Maternal Exposures to Indoor Air Pollutants during Pregnancy and Lung Function in Early Infancy

Mark Swee Ping Tan
BSc, MSc (Infectious Diseases)

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Declaration

This thesis is submitted for a research degree conducted between 2010 and 2013 at the University of Western Australia under the supervision of A/Prof Peter Franklin, Prof Graham Hall and A/Prof Guicheng Zhang.

The work presented in this thesis is original and has not been submitted for a degree at this or any other institution.

Mark Swee Ping Tan

Signed:
Abstract

A large number of studies have shown that exposure to air pollutants during the early stages of lung development can result in poor postnatal respiratory health. While some studies have linked fetal exposure to air and traffic pollutants with poor respiratory health in early postnatal life, there has been very little research into the potential for exposure to indoor air pollutants to influence fetal lung development. The indoor environment may be important in influencing lung development as it contains numerous air pollutants, some that have previously been associated with poor respiratory health outcomes. As events occurring during the fetal period, such as in-utero smoking, have been shown to influence long-term postnatal outcomes, maternal exposure to indoor air pollutants during pregnancy has the potential to affect fetal lung development, subsequently resulting in poor lung function early in, and possibly throughout, postnatal life.

Factors that contribute to the degree to which indoor air pollutant exposure may influence fetal and early life lung development include: the amount of time spent in the indoor environment; the variety of indoor chemicals, including many that have been linked to adverse health outcomes; the potential for increased pollutant exposure due to low ventilation rates and levels; and a large range of emission sources. As a result of these factors, the indoor environment can be a major source of personal exposure for many air pollutants. This thesis examines whether maternal exposure to several domestic air pollutants in the indoor environment can influence early life lung function.
A population of non-smoking pregnant women living in urban and rural areas south of Perth, Western Australia was recruited for a cohort study prior to 18 weeks of pregnancy. Questionnaire data were administered at 18 and 34 weeks, providing information on environmental, housing and behavioural factors. Distances from homes to main roads were obtained using geocoding as a proxy for traffic pollutant levels. Monitoring of indoor air pollutants were conducted in participant homes at 34 weeks gestation. Data on length, weight and head circumference were collected at birth. Lung function tests were conducted on infants between five and seven weeks after birth, subject to inclusion criteria at birth.

There are three results chapters for this thesis (Chapters 3 – 5). Chapter Three explores how environmental and behavioural factors can influence air pollutant levels in the monitored houses. Data obtained from questionnaires, information on geographical conditions and seasonal data was obtained to investigate the factors that could affect indoor pollutant exposure of the study population. 310 homes were monitored. Indoor pollution levels in this population were low for pollutants investigated. Indoor NO₂ levels were significantly higher in winter compared to other seasons, attributable to unflued gas heater usage during the cooler months. No associations were found between presence of unvented gas stoves and indoor NO₂ levels. Levels of formaldehyde and VOCs did not differ based on season, home age or recent renovation. Common household chemical exposure (CHCE) questionnaire scores showed good agreement with indoor VOC levels.

Chapter Four explores the relationships between indoor air pollutant levels and various physical parameters at birth. These included birth length, weight and head circumference. Analyses indicate that increased exposure to indoor formaldehyde and
VOCs during the prenatal period could result in decreased head circumference and increased length at birth respectively. No other associations were found. These results suggested that even at low exposure levels, increased pollutant exposure during the prenatal period may influence fetal growth and development.

Finally, in Chapter Five, the effects of maternal exposure to indoor air pollutants on early life lung function were explored. Formaldehyde exposure during pregnancy was linked to reduced ratio of time to peak tidal expiratory flow to total expiratory time (tPTEF/tE) in univariate analysis, as well as ratio of inspiratory time to total breath time (Ti/Ttot) after adjusting for other pollutants. Domestic NO₂ levels during peak emission periods were inversely associated with functional residual capacity (FRC) levels. CHCE questionnaire scores also displayed a positive association with FRC and lung clearance index (LCI). These results suggest that exposure to indoor pollutants, even at low exposure levels, may result in lung stiffness, airway obstruction and ventilation inhomogeneity.

Overall, this thesis demonstrates that maternal exposure to indoor pollutants has the potential to influence fetal development, including that of the respiratory system. Although threshold levels for health effects remain unknown, these associations may be present even in populations where exposure levels are low.
Acknowledgements

This research was part of the Peel Child Health Study, a cohort study involving investigators from Murdoch University, the University of Western Australia, Curtin University, Edith Cowan University and the Telethon Institute for Child Health Research. Funding for this research study was granted by the Australian Research Council (ARC) and the National Health and Medical Research Council (NHMRC).

Recruitment of study participants were done by a team of research staff. This team included study manager Martinique Sandy, administrative officers Jayne Delves and Julie Rosier, research assistants Natasha Cunningham, Deborah Soanes, Stephanie Sutton, Laura Bailey, Sue Williams and Lisa Tai, as well as additional staff members Des Cox and Nil Chitre.

Geocoding of study participants’ homes by Geographic Information Systems (GIS) was performed by Dr Stephen Ball. Analysis of indoor air monitors were performed by Dean Pemberton, Dennis Zienkiewicz, Andrea Sciubba, and other staff at the Chemistry Centre of Western Australia.

Infant lung function tests were conducted by the candidate and research staff at the Peel community health centre. Training for data collection was conducted by Prof Graham Hall. Equipment and consumables for lung function testing was provided by Prof Graham Hall’s research laboratory.

Assistance with statistical analysis was provided by A/Prof Guicheng Zhang, who carried 10% of the supervisory load and Prof Nazim Khan from the statistics office at
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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>ASCII</td>
<td>American standard code for information interchange</td>
</tr>
<tr>
<td>ATPS</td>
<td>atmospheric temperature and pressure</td>
</tr>
<tr>
<td>ATS</td>
<td>American thoracic society</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BDL</td>
<td>below detectable levels</td>
</tr>
<tr>
<td>BTEX</td>
<td>benzene, toluene, ethylbenzene and xylene</td>
</tr>
<tr>
<td>BTPS</td>
<td>barometric temperature and pressure</td>
</tr>
<tr>
<td>CFA</td>
<td>colour forming agent</td>
</tr>
<tr>
<td>CHCE</td>
<td>composite household chemical exposure</td>
</tr>
<tr>
<td>DNPH</td>
<td>dinitrophenylhydrazone</td>
</tr>
<tr>
<td>ERS</td>
<td>European respiratory society</td>
</tr>
<tr>
<td>FEF_{25-75}</td>
<td>forced expiratory flow</td>
</tr>
<tr>
<td>FeNO</td>
<td>Fractional exhaled nitric oxide</td>
</tr>
<tr>
<td>FEV_{1}</td>
<td>forced expiratory volume in the first second</td>
</tr>
<tr>
<td>FEV_{1}/FVC</td>
<td>ratio of forced expiratory volume in the first second to forced vital capacity</td>
</tr>
<tr>
<td>FRC</td>
<td>functional residual capacity</td>
</tr>
<tr>
<td>FVC</td>
<td>forced vital capacity</td>
</tr>
<tr>
<td>GC/MS</td>
<td>gas chromatography and mass spectroscopy</td>
</tr>
<tr>
<td>GIS</td>
<td>geographic information systems</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>IAP</td>
<td>indoor air pollutant</td>
</tr>
<tr>
<td>IARC</td>
<td>International agency for the research of cancer</td>
</tr>
<tr>
<td>ILF</td>
<td>infant lung function</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LCI</td>
<td>lung clearance index</td>
</tr>
<tr>
<td>MBW</td>
<td>multiple breath wash out</td>
</tr>
<tr>
<td>NEDA</td>
<td>N-1-Naphthyl ethylenediamine dihydrochloride</td>
</tr>
<tr>
<td>NO2</td>
<td>nitrogen dioxide</td>
</tr>
<tr>
<td>PEF</td>
<td>peak expiratory flow rate</td>
</tr>
<tr>
<td>PM</td>
<td>particulate matter</td>
</tr>
<tr>
<td>ppb</td>
<td>parts per billion</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>RR</td>
<td>respiratory rate</td>
</tr>
<tr>
<td>SF6</td>
<td>sulphur hexafluoride</td>
</tr>
<tr>
<td>TB</td>
<td>tidal breathing</td>
</tr>
<tr>
<td>TEA</td>
<td>triethanolamine</td>
</tr>
<tr>
<td>Ti/Ttot</td>
<td>ratio of inspiratory time to total breath time</td>
</tr>
<tr>
<td>tPTEF</td>
<td>time to peak expiratory flow</td>
</tr>
<tr>
<td>tPTEF/tE</td>
<td>ratio of time to peak expiratory flow to total expiratory time</td>
</tr>
<tr>
<td>UHPW</td>
<td>ultra-high pure water</td>
</tr>
<tr>
<td>USFM</td>
<td>ultrasonic flowmeter</td>
</tr>
<tr>
<td>V'E</td>
<td>minute ventilation</td>
</tr>
<tr>
<td>VmaxFRC</td>
<td>maximal flow at functional residual capacity</td>
</tr>
<tr>
<td>VOCs</td>
<td>volatile organic compounds</td>
</tr>
<tr>
<td>VT</td>
<td>tidal volume</td>
</tr>
<tr>
<td>VT/ti</td>
<td>mean tidal inspiratory flow</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
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Chapter 1

