5}$ has atmospheric lifetimes in the order of days to weeks. These particulates, and hence the metals attached to them, can conservatively travel for hundreds of kilometres (Figueroa, et al., 2006). As such, there are salient concerns that community exposures could resemble occupational exposure levels. This is especially distressing for more vulnerable or susceptible individuals such as children, the elderly, pregnant women and individuals already affected by health impairments who might require exposure levels significantly lower than occupational levels to elicit a health response. Chronic inflammation leading to cardiovascular death has been observed in haemodialysis patients with impaired ability to deal with exposure to environmental cadmium (Hsu, et al., 2009). A clinical study of 954 patients showed a positive relationship between Cd concentrations and high-sensitivity C-reactive proteins (CRP), which are an inflammation marker indicative of cardiovascular risk (Hsu, et al., 2009).

Using particulate matter samples collected from Chapel Hill, North Carolina, Huang, et al., (2009), not only suggest that PM$_{2.5}$ particulate matter increased genotoxicity in isolated human alveolar macrophages and bronchial epithelial cells, but also that the metal components within may be important in inducing gene changes independent of oxidative stress. Microarray analysis indicated that approximately a third of the differentially expressed genes in alveolar macrophages challenged with PM$_{2.5}$ had molecular functions related to metal binding, or encoded metallothioneins suspected of being involved in the detoxification and the homeostasis of essential trace metals (Huang, et al., 2009). It is thought that these intracellular proteins may be responsive to some of the heavy metals present in particulate matter, attempting to protect the cells from oxidative injury induced by heavy metal toxicity (Huang, et al., 2009). Other studies by Alflanie, Muhyi & Suhartono (2015), and Wei, et al., (2010), concurred with these findings, reporting that cultured endothelial cells showed signs of cytotoxicity when exposed to PM$_{2.5}$ samples highly enriched with metals such as Cd, Hg,
As, Fe, Zn and Pb. The results indicated that the observed cellular morbidity and mortality occurred via oxidative stress encouraged by the increased reactive oxygen species.

Although the entire mechanisms through which metals in particulate matter affect respiratory and cardiovascular health is not clear, clinical studies are now beginning to elucidate their correlation with the inflammation expressed by cells constantly exposed to them (Wei, et al., 2010; Huang, et al., 2009; Hsu, et al., 2009; Lynes, et al., 2007). Regardless of whether the metals taken into the body are essential or nonessential, metals generally elicit an effect on cellular targets of tissues within organs such as kidneys, liver, heart, and the immune and nervous systems (Zhao, et al., 2014; Lynes, et al., 2007). One of the main discoveries why nonessential toxic heavy metals can interfere with cellular processes is because depending on the specific metal, they may share similar valencies with essential metals normally utilised by the body for co-factors in important enzymatic function or the construction of cellular structures. As a result, not only are normal biological processes compromised, but as toxic metals accumulate within the organism, reactive oxygen species generated at a cellular level through PM$_{2.5}$ mediated mitochondrial apoptotic pathways inadvertently initiate an interaction between the accumulated heavy metals and sulphhydrils of protein structures (Shah, et al., 2008). Studies show that damage at the endothelial cell level precede the onset of cardiovascular morbidity and mortality (Wei, et al., 2010). The understanding from these studies is also that, the accumulation of heavy metals to a toxic level occurs when metallothioneins used to neutralise these metals cannot meet the demand. Examples of these include cytokines such as superoxide dismutase (SOD) found in the liver (Wei, et al., 2010; Choudhary, et al., 2007).

2.9 Susceptible Populations and Exposure to Particulate Matter

Research over the last ten years has clearly demonstrated the impact of ambient air particulates on children’s health. Specifically, epidemiological studies of young children have indicated that exposure to particulate matter in ambient air consistently showed an association with respiratory health effects (Fritz, & Herbarth, 2001). Studies such as Wheeler, et al., (2000) helped establish the association between exposure to particulate matter and the observed respiratory health effects in children. Short term investigations have reinforced this association by showing a correlation between exposure to air pollution containing PAHs and metals, and oxidative stress responses in children. The capacity of PM$_{2.5}$ particles to reach the
alveolar region and the subsequent toxic effects of its components could be a lethal combination for some individuals exposed either chronically or acutely (Wang, et al., 2015; Karr, et al., 2009; Rouse, et al., 2008). Individual susceptibility and reactivity are important factors to consider in PM toxicity and evidence gathered over the years has shown that the serious health consequences of community air pollution are not distributed equally in the population. In particular, infants, children, the elderly or individuals with respiratory diseases such as asthma or bronchiolitis, or cardiopulmonary or diabetic diseases are among the most susceptible to adverse health effects from air pollution and fine particulate matter (Wang, et al., 2015; Parker, et al., 2009; Filho, et al., 2008; Chahine, et al., 2007; Committee on Environmental Health, 2004; Fritz, & Herbarth, 2001).

According to Wang, et al., (2015) and Fang, et al., (2009), many studies have noted changes in heart rate variability in elderly populations and in some cases, even younger populations and workers after short-term exposures to PM air pollution. These studies show that individuals already exhibiting pre-clinical indicators of susceptibility or underlying impairments in vascular health and increased systemic inflammation will experience PM-associated health effects with greater severity. Yet, the majority of the data available providing evidence of air pollution associated cardiovascular morbidity relates to the population in general and not susceptible sub-groups. It is only in recent years that studies have been conducted to explore the vulnerability of populations that are more susceptible (Peel, et al., 2007).

The issue of PM sensitivity was investigated by Wang, et al., (2015), Parker, et al., (2009), Hsu, et al., (2009), and Loyo-Berrios, et al., (2007). Their findings have shown stronger associations among individuals compromised by diabetes, mental impairment, hypertension, congestive heart failure, respiratory conditions, and morbidity and mortality associated with exposure to respirable dust from air pollution, as compared with healthy individuals.

While what accounts for such enhanced susceptibility is not completely understood, it is evident that prior health impairments may be important determinants of greater risk for particle-related declines in health (Schwartz, 2004). In a ground breaking study conducted in Mexico, young dogs exposed to air pollution under experimental conditions exhibited elevated cyclooxygenase-2 (COX2), and interleukin-1beta (IL-1b), which correlated with chronic inflammation of the upper and lower respiratory tracts, and breakdown of the nasal
respiratory epithelial barrier and pre-frontal white matter hyperintense lesions in their brain. It was found that alterations in circulating inflammatory marker levels correlated with observed physiological damage (Calderon-Garciduenas, et al., 2008).

A number of researchers, Numan, Brown, and Michou, (2015), Suresh, et al., (2009), Hornung, et al., (2009), Hsu, et al., (2009), Liu, et al., (2009), Melén, et al., (2008), Liu, et al., (2007), have presented data indicating that individuals with chronic kidney disease as well as developing children presented significantly higher levels of oxidative stress markers such as tumor necrosis factor (TNF superfamily, member 2) polymorphisms, malondialdehyde, thiobarbituric acid reactive substances (TBARS), and C-reactive proteins, along with the exacerbation of inflammation in target organs, in response to their exposure to PM air pollution containing PAHs and heavy metals.

Of the susceptible sub-groups, children in particular, tend to be in a position where they are more vulnerable for a number of reasons. In most cases, they are exposed to a higher level of PAHs and metals in fine particulate matter when they spend more time outdoors (Sly, et al., 2009; Vyskocil, et al., 2000). They invariably have a higher PAH and metals exposure from air than adults, because they have a higher inhalation rate on a per unit body-weight basis compared with adults (Kunzli, and Perez, 2009; Mucha, et al., 2006; Wheeler, et al., 2000). Additionally, their susceptibility is also a consequence of their developing metabolism, physiology and immunity (Sly, et al., 2009; Schwartz, 2004). Eighty percent of their lungs are formed postnatally with development continuing through to adolescence, as such, children are prone to damage via external environmental toxic exposure (Committee on Environmental Health, 2004; Schwartz, 2004). Studies on children’s health have linked infant mortality, from increased respiratory infections and respiratory symptoms, to PAHs and metals associated with air pollution exposure (Karr, et al., 2009; Parker, et al., 2009; Suresh, et al., 2009; Hertz-Picciotto, et al., 2007 & Schwartz, 2004).

2.10 Environmental exposure Assessment Methods

Exposure studies of particulate matter take on a number of different designs, and the options of exposure assessment methods available are dependent on the emphasis and limitations of the study. A number of different methods of exposure assessment are available and data can be analysed utilising a number of different statistical techniques. Environmental or ecological exposure estimates of air pollution data derived from point sources or fixed-site
monitoring stations of a stipulated region, conducted over the duration of the study are often used to represent exposure estimates (Zanobetti & Schwartz, 2009; Rudez, et al., 2009; Rojas-Martinez, et al., 2007; and Chuang et al., 2007; Karr et al., 2006). In long-term or panel studies, surrogates are used to represent exposure estimates of the target population in the selected study location.

The preferred way to assess exposure is through active sampling. There are a number of options for active sampling and these are generally stratified into area sampling or micro-environment sampling, and personal sampling (Harrison, et al., 2002). There are a number of instruments available for area sampling and these fixed positional monitors rely on different methods of analysis or rates of uptake. Zota et al., (2009) collected PM$_{2.5}$ particles on Teflon filters 2-μm in pore size and 37 mm in diameter, using Harvard impactors attached to Medo air pumps running at 4 Lmin$^{-1}$ over 7 twenty four hour days of sampling. Huang et al., (2009) utilized a ChemVol Model 2400 high volume cascade impactor, analyzing metals in particulate matter, collected on a filter over 7 day period. Sharma et al., 2007, utilized an electrostatic sampler, actively collecting PM$_{2.5}$ particulates on a 37 mm Teflon filter at 1.5 Lmin$^{-1}$.

Using environmental or ecological exposure estimates derived from point sources or fixed-site monitoring stations that already exist, reduces cost and collection time for large sample sizes while compromising on accuracy in assessment of individual exposure levels (Nieuwenhuijzen, 2003). There can also occasionally be inconsistencies in data sets between monitoring sites. For example, in some cases when PM$_{2.5}$ data is not available as instruments may be designed to collect PM$_{10}$ or overall PM data. Furthermore, there may be a lack of standardized methodologies in obtaining the data as shown by Zanobetti and Schwartz, (2009), Rojas-Martinez, et al., (2007), Chuang et al., (2007), and Karr et al., (2006). This leads to a reduction in accuracy and/or increases in the number of confounders present in such population exposure surrogate data sets, thus limiting the value of the data. Actively collecting the required pollutant exposure estimates, can provide a more accurate and informative data set as shown by Zota et al., (2009), Huang et al., (2008) and Sharma et al., (2007). However, as these studies use different methods and instruments to serve the different purposes of their respective studies, there is little opportunity for comparison between studies and study sites.
It is therefore evident that environmental exposure estimates are often used to describe personal exposure levels, however, people generally traverse through high and low exposure areas throughout the day and do not remain in one position for extended periods of time. Stationary sources cannot accurately assess such exposures, thus necessitating the use of personal monitors or samplers which are worn by the study population. Personal sampling provides greater accuracy for subsequent exposure measurement. When collecting personal samples, the sampling equipment is worn by an individual and air is collected from the breathing zone, thus providing a more realistic assessment of exposure levels. There are a number of different personal samplers available and these should be selected based on cost, size, weight and comfort, especially when asking children to wear the instruments. Kim et al., (2009a), collected active personal exposure estimates of PM$_{2.5}$ on Teflon filters via a Cyclone environmental monitor attached to a 2 L min$^{-1}$ pump for 12 hour periods while Liu et al., (2007), used a passive collector, DataRAM model pDR-1000AN and a real-time photometric PM$_{10}$ monitor in their correlation studies. Another alternative used in collecting PM$_{2.5}$ was the GK2.05 cyclone which runs at 4 L min$^{-1}$ and is capable of collecting samples for 8 hours per day over a weekly period (Nieuwenhuijsen, 2003).

For this study, personal monitors were deemed to be the most appropriate samplers.

### 2.11 Biomarkers of exposure and effect as exposure assessment tools

Although labour intensive and expensive, biological exposure monitoring provides more accurate, precise and specific data of exposure estimates for the targeted sample populations (Kim et al., 2009a; Nieuwenhuijsen, 2003). There are a variety of different methods currently in use (Kim et al., 2009a; Liu et al., 2007; Nieuwenhuijsen, 2003). However, these all have a limitation in that they measure total exposure which could be influenced by diet and secondary sources such as smoking.

When the uptake of a specific compound can be identified and used to trace personal exposure, the possibility of using biomarkers for exposure assessment arises. Suresh et al., (2009) analysed a series of PAHs in blood samples of participants to quantify absorbed dose. Blood samples were centrifuged and analysed with a reversed phased HPLC-FD. This option
however, is problematic as it involves significant analytical costs and ethics approvals, particularly when sampling children. An alternative option is to measure a singular metabolic product of PAH uptake, using a less intrusive method. Mucha, et al., (2006), utilized 1-hydroxypyrene (1-OHp) derived from urine as a biomarker for PAH uptake. The analysis of samples was also carried out with reversed phased HPLC-FD and adjusted for creatinine clearance. Where metal uptake is concerned, the metal itself serves as a good biomarker. Studies by Huang, et al., (2009), and Hsu, et al., (2009), demonstrated a positive correlation between the uptake of metals found in ambient particulate matter identified by their presence in tissue, blood and urine.

There are a variety of ways in which health effects are measured in air pollution exposure studies. With regard to health effects from PM, long term and cohort studies rely on pre-existing relevant health data of the targeted sample population often sourced from insurance or medical institute data. Alternatively, studies that depend on smaller sample sizes have the option of actively measuring health effects in relation to levels of NO2, O3 and PM in air pollution. In studies investigating cardiopulmonary health effects in relation to PM2.5, forced volume capacity (FVC) and forced expiratory volume (FEV) and heart rate variability (HRV) have been used (Roche, et. al., 2008; Salo, et al., 2001). Spirometry in particular, is a common pulmonary test used to measure the functional capacity of lungs and more specifically the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled. The ratio of forced expiratory volume in one second and forced volume capacity, derived from volume and flow rate, assist in diagnosing pulmonary health status (Innocor®, 2009).