A review of

the literature
1.1 Introduction

The negative health impact arising from exposure to air pollution has been well documented, with evidence linking increased pollutant exposures to adverse health outcomes [1-3]. Exposure to air pollutants has been linked to a myriad of poor health outcomes, including poor respiratory health [4-10]. Poor respiratory health outcomes resulting from increased pollutant exposure include increased respiratory symptoms and poorer lung function in both adults [5, 11-13] and children [14-16]. Air pollution has also been associated with the development of respiratory disease such as asthma as well as increases the risk of lung cancer [2, 17-19].

Although research on the impact of air pollution on human health has typically focused on outdoor pollutants, there has been increasing concern about the impact of pollutant exposure from the indoor environment, especially in the developing world [1, 20-23]. Increased exposure to indoor pollutants is also likely to influence those living in the developed world, where over 80% of time is spent indoors [24-32].

Current evidence suggests that exposure to indoor air pollutants may adversely influence both short and long-term respiratory outcomes, particularly for infants and children [20, 32-43]. Exposure to air pollutants is an important issue for children as their lungs and immune system are still developing [35, 39, 40, 44-47].

Lung development commences in-utero and it is possible that maternal exposure to air pollutants during the prenatal period may also adversely influence lung development. There is growing evidence that maternal exposure to ambient air pollution can affect lung function in infancy [48] and early childhood [49-51]. Only one study has investigated the maternal exposure to pollutants in the indoor environment [52]. In that
study, increased fetal exposure to indoor chemicals was associated with increased wheeze and impaired lung function during childhood.

No studies have looked specifically at fetal exposure to indoor air pollutants and its impact on lung development. An understanding of the impact of outdoor and indoor air pollution on lung growth and development will improve our understanding of how exposures during the prenatal period may influence long-term postnatal lung function and respiratory health.

1.2 Early life determinants of long-term lung function and respiratory symptoms

There is growing evidence that lung development in early life can be a key determinant of respiratory health throughout the life course. Low lung function during infancy has been linked to poorer respiratory outcomes such as increased airway hyperresponsiveness, wheezing, cough and asthma during childhood [53-57]. Other studies have also shown that early life lung function can affect respiratory function in later life [53, 58-61]. This was most clearly demonstrated in large scale longitudinal studies which have reported that subjects with low maximal flow at functional residual capacity (VmaxFRC) during infancy had reduced mean forced expiratory volume in the first second (FEV₁) and a measure of forced expiratory flow (FEF₂₅₋₇₅) when followed up throughout childhood into early adulthood [53, 58]. Similarly, Pike et al. [61] reported that low VmaxFRC during early infancy was associated with wheeze in both the second and third years of life.

While current evidence has linked early life lung function to long-term respiratory health, there has been increasing interest in investigating the long-term influences of the
prenatal period, when the early stages of lung development is known to occur [62, 63]. Lung development during the prenatal period is influenced by several key factors, such as genetics, epigenetics, hormonal, nutritional and environmental influences [64-66]. The importance of environmental influences on fetal lung development have been demonstrated in several studies that have linked maternal smoking to increased airway inflammation, poorer lung function and increased risk of recurring wheeze during early childhood [67-81]. Longitudinal evidence has also demonstrated that the effects of fetal tobacco smoke exposure can also extend to adulthood, with outcomes such as poor lung function, increased risk of chronic obstructive pulmonary disorder, and increased asthma risk [82-86].

Apart from maternal smoking, exposure to a wide range of airborne pollutants during pregnancy has also been linked to poor respiratory outcomes in early postnatal life [50, 52, 67, 72-75, 87-89]. In a recent case-control study, asthma risk during early childhood was shown to be increased with elevated levels of nitrogen dioxide (NO2), particulate matter (PM), black carbon and sulphur dioxide (SO2) during the prenatal period [90]. Another longitudinal study found positive associations between the frequencies of household chemical usage and wheeze outcomes during early childhood [91].

At present, there is a paucity of data linking fetal air pollution exposure and lung function in early postnatal life. A small number of studies have suggested that maternal exposure to increased levels of outdoor air pollutant can adversely influence lung function during infancy and childhood [50, 87, 92]. Latzin et al. [87] demonstrated in a cohort study that elevated PM exposures during pregnancy was associated with increased ventilatory requirement (V’E) at six weeks of age. The authors also reported associations between increased prenatal exposure to NO2 and elevated levels of
fractional exhaled nitric oxide (FeNO) – a proposed marker for inflammation [87]. Similarly, Jedrychowski et al. [50] demonstrated in a cohort study that exposure to higher levels of PM during the fetal period resulted in lower FEV\textsubscript{1} and forced vital capacity (FVC) at five years of age.

1.3 Outdoor air pollution

The World Health organization has recognised outdoor pollutants as a contributor to adverse health outcomes [1]. The main contributors to air pollutants are industrial processes and traffic exhaust fumes, particularly in urban areas [93, 94]. There is ongoing concern about the impacts associated with these pollutants, with mounting evidence linking elevated levels of pollutants with adverse health outcomes. In terms of exposure levels, vehicle exhaust is of particular concern due to the increased amount of traffic worldwide [1]. Studies have found that high concentrations of vehicle exhaust pollutants can be found in the proximity of freeways and busy roadways [95, 96].

Adverse health outcomes that have been associated with outdoor air pollution include increased allergies, acute respiratory infection rates, cancer incidence and altered neurobehavioral development [1]. As air pollution primarily affects the respiratory system, a wide body of research has focused on the impact of increased pollutant exposure on respiratory health [5-10]. Evidence suggests that increased exposure to air pollution is associated with both increased respiratory symptoms and impaired lung function [14-16, 97-101]. Furthermore, several studies have demonstrated that increased pollutant exposure may significantly result in impaired respiratory health on children [1, 5, 18, 90, 102-104] and infants [93, 105-108]. In addition, maternal exposures to pollutants have also been shown to influence physical parameters as birth, as well as respiratory function in early life [50, 87, 92, 109-113]. It is currently accepted that
increased exposure to outdoor pollutants can have a negative effect on the respiratory health of children, although the weight of the evidence varies between specific pollutants and outcomes [1].

1.3.1 The impact of air pollution on the respiratory symptoms and disease in children

Compared to adults, children may be more susceptible to the effects of pollutants as their lungs are still developing [1]. Reviews that have explored the link between air pollutant exposure and respiratory outcomes in children suggest that there is some evidence linking prolonged air pollutant exposure to poor respiratory health, although these findings have not been consistent [1, 5, 102, 103]. Outcomes associated with elevated air pollutant exposure in children include increased hospital admissions, an increase in school absences attributed to respiratory illness, increased prevalence of pneumonia and bronchitis, asthma incidence, prevalence and exacerbation, as well as increased lung inflammation [14, 97-99, 114, 115]. These associations have also been found with young children and infants [93, 98, 104, 107, 114, 116].