Heart rate variability (HRV) is a measure of variation in heart rate, which is determined by assessing the interval between the R waves (RR intervals) as shown on an electrocardiogram. RR intervals are analysed to quantify effects on the autonomic nervous system. The autonomic nervous system consists of two main components: the sympathetic system and the parasympathetic system. The relative influence of these two components on the sino-atrial node of the heart determines the heart rate. A number of physiological factors, including blood pressure and respiratory rate, can affect the normal function of the autonomic system (Salo, et al., 2001). HRV analysis therefore provides a non-invasive method for investigating the dynamic influence of changing physiological parameters on cardiac regulation.
Respiratory and cardiovascular systemic health effects can also be measured using health biomarkers that register signs of inflammation in response to oxidative stress. Physiologically based pharmacokinetic models have been used to assess respiratory and cardiovascular health outcomes associated with exposure to particulate matter (Kim et al., 2009b; Bell et al., 2009; Graff, et al., 2009; Hoffmann et al., 2009; Suresh et al., 2009; Rouse, et al., 2008; Chuang et al., 2007; Mucha, et al., 2006; Penn et al., 2005).

When oxidative stress occurs in target tissues, it is due to inherent chemical activity induced by the inhaled pollutants that result in the generation of reactive oxygen species which in turn result in inflammation (Donaldson et al., 2001). Several blood biomarkers of health effects have been used in air pollutant exposure studies, these include tumour necrosis factor–alpha (TNF-α), Interleukins -6 and -8 (IL-6 & -8) and high sensitivity C-reactive protein (HS-CRP) (Miller, Shaw & Langrish, 2012; Delfino, et al., 2009; Suresh, et al., 2009; Samet, et al., 2009; Chuang, et al., 2007; Sharma et al., 2007 & Sullivan et al., 2007). Alternatively, studies by Alflanie, Muhyi & Suhartono (2015), Kim, et al., (2009a), Kim, et al., (2009b), & Liu, et al., (2007), investigating the relationship between particulate matter and its constituents, and accompanying oxidative stress have used urinary biomarkers such as Malondialdehyde (MDA), reduced Glutathione (GSH) and 8-hydroxy-2’deoxyguanosine (8-OHdG), and they observed a positive correlation with exposure to PM2.5 particulates.

As with exposure profiling from environmental sampling programs, the use of pre-existing health data such as hospital admissions help to reduce cost and collection time when the sample size is large, the drawback to this source of information is a significant limitation in terms of accuracy, precision and relevance of the data. Conversely, although actively measured health effects can provide a more refined, informative and representative study of the correlation between the air pollutant in question and the measured health effect for specific groups, they incur high financial and temporal cost. Physical health measures such as FVC and FEV₁ are taken for spirometry readings while systolic and diastolic readings help determine Heart Rate Variability in individuals. Both these indicators of respiratory and cardiovascular health effects utilize diagnostic tools prone to human errors and variability of observed physical differences in individuals, often resulting in inadequate consistency and reproducibility (Barr, et al., 2008; Roche, et. at., 2008; Salo, et al., 2001). Further, these tools do not consider ethnicity and age differences (Juan, et al., 2009; Eizaguirre, et al., 2008; Innocenti, et al., 2007). The effective use of a spirometry test is highly dependent on patient cooperation and effort. As such, it is not uncommon to underestimate FEV₁ and FVC values,
particularly among children. Heart rate variability as a means of estimating the magnitude of cardiac sympathetic activation in individual patients with heart failure has also proved inconsistent and this method is influenced by a variety of arrhythmias, often present in cardiac patients, especially in the elderly and in those with impaired LV function. (Abildstrom, et al., 2003; Kleiger, et al., 1987).

Biomarkers of exposure that can be used to assess the amount of a specific pollutant being absorbed, distributed or eliminated from individuals can serve as a complement to the above mentioned methods to provide a better assessment of exposure. Studies that utilise biomarkers as indicators of exposure and/or oxidative stress in response to external stimuli provide a more accurate estimate of exposure as well as health effects, as they exploit the underlying mechanisms involved in cardiopulmonary responses to pollution exposure (Kim et al., 2009b). The use of biological markers of exposure therefore offers an alternative method that can be used to quantify personal exposure to air pollutants (Rainska, 2007; Mukherjee, 2005; Schuhmacher, 2002). Biomarkers can be used to assess personal exposure to both PAHs and metals as they provide an assessment of biologically absorbed doses via external exposure routes including inhalation (Rainska, 2007; Mukherjee, 2005).

Blood and urine are examples of two effective matrices from which personal exposure to chemicals can be measured (Rainska, 2007; Nieuwenhuijsen, 2003). While there are many airborne inhaled substances that can be detected in blood, urine is a preferred sample medium because blood is considered an invasive matrix and is negatively associated with participation rates. Urine on the other hand is relatively easy to collect and is more feasible, especially when repeat sampling is required and/or the participants are young children, (Mukherjee, 2005; Nieuwenhuijsen, 2003 & Smolders, et al., 2009).

Suresh, et al., (2009), analyzed a series of PAHs from the EPA priority list, in blood. However PAH exposure has also been assessed by analysing urinary 1-OHp, which is an accurate and less invasive option (Mucha, et al., 2006; Schuhmacher, 2002; Bouchard, 1998).

Where metals are concerned, the metal itself can serve as an appropriate biomarker in both blood and urine. Measuring the respective metals provide an accurate assessment of personal metal exposure (Rainska, 2007; Mukherjee, 2005). Studies by Sarmiento-Gonzalez, et al., (2008), Huang, et al., (2009), Hsu, et al., (2009), and Mukherjee, (2005) demonstrate positive associations between the metal concentrations found in ambient particulate matter and measured concentrations in tissue, blood and urine in participants.
A number of different biomarkers of health effects have been used in air pollutant exposure studies to show a positive relationship between PM$_{2.5}$ particulates and health effects. In studies by Alflanie, Muhyi & Suhartono (2015), Delfino, et al., (2009), Suresh, et al., (2009), Samet, et al., (2009), Chuang, et al., (2007), Sharma, et al., (2007), and Sullivan, et al., (2007), tumour necrosis factor–alpha (TNF-α), interleukins -6 and -8 (IL-6 & -8), high sensitivity C-reactive protein (HS-CRP), malondialdehyde (MDA), reduced glutathione (GSH) and 8-hydroxy-2’deoxyguanosine (8-OHdG) were used as blood biomarkers of cardiopulmonary oxidative stress.

As a non-invasive medium, urine has the potential to be the preferred sample medium where participation rates are paramount. Alflanie, Muhyi & Suhartono (2015), Kim, et al., (2009a), and Liu, et al., (2007), investigated the relationship between particulate matter, its constituents, and oxidative stress using health markers such as Malondialdehyde (MDA), reduced Glutathione (GSH) and 8-hydroxy-2’deoxyguanosine (8-OHdG) detectable in urine and showed a positive relationship between them. Aside from its convenience in collection, measured concentrations of oxidative stress markers MDA and 8-OHdG were shown to present a more accurate correlation with personal exposure to toxic chemicals as diet and normal cell turnover did not influence oxidative stress levels (Ściskalska, et al., 2014; Ren, et al., 2011; Lagadu, et al., 2010).

While metal detection in general produces high analytical reproducibility, complex chemicals that are metabolized may produce higher variability in their detected concentrations depending on the measurement techniques used. Biological markers of both exposure and/or oxidative stress cannot distinguish between concentrations attributed to different sources of exposure and even sources of inflammation respectively (Nieuwenhuijsen, 2003) therefore these limitations need to be considered during study design and data interpretation. Using internationally recognized validated standard methods, such as those developed by the U.S. EPA, helps to enhance the accuracy, reproducibility and comparability of biomonitoring data (WHO, 2000). Minimizing or controlling and accounting for the number of extraneous variables that could be associated with the investigation is a challenge. Occupation, dietary or smoking habits may all be potential sources of confounders (Smolders, et al., 2009). When these confounders are adequately controlled, biological markers have been shown to be proficient alternative measures of environmental health impact assessments.
2.12 Summary

Exposure to PM is currently recognised as one of the most widespread health threats to date. Whilst there has been a significant decrease in overall ambient levels of air pollution, increases in industrialisation has meant that people residing close to fixed sources of pollution are being exposed to short-term particulate matter peak exposures that have been shown to aggravate bronchitis, asthma and other respiratory diseases (Uzoigwe, et al., 2013; Glinianaia, et al., 2004; Sorensen, et al., 2003). Consistent efforts to reduce exposures to PM$_{2.5}$ in the last decade have seen no reduction in associated health effects (WHO, 2013).

The literature reviewed presents evidence from epidemiological, animal and clinical studies substantiating this relationship and the potential health effects that occur even at lower levels on susceptible individuals.

The chapter also addresses the limitations and confounders associated with epidemiological studies that have utilised non-specific air pollution and health data. The significance of lifestyle factors, age of participants and living conditions, in many cases while acknowledged, are often unable to be accounted for, often resulting in an inaccurate representation of the correlation, or in the case of children, elderly or health impaired individuals, misrepresenting air pollutant effects on their health.

This chapter outlines the direction that research in this area of air pollutant related health effects has taken in order to address these limitation. Recent studies have shown that in particular, PAHs and heavy metal components in PM$_{2.5}$ contribute significantly to the respiratory and cardiovascular health effects experienced by susceptible individuals (Wei, et al., 2010; Bartoli, et al., 2009; Huang, et al., 2009; Grahame, 2009; Hanno, et al., 2008; Stracquadanio, et al., 2007). As such, the risk of suffering air pollutant related health effects increases for susceptible individuals such as children, the elderly and those already impaired by ill health (Ranzi, et al., 2011).

Many of the recent air pollution studies utilised personal exposure to PAHs and heavy metals, as additional measurements of air quality. The relationship between these measurements and biomarkers of health effects indicative of corresponding oxidative stress from localised inflammation in affected tissues, are shown to be novel approaches to assess the effects of air pollution exposure.
In this study the relationship between exposure to respirable PM$_{2.5}$ and its chemical composition, in terms of polycyclic aromatic hydrocarbons (PAHs) and metals, on children was explored.
3. Materials and Methods

3.1 Study Design

This cross-sectional study assessed children’s personal exposures to air pollution and associated health effects at Collie and Dalyellup, in Australia’s South West. The Shire of Collie is located in close proximity to a range of integrated industries such as coal and bauxite mining, coal fired power stations and fertilizer manufacturing plants. Residents of Collie are impacted by industrial emissions, as well as high urban traffic loads. Dalyellup, which is located in the Shire of Capel, was selected as a control site as it is relatively unexposed to air pollution from industrial sources.

In this study, ambient and personal PM$_{2.5}$ samples were collected from participating children who also provided urine samples and completed a questionnaire with a daily activity sheet (diary).

3.2 Study Area Description

The South Western region of Australia is home to both small picturesque townships, dairy farms and wineries as well as a large regional port city (Bunbury). The population of the south west region of Western Australia is the largest in WA outside of the Perth metropolitan area (ACIL Tasman, 2004, Australian Bureau of Statistics report, 2006). This region represents a juxtaposition of both popular tourist destinations such as the state forests, native bushland, coastal and port areas, and integrated industries including coal mines with coal fired power stations and bauxite mining with aluminium production plants and chemical and fertiliser manufacturing industries.

Collie is a town in the South West region of Western Australia, 213 kilometres south of the state capital, Perth, and 59 kilometres inland from the regional city and port of Bunbury. The township of Collie was selected as the study location as it represents a community in close proximity to both industrial plants and mining areas with associated traffic and it is expected that activities from these premises will contribute to a higher concentration of airborne particulate matter (Worsley Alumina Pty. Ltd. Bauxite – Project Expansion, 2005).

According to a predictive air quality assessment, that was conducted in 2009, for the proposed Collie Urea Project, significant exceedances of the Air National Environment Protection Measures (NEPM) and the Kwinana Environmental Protection Policy (EPP)
standard were anticipated to occur within the Collie airshed for 24 hour PM$_{10}$, 24 hour PM$_{2.5}$, and 1 hour SO$_2$ (Perdaman Chemicals and Fertilizers, 2009). Emissions from the Muja coal power stations, as well as those from Bluewaters III and IV also contributed to the predicted exceedances of PM$_{2.5}$, with Muja A and B being the main sources of particulate emission (Worsley Alumina Pty. Ltd. Bauxite – Project Expansion, 2005). Tabulated predicted 24-hour average ground level concentrations were used to model PM$_{2.5}$ particulate matter emissions in the Collie area (figure 3.1) and movement patterns showed that the community could be potentially affected by exposure to particulate matter from these sources (Perdaman Chemicals and Fertilizers, 2009).

Figure 3.1 illustrates the predicted 24-hour average ground level concentrations of PM$_{2.5}$ in the Collie area. The predicted concentrations are represented by light blue contours with the Air NEPM 24hour PM$_{2.5}$ standard of 25 µg/m$^3$ represented by red contours. Yellow crosses represent sensitive receptors and orange crosses emission sources. Areas within the red contours represent areas where the Air NEPM standard is predicted to be exceeded.
Dalyellup is a coastal community south of Bunbury in the Shire of Capel. This township is surrounded by the Usher-Dalyellup Regional Park to the South and Bunbury to the North. The area is residential and not in the path of particulate matter plumes or point sources of air pollution. Furthermore, the area is not close to busy roads and experiences frequent oceanic winds. Air monitoring data collected in Bunbury, which is close to Dalyellup, indicates that air quality is of a high standard and Dalyellup would be expected to have even less exposure to air pollution (Technical Report AQM 4, 2009, Western Australia Air Monitoring, Report, Department of Environment and Conservation).

Table 3.1 Summary of PM$_{2.5}$ emissions for Bunbury (1999 - 2008) close to Dalyellup.

<table>
<thead>
<tr>
<th>Year of Monitoring</th>
<th>Number of Exceedences (days)</th>
<th>Highest Recorded 24 hour average exceedence ($\mu$g/m$^3$)</th>
<th>Annual Average ($\mu$g/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>1</td>
<td>30.0</td>
<td>9.3</td>
</tr>
<tr>
<td>2000</td>
<td>3</td>
<td>29.2</td>
<td>9.3</td>
</tr>
<tr>
<td>2001</td>
<td>2</td>
<td>47.3</td>
<td>8.7</td>
</tr>
<tr>
<td>2002</td>
<td>4</td>
<td>36.1</td>
<td>9.0</td>
</tr>
<tr>
<td>2003</td>
<td>3</td>
<td>37.6</td>
<td>8.6</td>
</tr>
<tr>
<td>2004</td>
<td>5</td>
<td>94.8</td>
<td>9.2</td>
</tr>
<tr>
<td>2005</td>
<td>5</td>
<td>64.2</td>
<td>8.6</td>
</tr>
<tr>
<td>2006</td>
<td>8</td>
<td>113.5</td>
<td>8.7</td>
</tr>
<tr>
<td>2007</td>
<td>3</td>
<td>34.5</td>
<td>7.8</td>
</tr>
<tr>
<td>2008</td>
<td>2</td>
<td>27.8</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Table 3.1 Presents a summary of the PM$_{2.5}$ 24 hour emission for the region of Bunbury where Dalyellup is situated. The data analysis by the Department of Environment and Conservation shows that this region is generally not frequented by persistent high levels of ambient PM$_{2.5}$ and has seen rare and infrequent occasions where 24 hour PM$_{2.5}$ emissions have progressed above AAQ NEPM advisory standards.