Several studies have examined the effect of air pollution on hospitalization and school absenteeism at a population level. Positive associations have been reported between respiratory-related hospitalizations in children and varying levels of pollutant exposure [14, 97-99, 114, 117-135]. A number of studies have also linked outdoor air pollution levels with school absenteeism, some of which may be attributable to respiratory illnesses [14, 99, 119, 129]. Cumulatively, the weight of the evidence suggests that acute exposure to high levels of pollutants could result in respiratory outcomes which may be sufficiently severe to result in hospitalisation.
A large body of literature has linked changes in air pollution with respiratory symptoms in children [18, 90, 104, 136-141] and infant [93, 105-108] populations. Recently, Hoek et al. [140] demonstrated in a large-scale cohort study from several European cities that increased exposure to PM in children was linked to poorer respiratory outcomes such as increased phlegm production, prevalence of hay fever, bronchitis and coughing. Ward and Ayres [142] performed a meta-analysis of available studies and showed that PM exposure during childhood was linked to increased coughing episodes and lower respiratory infections. While some studies have reported that increased pollutant exposure were associated with poorer respiratory outcomes in children, other studies did not [143-149]. These were likely to be due to a combination of various factors such as inappropriate study design [145], insufficient statistical power [143], low exposure levels [143], or overshadowing effects by other pollutants [144-146, 148].

A number of cohort studies have also presented evidence of associations between increased exposures to outdoor pollutants and asthma incidence in school-aged and young children [90, 95, 116, 138, 139, 150, 151]. McConnell et al. [116] reported in a cohort of kindergarten-aged children that increased exposure to NO₂ was associated with new-onset asthma. This was in agreement with a retrospective cohort study by Clark et al. [90], who reported that exposure levels to NO₂, nitrogen oxide, carbon monoxide (CO) and PM during the first year of life were independently associated with asthma development during childhood. Carlsten et al. [151] also showed that traffic exposure during infancy was linked to new onset asthma at the age of seven.

Taken together, the evidence from longitudinal cohort studies of a similar design suggests a positive relationship between early life exposure to traffic air pollutants and asthma incidence. Recent reviews have suggested that increased exposure to air
pollution is likely to influence respiratory symptoms and asthma incidence [17, 19, 152]. Data from epidemiological studies on asthma prevalence, however remains less convincing, possibly due to asthmatics avoiding areas with high levels of air pollution [17].

1.3.2 The impact of air pollution on impaired lung function and lung function growth in children

The association between outdoor air pollutants and both acute and chronic impairment of lung function during childhood has also been extensively studied [15, 16, 76, 100, 101, 116, 153-159].

Several studies have shown that short term pollutant exposure may result in acute lung function decrements in children [11, 155, 157, 158, 160, 161]. In a series of summer camp studies, Kinney et al. [155] demonstrated that increased daily exposure to ozone (O₃) was correlated with decreased FEV₁ in children aged 8 to 15. Similarly, Correia-Deur et al. [160] also reported that 24-hour average exposure to pollutants such as PM, NO₂ and O₃ were linked to decrements in peak expiratory flow (PEF) in children. Other studies, however, have not reported similar findings [143, 162]. In a large scale European multi-centre study conducted by Roemer et al. [143], the authors reported that daily variations of several pollutants did not influence PEF levels in asthmatic children. Despite the various strengths of that study, including the high statistical power, it was possible that differences in data collection between centres may have been a factor influencing the results.

Current evidence suggests that lung function in childhood has been associated with exposure to air pollution throughout life  [5, 15, 16, 45, 100, 101, 103, 136, 144, 159,
In a series of studies in children, Barraza-Villareal et al. [16, 100] found significant inverse relationships between exposure to airborne pollutants and lung function indices of FEV₁, FVC and FEF₂₅-₇₅, although the strength of the association varied based on asthma and atopic status. Similarly, another recent cohort study in children reported strong associations between NO₂ exposure and FEV₁, FVC, FEF₂₅-₇₅ and PEF [144]. A few studies, however, failed to find significant associations between pollutant exposure and lung function measures in children [140, 167, 169]. The lack of associations found in these studies was likely to be due to low pollutant levels found in their study regions.

In addition to being linked with poor respiratory function, there is strong evidence that long-term exposures to air pollutants can potentially affect lung function development in children and adolescents [99, 116, 153, 154, 166, 170-177]. A Polish study conducted in two areas of differing pollution levels indicated that children who live in an area with high PM and SO₂ pollutant levels are more likely have reduced lung function growth between the ages of eight and ten compared to those living in a low pollutant area [171]. Children who lived in more polluted urban areas between the ages of eight and ten were twice as likely to be in the lowest quintile of lung growth for FEV₁ (OR 2.15, 95% CI 1.25-3.69) and FVC (OR 1.90, 95% CI 1.12-3.25) compared to those living in a less polluted area. Other studies which have measured lung function growth over this period have also reported similarly consistent findings [154, 172, 173, 175-177].

Taken together, these studies suggest that long-term exposure to environmental pollutants has the potential to influence lung function development from childhood into adulthood, which is a period representing lung function growth.
1.3.3 The impact of in-utero air pollution exposure on birth outcomes and early life lung function

The prenatal period, representing a period when fetal development occurs, can have an impact on birth outcomes such as birth length, weight, and head circumference. It may also affect more specific outcomes such as the development of the respiratory system. Several studies have demonstrated that increased exposure to pollutants during the prenatal period is linked to reduced length, weight and head circumference at birth [109-113]. These findings are relevant, as studies have reported significant associations between birth parameters and poorer lung function in early life [178-181], with its effects shown to track through to later life, as demonstrated in several longitudinal studies [182-188] and discussed in the sections above.

To date, the relationship between maternal exposure to pollutants and lung function outcomes in early life has not been extensively explored. The few studies that have been published in this area suggest that associations exist between pollutant exposure and fetal lung development, as reflected by poorer lung function in early life [50, 51, 87, 92]. A recent study birth cohort study reported that maternal exposure to certain pollutants throughout pregnancy may influence several measures of lung function in the first six weeks of life [87]. In that study, exposure to increased environmental PM throughout pregnancy was shown to be positively associated with increased minute ventilation in early life. Similarly, higher NO₂ exposure during the fetal period was linked to increased levels of FeNO – a marker for lung inflammation at six weeks of age. Pollutant exposures during the third trimester of pregnancy had stronger associations with early life decrements in lung function compared to exposure levels during early pregnancy [87].
Prenatal exposure to environmental pollutants may also influence lung function in early childhood. A cohort study in Poland demonstrated that pregnant women exposed to the highest quintile of PM throughout pregnancy had children with significantly lower FEV$_1$ and FEF$_{25-75}$ at 7.5 years of age compared to those in the lowest quintile [50]. Another longitudinal study by Mortimer et al. [92] also reported that prenatal exposure to CO was also linked to reductions in FEF$_{25-75}$ during childhood. While current evidence suggests that increased pollutant exposure during the prenatal period has the potential to adversely influence lung development in early life, the research to date remains insufficient to draw reliable conclusions.

Although there is a wide body of evidence linking outdoor air pollution to respiratory health outcomes, there are valid reasons to investigate the effects of indoor air pollution, which has been recognized to potentially represent a higher proportion of an individual’s overall pollutant exposure [20, 30]. Furthermore, recent evidence has shown that over the past few decades, the number of pollutant sources in the indoor environment has been steadily increasing, and is thought to be primarily driven by increasing technological advancement [26, 27, 29]. These observations, taken together with the finding that people in developed countries spend a high proportion of their time indoors [24, 189-191], suggests that the indoor environment may contribute to the overall influence of air pollution on respiratory health.