Both the study communities have similar socio-economic and age distribution profiles, as determined by the Australian Bureau of Statistics, thus limiting the number of confounders between the groups (Australian Bureau of Statistics, 2013).
3.3 Study Population and Recruitment

3.3.1 Study Population

The study population consisted of children aged between 7 to 12 years currently residing in the Collie area, with a control group recruited from Dalyellup. Children were recruited for this study as they represent the group that is potentially most vulnerable to respiratory health effects from exposure to ambient PM$_{2.5}$ (Lee, et al., 2011, Gamboa, et al., 2008).

3.3.2 Method of Recruitment

Upon receiving consent from the ECU Human Research Ethics Committee, recruitment at both the study and control locations was initiated.

Shire Councils, General Practitioners, parental groups, schools and sporting organizations within the local community were approached to seek their assistance in advertising the study. Advertisements were also placed at shopping centres, community centres, and the study was advertised in the local newspapers. Information flyers, questionnaires, time activity diaries, an example of the personal particulate sampler and consent forms were made available to help families and children to better understand the study, and what participation would entail (Appendices A, B, C, D & E).

Volunteering children and their parents were informed of the aims of the study and they were requested to complete a written consent form prior to data collection. Participants and their parents were issued with:

- a questionnaire,
- daily activity diary
- calibrated and fully charged personal particulate sampler (SKC dust pump),
- 120 ml container for urine collection and
- a diagrammatic protocol to help remind participants what they needed to do.
3.4 Sample Size

To establish an appropriate sample size for the study, a calculation was performed based on an understanding of expected confidence and significance values as well as data collected from previous studies (Samet, et al., 2009, Suresh, et al., 2009, Calderon-Garciduenas, et al., 2009, Fang, et al., 2009, Liu, et al., 2007, Chahine, et al., 2007, and Chuang, et al., 2007).

In a study conducted by Suresh et al., (2009), PAH phenanthrene was analysed in both exposed and unexposed individuals giving mean blood concentrations of 63.11 ppb and 4.2 ppb respectively. As the proposed study intended to investigate the relationship between PM$_{2.5}$ and health effects, the number of participants to be recruited for this study was determined by applying a web-based sample size calculator sourced from the Department of Statistics of British Columbia, Canada (Brant, 2010), on differences in mean concentrations of PAH. Assuming an alpha level of 0.05 and power of 0.8, the calculated sample size for this study required 20 participants in both exposure groups. Previous studies performed by other investigators have demonstrated an ability to observe meaningful alterations in their study outcomes with similar (or even fewer) subjects (Suresh, et al., 2009).

Initially it was proposed to recruit 30 children from both the study area and the control area. However, this proved to be difficult as most parents/children were not willing to wear personal dust samplers for a whole day and/or they did not want to provide urine samples. At the conclusion of the defined recruitment phase 18 children had been recruited from the study location and 15 from the control site.

Various attempts were made to increase participation rates through personal visits, shopping centre information sessions and liaison with schools and clubs. However, these were unsuccessful and it appeared as if the maximum participation rate had been achieved, therefore a decision was made to discontinue recruitment and to move ahead with sample collection and analysis. The power value obtained using the collected samples indicated that the sample size achieved for this study was acceptable.
3.5 Data Collection

3.5.1 Equipment Preparation

3.5.1.1 Ambient Environmental PM2.5 Samples

Ambient environmental PM$_{2.5}$ samples were collected in accordance with AS/NZS and measured in accordance with NIOSH 0600. Polytetrafluoroethylene teflon filters were used for micro-environment PM$_{2.5}$ sample collection. All Teflon filters were prepared in a dedicated temperature and relative humidity-controlled clean balance room. After a 24 hour period of conditioning, the filter mass was determined using a 4 decimal place Mettler XP6 Micro Balance in milligrams. Filters were subsequently loaded into the calibrated high volume impactor (Quest EVM-07) which was used to collect the environmental 8 hour daily average PM$_{2.5}$ levels. The instrument was packed into a custom designed carry case for transport to the sampling area.

3.5.1.2 Personal PM$_{2.5}$ Samples

All personal PM$_{2.5}$ samples were collected and measured in accordance with NIOSH 0600 using calibrated SKC Aircheck® 52 personal sampling pumps. Polytetrafluoroethylene teflon filters were again used to collect personal PM$_{2.5}$ exposure samples. All Teflon filters were prepared in a dedicated temperature and relative humidity-controlled clean balance room. After a 24 hour period of conditioning, the mass was determined using a 4 decimal place Mettler XP6 Micro Balance in mg.

3.5.1.3 Questionnaire

Participating children and their parents were asked to complete a self-administered questionnaire which was adapted from an instrument used by Van Wijnen, Slob, Jongman-Liedekerken, van de Weerdt, and Woudenberg, (1996). The questionnaire was designed to collect data on demographics, medical history and environmental exposures, including consumption of home grown fruit and vegetables. The questionnaire was designed to also identify any potential confounding factors that could contribute to levels of oxidative stress biomarkers within their urine samples. Their self-reported health status was used to ensure that recruited participants did not have any existing ailments. Questionnaires were completed on the morning of sample collection.
3.5.2 Micro-Environment PM$_{2.5}$ Sampling

External monitoring for environmental particulate matter (PM$_{2.5}$) was conducted with the aid of a Quest™ Technologies EVM-7 high volume active sampler (ISO 12103-1, A2), loaded with 37 mm Zeflour™ Teflon filters, with a pore size of 2.5 µm. Average PM$_{2.5}$ levels were determined over 8 hour sampling periods. The mass concentration of the PM$_{2.5}$ dust captured on the filters was determined using the approved NIOSH Method 0600 (NIOSH, 1994). 33 PM$_{2.5}$ samples were collected from both the township of Collie as well as Dalyellup, on the days participating children were asked to wear personal particulate samplers. Personal sampling was conducted in terms of the methods specified in AS 2724.2-1987.

Figures 3.2 and 3.3 are maps indicating the locations where samples were collected. In Collie, samples were collected from three sites;

- Allanson primary school,
- Preston farmland, and
- Collie District Hospital (figure 3.3).

The local industries in the Collie location were operating under normal conditions and the prevailing wind direction was predominantly in a Northwest direction during each period of sampling for PM$_{2.5}$. The EVM-7 sampling instrument was placed more than 3 metres above the ground as per AS 2922-1987/ NAAQS guidelines. The 3 locations were selected as they were deemed to be downwind from point sources of pollution and close to participating resident’s homes.
In Dalyellup, ambient respirable particulate matter samples were taken from two sites, these were:

- Dalyellup Lake, and
- Dalyellup Sport Complex (figure 3.3).

Again the EVM-7 sampler was placed 3 metres from the ground as per AS 2922-1987/NAAQS guidelines. These areas were chosen because they were also close to the participating residents homes, and were appropriate sensitive receivers in line with the local wind directions therefore represent ambient conditions in the area.
3.5.3 Personal Sampling

3.5.3.1 Respirable dust

Personal exposure data was collected on 2 occasions from each participant. Sampling for every child was conducted for 8 hours on 2 separate days over a period of a fortnight (one weekday in a week for two weeks).

On each sampling occasion an SKC Aircheck® 52 personal sample pump was connected via a silicon tube to a PM$_{2.5}$ cyclone elutriator. The pump was clipped to a waste belt and the cyclone sample head was positioned in the breathing zone directly beneath the mouth and nose for the duration of the 8 hour sample period (figure 3.4). This was in accordance with the Australian Standard AS 2985-2009, “Workplace atmospheres – Method for sampling and gravimetric determination of respirable dust” which describes the methodology to be used in Australia for the collection of respirable dust samples. The prescribed sample flow rate was 2.2 litres per minute (Lmin$^{-1}$) and pumps were accordingly calibrated. The cyclone elutriators were loaded with pre-weighed Teflon filters; children wore the sample pumps from 9am to 5pm (8 hours) on sampling days. At 5pm the pumps were turned off and the cyclone elutriator was detached from the silicon tube and stored in a zip-lock bag, which was provided to participants. Pumps were calibrated after sampling to ensure the flow rates had remained constant.
3.5.3.2 Questionnaire data

On sampling days children / parents were requested to complete a respiratory heath questionnaire and a Time Activity Diary where all activities throughout the day were logged at 30 minutes intervals in order to identify any activities that may have influenced results throughout the day.

3.5.3.3 Urine samples

Urine samples were obtained from all participating children on the morning after the sample pumps were worn. The samples were collected in 120ml sterile screw cap containers which were provided to children and were stored in sealed biohazard bags and refrigerated while awaiting collection later in the morning. Samples were maintained at a temperature below 4 °C during transport to the ECU Joondalup campus where they were frozen while awaiting analysis.

3.6 PM$_{2.5}$ Sample Analysis

3.6.1 Determination of Particulate Matter concentration

Both personal and environmental sample filters were transported to the university micro-balance room on Joondalup campus after sampling and left to equilibrate for 24 hours prior to re-assessing their mass and calculating concentration in mg.m$^{-3}$ as prescribed by
relevant standards of the US EPA, NIOSH, and the Australian standard AS 2985-2009. Subsequently the weighed filters were packed into individual plastic filter holders for transport to a National Association of Testing Authorities (NATA) accredited analytical laboratory in New South Wales (TestSafe Australia - Chemical Analysis Branch of WorkCover Laboratories). The date of collection and storage were logged on both field data sheets and the laboratory logbook.

3.6.2 PAH Extraction and Analysis

All Teflon filters were analysed for the 16 priority PAHs in air by Gas Chromatography/Mass Spectrometry according to EPA method WCA.178. PAHs on the residue of the filters were desorbed in the laboratory using cyclohexane and the extracts were analysed by GC/MS in SIM mode with an isotopically labeled internal standard. The eluate was then analysed for PAHs with a detection limit of 0.1µg/sample for all analytes (WCA.178).

3.6.3 Metal Analysis

Teflon filters allocated for metal analysis were analysed using Direct Determination of Elements in inhalable dust by X-Ray fluorescence spectrometry according to method WCA.181. Teflon filters were analysed for 16 metals with a detection limit of 0.1µg/element/25 mm filter except for Tin (Sn) and Cadmium (Cd) which were assigned a detection limit of 2µg/element/25 mm filter each as per the method WCA.181.

3.7 Quality Assurance

The techniques employed to determine PM$_{2.5}$ and Respirable dust levels were accepted and validated techniques which complied with Australian standards (AS 2985-2009; AS and associated methodologies. A NATA approved laboratory was used to analyse samples.

The results of this report were derived in accordance with NATA’s accreditation requirements and approved by a NATA signatory. A high degree of accuracy and precision in the analytical results was ensured by extensive intra- and inter laboratory quality assurance (QA) implemented by the analytical laboratory. Field blanks were analysed alongside duplicate and repeat analysis of samples. Spiked QA samples were also included routinely in each sample run to ensure the accuracy of the analyses. WorkCover Laboratory Services also
participates in several national and international inter-laboratory comparison programs (NATA-accreditation no. 3726, ILAC-MRA).

3.8 Urinalysis

Urine samples were acidified with dilute nitric acid and stored below -20 °C until they were shipped to the laboratories in NSW via courier for analysis. Individual urine samples from each participant were analysed for both biomarkers of oxidative stress and creatinine.

3.8.1 Urinary 1-OHpy

Reverse-phase high performance liquid chromatography with enzymatic hydrolysis using β-glucuronidase and arylsulphatase was used to analyse samples for 1-OHpy according to the NATA approved TestSafe Australia’s WCA.158 method.

3.8.2 Urinary Metals

Urine samples were also analysed for metals using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) according to method 200.7, (rev. 5) by the USEPA, (2001).

3.9 Supplemental data

Historical air pollution data for the South Western Australia region was sourced from the Department of Environment and Conservation (DEC). These 24 hour daily and annual average results for ambient PM$_{2.5}$ and PM$_{10}$ were collected over the course of 3 years from 2007 – 2011.
4. RESULTS

4.1 Micro-Environment PM$_{2.5}$ Samples

4.1.1 Ambient Micro-Environment Particulate Matter Data

During the course of the study, ambient micro-environment PM$_{2.5}$ samples were collected with the aid of a calibrated Quest EVM-07 monitor. Environmental samples were collected at strategic locations in close proximity to the homes of participating children, on the days when children were wearing personal particulate samplers. Collection was carried out for 8 hours on each sampling day to provide average values in both the study location (Collie) and the control area (Dalyellup) and these data are presented alongside data collated by the DEC for those regions in table 4.1.

<table>
<thead>
<tr>
<th></th>
<th>Quest EVM-07 8-hr Daily Average in Collie</th>
<th>Quest EVM-07 8-hr Daily Average in Dalyellup</th>
<th>DEC 24-hr Daily Average for South Western Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM$_{2.5}$ (µg/m$^3$)</td>
<td>31.25</td>
<td>7.58</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Table 4.1 Mean ambient levels of PM$_{2.5}$

Table 4.1 shows the mean PM$_{2.5}$ values for particulates collected at both the study and control locations.

An independent one-tailed T-test confirms that these mean concentrations are significantly different (p= 0.015). Annual air quality monitoring data sourced from the DEC of Western Australia indicated that the 24 hour daily average PM$_{2.5}$ values for the south west region of Western Australia over the period of 2011 were significantly higher at 45.5µg/m$^3$. However, insufficient data were available from the DEC air quality report to determine variances in their concentrations between both study locations. Further to this, local climatic conditions which could not be controlled may also have influenced results on the days of monitoring.
4.1.2 Ambient Heavy metals and PAHs

The filters used for the collection of particulates were shipped to the Chemical Analysis Branch, of the Work Cover laboratory, TestSafe Australia, in New South Wales for further analysis and they were tested for all 16 priority PAHs and heavy metals. However all samples (100%) were found to be below the level of detection of the X-ray fluorescence spectrometry analytical method that was used, thus no ambient airborne heavy metal and PAH data could be reported.

4.2 Personal monitoring

A total of 33 children were recruited from the study location of Collie (n=18) and the control location at Dalyellup (n=15). Unfortunately participation rates were very low and the target sample size was not achieved. Personal PM$_{2.5}$ and urine samples were assessed for PAHs and heavy metals. Children also completed a self-administered questionnaire and a daily activity diary.

4.2.1 Profile of Participating children

The demographic data for the participants in the study and control groups present no significant difference between genders (Table 4.2).

<table>
<thead>
<tr>
<th></th>
<th>Collie</th>
<th>Dalyellup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Participants</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Mean Age (Years) and Std. Deviation</td>
<td>8.375 ±1.34</td>
<td>8.867 ±1.68</td>
</tr>
</tbody>
</table>

Table 4.2 presents the profiles of children from the study locations

4.2.2 Personal PM$_{2.5}$ exposure concentrations for children in Collie and Dalyellup

Mean values of personal PM$_{2.5}$ exposure from Collie and Dalyellup participants were 11.91µg/m$^3$ and 7.16µg/m$^3$ respectively. Although the differences between the means were not deemed to be statistically significant (p=0.169), the trend indicates that personal particulate exposure levels in the Collie cohort were higher than those measured in the
Dalyellup group (Figure 4.1). This finding is consistent with the mean ambient micro-environmental levels of PM$_{2.5}$ collected at both the study and control locations (Table 4.1).

**Figure 4.1 PM$_{2.5}$ Exposure of Participating Children**

![Figure 4.1 PM$_{2.5}$ Exposure of Participating Children](image)

Figure 4.1 presents a scatterplot indicating the concentrations of personal PM$_{2.5}$ particulates in $\mu$g/m$^3$ collected from the personal monitors of children located in both Collie and Dalyellup.

### 4.2.3 Heavy metal and PAHs in personal PM$_{2.5}$ exposure samples

Personal sample filters were submitted to the Chemical Analysis Branch, Work Cover laboratory, Test Safe Australia, in NSW for further analysis to determine concentrations of 16 priority PAHs and heavy metals. All samples indicated that concentrations of both PAHs and heavy metals were below the level of detection and were therefore also insignificant. As such it was therefore not possible to determine if any difference existed between samples collected from Collie and Dalyellup.

### 4.2.4 Urinary 1-OHpy

All participating children from both the study and control areas provided early morning urine samples on the day following the personal monitoring and these were analysed for 12 heavy metals and 1-OHpy, a metabolite and indicator of exposure to PAHs.
All urine samples from children in both locations, showed non-detectable levels of 1-OHpy and it was therefore not possible to determine if there were any significant differences between the 2 groups as both groups showed no significant PAH exposure.

### 4.2.5 Urinary Metals

The following heavy metals were detected in urine samples; cadmium, chromium, copper, nickel, selenium, thallium and vanadium.

**Figure 4.2 Comparison of Heavy Metals Detected In Urine Samples from Participating Children from Collie and Dalyellup**

![Figure 4.2](image_url)

Figure 4.2 presents results of creatinine corrected concentrations of heavy metals that were detected in the urine samples collected from children in both Collie and Dalyellup.

As can be seen in figure 4.2 and table 4.3, urinary copper and selenium concentrations were significantly elevated among the Collie study group compared to the Dalyellup group of participants. Mean concentrations of copper (22.53 μmols/mol cr in Collie and 14.87 μmols/mol cr in Dalyellup) and selenium (77.37 μmols/mol cr, Collie) and 41.23 μmols/mol cr for Dalyellup) were detected respectively. A one-way Analysis Of
Variance (ANOVA) showed that, for copper and selenium, the concentrations between Collie and Dalyellup samples were significantly different ($P < 0.05$).

Urinary nickel concentrations were higher in Collie than in the control location, however, the difference was not significant ($p = 0.164$) with mean values of 7.01 µmols/mol and 4.74 µmols/mol cr respectively. Urinary Selenium and Nickel were the only metals that presented concentrations above their biological exposure limits (BOEL) of 43 µmols/mol cr and 3.9 µmols/mol cr respectively (TestSafe Australia, 2014). Nickel is of particular concern as it was elevated in both locations. Other heavy metals detected were neither significantly different between the 2 groups of children nor were they elevated above the non-hazardous BOEL (TestSafe Australia, 2014).

An analysis of co-variance, which included duration in years participating children had resided in their current locations, also showed significant differences in urinary copper ($p=0.003$) and selenium ($p=0.006$).

Table 4.3 Mean and Standard Deviation levels of urinary heavy metals

<table>
<thead>
<tr>
<th>Location of Participant</th>
<th>Urine Cd, µmols/mol cr</th>
<th>Urine Cr, µmols/mol cr</th>
<th>Urine Cu, µmols/mol cr</th>
<th>Urine Ni, µmols/mol cr</th>
<th>Urine Se, µmols/mol cr</th>
<th>Urine Th, µmols/mol cr</th>
<th>Urine V, µmols/mol cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collie</td>
<td>Mean 1.84</td>
<td>1.56</td>
<td>22.53</td>
<td>7.01</td>
<td>77.37</td>
<td>0.18</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation 4.46</td>
<td>4.17</td>
<td>5.15</td>
<td>5.28</td>
<td>39.33</td>
<td>0.567</td>
<td>3.23</td>
</tr>
<tr>
<td>Dalyellup</td>
<td>Mean 1.17</td>
<td>0.70</td>
<td>14.87</td>
<td>4.74</td>
<td>41.23</td>
<td>0.08</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation 2.01</td>
<td>1.24</td>
<td>8.18</td>
<td>3.45</td>
<td>27.10</td>
<td>0.16</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Table 4.3 presents a summary of urinary heavy metals levels in µmols/mol cr of participating children categorised according to location.

The urinary heavy metal data were also compared to residential areas, household heating methods, cooking fuel used in the home (gas or electricity), consumption of home grown fruit, however no associations were identified and the sample size was too small to stratify to this level of analysis.
4.2.6 Urine Malondialdehyde and 8-hydroxy-2-deguanosine

Although it was intended to analyse for biomarkers, MDA and 8-OHdG the analytical laboratory was unable find concentrations at detectable levels in urine samples provided for analysis.

4.3. Questionnaire Data

No significant association was detected between mean personal PM$_{2.5}$ exposure concentrations and self-reported respiratory health effects; this finding is not surprising given that personal PM$_{2.5}$ levels detected also showed no significant difference between children from Collie and Dalyellup.

Further analysis was conducted by aggregating the data sets (study and control areas) and determining if there were other factors that could have contributed to differences in personal PM$_{2.5}$ exposures (other than study area) within the larger cohort. Risk factors such as proximity to major roads, number of hours spent outdoors, household heating method and symptoms of colds or flu were explored but the sample size was too small to allow for stratification to this level.

Questionnaire data were also collated to investigate if parents’ smoking habits were reflected in their children’s PM$_{2.5}$ exposure concentrations. However, all parents claimed that they did not smoke indoors or around their children, as such statistical analysis found no evidence of effect modification by smoking on the personal PM$_{2.5}$ exposure concentrations either by location ($p = 0.27$) or by residential area description ($p = 0.076$). Finally, no significant difference existed between personal PM$_{2.5}$ exposure concentrations when categorised according to different cooking fuels used ($p = 0.51$).
5. Discussion

The Department of Environment and Conservation (DEC) in Western Australia monitors PM$_{2.5}$ concentrations annually to determine 24 hour daily averages as well as annual levels through fixed-site monitoring stations. When comparing the variances in PM$_{2.5}$ concentrations between Collie and Bunbury (close to Dalyellup), the 24 hour daily average for PM$_{2.5}$ in the year 2011 was noted to be 45.5 µg/m$^3$ for the region in close proximity to Collie. Exceedances in particulate matter emission have also previously been noted in technical reports. These included reports from the DEC (DEC Air Quality Report, 2011; Perdaman Chemicals and Fertilizers, 2009; Worsley Alumina Pty. Ltd. Bauxite – Project Expansion, 2005). By contrast, the region of Bunbury seldom showed exceedances in ambient PM$_{2.5}$ concentrations in its airshed (DEC Air Quality Report, 2011) and so was deemed to be an appropriate control location, particularly since the predominantly prevailing winds in Dalyellup were those from Bunbury and are expected to have even lower pollution levels. This assumption was confirmed as the average levels of 8 hour ambient micro-environment PM$_{2.5}$ particulate matter were found to be significantly higher in Collie as compared to Dalyellup (Table 4.1), thus validating the selection of Dalyellup as an appropriate control area.

From the scatter plot (Figure 4.1) it is apparent that personal PM$_{2.5}$ samples collected from children residing in Collie were higher than for those children residing in Dalyellup, this would therefore suggest congruity between the 3 data sets derived from;

- DEC’s fixed-site monitoring stations,
- this study data collected from mobile micro-environment samplers,
- and personal exposure monitoring data.

The environmental sampling for PM$_{2.5}$ and the personal exposure monitoring of respirable dust was conducted with calibrated equipment and strictly according to accepted and validated methods, and therefore were considered valid results.

Prima facie, all three sources of data confirm that the study location (Collie) presented slightly higher mean concentrations of PM$_{2.5}$ than the control location of Dalyellup (Table 4.1). However, a T-test on data from personal PM$_{2.5}$ samples indicated no significant difference between personal exposure sample measurements from the two locations.
Concentrations of all 16 priority PAHs and heavy metals in personal PM$_{2.5}$ samples were found to be below the level of detection, this was not an unexpected result as outdoor ambient samples analysed also showed insignificant levels of PAHs and heavy metals in air. This finding, although restricted the capacity for data analysis, is positive and should be welcomed by the Collie community.

The presence of 1-OHpy in urine is an established marker that can be used to determine the amount of PAH an individual has absorbed into their body as it is metabolized into 1-OHpy and excreted via micturition (Mucha, et al., 2006). Urine samples obtained from all participants from both locations were therefore analysed to determine levels of 1-OHpy as an indicator of PAH exposure, however, no levels were detected, thus confirming the validity of the air sampling data, where no PAHs were detected.

Metal urinalysis only showed the presence of toxic heavy metals, Cadmium, Chromium, Thallium and Vanadium in all urine samples (Figure 4.2), however, these metals were well within acceptable environmental biological exposure limits according to TestSafe Australia (2014).

Copper and Selenium levels were higher for the Collie cohort, as compared to Dallyelup (Copper 22.53 and 14.87 µmols/mol cr, (p=0.003) Selenium 77.37 and 41.23 µmols/mol cr (p=0.006) respectively. These levels were within the normal levels expected for urine copper but slightly higher than the normal range for urine selenium (Copper 15 – 60 µmols/mol cr ; Selenium 13 – 55 µmols/mol cr) expected in urine (MayoClinic, 2016). Mean concentrations of Nickel were 7.01 µmols /mol cr for Collie and 4.74 µmols/mol cr for Dalyellup (Figure 4.3). Although mean urinary nickel concentrations were not significantly different between the 2 locations, both these levels exceeded the biological exposure level of 3.9 µmols/mol cr as recommended by TestSafe Australia (2014); National Environment Protection (Air Toxics) Measure (2004).

While self-reported respiratory health effects data were collected in the questionnaires, there was no significant variance in responses between the Collie and Dalyellup cohorts. As such no significant association could be detected between mean personal PM$_{2.5}$ exposure concentrations and self-reported respiratory health effects. This response is also indicative of the low personal PM$_{2.5}$ exposure concentrations presented by participants in both Collie and Dalyellup.
Variables such as proximity to major roads, average number of hours spent outdoors, household heating method, symptoms of colds or flu and parental smoking were also explored in this study. However, the sample size of recruited participants in this study was deemed insufficient for stratification across these variables listed in the questionnaire and daily activity log sheet.

The association between early exposures to passive smoking and increased respiratory symptoms such as asthma in children has been well established (Miller, et al., 2004). However, all smoking parents reported that they did not smoke indoors. In this study, no association between parental smoking and respiratory symptoms could be established. No correlation was also apparent with heavy metals in urine.

Specific lifestyle factors such as choice of fuel used for cooking and heating, water source and food types consumed were also investigated. No significant associations were detected reinforcing that diet and normal cell turnover are not expected to influence oxidative stress levels (Ściskalska, et al., 2014; Ren, et al., 2011; Lagadu, et al., 2010). The insignificant levels of these respective markers in susceptible participants such as children were therefore also a clear indication of no exposure to any toxic levels of PAHs or heavy metals within their personal PM\textsubscript{2.5} exposure. Detected MDA and 8-OHdG concentrations in urine samples therefore have the potential to be effective markers of health effects in response to personal exposure to toxic levels of pollutants.

### 5.1 Limitations

A number of confounders may have contributed to the limitations of this study. The major setback in this study was the less than optimal sample size. Participation rates were lower than expected, despite significant efforts to increase recruitment through advertising, community meetings and use of local media, sporting and recreational groups, schools and medical professionals in the area. The issues associated with wearing of sampling pumps while having to attend school or sporting commitments and also the need to complete daily diaries and provide urine samples may have been a deterrent, in spite of a high level of interest initially shown by families. It is also probable that community concern was not as high as anticipated and therefore there was no perceived benefit from participation. Recruitment of control subjects proved to be even harder as there were clearly no benefits to
be derived for residents of the coastal community of Dalyellup as they perceived that they lived in a ‘clean-air’ environment.

Other limitations included the inability to consider exposure levels across seasonal variations as a result of the insufficient sample size in the study. As questionnaires and daily diary data were self-administered, the data provided could have contained personal bias from the participating families affecting its accuracy. Many of the air sampling results (PAH and heavy metals) were below the level of detection and although this is a positive finding for the exposed community in Collie, it limited the data analyses.

The urine samples were also collected by participating children and although they were asked to provide the specimen in the morning and place them in iced cooler boxes, this was not controlled so the time lapse between sample void and cold storage may have been an issue in sample integrity. It has been established that, storing urine samples at 25 °C for 24 hours does not affect the quality and concentrations of oxidative stress markers measured in samples (Matsumoto, et al., 2008). Transport of urine samples from participants to the laboratory for storage, and analysis required less than 24 hours under 25 °C.

The levels of exposure to PM$_{2.5}$ and toxic heavy metals in this community based study were very low and even if a larger sample had been recruited it is unlikely that a significant effect would have been noted between the study cohorts.