### 1.4 Indoor air pollution

Indoor air pollution has been described as a complex mixture of pollutants migrating indoors from ambient air and pollutants generated inside the home [192]. Research on the impact of indoor air pollution has gained interest over the past few decades with current evidence linking increased exposure to poorer health outcomes. Adverse health
effects commonly associated with short-term indoor pollutant exposure include sensory irritation, sick building syndrome, and asthma [192-194]. In the long term, exposure to increased concentrations of certain pollutants may also put individuals at risk of developing more severe outcomes such as cancer and cardiovascular-related diseases [195-198].

The World Health Organisation [1, 94, 199] has recognized the global impact of indoor air pollution, with estimates that in 2004 alone, indoor air pollution contributed to 2.7% of total deaths worldwide. Most of these, however, can be attributed to the use of biomass fuels in the developing world [8, 200-204]. However, in the developed world indoor air pollution is also of concern, albeit generally with less severe consequences, as indoor air is the major source of personal exposure to many pollutants [20, 30, 200, 205, 206]. This can be explained in part by several main factors, including the amount of time spent indoors, number of pollutant sources, overall quantity of pollutants and decreased ventilation rates.

1.4.1 Time spent in the indoor environment and personal exposure

Since the 1980s, time-activity studies have shown that people in the developed world are spending an increasing amount of time in the indoor environment [24, 189]. Furthermore, a large proportion of time spent in the indoor environment occurs in the domestic setting [24, 25, 191]. This trend has also been shown to apply across several categories such as age and socioeconomic status [32, 190, 192]. Over the past decade, an Australian study conducted in ten urban cities showed that on average, people across all age groups typically spend less than five hours of their day outdoors [191].
As the current study examines in-utero exposures, the time-activity patterns of pregnant women are of primary interest. Studies that have specifically investigated the time-activity patterns of women during pregnancy have concluded that pregnant women in the developed world spend a significant amount of time in the indoor environment, especially at home [189-191]. Nethery et al. [190] reported that pregnant women spent an increasing amount of time in the home environment as pregnancy progressed, with an average of 14.4 hours per day for the first trimester and 16.9 hours per day in the final trimester [190].

Personal exposures to several pollutants have been shown to be more closely related to indoor levels compared to outdoor levels [207-213]. Pooled data from the European Union has also shown that indoor inhalation exposure was a key contributor to overall exposure to several pollutants such as benzene, formaldehyde and acetaldehyde [206, 214]. Due to the increased proportion of time spent in the indoor environment [189, 190, 215], it is possible that pregnant women and young children also have high levels of personal pollutant exposures in the indoor environment [212, 214, 216-218].

1.4.2 Pollutant sources in the indoor environment

With increases in technological advancement, the number of pollutant sources within the indoor environment is known to be increasing [26, 27, 29, 30]. Indoor environments are known to contain more pollutant sources than outdoor environments [219-221]. Sources of indoor pollutants are known to be numerous and include building materials, cooking and heating appliances, as well as numerous liquid and aerosol products such as cleaning agents, air fresheners and perfumes [27, 29, 32, 221].
1.4.3 Decreased ventilation

Decreased ventilation is an important factor that may influence indoor pollution levels [31, 32]. While some homes contain reverse cycle air conditioners, many modern homes are often ‘sealed up’ and thermally insulated in order to conserve energy, leading to low ventilation rates [222]. Subsequently, a build-up of pollutants over time within a confined space might occur [223]. Sharpe et al. [30] estimates that the release of a specific quantity of pollutants in the indoor environment may result in up to 1000-fold increase in exposure for some pollutants compared to a similar exposure in the outdoor environment. Other studies have also reported an inverse relationship between domestic levels of indoor pollutants and ventilation rate [223, 224]. A comprehensive investigation in several urban areas by the California Air Resources Board [32] concluded that although emission levels of air pollutants are generally higher in the outdoor environment, overall exposure levels to indoor pollutants remained as high due to its slow dilution rate.

1.4.4 Pollutant concentrations in the indoor environment

For some pollutants concentrations are higher in the indoor environment compared to the outdoor environment [39, 208, 225-228]. Several studies have shown that indoor levels of certain pollutants, such as formaldehyde and certain classes of volatile organic compounds (VOCs) can be up to 12 times as concentrated in the indoor environments [211, 229, 230]. Combined with other factors, this is likely to result in increased exposure to indoor pollutants.

1.4.5 Common indoor air pollutants

In the developed world, indoor air pollutants can be derived from a variety of sources such as outdoor air, ventilation systems, construction and renovation, building
materials, outside water, heating sources, household chemicals, vacuum cleaners, candles and indoor smoking [30, 32, 220]. A wide range of pollutants have been measured in indoor air, including formaldehyde, nitrogen dioxide (NO₂) and volatile organic compounds (VOCs), carbon dioxide (CO₂), CO, hydrogen chloride (HCl), nitrous acid (NHO₂), nitric acid vapour (HNO₃), chlorinated solvents, chlorinated pesticides, and phthalate esters [21, 26, 205]. While indoor air pollution is known to comprise of a wide variety of individual pollutants which have been linked to adverse health effects, specific pollutants found ubiquitously in the domestic environment include formaldehyde, nitrogen dioxide (NO₂) and volatile organic compounds (VOCs).

1.4.5.1 Formaldehyde

Formaldehyde is a colourless gas that is flammable and highly reactive at room temperature [199]. Pure formaldehyde has a molecular mass of 30.03 g/mol, relative vapour density of between 1.03 and 1.07, melting point of -92°C and boiling point of -19.1°C. Although naturally found as a gas, formaldehyde is also soluble in water (around 400 g/l at 20°C).

Formaldehyde is emitted as a primary pollutant from environmental sources [195]. In the industrial setting, formaldehyde is used in a variety of processes such as manufacturing of urea-formaldehyde resins, rubber, plastics, plywood, fabric coatings and adhesives [195, 231, 232]. It is also produced secondarily in the indoor environment as a by-product of oxidation processes by VOCs [199, 233].

In the domestic setting, formaldehyde is emitted by building materials, carpets, resins, paints, wallpapers, glues, adhesive, varnishes, lacquer, as well as various household products such as pesticides, detergents, disinfectants, softeners, carpet cleaners, soaps
and nail varnish [195, 199, 205]. Major sources of formaldehyde in households include emissions from particle and fibre boards, as well as pressed or glued wood products [195, 199]. Small amounts of formaldehyde are also known to be emitted by a wide variety of electronic equipment including computers and photocopiers [195, 199].

Although there are no standard guidelines for domestic formaldehyde exposure, the World Health Organisation [199] recommends exposure of no more than 0.1 mg/m$^3$ (80 ppb) over a 30 minute period in the indoor setting for the prevention of sensory irritation. Levels of formaldehyde in households of developed countries have been found to be consistently lower than recommended levels, with mean levels such as 21.4 ppb (range 7.6 to 40.3 ppb) in Finland [211], 17.2 ppb (SD 4.49) in the United States [226], 23.6 ppb (range 7.7 to 72 ppb) and Canada [234]. An Australian study has reported a mean level of 22.8 ppb (range 3.0 to 92.3) [235], with data showing steady decreases in recent years [228, 236].

1.4.5.1.1 Health impacts of formaldehyde

Formaldehyde is widely accepted to be an irritant of the eyes, nose, throat and upper respiratory tract [20, 237-241]. Several reviews have shown that 1 ppm is the most commonly reported threshold level for irritant effects, although some studies have reported sensory irritation at concentrations as low as 100 ppb [199, 237]. One study also reported a dose-response relationship for known irritant symptoms [238]. Early studies have also shown that increased formaldehyde exposure is associated with other non-specific symptoms such as fatigue, headache, nausea, chest tightness, and shortness of breath [224, 240, 241]. Chamber studies have also demonstrated a dose-response relationship between short-term formaldehyde exposure and irritant effects [231].
Furthermore, a recent study showed that increased formaldehyde exposure at work was linked to increased symptoms characteristic of sick building syndrome [242].