5.2 Conclusions and recommendations

Over the last 30 years, air pollution research has focused on particulate matter and its effects on human morbidity and mortality. Currently the guidelines for permissible concentrations of particulate matter are governed by its size fractions. However, a number of studies have shown that health effects are also associated with the chemical components that constitute particulate matter, such as PAHs and heavy metal compounds. This study therefore analysed ambient air levels and personal PM$_{2.5}$ samples for these contaminants, and also quantified the body burden of contaminants in a presumed exposed and control population through urine analysis. While the data presented an expected significant difference in PM$_{2.5}$ exposure between study locations, no differences in PAH and heavy metal concentrations were detected. The collection of self-reported respiratory health data showed no correlation with air monitoring data. The presence of certain heavy metals in urine suggests possible
accumulation via alternative routes of entry other than inhalation, such as skin contact or food and water consumption.

Findings from this study suggest that the Collie population is not exposed to significantly higher levels of PAHs and heavy metals in its PM$_{2.5}$ air pollution than people living in Dalyellup, and that the health of children were not impacted by their proximity to industrial sources of pollution.

It is recommended in future air pollution studies that environmental data be correlated to personal exposures and that the use of biomarkers of pollution also be included in the study design. Such comprehensive and integrated studies will generate more robust data which would aid in providing greater detail to the interpretation of the relationships.

Although sample size was considered suboptimal to allow for stratification, some associations were noted that require further investigation in larger cohorts. Children in houses with wood fired heating / fireplaces and electric heating sources had elevated Se and Cu levels when compared to those with gas heaters. Selenium in particular was significantly higher in participants in houses using wood fuels. The consumption of home grown fruit and vegetables also showed an association (although not significant) with urinary Copper and Selenium. Questionnaire data on self-reported respiratory health effects showed no associations with personal monitoring results or ambient PM$_{2.5}$ exposures; with no differences between the 2 study locations.
Study 2:

6. Urinary levels of malondialdehyde and 8-deoxyguanosine as biomarkers of oxidative DNA damage induced by exposure to nickel and cobalt in metal refinery workers.

Reason for Study 2:

The initial study, conducted in a community setting in Collie found that ambient air contained relative low concentrations of PM$_{2.5}$ which was consistent with the low personal exposures to respirable dust, and negligible PAHs and heavy metals in the respirable dust. Concentrations of urinary 1-OHpy indicative of PAH absorption, as well as heavy metals, were determined to be below their level of detection, thus corresponding with the low levels of exposure to ambient PM$_{2.5}$ and respirable dust.

As the original study was unable to determine whether 8-OHdG and MDA were good indicators of oxidative stress due to the negligible exposure to PAHs or heavy metals, it was necessary to conduct a second study in an occupational cohort known to be exposed to Nickel and Cobalt, with the specific objective of examining the relationship between urinary nickel and cobalt and urinary levels of the oxidative stress biomarkers MDA and 8-OHdG.
6.1 Abstract

Metal mining and refinery workers have the potential to be occupationally exposed to quantities of heavy metals that may be associated with health impacts affecting major organ and immune systems (Li, et al., 2014). To ensure the safety of personnel in such occupations regulatory and internal company policies and guidelines require regular monitoring of occupational exposures of employees through a combination of airborne sampling as well as biological monitoring for heavy metals.

Toxic levels of heavy metals accumulated in the body have been shown to elicit inflammatory responses linked to exacerbated health effects impacting the respiratory, cardiovascular and nervous systems (Tchounwou, et al., 2012). There are many studies that have established a significant link between heavy metal exposure and increased oxidative stress (Aflanie, et al., 2015; Ghasemi, Rostampour, Ranjbar, 2014; Pizzino, et al., 2014; Valko, Morris, and Cronin, 2005; Sørensen, et al., 2005). In light of these observations, this study investigated urinary levels of Nickel (Ni) and Cobalt (Co) and oxidative stress markers of cellular and DNA damage, Malondialdehyde (MDA) and 8-deoxyguanosine (8-OHdG) respectively among Ni and Co refinery workers.

The traditional methods of assessing workers exposure using airborne dust concentrations do not consider variations in individual susceptibility. The results of this study showed a positive correlation between urinary Ni and Co exposure and oxidative stress markers, MDA and 8-OHdG, among refinery workers. This finding has implications for occupational health management as individual responses to exposures can now be identified providing an accurate estimate of potential long term health impacts.

6.2 Introduction

In Australia, minerals extraction has been an integral part of the country's culture and development since Europeans first colonised the continent. The first metals mined in Australia were silver and lead at Glen Osmond in South Australia in 1841 (ABS, 2001). Australia has since become one of the world's leading mineral resources nations with the mineral industry being one of the biggest contributors to Australia's export trade.

With Australia, and in particular Western Australia being the largest holder of high-grade nickel and cobalt economic resources worldwide, these metals contribute significantly to the
countries revenue. The location of current Western Australia nickel-cobalt laterites at remote locations dictates the need for metal refineries to also be located remotely (Elias, 2013). This integrated infrastructure also means that workers have contact with both the metal ore as well as its refinery process. The issue is further complicated by the extended shifts and longer work days that workers spend on site, as well as the fact that the accommodation facilities are also located in close proximity to the work site and workers return to the mine village in their work uniforms thus potentially transporting contaminated dust to their sleeping accommodation.

6.2.1 Suggested source of metal exposure

Experimental evidence from studies analysing heavy metals emitted from coal fired power stations suggests that the metal constituents such as As, Cu, Cd, Se, Hg, Pb, V, Zn, Fe and Ni are often present in particulate matter, and it is common for them to undergo speciation to form reactive oxygen species when present in excess (Shah, et al., 2008). Chronic levels of exposure in metal refinery workers may therefore be associated with the high prevalence of the metals in the inhalable particulate matter. There is accumulating evidence in experimental and epidemiological studies indicating that atmospheric pollution based toxic metal exposures can contribute to the induction and alteration of epigenetic markers through cellular oxidative stress (Numan, et al., 2015).

To protect its workforce, these industries adhere to strict guidelines to ensure that their personnel do not suffer from metal toxicity. In Western Australia, metal mining and refining practices are monitored strictly by the Department of Mines and Petroleum (DMP) with up to date regulations in order to maintain a safe and efficient work place for their workforce (DMP, n.d).

6.2.2 Heavy Metal Toxicity

Heavy metal poisoning occurs when toxic amounts of the metals accumulate within the tissues of the body and the usual mechanisms of elimination are impaired resulting in subsequent health effects (National Organization for Rare Disorders, [NORD], 2006). Through establishing and maintaining epigenetic chromatin states, environmental metals may exert their effects on gene expression (Martinez-Zamudio & Ha, 2011).
Different toxic metals produce varying symptoms which may lead to serious damage, in both people and animals exposed to them in concentrations sufficient to cause poisoning. From an occupational health perspective, work force heavy metal exposures have been investigated extensively Nelson, et al., (2010), particularly because of their association with health effects affecting the lungs, kidney, liver, heart and nervous systems and are known to evoke an immune response in individuals exposed to significant quantities (Rouse, et al., 2008, Lynes, et al., 2007).

Arsenic, cadmium, chromium, lead, and mercury are ranked among the priority metals that are of health concern as a result of their high degree of toxicity (Tchounwou, et al., 2012). Such heavy metals have been shown to elicit inflammatory responses in the respiratory system, as well as impact cardiovascular and nervous systems when present at toxic levels (Aflanie, et al., 2015). Their propensity to exacerbate health effects have therefore linked them with a significant amount of respiratory and cardiovascular health burden associated with particulate matter exposures (WHO, 2013, Nelson, et al., 2010, Huang, et al., 2009, Zota, et al., 2009, Shah, et al., 2008).

The tendency for all of these systemic toxicants to induce multiple organ damage even at chronic low levels of exposure have led to their classification as known or probable human carcinogens by the U.S. Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC), and part of Australia’s National Pollution Inventory (NPI) Air Toxic program as they are most commonly associated with poisoning of humans.

6.2.3 Nickel Toxicity

Nickel is an essential trace metal required for various biochemical and physiological functions (Tchounwou, et al., 2012). However, in excess, such metals may also produce cellular and tissue damage which may lead to a variety of adverse effects and human diseases. Dose, duration and route of exposure to essential metals such as Cu, Zn, Ni, Fe and Co, can permit these metals to reach levels that are also toxic in individuals, eliciting very similar health effects on cellular targets within these organ systems (Lynes, et al., 2007). Due to its wide use in industry, nickel (Ni) has been deemed as a toxic and carcinogenic metal of environmental concern (Martinez-Zamudio & Ha, 2011; Das, et al., 2008). The health effects of nickel toxicity depend on the route of exposure (inhalation, oral, or dermal) (Das, et al., 2008). The most common health effect of nickel in humans is an allergic skin reaction in sensitive individuals. However, the inhalation of nickel particles represents another major
route of human exposure, where nickel has been suggested to exert its toxic effects through non-genotoxic mechanisms such as DNA methylation (Martinez-Zamudio & Ha, 2011).

Nickel transformation has been shown to suppress the DNA repair gene, O6-methylguanine DNA methyltransferase (MGMT) expression in lung cancer cells (Martinez-Zamudio & Ha, 2011). As a potential immunomodulatory and immunotoxic agent in humans, nickel compounds, except for metallic nickel, have therefore also been classified as human carcinogens by the International Agency for Research on Cancer (IARC) and the U.S. Department of Health and Human Services (DHHS, National Institute of Environmental Health Sciences; 1994, IARC; 1990).

6.2.4 Cobalt Toxicity

Cobalt is an element that occurs naturally in many different chemical forms throughout our environment (Lisbon et al., 2001). The inhalation of Co alone can cause asthma with toxic effects in higher concentrations affecting mainly the lungs, leading to pneumonia, wheezing and pulmonary oedema (Barceloux, 1999). Other health effects linked to overdosing on cobalt (>5 mg/day) include broad and unspecific effects such as, abnormal thyroid functions, polycythemia and overproduction of red blood cells (erythropoiesis), with increased production of the hormone erythropoietin which may lead to peripheral vascular thrombosis and optic nerve atrophy (Hengstler, et al., 2003).

It is also well-established that cobalt is a potent inducer of oxidative stress causing free radical generation, which in turn induces DNA damage, inhibits DNA repair mechanisms and the exchange of DNA between sister-chromatids and aneuploidy contributing to its toxicity and carcinogenicity (Galanis et al., 2009). Recent experimental studies confirm its interference with DNA repair processes, and its direct induction of DNA damage, DNA-protein crosslinking, and sister-chromatid exchange (Jomova and Volko, 2011). As such, the IARC also classified cobalt as “possibly carcinogenic to humans” (IARC, 2006).

6.2.5 Proposed mechanism of heavy metal toxicity

The accumulation of heavy metals to a toxic level has been shown to occur when metallothioneins that normally neutralise these metals are insufficient to restrict their interference with cellular mechanisms (Mohamed, et al., 2014, Wei, et al., 2010).
It is proposed that, when in excess, one of the main reasons heavy metals can interfere with cellular processes is because, many non-essential toxic metals share similar valencies with essential metals normally utilised by the body for co-factors in important enzymatic functions or the construction of cellular structures (Choudhary, et al., 2007). Studies have shown that the toxic accumulation of metals within the organism not only produce reactive radicals that compromise normal biological processes, but also result in DNA damage, lipid peroxidation, depletion of protein sulfhydryls and other effects (Shah, et al., 2008, Valko, et al., 2005).

Research over the past two decades has established that it is possible even for essential common transition metals such as Cu, Zn, Ni and Fe, to disturb normal biological functions, by generating reactive oxygen species at a cellular level through mitochondrial apoptotic pathways, evoking similar cellular responses, when present in excess (Tchounwou, et al., 2012; Martinez-Zamudio & Ha, 2011; Bell, et al., 2009; Wallenborn, et al., 2009; Lynes, M.A., et al., 2007; Tjalve, et al., 1999). At a molecular level, the main factor that defines the toxicity or carcinogenicity of a metal is its ability to generate reactive oxygen and nitrogen species (Ghasemi, Rostampour, Ranjbar, 2013; Das, et al., 2008; Valko, et al., 2005). These free radicals then activate redox sensitive signalling pathways of transcription factors, resulting in a number of modifications to DNA bases, lipid peroxidation, and altering the calcium and sulphydryl homeostasis (Rahman, 2007; Valko, et al., 2005).

6.2.6 Malondialdehyde (MDA)

Malondialdehyde is an example of a reactive aldehyde resulting from lipid peroxidation of polyunsaturated fatty acids. It is a prominent product in Thromboxane A2 synthesis wherein arachidonic acid is metabolized to prostaglandin H2 by platelets and a wide array of other cell types and tissues by cyclooxygenase 1 or 2 and subsequently to Thromboxane A2, 12-Hydroxyheptadecatrienoic acid, and malonyldialdehyde by Thromboxane synthase. As a major bioactive electrophile species, malondialdehyde causes toxic stress in cells by reacting with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, which are mutagenic. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. In human nutrition and biology, advanced glycation end products, known as AGEs, are substances that can be a factor in the development or worsening of many degenerative diseases, such as diabetes, atherosclerosis, chronic renal failure, and Alzheimer's disease (Ghasemi, Rostampour, Ranjbar, 2013; Das, et al., 2008; Valko, et al., 2005).
Malondialdehyde is an advanced lipoxidation end-products (ALE), in analogy to AGE (Jomova, K., Valko, M., 2011). These harmful compounds can affect nearly every type of cell and molecule in the body and are thought to be one factor in aging and in some age-related chronic diseases. They are also believed to play a causative role in the blood-vessel complications of diabetes mellitus. AGEs are seen as speeding up oxidative damage to cells and in altering their normal behaviour (Rahman, 2007; Valko, et al., 2005).

6.2.7 8-hydroxy-deoxyguanosine

8-hydroxy-2′–deoxyguanosine has been widely accepted as an indicator for oxidative stress and carcinogenesis with extensive studies showing urinary 8-OHdG to be a good biomarker for risk assessment of various cancers, cardiopulmonary events and degenerative diseases as it is not influenced directly by either diet or cell turnover (Ściskalska, et al., 2014; Ren, et al., 2011; Lagadu, et al., 2010). Although findings have determined that the presence of 8-OHdG alone is insufficient for the formation of tumours, 8-OHdG is considered to be a potential intermediate marker of disease end-point and is likely to play a role in many pathological conditions where it has been shown to be elevated (Cooke, et al., 2003). At the cellular level, 8-OHdG formed as a result of oxidative stress is released into the circulatory system by the DNA base excision repair pathway and excreted directly into urine (Ren, et al., 2011).

Studies have also established a significant relationship between the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a predominant form of free radical-induced oxidative lesions in nuclear and mitochondrial DNA and heavy metal toxicity (Ściskalska, et al., 2014; Valavanidis, Vlachogianni, Fiotakis, 2009). Occupational health studies have therefore utilised the significant and consistent linear associations between urinary heavy metals and 8-OHdG as a measure of oxidative stress from heavy metal exposure (Wang, et al., 2015).

MDA and 8-OHdG were therefore deemed to be appropriate biomarkers that would potentially be correlated with levels of nickel and cobalt in urine.
6.3 Materials and Methods

6.3.1 Study Population

This cross-sectional study sought to assess the oxidative stress levels of Ni and Co refinery workers (n=77) by measuring urinary Ni and CO as well as MDA and 8-OHdG concentrations. Personnel were recruited from all sectors of the refinery. Participants were informed of the study via a company information session and through the disseminated of an information brochure (Appendix F). Interested volunteers were briefed on the study procedures and any risks, and subsequently given the option to sign an informed consent form (Appendix G) to participate. Recruited participants completed a questionnaire providing demographic information on their age, weight, sex, smoking status and frequency, alcohol consumption, dietary habits, as well as information on their work profile, such as hours worked outdoors in the refinery and tasks performed. Participants were then required to provide urine samples for analysis. All participant consent forms, accompanying questionnaires and urine samples were de-identified.

The study was approved by the Edith Cowan University Human Research Ethics Committee as well as management of the refinery.

6.3.2 Data Collection

6.3.2.1 Questionnaire

All participants were asked to complete a self-administered questionnaire at the end of their work shift which was adapted from an instrument used by Van Wijnen, Slob, Jongman-Liedekerken, van de Weerdt, and Woudenberg, (1996), to more accurately address the occupational exposures of workers in a refinery setting (Appendix H). The questionnaire was designed to collect data on demographics, a brief medical history and environmental exposures, including consumption of fruit and vegetables. Questionnaires were completed after urine sample collection.

6.3.2.2 Urine Samples

Participants were issued with 120ml sterile screw cap cryostorage containers to provide post shift urine samples which were then sealed in biohazard bags and refrigerated before being transported by air to Perth in cold storage on the same day of collection. Samples were subsequently stored at Edith Cowan University at -80°C prior to their frozen transport to the
Central Laboratory, Beijing Center for Disease Prevention and Control, in China for analysis of Creatinine, Ni, Co, MDA and 8-OHdG.

6.3.3 Urinalysis

6.3.3.1 Ni and Co urinalysis

For the measurement of Cobalt and Nickel, the total urine volume was recorded and approximately 5 mL of each sample was treated with 4% HNO3(aq), and digested in a microwave oven. Urine samples were analysed for Ni and Co, using an Agilent 7700 ICP-MS instrument equipped with a quartz spray chamber, glass ICP torch, and a micro mist nebulizer and X-len ion lens.

The results in the form of descriptive statistics were expressed in ng/ml. All samples showed urine creatinine levels within 0.3 and 3.0 g/L, which is the range recommended by the World Health Organization as a criterion for spot urine samples to be considered valid (Bader, Messerer and Will, 2013). Both creatinine-normalised analytical data and data that were not normalised using creatinine levels presented very similar values and presented the same correlation.

6.3.3.2 Urine MDA and 8-OHdG urinalysis

Urinary MDA and 8-OHdG concentrations were determined using an Acquity ultra performance water chromatography system (UPLC) coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). UPLC separation was conducted with an Acquity HSST3 column (2.1 mm×100 mm; 1.8 µm; (Waters), and the mobile phase was ACN and 0.1% formic acid water (0.1% FA), with the flow rate set at 0.3 mL/min and the injection volume at 5 µL. Extraction was performed on a Positive Pressure-96 Processor (Solid Phase Extraction Manifold, Waters, Milford, MA, USA).

6.3.4 Statistical Analysis

Statistical analysis was performed using STATGRAPHICS – Centurion, V.1.10, 2015. The association of urinary MDA and 8-OHdG concentrations with the following variables: urinary metals (Ni and Co), self-reported age, weight, sex, smoking status and frequency, dietary habits, hours outdoors, tasks during working hours were investigated. An analysis of variance (ANOVA) was performed between MDA and 8-OHdG urinary concentrations (dependent variables), and Ni and Co urinary concentrations, smoking status and frequency,
age, weight, to determine the significance of the relationship between MDA and 8-OHdG and each respective independent variable listed.

A simple regression was then subsequently used to investigate the strength of the linear relationship between each oxidative stress marker and each urinary metal and covariates.

Multiple variable analysis, was used to investigate the bivariate and multivariate relationships between the urine metals (Ni and Co) and oxidative stress markers (MDA and 8-OHdG) in the urine samples. A multiple regression analysis was also performed to construct a statistical model to best describe the impact of the independent variables and the covariates on the dependent variables. Formal statistical significance was defined at the conventional 5% level.

6.4 Results

6.4.1 Participant Characteristics

Table 6.1 summarises the basic characteristics of the recruited participants from the refinery workforce. In total, 77 refinery workers volunteered to take part in this study. Although the youngest participant was 35 and the oldest participant was 71 years of age, most were closer to the median age of 55 which also is a reflection of the usual work environment of experienced refinery personnel. The percentage of participants that indicated that they were smokers was approximately 18%. The range of average hours worked outdoors was broad (1 – 12 hrs).

Table 6.1 Subject Demographics of Refinery Workers, Western Australia

<table>
<thead>
<tr>
<th>Age (Median and Range)</th>
<th>55, 35-71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Smokers</td>
<td>18%</td>
</tr>
<tr>
<td>Daily Frequency of Smoking</td>
<td>Daily</td>
</tr>
<tr>
<td>Range of Indv. average hours outdoors during the day over the working week.</td>
<td>1 - 12</td>
</tr>
</tbody>
</table>
6.4.2 Urinary Oxidative biomarkers and Metal Concentrations

Scatterplots of bivariate analysis between Oxidative Stress marker concentrations (MDA and 8-OHdG) and Ni and Co concentrations in urine samples.

Figure 6.1. Shows the scatterplots of the bivariate analysis between urine MDA and urine Ni (a) and Co (b) concentrations, as well as 8-OHdG concentrations and urine Ni (c) and Co (d) concentrations, from all study participants.

The primary focus of this study was to investigate the relationship between personal exposure to Ni and Co and oxidative stress markers in urine, indicative of systemic oxidative stress. To determine the significance of association, a bivariate analysis was performed for urine Ni and Co concentrations separately in relation to concentrations of MDA and 8-OHdG (Figure 6.1). The graphs illustrate their associations respectively. For all four associations, the Pearson product-moments showed P values of < 0.05, indicating that there was a significant relationship between oxidative stress markers (MDA and 8-OHdG) and urine metals (Ni and Co) concentrations in the analysed urine samples. Although elevated concentrations of both MDA and 8-OHdG were also apparent at lower levels of both urinary Ni and Co concentrations in participating refinery personnel, these points were statistically insignificant.
Simple regression linear models illustrating the relationship between the concentrations of oxidative stress markers and Ni and Co concentrations in urine samples.

Figure 6.2. Shows the respective results of fitting a linear model to describe the relationship between urine MDA and Ni (a), MDA and Co (b), 8-OHdG and Ni (c) and finally 8-OHdG and Co (d). All four correlations showed patterns with slopes presenting a significant relationship at the 95.0% confidence level.

To determine the strength of these correlations, a simple regression analysis was conducted on both urine MDA and 8-OHdG concentrations in relation to Ni and Co concentrations in urine separately (Figure. 6.2). All four models showed a linear regression pattern with slopes for all cases presenting a significant association between the oxidative markers (MDA and 8-OHdG) and metal concentrations.

P-values in their ANOVAs were all less than 0.05, indicating statistically significant relationships for all simple regressions constructed, at the 95.0% confidence level (Figure 6.2a, 6.2b, 6.2c & 6.2d). All four models presented coefficients ranging from 0.67 to 0.75, indicating moderately strong relationships, accounting for approximately 50% of the variability in urine MDA and 8-OHdG concentrations (R-squared values ranged from 45.4 to 55.9).

MDA concentrations were significantly correlated with Ni as well as Co concentrations in the urine samples (Fig. 6.1a, 6.1b and 6.2a, 6.2b; P < 0.005, $R^2 = 45.4 - 47.2$). Similarly, 8-
OHdG concentrations were also significantly correlated with Ni as well as Co concentrations in the urine samples. (Fig. 6.1c, 6.1d and 6.2c, 6.2d; P < 0.005, R² = 47.8 – 55.9).

This study also considered the significance of the relationships between other independent variables and MDA and 8-OHdG to determine if there were any confounding factors such as smoking status or age affecting these results.

An analysis of variance performed on urine MDA concentrations and smoking status, as well as participant age, both provided P-values greater than 0.05. This indicated that there were no statistically significant differences in urine MDA concentrations based on smoking status, or participant age at the 95% confidence level.

This was also confirmed through simple regression performed separately for smoking status, and participant age, with urine MDA concentrations which indicated that both variables only explained 0.55% and 0.29% of the variability in MDA concentrations respectively and were therefore not statistically significant.

Similarly, an analysis of variance performed on urine 8-OHdG concentrations and smoking, as well as participant age both also provided P-values greater than 0.05. This also indicated that there were no statistically significant differences in urine 8-OHdG concentrations based on smoking status, or participant age at the 95% confidence level.

Through simple regression performed separately for smoking status and participant age, with urine 8-OHdG concentrations, it was indicated that their association only explained 0.06 % and 0.13 % of the variability in 8-OHdG concentrations respectively and were therefore not statistically significant.

However, an analysis of variance performed on urine MDA concentrations and average hours outdoors in a day provided a P-value less than 0.05, indicating a statistically significant relationship between mean urinary MDA concentrations and average hours working outdoors in a day during their shift at the 95% confidence level.

Simple regression on urinary MDA concentrations and average hours outdoors in a day indicated that there was a moderately strong relationship between the variables which explained 63.8% of the variability in MDA concentration observed.

An analysis of variance performed between mean 8-OHdG concentrations and average hours outdoors in a day also showed a statistically significant relationship at the 95% confidence
level, with simple regression on urinary 8-OHdG concentrations and average hours outdoors in a day also indicating a moderately strong relationship between the variables explaining 39.3% of the variability in 8-OHdG concentrations observed in the refinery personnel.

This finding reflected the exposure profile of workers, as those that spent most of their time in a control room had lower exposures than those who worked on maintenance tasks in the actual refinery. Other variables such as, smoking frequency and diet were also not shown to contribute significantly to the variation in urine MDA and 8-OHdG concentrations.

Taking into consideration the significant relationship with hours worked outdoors, a multiple regression analysis was performed to analyse the combined effects of Ni and Co exposure, measured from their concentrations in the urine samples, and the average hours participants worked outdoors during their shifts, on urine MDA and 8-OHdG concentrations.

The equation of the fitted model assumed the equation:

\[
\text{MDA Concentration} = 1.79495 + 0.0720098\times \text{Ni Concentration} + 1.21732\times \text{Co Concentration} + 2.09334\times \text{Average hours outside in a day}.
\]

For this model, the P-value in the ANOVA showed a value < 0.05, indicating a statistically significant relationship between the variables at the 95% confidence level. The R-squared statistic also indicated that the model as fitted explains approximately 75% of the variability in MDA concentrations in the urine samples.

Although this attributes approximately 25% of the MDA concentration variability to other factors not included in the model, a Lag 1 residual autocorrelation value that was close to zero (-0.08) indicated no significant variable was not accounted for in the model. In addition, a Durbin-Watson statistic of 2.14, and a P-value greater than 0.05, also indicated that residuals tested had no indication of serial autocorrelation at a 95% confidence level.

Similarly a multiple regression analysis was subsequently performed on urine 8-OHdG concentrations in relation to Ni and Co concentrations in the urine samples, as well as the average hours participants worked outdoors during their shifts, smoking frequency and their age, to analyse the significance of their combined effects on the variation of 8-OHdG concentrations in the urine samples.

The result indicated that only Ni and Co concentrations showed statistically significant associations at the 95% confidence level between both independent variables and the
variations observed in urine 8-OHdG concentrations as indicated by P-values in the ANOVA (< 0.05).

Variables such as average hours outside during their shift, smoking frequency and age were shown not to be associated significantly with urine 8-OHdG concentrations and therefore were excluded from the analysis without any consequence to the multiple linear regression model describing their relationship.

From the fitted model the R-squared statistic also indicated that the model as fitted explains approximately 70% of the variability in 8-OHdG concentrations in the urine samples, leaving approximately 30% of the 8-OHdG concentration variability to other factors not included in the model. However, a Lag 1 residual autocorrelation value that was close to 0 (-0.08) indicated no significant structure not accounted for in the model. In addition, a Durbin-Watson statistic of 1.77, and a P-value greater than 0.05, also indicates that residuals tested had no indication of serial autocorrelation at a 95% confidence level.

Potential confounders for this study include smoking and diet. Self-reported questionnaire data indicated that smoking prevalence was low at 18%.

Personnel in the refinery participating in this study had very similar food consumption patterns. This could be expected as all personnel at this remote site during their shift are residential and they all ate food provided from the same mess hall. From this perspective diet can be excluded as a confounder and this assumption was confirmed by participant responses to the dietary questions in the survey.

6.5 Discussion

The currently employed method of exposure assessment for occupational cohorts is to determine a time weighted average (TWA) concentration of a contaminant that workers are exposed to over the length of a shift. The sampling method utilises a pump to collect the total particulate matter exposure across the shift giving an average result. Therefore, in cases where the exposure profile is not homogenous peak exposures are not identified. The complexity and cost of TWA exposure assessments are also limiting factors that make it unlikely that monitoring will be conducted more than once or twice per year for a particular exposure cohort and so the likelihood of missing high exposures is relatively high. Exposure standards for TWA’s are generally based on toxicological data obtained from animal
experimentation or epidemiological studies. The standards are designed to protect the “average” workers from harmful effects, and so do not compensate for variations in individual susceptibility, this is a fundamental flaw of the TWA exposure assessment method (ACGIH, 2015; Safe Work Australia, 2016).

In some cases health monitoring or health surveillance is also prescribed by the regulators as an additional check on workers exposures (Safe Work Australia, 2013; DMP, 2010). In the case of nickel and cobalt, exposure is assessed as total metal concentration in urine. However, these traditional measurements also do not account for the specific effects of exposure and do not consider other risk factors such as individual susceptibility and lifestyle factors that could also contribute to personal susceptibility.