While short-term exposure to high levels of formaldehyde is known to primarily cause irritant effects, chronic formaldehyde exposure can result in more severe outcomes. This was shown to be more apparent with occupational exposure, which has been linked to an increased risk of nasopharyngeal, leukaemia, sinonasal and other cancers [226, 243-247]. The evidence, however, is inconsistent, with other reviews commenting on the lack of strength of available evidence linking formaldehyde exposure with various cancers [248-251]. Nevertheless, various lines of evidence from animal and human studies have resulted in the International Agency for Research of Cancer (IARC) officially recognising formaldehyde as a probable human carcinogen, within the group 1 carcinogens [195].

As formaldehyde is primarily an irritant of the airway and respiratory system, much research on its health effects have focused on its effects on respiratory health. A large number of studies have examined the effects of increased formaldehyde exposure on airway and lung tissue inflammation, respiratory symptoms, asthma-related outcomes, and lung function. These are discussed below.

**1.4.5.1.2 Formaldehyde exposure and inflammation**

Irritant effects arising from increased exposure to formaldehyde may be accompanied by inflammation in the respiratory tract. The key mechanism underlying this process occurs via the mediation of oxidant and antioxidant enzyme levels in response to formaldehyde antagonism [252]. In-vitro studies have also linked increased exposure to formaldehyde with elevated levels of inflammatory markers such as IL-8 [253], MCP-1
[254] and IL-11 [255] in human lung tissue. Animal studies have supported in-vitro findings, with increased formaldehyde antagonism being linked to increases in various inflammatory markers [256-261]. In human adults, several studies have also linked exposure to higher levels of formaldehyde to higher eosinophil levels [262-265].

While the inflammatory effects of increased formaldehyde exposure have been demonstrated in in-vitro, animal, and human adult studies, this relationship remains relatively unexplored in children. Existing evidence, however, indicates that even at low levels, formaldehyde exposure may influence inflammation in children. Two studies have independently demonstrated that low level formaldehyde exposure was positively associated with levels of FeNO – a proposed marker for inflammation [266, 267]. Franklin et al. [266] showed using a 50 ppb cut-off value, that children living in homes with low formaldehyde levels (8.7 ppb, 95% CI 7.9 – 9.6 ppb) had significantly lower FeNO levels than those living in homes with higher formaldehyde levels (15.5 ppb, 95% CI 10.5 – 22.9 ppb). Kovesi and Dales [268] showed that the use of formaldehyde-emitting electric baseboards in homes was also linked to increased FeNO levels in children.

1.4.5.1.3 Formaldehyde exposure and respiratory symptoms

There is some evidence linking formaldehyde exposure to respiratory symptoms, with most studies finding that increased levels of formaldehyde may increase symptoms such as prevalence of wheeze and nocturnal respiratory symptoms in children [33-38, 147, 149]. Venn et al. [33] showed that children differentially exposed to formaldehyde had a different wheeze prevalence, with a positive relationship observed with increasing quartiles (OR 1.45, 95% CI 1.06 – 1.98 per quartile). Similarly recent time-series study by Raaschou-Nielsen et al. [38] reported that infants with high formaldehyde exposure
had more days with wheeze symptoms compared to those with low exposure levels. However, while such associations may exist, a number of these studies acknowledged that the potential for a causal relationship remain unclear [38] and further evidence is required to understand the associations better [36].

1.4.5.1.4 Formaldehyde exposure and asthma prevalence

The influence of formaldehyde exposure on asthma prevalence is unclear, although current evidence suggests that effects could be found at sufficiently high exposure levels. Most of the evidence in this area have shown that occupational exposure to high formaldehyde levels could be associated with increased asthma incidence and prevalence, although even the role of formaldehyde as an occupational asthma allergen remains controversial [239, 269-273]. Subjects of these studies, however, tend to be occupationally exposed to formaldehyde levels that are often higher than indoor home exposures, primarily due to direct emission source exposure.

Research on formaldehyde exposure and asthma prevalence in the domestic setting, where exposure levels are low, also remain equivocal, with several studies showing that exposure to increased formaldehyde was associated with asthma prevalence [35, 274-276], and others not finding an association [36, 277]. A case control study in Perth conducted by Rumchev et al. [274] found that young children admitted to a hospital with asthma as a primary diagnosis had, on average, higher formaldehyde levels at home when sampled across two seasons. Similarly, Hulin et al. [275] also reported significant positive associations between formaldehyde levels and asthma prevalence in children living in rural areas, where levels were generally low.
McGwin et al. [278] recently conducted a meta-analysis of available data, with the authors reporting that each increasing interval of 10 µg/m³ of formaldehyde was linked to modest increases in asthma prevalence in both a fixed model (OR 1.03, 95% CI 1.02 - 1.04) and a random effects model (OR 1.17, 95% CI 1.01 – 1.36). A series of reviews by Wolkoff et al. [279, 280] have suggested that a 100 µg/m³ threshold be used as the point at which formaldehyde exposure may have an effect on asthma incidence in children.

1.4.5.1.5 Formaldehyde exposure and lung function

While existing evidence suggests that increased formaldehyde exposure may influence asthma outcomes and respiratory symptoms, its effect on lung function outcomes have been less clear, with various experimental [281-283] and epidemiological [35, 44, 263, 284, 285] studies reporting conflicting findings. Experimental chamber studies generally showed that 90 minute exposures to low (1 ppm or less) formaldehyde levels did not result in impaired lung function in adults [281-283]. The evidence arising from epidemiological studies of various designs, however, has been inconsistent with some studies finding associations between formaldehyde exposure levels and long-term lung function outcomes such as FEV₁, FVC and PEF [35, 44, 266] while others did not [263, 284, 285]. As the evidence remains equivocal, there is a need for further studies to investigate the relationship between formaldehyde exposure, particularly during the prenatal period and lung function outcomes. Furthermore, there is also insufficient longitudinal data in this area of research.

1.4.5.1.6 In-utero and early life formaldehyde exposure

To date, there are no known studies investigating the role of formaldehyde exposure during the fetal period on lung function development. One study, however, has linked
low levels of formaldehyde to reduced birth weight [286], although the potential mechanisms of this association was not discussed. However, as birth size may influence postnatal lung function [183, 184, 187], it is possible that maternal exposures to formaldehyde during pregnancy may influence lung function outcomes in early infancy.

1.4.5.2 Nitrogen dioxide (NO₂)

Nitrogen dioxide (NO₂) exists naturally as a colourless gas in air, although at high concentrations, it presents as a reddish brown gas with a pungent odour. It is formed naturally by the oxidation of nitric oxide in air. NO₂ has a molecular weight of 46.05 g/mol⁻¹, relative vapour density of 1, melting point of -11.2°C and boiling point of 21.1°C. As a strong oxidant, it is also known to be corrosive. While NO₂ is known as an indoor air pollutant, it is also an outdoor pollutant which is produced by combustion processes in motor vehicles [1, 94, 199, 287].

In the domestic environment, the biggest sources of NO₂ are fuel-burning stoves such as natural gas and propane used for cooking purposes, and unflued gas heaters used as a source of heating [199, 228]. Although NO₂ is primarily formed by oxidation of NO by other oxides, this swift oxidation process usually results in NO₂ being considered a primary pollutant [199].

In the developed world, background domestic NO₂ levels are generally low. Numerous studies have reported levels of NO₂ that fall within the World Health Organisation’s [199] air quality guideline level of 200 µg/ m³ hourly, or an annual average of 40 µg/ m³. Mean average levels that have been reported include 8.3 (range 3.3 to 29.1) µg/ m³ in Canada [234], 19.7 (SD 11.8) µg/ m³ in Spain [288], and 28 (SD 12.6) µg/ m³ in the United States [227]. However, peak exposures during the operation of unflued gas
appliances can far exceed outdoor standards [212, 236, 289, 290] and can reach levels up to one part per million as shown in a recent Australian study [291].