The nickel and cobalt refinery plant offered a unique opportunity to investigate the impact of heavy metal exposure on individuals under chronic occupational exposure conditions. In this study the relationship between oxidative stress markers MDA and 8-OHdG and biomarkers of metal exposure were investigated in workers exposed to Ni and Co. Occupational exposure to both metals can be attributed to the inhalation of heavy metal residue in a number of pollutants transported by dust through the air, by hand to mouth contact, or by the consumption of contaminated drinking water and food.

This study has shown that the refinery workers demonstrated varied concentrations of Ni and Co exposure during their workweek, as indicated by their post-shift urine samples. Corresponding to these urine metal concentrations are concentrations of MDA and 8-OHdG detected in the respective urine samples that are significantly correlated. It was evident from the study that personnel with higher concentrations of Ni and Co also presented higher concentrations of MDA and 8-OHdG in their urine.

Another variable that was shown to affect the MDA and 8-OHdG concentrations in the urine samples was the average number of hours personnel spent outdoors in the refinery as opposed to working in the control rooms in-doors. Multiple regression analysis reinforced this association showing that as workers were occupationally exposed to Ni and Co for longer periods, metal levels in their urine were invariably higher, resulting in associated increases in oxidative stress as evidenced by elevated MDA and 8-OHdG levels.

Urinary Ni and Co concentrations have been reported in other occupational cohorts as being predictive of urinary MDA and 8-OHdG with associated DNA damage (Mukherjee, et al.,
This study therefore provides further evidence to support the correlation between urinary metals, Ni and Co, and urinary MDA and 8-OHdG without being influenced by confounding variables such as smoking, alcohol, or age.

This study has shown that the potential exists to develop a simple and non-invasive screening tool to ascertain the physiological response of workers to a range of workplace contaminants and to work towards developing a new health surveillance regime that is personalised and tailored to suit individuals rather than relying on TWA and metal concentrations in urine as an indicator of potential for adverse health effects. Research should be directed at ascertaining what levels of 8-OHdG and MDA biomarkers are associated with DNA damage and workers should be screened regularly in order to ensure they remain working within acceptable parameters. Post shift levels of 8-OHdG and MDA should also be analysed over time in order to determine how long workers require to recover from their period of time on-site as this could help determine the ideal FIFO swing period that will allow recover and repair after a swing on-site.

6.5.1 Limitations

The workplace questionnaire only focused on the on-site activities and did not take into consideration other the lifestyle activities (with the exception of smoking) of the workers particularly during their rest and recreation time away from the refinery. These lifestyle (confounding) factors may well have accounted for the elevated oxidative stress markers (MDA and 8-OHdG) seen in figure 6.1 where the oxidative stress markers were elevated even at low levels of urinary Ni and Co.

The role of oxidative stress markers such as MDA and 8-OHdG require further research. While more post shift urine samples of refinery workers from a larger sample size, and over longer work periods will help build confidence in the predictive value of oxidative stress biomarkers through epidemiological studies; it is recommended that in-vitro cell culture techniques could be utilised to help establish the predictive value of oxidative stress biomarkers for specific pollutants which will provide a model for occupational disease surveillance/ screening. It may be possible then to predict occupational exposure standards based on an in vitro model rather than waiting for the onset of symptoms due to exposure to heavy metals.
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Appendix A

INFORMATION

Children’s Exposure to air pollution in the South West of WA

Researchers from Edith Cowan University are conducting a study of children’s exposure to some air pollutants such as very small particulate matter (dust), hydrocarbons and metals in the South West of WA.

We are currently recruiting school-going CHILDREN between the ages of 8 -12.

Participation is VOLUNTARY.

If you would like to find out more information or get involved, Call Dev Menon on 6304 5714.

Human exposure to polycyclic aromatic hydrocarbons and metals in Particulate Matter 2.5

From a variety of activities including urbanization, industrial development, mining, agriculture and environmental change, communities are experiencing increasing amounts of air pollution. In particular, very small invisible particulate matter with a diameter of 2.5µm or less, also known as PM2.5. These particles can contain polycyclic aromatic hydrocarbons (PAHs) and metals and the concentrations of these pollutants tend to be higher in close proximity to industries or urban traffic.

PAHs are a group of organic compounds that could be contributing to an increase in cases of asthma and other respiratory diseases among children. When metals are present in significant quantities, they have been associated with a range of health effects among children and adults.

While PAHs and metals are known to be toxic in relatively large concentrations, it is unclear whether the relatively minor increases in ambient air concentrations are sufficient to trigger a biological response, particularly among children.

How can I help?

We hope to recruit school-going children between the ages of 8 – 12 yrs, from the township of Collie to represent a community in close proximity to an industrial exposure source. These children’s exposure measures will be compared to those of children recruited from the community in Dalrymple (near Bunbury). Owing to its location close to the coast, Dalrymple is not exposed to any specific sources of air pollution. As such, participants from Dalrymple can contribute to the project by representing the control group unexposed to ambient air pollution specific to industry.

Children participating in the study will be interviewed and together with their parents will complete a questionnaire to help us understand their lifestyle activities which could influence their potential interaction with outdoor air pollution. Children will be asked to wear a personal particulate exposure monitoring device for 8 hours on 2 days over a period of a fortnight.

Researchers will collect a morning urine sample following each personal particulate exposure sampling day. From these analyses, the researchers will be able to determine if the concentrations of particulates, PAHs and metals in the ambient air have contributed to some of measureable biological effects in the children.

Confidentiality

We will provide each family with individual results if required.

Your personal information will be kept in locked storage cabinets and accessed only by researchers ethically approved to work on this project. The results of this study will be published without any personal information. In other words data will be pooled and discussed in group format, hence preventing the identification of any individuals in any report.

This study has been approved by the ECU Human Research Ethics Committee. If you have any concerns about this project, an independent ECU Ethics Officer may be contacted on (08) 6304 2170.
Appendix B

INFORMATION LETTER

Children’s Exposure to air pollution in the South West of WA

Dear Parent/Guardian

Researchers from Edith Cowan University are conducting a study of children’s exposure to air pollution in South West of WA as part of a PhD project. The study aims to find out the concentrations of small particulate matter (dust) in the air. These particles will be further analysed for hydrocarbon (PAHs) and metal concentrations. In addition we would like to analyse urine samples to see if we can detect any indication of a biological response to air pollution.

We would like to invite your child to participate in the study by volunteering to wear a dust sampler for 8 hours on 4 days. (twice in the summer season and twice in the winter season). We would also like you to complete a questionnaire together with your child to determine respiratory symptoms and other possible sources of exposure such as the type of heating and cooking appliances used in the home. Furthermore, we would like to request that your child provide a morning urine sample on the days after wearing the dust pump. All participation is completely voluntary and you may withdraw your child from the study at any time should you no longer wish for your child to participate.

Background Information

Very small invisible particulates are found in air associated with a wide variety of activities. When they are produced from industry or vehicles, they can contain a variety of pollutants. Polycyclic aromatic hydrocarbons (PAHs) and metals are two groups of chemicals that are often found associated with particulate matter. PAHs are a group of organic compounds that can contribute to the increase in the prevalence of asthma and other respiratory complaints. Metals in large concentrations can also be associated with a variety of health problems.

We do not know exactly how much respirable particulate matter children in the South West are exposed to and if these particulates may contain PAH’s or metals, or if there is a measurable biological response to air pollution among children in the region. This research is attempting to answer these questions.

What Your Child Will Be Doing

Should you allow your child to participate, you will be asked to complete a consent form on behalf of your child permitting her/him to volunteer. With your help your child will complete a questionnaire that will help us understand his/her lifestyle activities that could influence exposure
to outdoor air pollution. Your child will also be provided with a personal particulate matter sampler, (dust pump) a diagrammatic protocol which will remind them how to use it and what to do on that day, a time activity diary, and a 100ml container for urine. Your child will be asked to wear the sampler on 4 occasions over a year for eight hours on each occasion. Finally you will be asked to assist your child in completing a time activity diary and your child will be asked to provide a urine sample on the morning after each sampling day.

For further details please see the instructions to participants attached.

The time commitment for the participants and any travel involved
The research will be conducted for 2 days in the summer season and 2 days in the winter season. Children are expected to go about their normal routine on these days.

Possible Effects of Research Procedures
Should your child find the air pumps of the personal particulate sampler too heavy or cumbersome to carry they may remove the sampler while indoors as long as they do not turn the pump off. Children are also free to withdraw from the study at any time should they no longer wish to participate.

Should you or your child have queries regarding the project at any stage, the researcher and his supervisors can be contacted to answer any questions or concerns. Should participants have any concerns or complaints and need to speak to an independent person, the ECU human research ethics office can also be contacted. Please bear in mind that the study will not diagnose any medical/clinical conditions.

Potential Benefits
The study will inform the research community of the significance of components of air found in respirable particulate matter which is an area of considerable interest nationally and internationally. Furthermore, this study will provide the communities, industries and government regulators with knowledge about exposure to particulates and its significance, if any, on children’s health.

Confidentiality

All information provided, as well as individual sample results will be kept confidential. Only summarized information will be made available in reports no individual will be identifiable from the research reports. However each participant will be provided with their own results.
Appendix C

Consent Form

Personal ID: □□□□

Children’s Exposure to air pollution in the South West of WA

Chief Investigator: Dev Menon  (PhD Student)
Supervisor: Dr. Jacques Oosthuizen

Thank you for your interest and assistance with this study.

Before signing this form, please ask any questions on any aspects of the study that are unclear to you.

Statement of consent

My child has been invited to participate in this study after having had their rights and obligations explained to my satisfaction. I give my consent by signing this form on the understanding that:

1. I comprehend the general purposes, methods and possible inconvenience of the study.
2. All tests described in this document which are performed as part of the research program will be performed at no cost to me.
3. Individual results will also be made available to volunteering children and their families.
4. If requested, a summary of the results will be provided at the end of the study.
5. In giving my consent I acknowledge that my participation in this study is voluntary, and that I may withdraw my child at any time and that I may be asked by the investigators to withdraw at any time.
6. I have read the information letter and any questions I have asked have been answered to my satisfaction.

Participant (Parent/Guardian): ___________________________________________ Date: ____________

Child (Signature Optional*): ___________________________________________ Date: ____________

Please allow your child to sign if they wish to do so.
Witness: ___________________________________________ Date: ____________

I do/do not wish to be informed of the results of this study (please circle).

If you would like to be informed of the results, please provide a contact address (below).

______________________________________________________________________
______________________________________________________________________
Appendix D

Questionnaire

Personal ID: ☐☐☐

Date: ___ / ___ / ___

Sample Collection Date: ___ / ___ / ___

Children's Exposure to air pollution in the South West of WA

Edith Cowan University

Instructions
1. Please complete on behalf of your child or assist your child to complete this questionnaire.
2. Please read each question carefully.
3. Please provide your answer in the box or space provided.

All information provided in this questionnaire will be treated confidentially.

The purpose of this questionnaire is to obtain information about your child and their daily activities that may influence exposure to air pollution.

If you have any concerns or complaints about the research project and wish to talk to a research personnel, you may contact:

Dev Menon
PhD Candidate
School of Exercise, Biomedical & Health Sciences
Edith Cowan University
270, Joondalup Drive
JOONDALUP WA 6027
Phone: (08) 6304 5714
Email: d.menon@ecu.edu.au

OR

Associate Professor Jacques Oosthuizen
School of Exercise, Biomedical & Health Sciences
Edith Cowan University
270, Joondalup Drive
JOONDALUP
WA 6027
Phone (08) 6304 5876
Email: j.oosthuizen@ecu.edu.au
**Part A: Personal and family information**

| Q1. What is your age in years? | □  □ years |
| Q2. What is your gender? | BOY □ 1 GIRL □ 2 |
| Q3. What is your post code? | □ □ □ □ |
| Q4. What is the occupation of your father or male carer? | |
| Q5. What is the occupation of your mother or your female carer? | |
| Q6. Did anyone in your household smoke in the last seven days? If no, please proceed to Q9. | Yes □ 1 No □ 2 |
| Q7. Did they smoke indoors or outdoors? (Please tick one) | Indoors □ 1 Outdoors □ 2 Both □ 3 |
| Q8. How often did they smoke in the time they were there? (Please tick one) | Daily □ 1 Twice in the week □ 2 Three times in the week □ 3 Four times in the week □ 4 Five times in the week □ 5 Six times in the week □ 6 |
## Part B: Dwelling Information

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q9. What type of dwelling do you live in?</td>
<td>House □1, Flat □2, Other (Specify) □3</td>
</tr>
<tr>
<td>Q10. What type of location best represents your dwelling?</td>
<td>Near traffic lights □1, Near the freeway or Highway □2, Near an urban area □3, Near a country side □4, Near an industrial area □5</td>
</tr>
<tr>
<td>Q11. How many years have you been living at your current address?</td>
<td>□ yrs and □ months</td>
</tr>
<tr>
<td>Q12. What was your suburb postal code of your previous residence?</td>
<td>□ □ □ □ □</td>
</tr>
<tr>
<td>Q13. What is the main type of heating used in your home during winter?</td>
<td>Central heating □1, Fire place or wood heater □2, Oil Heater □3, Electric Heater □4, Don’t know □5</td>
</tr>
<tr>
<td>Q14. How often does your heating unit get used each day?</td>
<td>1 – 2 hours □1, 2 – 4 hours □2, 4 – 8 hours □3, All day □4, Don’t know □5</td>
</tr>
<tr>
<td>Q15. What type of fuel/energy do you use for your cooker/stove?</td>
<td>Gas □1, Electric □2, Wood □3, Don’t know □4, Others (Specify) □5</td>
</tr>
<tr>
<td>Q16. Is a ventilation fan used with the cooker/stove?</td>
<td>Yes □1, No □2</td>
</tr>
</tbody>
</table>
### Part C: Commuting Information

| Q18. What is the main transport method you take to school each day? | Motorcar ☐1  
Motorcycle ☐2  
Bus ☐3  
Bicycle ☐4  
Public Transport ☐5  
Walk/Jog ☐6  
Others (Specify) ☐7 |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Q19. How long on the average does it take you to reach school?</td>
<td>☐ hrs and ☐ mins</td>
</tr>
</tbody>
</table>