1.4.5.2.1 Health effects arising from increased NO$_2$ exposure

Increased exposure to NO$_2$ in the short term is known to cause irritant effects, primarily affecting the eyes, nose and throat mucosa, and respiratory tract [200]. At higher levels, increased exposure to NO$_2$ has been implicated with a wide range of disorders, most of which affect the respiratory system [94, 199]. These include pulmonary oedema and bronchiolitis fibrosis, which may ultimately result in death with long-term exposures [292].

Many studies have investigated the relationship between increased NO$_2$ exposure levels and respiratory effects such as inflammation, respiratory symptoms, asthma-related outcomes, and lung function. The respiratory effects arising from increased NO$_2$ exposure is discussed below in further detail.

1.4.5.2.2 NO$_2$ exposure and inflammation

NO$_2$ is an oxidant gas and increased NO$_2$ may have the potential to trigger an inflammatory response in the airways. Although its physiological mechanisms have not been well elucidated, in-vitro studies have shown that it is likely to occur via increased oxidant damage or signalling pathways in epithelial cells [293-301]. This has also been supported by animal studies, which have shown that direct exposure to elevated levels of NO$_2$ was associated with increases in levels of inflammatory markers [298, 302-307].

Human chamber studies have demonstrated that increased exposure to NO$_2$ may result in higher levels of inflammatory markers such as neutrophils, eosinophil cationic
protein, IL-5, IL-6, IL-8, IL-10, IL-13, alpha 1-antitrypsin and ICAM-1 [10, 295, 308-312], with the effect being especially apparent in asthmatic subjects [294, 313]. Concentrations sufficient to elicit an inflammatory response in these chamber studies have been reported in homes during the active operation of unvented gas stoves [234, 290, 314, 315] and unflued gas heaters [236, 289, 290, 316-318].

Over the past decade, several investigators have examined the potential for NO2 to influence lung inflammation in children. Current understanding, however, remains inconclusive, with some studies reporting significant associations [11, 47, 267, 319, 320] and others failing to do so [100, 321]. Steerenberg et al. [11] showed that children living in an urban environment with higher outdoor NO2 exposure had significantly higher IL-8 levels compared to those living in low exposure environments. A recent longitudinal study by Sarnat et al. [319] also reported small but consistent associations between NO2 exposure and FeNO levels in children. Conversely, Berhane et al. [321] was unable to find any association between NO2 exposure and FeNO levels in school-aged children. However, this could have been due to the cross-sectional nature of the analysis, which may preclude the independent effects of short-term fluctuations of air pollution on FeNO [321].

Despite the findings discussed above, the potential for NO2 exposure commonly encountered in outdoor environments to influence inflammation remains unclear. A recent review has shown evidence that FeNO is associated with eosinophilic inflammation [322]. Existing studies however, have shown that inflammation arising from increased NO2 exposure is known to be neutrophilic in nature [295, 298, 307, 311]. Therefore, it is unclear whether FeNO is the most suitable indicator for measuring
inflammatory responses arising from increased NO$_2$ exposure. Furthermore, the relationship between NO$_2$ exposure and inflammation could also be influenced by dose-response dynamics which remain unclear at present.

1.4.5.2.3 Indoor NO$_2$ exposure and respiratory symptoms

There is substantial evidence surrounding the influence NO$_2$ on respiratory symptoms in children. Numerous studies have found that increased NO$_2$ exposure was associated with an increase in respiratory symptoms such as difficulty breathing, cough, wheeze, chest tightness, and nocturnal symptoms [39-41, 47, 323, 324]. A large-scale cohort study in the late 1980s showed that every 15 ppb increase in annual household NO$_2$ levels was associated with an increased cumulative incidence of one or more lower respiratory symptoms such as shortness of breath, wheeze, cough, phlegm, or bronchitis (OR 1.4, 95 % CI 1.1 – 1.7) [323]. A recent analysis of a community trial in asthmatics reported that higher indoor NO$_2$ levels were associated with modest increases of lower (OR 1.14, 95% CI 1.12 – 1.16) and upper respiratory tract symptoms (OR 1.03, 95% CI 1.00 – 1.05), more frequent cough and wheeze, and more frequent reliever use during the day [39].

The use of NO$_2$ emission sources such as gas stoves and unflued gas heaters have also been linked to poor respiratory outcomes in children. Studies conducted in schools and homes have shown that increased usage of unflued NO$_2$ emission sources were linked to more frequent respiratory symptoms such as increased cough, difficulty breathing, chest tightness and wheezing [141, 317, 325-329]. In a double-blind cross-over study in 22 Australian schools, the presence of an unflued gas heater was associated with increased cough in the evening (OR 1.16, 95% CI 1.01 - 1.34) and wheeze in the morning (OR 1.38, 95% CI 1.04 – 1.83), with the association stronger in atopic children [325].
Similar results were observed in a home-based intervention study conducted in New Zealand [327], where children living in homes without unflued gas heaters had reported less wheeze-related sleep disruptions (OR 0.55, 95% CI 0.35 – 0.85), less dry nocturnal dry cough (OR 0.52, 95% CI 0.32 – 0.83), and reduced lower respiratory tract symptoms (OR 0.77, 95% CI 0.73 – 0.81) than those in homes with unflued gas heaters.

Despite mounting evidence suggesting that NO₂ exposure can influence respiratory health in children, other studies have not been able to find significant associations between increased NO₂ exposure and more frequent or severe respiratory symptoms [330-333]. A study by Hosein et al. [330] found that children living in households with unflued gas stoves had similar prevalence of cough, phlegm, wheeze and dyspnoea as those in households utilizing electrical cookers. Similarly, the authors found no differences in respiratory symptoms between homes with different forms of heating. More recently, a Dutch cohort study also reported a lack of association between home gas use and nasal symptoms or wheeze outcomes [331]. In light of the current evidence, recent reviews have suggested that while increased outdoor NO₂ exposure might be linked to increased respiratory symptoms in children, the weight of the evidence is insufficient to infer a direct causal relationship [334, 335].

1.4.5.2.4 NO₂ exposure and asthma-related outcomes

As NO₂ is known to be both an indoor and outdoor air pollutant, numerous studies have explored its potential to influence various outcomes related to asthma in children. However, the evidence of the impact of indoor NO₂ exposure on asthma incidence, prevalence and asthma-like symptoms remains inconclusive.
Several studies have provided evidence linking elevated indoor NO₂ levels to more frequent hospital visits from asthma attacks and increased episodes of asthma-related symptoms [317, 324, 327, 329, 332, 336]. Mi et al. [332] demonstrated a relationship between classroom NO₂ levels with current asthma (OR 1.51 for every 10 µg/m³ interval, p < 0.01) and use of asthma medication (OR 1.45 for every 10 µg/m³ interval, p < 0.01) in children. Similarly, Pilotto et al. [317] showed that replacement of unflued gas heaters with non-NO₂ emitting sources led to the reduction of asthma-like symptoms such as reduced difficulty breathing during the day (OR 0.41, 95% CI 0.07 – 0.98) and night (OR 0.32, 95% CI 0.14 – 0.69) and chest tightness during the day (OR 0.45, 95% CI 0.25 – 0.81).

Other studies, however, have not been able to replicate similar findings, reporting a lack of association between NO₂ levels and outcomes such as asthma incidence [325, 331] and prevalence [139, 270] during childhood. Diette et al. [337] found similar levels of long-term indoor NO₂ exposure in asthmatic as well as non-asthmatic children living in similar geographical areas. Willers et al. [331] also found similar incidences of asthma in young children with different exposures to gas cooking in the household. Consistent with such findings, several reviews have concluded that while there is evidence that increased NO₂ exposures can exacerbate asthma, its influence on asthma-related outcomes remains inconclusive [194, 335, 338, 339].

1.4.5.2.5 NO₂ exposure and lung function

The potential impacts of NO₂ exposure on lung function in children has been explored in several studies, which some reporting an inverse relationship [39, 40, 45-47] and others not finding any significant associations [323, 340]. The significance of indoor exposures, in particular, were stronger, with a recent study by Gillespie-Bennett et al.
reporting that decreases in morning and evening FEV₁ was linked to indoor, but not outdoor NO₂ levels. Nitschke et al. [40] also reported that daily NO₂ levels in domestic kitchens were linked to reductions in FEV₁ in school-aged children. A study in six cities from the United States, however, did not find any relationship between NO₂ levels and various measures of lung function such as FEV₁, FVC and FEF₂₅₋₇₅ [323]. Similar results were reported in an early study by Florey et al. [340], who also found no correlations between bedroom or kitchen NO₂ levels and lung function measurements such as forced expiratory volume during the first three-quarters of a second (FEV₀.₇₅), PEF or FEF₂₅₋₇₅. Although the relationship remains unclear, the studies which have found no associations tend to be older, and may have reflected differences in time points.

The relationship between NO₂ exposures by surrogate and lung function in children have similarly resulted in similarly inconclusive findings. Several studies have reported that increased usage of wood or gas heating, gas water systems and gas cooking led to significantly reduced FEV₁ and FVC and ratio of FEV₁ to FVC (FEV₁/FVC) in children and teenagers [314, 330, 341-343]. A study by Hosein et al. [330] found that usage of hot water systems and gas cookers strongly influenced the FEV₁ of children, with the effect especially pronounced in boys. Similarly, Jedrychowski et al. [342] demonstrated in a cohort study that pre-adolescents living in homes with gas heaters had significantly lower FEV₁ and FVC compared to those living in homes without gas heaters. A number of studies, however, did not find significant associations between usage of gas heating and lung function in children [317, 325, 327]. Out of these, two studies measured interventions in classrooms [311, 319] while the third [321] was a randomised control trial in asthmatic children. It was possible that a lack of statistical power accounted for a lack of significant findings in the randomised control trials [327]. Overall, it is likely
that increased exposure to NO\textsubscript{2} emitting sources in homes could result in reduced lung function in children, although the extent of reduction is likely to be small [314].

1.4.5.2.6 Effects of prenatal and early life NO\textsubscript{2} exposure on early life respiratory health

Although the role of NO\textsubscript{2} in influencing respiratory health has been extensively studied in children, few studies have examined its impact on fetal and early life exposures. Studies which have examined the effects of prenatal and early life exposure to indoor and outdoor NO\textsubscript{2} on respiratory health, however, have reported inconsistent findings.

Studies relating NO\textsubscript{2} exposure to respiratory-related hospitalisations seem to display strong positive associations [98, 114, 122]. In a time-series analysis, Dales et al. [98] reported slightly increased odds of neonatal respiratory hospitalization with each interquartile range of NO\textsubscript{2} exposure (OR 2.85, 95% CI 1.68 – 4.02). This was consistent with another study which showed small but significant increases in asthma-related emergency visits with every 10 µg/m\textsuperscript{3} increase in NO\textsubscript{2} pollutant exposure [122]. Karr et al. [115], however, failed to link acute NO\textsubscript{2} exposures to hospitalization due to bronchiolitis in the first year of life. This could, however, be the effect of the case-crossover design used by the authors, which would be useful only for short-term acute exposures [115].

Increases in NO\textsubscript{2} have also been linked to non-hospital-related respiratory symptoms [108, 323, 344]. A cohort study reported that infants living in homes with the highest quartile of NO\textsubscript{2} concentrations had an increased frequency of days with wheeze (OR 2.2, 95% CI 1.4 – 3.4), persistent cough (OR 1.8, 95% CI 1.2 – 2.7), and shortness of breath (OR 3.1, 95% CI 1.8 – 5.6) compared to infants with the lowest quartile of exposure in the first year of life [108].
Several studies have reported the potential for increased indoor NO2 to influence respiratory symptoms during infancy by observing that increased use of gas stoves in homes were associated with increased respiratory symptoms such as cough and wheeze [46, 345-347]. Although the amount of research in this area is limited, other studies which have focused on outdoor NO2 levels have also linked outdoor exposure to increased respiratory symptoms during early life [93, 104-106]. On the contrary, however, a few cohort studies did not find any relationship between NO2 levels and respiratory symptoms in children and infants [38, 148, 189, 344]. Sunyer et al. [148] showed that NO2 exposure did not influence the incidence of lower respiratory tract infection in the first year of life, while Raaschou-Nielsen et al. [38] reported a lack of association between NO2 exposure and wheeze prevalence during early life.

The potential for increased NO2 exposure during infancy and young age to influence asthma-related outcomes during childhood remains a poorly researched area, although current evidence suggests that increased levels could be linked to increased asthma prevalence in childhood. Carlsten et al. [151] found in a recent birth cohort study that traffic-related NO2 exposure in the first year of life was linked to asthma prevalence at seven years of age. These findings were in agreement with a case-control study in Canada which demonstrated that prenatal and early life exposure to traffic-related NO2 was linked to asthma prevalence at three years of age [90]. Another large-scale birth control has also linked early life NO2 exposure with asthma incidence during the preschool ages [116].

At present, only one study has examined the relationship between maternal NO2 exposure and lung inflammation and function during early infancy. Latzin et al. [87] reported that on average every 1µg/m3 increase in maternal NO2 exposure throughout
the pregnancy period was significantly associated with a 0.98 part per billion (ppb)
increase in FeNO. Overall, the evidence presented suggests that increased indoor NO₂
exposure during the fetal development period could have the potential to result in poor
lung function in early life.

1.4.5.3 Volatile organic compounds (VOCs)
Volatile organic compounds (VOCs) refer to a wide range of gaseous organic
compounds that have high vapour pressure, and are likely to sublime at room
temperature. This blanket term includes various functional groups of organic
compounds such as aliphatic, aromatic and halogenated hydrocarbons, alcohols,
phenols, ethers, amines, aldehydes, ketones and terpenes [22, 348, 349]. VOCs
generally have large molar masses, vapour pressures of 101.3 kPa at 20°C, and boiling
points within two ranges - between 50-100 and between 240 - 260° C [21, 350]. VOCs
also often include very volatile organic compounds (VVOCs) and semi volatile organic
compounds (SVOCs), which often have different physical properties and attributes
[348, 351].

VOCs originate from numerous indoor sources in the domestic environment. Examples
of such sources include aerosol sprays, solvents, glues, cleaning agents, paints,
humidification devices and air fresheners [29, 30, 223, 352-354]. Other common
sources of VOCs include building materials, furnishing materials, combustion
appliances and consumer products [348, 355, 356]. In addition, VOCs emitted from
outdoor sources may also enter the indoor environment and contribute to indoor VOC
levels [349, 357].
Studies in homes across cities in the developed world have reported a wide range of indoor levels of both individual compounds and total VOC (TVOC); the sum of all measured individual VOC levels. A review by the European Commission revealed that most non-industrial indoor environments contain less than 1000 µg/ m³ of TVOC, with few exceeding the 25000 µg/ m³ [350]. Mean average levels that have been reported include 230 to 482 µg/ m³ [358, 359] in Japan, 296 to 308 µg/ m³ in the United Kingdom [360], and 1626 µg/ m³ in the United States [215]. Australian studies have reported values such as 36.2 to 78.5 µg/ m³ [361], 170 µg/ m³ [362], and 190 to 206 µg/ m³ [228]. The wide variation observed between TVOC levels across studies was most likely due to the inclusion of different constituent compounds in each study. The most common indoor VOCs which are known to cause health effects include the BTEX and terpenes [221, 363].

1.4.5.3.1 Health impacts of VOCs

VOCs have varying effects on human health. Studies have linked acute and chronic exposure to high concentrations of VOCs to sensory irritation of the eyes, nose and throat, as well as unspecific symptoms such as nausea, headaches, dizziness, muscle weakness, dizziness, and allergic sensitisation [200, 203, 205, 224, 351, 354, 358, 360, 364-372]. Furthermore, chronic exposure to high levels of certain VOCs might lead to more severe outcomes, including central nervous system disorders and various forms of cancers [94, 197, 246, 373].