### Part D: Information on consumption of food and water

| Q20. Does your family grow vegetables/fruits/herbs in your home garden? | Yes ☐1  
No ☐2  
Don’t know ☐3 |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>If the answer is “No” go to Q24</td>
<td></td>
</tr>
</tbody>
</table>
| Q21. Do you eat the vegetables/fruits/herbs grown from your garden at your current address? | Yes ☐1  
No ☐2  
Don’t know ☐3 |
| Q22. How often do you eat vegetables/fruits/herbs grown from your garden at the moment, or over the last four months? | Daily ☐1  
A few days a week ☐2  
A few days a month ☐3  
< a few days a month ☐4  
Don’t know ☐5 |
| Q23. Do you or a parent wash the vegetables/fruits/herbs grown from your garden before eating? | Always ☐1  
Often ☐2  
Rarely ☐3  
Never ☐4  
Don’t know ☐5 |
### Q24. Are you currently using water from a rain water tank system at home?
- Yes ☐
- No ☐
- Don’t know ☐

### Q25. If you are using water from a rain water tank system, how often do you use it?
- Every day ☐
- Once a week ☐
- 4-5 times per week ☐
- 2-3 times per week ☐
- None ☐
- Don’t know ☐

### Q26. What is the main activity you spend time doing when you are outside? (Please specify)
- Physical sports like footy or basketball or cricket or running ☐
- Relaxed activities like chatting to friends or reading a book or walking ☐
- Others ☐

### Q27. On average how many hours do you spend outside during a day?
- ☐ ☐ hrs per day

### Q28. How many hours do you spend outside gardening or in contact with soil?
- ☐ ☐ hrs per day

---

### Part E. Information on health

#### Past Illnesses

<table>
<thead>
<tr>
<th>Q29. Have you seen a doctor in the last two weeks for any illness or health problems? If the answer is “No” go to Q34.</th>
<th>Yes ☐</th>
<th>No ☐</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q30. If you saw a doctor, please specify what the doctor said your main health problem was.</td>
<td>Asthma ☐</td>
<td>Chronic coughing ☐</td>
</tr>
</tbody>
</table>

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109
<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q31. Did you take any medication for your health problem specified in Q30?</td>
<td>☐ 1</td>
<td>☐ 2</td>
</tr>
<tr>
<td>Q32. What medication are you taking for your health problem?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q33. How long have you had this health problem?</td>
<td>1 day</td>
<td>☐ 1</td>
</tr>
<tr>
<td></td>
<td>Half a week</td>
<td>☐ 2</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>☐ 3</td>
</tr>
<tr>
<td></td>
<td>More than a week</td>
<td>☐ 4</td>
</tr>
<tr>
<td></td>
<td>Don’t know</td>
<td>☐ 5</td>
</tr>
<tr>
<td><strong>Nasal Congestion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q34. At the moment is your breathing through your nose blocked?</td>
<td>Yes ☐ 1</td>
<td>No ☐ 2</td>
</tr>
<tr>
<td>Q35. Are you taking any medication for this?</td>
<td>Yes ☐ 1</td>
<td>No ☐ 2</td>
</tr>
<tr>
<td><strong>Colds Symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q36. Are you currently suffering from a cold?</td>
<td>Yes ☐ 1</td>
<td>No ☐ 2</td>
</tr>
<tr>
<td></td>
<td>No ☐ 2</td>
<td>Don’t know ☐ 3</td>
</tr>
<tr>
<td>Q37. Do you frequently from cold and flu symptoms?</td>
<td>Yes ☐ 1</td>
<td>No ☐ 2</td>
</tr>
<tr>
<td></td>
<td>No ☐ 2</td>
<td>Don’t know ☐ 3</td>
</tr>
<tr>
<td>Q38. Are you taking any medication for this?</td>
<td>Yes ☐ 1</td>
<td>No ☐ 2</td>
</tr>
<tr>
<td>Question</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Breathlessness and Wheezing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q39. Are you ever troubled by shortness of breath when hurrying on level ground or walking up a slight hill?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q40. Do you ever get short of breath walking with other people of your age on level ground?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q41. Do you ever have to stop for breath when walking at your own pace on level ground?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q42. If you run, or climb stairs fast do you ever a. cough? b. wheeze? c. get tight in the chest?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q43. Was your sleep broken this morning a. by wheeze? b. difficulty in breathing?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q44. Do you wake up in the morning a. with wheeze? b. difficulty with breathing?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q45. Do you wheeze from being a. in a smoky room? b. in a very dusty place?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>If Yes to Q43:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q46. Are your symptoms better after leaving the smoky or dusty room?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q47. If you answered yes to any of the questions Q39 to Q45, do you take any medication for it?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cough</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q48. Do you frequently cough during the day or at night?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Q49. Are you taking any medication for this?</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
### If Yes to Q48:

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q50. Do you cough like this on most days for as much as three months each year?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**Phlegm**

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q51. Do you bring up phlegm from your chest first thing in the morning?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

### If Yes to Q51:

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q52. Do you bring up phlegm from your chest like this for as much as three months each year?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*Your assistance is greatly appreciated*
### Appendix E

**Daily Activity Diary**

<table>
<thead>
<tr>
<th>Date:</th>
<th>Location</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Where are you? (e.g., school, home, friend's house)</th>
<th>Can you provide an address of your location?</th>
<th>What activities are you doing?</th>
<th>Location</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As this time are you indoors or outdoors? (Please tick ✓)</td>
<td>Are you near any of the following (Please tick ✓)</td>
<td></td>
<td>Inside</td>
<td>Outside</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cooking</td>
<td>Heating</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Someone smoking</td>
<td>Vehicles driving by</td>
</tr>
</tbody>
</table>

---

Did you wake up with 1) Nasal congestion/Colds 2) Asthma attack 3) Coughs or any illness this morning?  
What time did you go to bed?  
Was the heater or Air-con on last night?  
Was this a typical school day?  
If not, how was it different?  
Extra comments or notes   
---
Dear Potential Participant,

INVESTIGATING THE RELATIONSHIP BETWEEN NICKEL, COBALT EXPOSURE AND OXIDATIVE STRESS MARKERS (MDA AND 8-OHdG) IN URINE SAMPLES OBTAINED FROM A COHORT OF REFINERY WORKERS

You are invited to participate in this research project, which is being conducted as part of the requirements of a PhD Study at Edith Cowan University.

The purpose of this project is to define and quantify the impact of nickel and cobalt exposure on individuals, by investigating their relationship with Malondialdehyde (MDA) and 8-deoxyguanosine (8-OHdG) which are produced by your body as biomarkers of oxidative stress. As both the trace metals in question as well as the oxidative stress markers are excreted from the body via urine, this study aims to utilise urine, volunteered by recruited participants, as a non-invasive method of determining if corresponding levels of oxidative stress are expressed in refinery workers exposed to varying levels of nickel and cobalt metal from the environment.

It aims to benefit the industry and researchers in defining the environmental conditions and trace metal exposure levels that may cumulatively contribute to a significant production of oxidative stress within the body.

If you choose to participate in this project, you will be asked to volunteer a sample of your urine for the analyses of trace metals, nickel and cobalt, and oxidative stress markers Malondialdehyde and 8-deoxyguanosine. This will be done over several sessions of approximately two hours each in duration. The captured images will be included in a database and electronically stored and will be destroyed five years after the completion of the project. There are no physical risks involved.

Any information will only be used for this research project and only the student and supervisor will have access to the information.

Any information or details given for this study will be kept confidential. You will not be identified in any report or presentation of the results of this research project. You will be provided with your individual results of this project should you choose to, verbally by the student at the time of participation.
Participation in this project is voluntary. If you choose to participate, you are free to withdraw from further participation at any time without giving a reason and with no negative consequences. You are also free to ask for any information which identifies you to be withdrawn from the research project.

If you have any questions or require any further information about the research project, please contact Desmond Menon (Chief Investigator) on +61 (08) 6304 3571 or email d.menon@ecu.edu.au or Jacques Oosthuizen (Supervisor) on +61 (08) 6304 5876 or email j.oosthuizen@ecu.edu.au.

If you would like to participate in this project, please complete and return the consent form.

Desmond Menon
PhD Student
School of Medical and Health Sciences
Edith Cowan University

This research project has been approved by the Faculty of Computing, Health and Science Ethics Subcommittee. If you have any concerns or complaints and wish to talk to an independent person, you may contact the Research Ethics Officer on +61 8 6304 2170 or email research.ethics@ecu.edu.au
Appendix G

Consent Form

Informed Consent Document

INVESTIGATING THE RELATIONSHIP BETWEEN NICKEL, COBALT EXPOSURE AND OXIDATIVE STRESS MARKERS (MDA AND 8-OHDG) IN URINE SAMPLES OBTAINED FROM A COHORT OF REFINERY WORKERS

Chief Investigator: Desmond Dev Menon
Supervisor: Prof. Jacques Oosthuizen
(PhD Student) tel. +61 8 6304 3571
tel. +61 8 6304 5544
email d.menon@ecu.edu.au
e-mail j.oosthuizen@ecu.edu.au

Thank you for your interest and assistance with this study.

Before signing this form, please ask any questions on any aspects of the study that are unclear to you.

Statement of consent

1. I have been provided with a copy of the Information Letter which explains the research study.
2. I have read and understood the information provided and have been given the opportunity to ask and have had questions answered to my satisfaction.
3. I am aware that I can contact the researcher with any further questions. I understand that my participation in the research project will involve volunteering my urine sample to be analysed for levels of trace metals, nickel and cobalt, as well as oxidative stress markers, Malondialdehyde (MDA) and 8-deoxyguanosine (8-OHdG).
4. I understand that this information will be kept confidential, and that my identity will not be disclosed without my consent.
5. I am also aware that my individual results can also be made available upon request.
6. If requested, a summary of the results will be provided at the end of the study.
7. I understand that the information provided will only be used for the purposes of this research project, and I understand how this information is to be used.
8. I understand that I am free to withdraw from further participation at any time, without explanation or penalty.
9. I freely agree to participate in this project.
10. In giving my consent I acknowledge that my participation in this study is voluntary, and that I may withdraw at any time and that I may be asked by the investigators to withdraw at any time.

Participant: ____________________________ Date: ______________

Witness: ____________________________ Date: ______________

I do/do not wish to be informed of the results of this study (please circle).

If you would like to be informed of the results, please provide a contact address (below).
Appendix H

Questionnaire

Personal ID: □ □ □

Date: ___ / ___ / ___

Sample Collection Date: ___ / ___ / ___

RELATIONSHIP BETWEEN NICKEL, COBALT EXPOSURE AND OXIDATIVE STRESS MARKERS (MDA AND 8-OHGD) IN URINE SAMPLES OBTAINED FROM A COHORT OF REFINERY WORKERS

Edith Cowan University

Instructions

1. Please complete on behalf of your child or assist your child to complete this questionnaire.
2. Please read each question carefully.
3. Please provide your answer in the box or space provided.

All information provided in this questionnaire will be treated confidentially.

The purpose of this questionnaire is to obtain information about your child and their daily activities that may influence exposure to air pollution.

If you have any concerns or complaints about the research project and wish to talk to a research personnel, you may contact:

Dev Menon
PhD Candidate
School of Medical & Health Sciences
Edith Cowan University
270, Joondalup Drive
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tel +61 8 6304 3571
e-mail d.menon@ecu.edu.au

OR

Associate Professor Jacques Oosthuizen
School of Medical & Health Sciences
Edith Cowan University
270, Joondalup Drive
JOONDALUP
WA 6027
Phone 61 8 6304 5544
e-mail j.oosthuizen@ecu.edu.au
### Personal and family information

| Q1. What is your age in years? | □ □ years |
| Q2. What is your gender? | Male □, Female □ |
| Q3. What is your weight? | □ □ □ kg |
| Q3. What is your occupation? | [ ] |
| Q4. Do you smoke? (Please tick one) | Yes □, No □ |
| Q5. How often do you smoke? (Please tick one) | Daily □1, Twice in the week □2, Three times in the week □3, Four times in the week □4, Five times in the week □5, Six times in the week □6 |

### Information on consumption of food and water

| Q6. What is your daily diet generally like? | Answer: |
| Q7. How often do you eat the vegetables/fruit? | Daily □1, A few days a week □2, A few days a month □3, < a few days a month □4, Don’t know □5 |
| Q8. How many liters of water do you drink in a day? | ½ lites | □1 | 1 liter | □2 |
| 1 and ½ liters | □3 | 2 liters | □4 |
| 2 and ½ liters | □5 | Don’t know | □6 |

| Q9. What is the main activity you spend time doing during your shift? (Please specify) | Answer: |
| Q10. On average how many hours do you spend outside working during a day during your shift? | □ □ hrs per day |

**Nasal Congestion**

| Q11. At the moment is your breathing through your nose blocked? | Yes □1 | No □2 |
| Q12. Are you taking any medication for this? | Yes □1 | No □2 |

**Colds Symptoms**

| Q13. Are you currently suffering from a cold? | Yes | □1 | No | □2 |
| Don’t know | □3 |

| Q14. Do you frequently from cold and flu symptoms? | Yes | □1 | No | □2 |
| Don’t know | □3 |

| Q15. Are you taking any medication for this? | Yes | □1 | No | □2 |

**Breathlessness and Wheezing**

| Q16. Are you ever troubled by shortness of breath when hurrying on level ground or walking up a slight hill? | Yes | □1 | No | □2 |

| Q17. Do you ever get short of breath walking with other people of your age on level ground? | Yes | □1 | No | □2 |
Q18. Do you ever have to stop for breath when walking at your own pace on level ground? Yes □ 1 No □ 2
Q19. If you run, or climb stairs fast do you ever
   a. cough? Yes □ 1 No □ 2
   b. wheeze? Yes □ 1 No □ 2
   c. get tight in the chest? Yes □ 1 No □ 2
Q20. Do you wake up in the morning
   a. with wheeze? Yes □ 1 No □ 2
   b. difficulty with breathing? Yes □ 1 No □ 2
Q21. Do you wheeze from being
   a. in a smoky room? Yes □ 1 No □ 2
   b. in a very dusty place? Yes □ 1 No □ 2

If Yes to Q18–21:
Q22. Are your symptoms better after completing your shift? Yes □ 1 No □ 2

Cough
Q23. Do you frequently cough during the day or at night? Yes □ 1 No □ 2

If Yes to Q23:
Q24. Do you cough like this on most days for as much as three months each year? Yes □ 1 No □ 2

Phlegm
Q25. Do you bring up phlegm from your chest first thing in the morning? Yes □ 1 No □ 2

If Yes to Q25:
Q26. Do you bring up phlegm from your chest like this for as much as three months each year? Yes □ 1 No □ 2

Your assistance is greatly appreciated