Current evidence suggests that increased exposure to several classes of VOCs may result in poor respiratory health. Adverse outcomes that have been linked to increased VOC exposure include increases in lung and airway inflammation, respiratory symptoms, asthma prevalence, and poorer lung function [374, 375].
1.4.5.3.2 Effects of VOCs on lung and airway inflammation

The potential for several classes of VOCs to trigger airway inflammation has been investigated with various study designs. In-vitro studies have shown that certain VOCs can induce oxidative stress in the epithelial cell lining of the airways and alveoli, leading to an inflammatory response [376, 377]. Increased exposure to compounds containing benzene, toluene, ethylbenzene and xylene (BTEX), in particular, has been linked to higher levels of inflammatory markers such as IL-8 and MCP-1 [371, 376-380]. Studies in mice have also demonstrated that exposure to higher VOCs were associated with increased oxidative stress and elevated levels of inflammatory markers such as IL-6 and GSTP-1 [381-385].

Although in-vitro and animal studies have suggested that exposure to various VOCs may induce inflammation in lung and airways, the relationship between both short and long-term VOC exposure and lung inflammation in humans remain inconclusive. Chamber studies have shown that short-term VOC exposure of adults result in lung tissue inflammation [374, 375] and can result in increased neutrophil counts for up to 18 hours post-exposure [386, 387]. While chamber studies have demonstrated the potential for VOC exposure to influence lung inflammation, exposure levels in such experiments are in the range of 25 mg/ m³, which do not accurately represent everyday VOC exposures that are rarely exceed the range of 1000 µg/ m³ [350].

While there is a paucity of data in this research area, two epidemiological studies have demonstrated weak but significant associations between VOC exposure and inflammation [262, 388]. Weichenthal et al. [388] found in a cohort study that each inter-quartile range increase in benzene exposure was associated with a 1.7 ppb (95% CI 1.1 – 2.3) increase in FeNO. Similarly, Wieslander et al. [262] reported that adults exposed
to newly painted surfaces had higher levels of blood eosinophils compared to those who were not. This was inconsistent with the results from chamber studies which have focused on neutrophilic responses. The data relating VOC exposure and inflammatory effects on children also remains scarce. However a recent cross-sectional survey conducted revealed that children living in an area with high VOC concentrations have, on average, 35% higher FeNO levels (90% CI 11.7 – 80.1%) compared to those who lived in areas with low-exposure levels [47]. However, as stated previously, the relationship between inflammation and FeNO levels still remains unclear.

1.4.5.3.3 Effects of VOCs on respiratory symptoms

There is some evidence to suggest that exposure to various VOC compounds can influence the respiratory health of children. A number of studies which have used proxies for exposure to several classes of VOC such as interior surface materials and plastic wall materials have linked increased exposure with symptoms commonly indicative of poor respiratory health. Increased VOC exposure from indoor sources such as flooring and textiles, as well as increased use of chemicals have been linked to having more frequent episodes of wheezing, breathlessness and lower respiratory tract symptoms in children [34, 43, 47, 91, 389-392].

Other studies have shown that proxies of VOC exposure have been linked to poor respiratory outcomes in children [34, 42, 263, 389, 390, 393]. In a series of cohort studies conducted on Russian schoolchildren, Jaakkola et al. [34, 389, 390] demonstrated that recent renovation activities such as having new linoleum flooring, synthetic carpeting, particleboard, wall coverings, furniture, as well as recent painting increased the risk of respiratory symptoms such as wheezing, bronchial obstruction, cough and phlegm in children. Similarly, Zhang et al. [393] reported that children living
in households with increased use of chemicals appeared to have a higher prevalence of wheeze (OR 1.16, 95% CI 1.03 – 1.29) compared to those who did not. Venn et al. [33], however, reported in a case-control study, that measured VOC levels were similar in households with wheezy and non-wheezy children.

It has also been shown that living in areas with high VOC levels such as petrochemical plants increases the risk of having more frequent respiratory symptoms such as wheeze, nocturnal cough and rhinitis in children [43, 47, 391, 392]. Ware et al. [43] reported that children enrolled in schools with high VOC exposure had a higher score on a composite indicator of five chronic lower respiratory symptoms (OR 1.13, 95 % CI 1.02 - 1.26) than children in schools with lower VOC exposure.

1.4.5.3.4 Effects of VOCs on asthma-related outcomes

The potential for VOCs to influence asthma outcomes in humans has been documented in studies which have linked occupational exposure to increased incidence, prevalence and frequency of asthma attacks [394-396]. While limited data exists in this area, non-occupational exposure to VOCs has also been linked to increased asthma prevalence and exacerbation in adults [262, 397, 398].

In children, exposure to higher levels of VOC, particularly BTEX compounds, have been linked to increases in asthma prevalence during childhood [43, 275, 285, 361, 370, 399, 400]. A case control study in Perth showed that children admitted to hospital for asthma attacks had significantly higher domestic levels of BTEX chemicals than those admitted for other reasons [361]. Similarly, Hulin et al. [275] showed that increased toluene exposure was linked to a higher risk of asthma attacks in children living in urban areas. Wichmann et al. [391] and Yang et al. [392] independently found that

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children living near petrochemical plants had higher asthma prevalence and more frequent asthma exacerbations than children who lived further away. Two studies, however, have not found significant associations between VOC exposure levels at home [285] or school [276] and asthma-related outcomes.

1.4.5.3.5 Effects of VOCs on lung function

The impact of short and long-term VOC exposures on the respiratory function of humans has been explored using various study designs and current evidence remains inconclusive. While some chamber studies have reported that increased VOC exposure influences short-term lung function outcomes in adults [367, 401, 402], other studies which have utilized similar concentrations and exposure times have not been able to replicate such findings [375, 403]. Current evidence relating longer-term VOC exposures on respiratory function also remains uncertain, with several occupational studies reporting significant associations [404, 405], while others did not [365, 366, 406, 407]. Adult studies on non-occupational exposure suggest small but significant associations between VOC exposure and lung function outcomes, particularly for certain VOCs such as terpenes and BTEX chemicals [263, 388, 408, 409].

The influence of increased VOC exposure on lung function in children is also unclear at present [44, 45, 47, 285, 391, 410]. Recent studies by Rusconi [47] and Martins [45] have independently reported significant associations between increased benzene exposure and decreased FEV₁ and FVC in children. Delfino et al. [285] demonstrated in a cohort study that exposure to higher concentrations of toluene and methylene chloride was associated with decreased PEF, while no associations were found in another panel study of asthmatic children [410]. While there is a paucity of data in this area, some
evidence suggest that increased VOC exposure may result in lung function decrements in both adults and children.

**1.4.5.3.6 Effects of prenatal and early life VOC exposure on respiratory health**

Early life postnatal exposure to VOCs have been shown to be linked to increased respiratory symptoms and increased susceptibility to infection during the first two years of life [147, 411-413]. A large scale birth cohort study showed that infants living in recently renovated homes had an increased risk of wheeze (OR 1.7, 95% CI 1.3 – 2.6) [147]. Similarly, Herr et al. [413] demonstrated that having a carpet covered floor in the child’s bedroom was linked to a greater risk of wheeze during the first 18 months of life (OR 1.39, 95% CI 1.12 – 1.73). A series of studies by Diez et al. [411, 412] also reported an increased risk of pulmonary infection in infants living in newly painted dwellings (OR 2.4, 95% CI 1.1 - 5.3), dwellings with restoration painting (OR 5.6, 95% CI 1.3 – 24), or homes with elevated styrene levels (OR 2.1, 95% CI 2.1, 95% CI 1.1 – 4.2).

The effects of maternal exposure to VOC and early postnatal respiratory health have also been investigated in several studies [52, 91, 414-416]. Sherriff et al. [91] linked maternal exposure to total chemicals during pregnancy with wheezing during early postnatal life (OR 1.06, 95% CI 1.03 – 1.09). In the same study, maternal usage of household chemicals were linked to increased early (OR 1.41, 95% CI 1.13 – 1.76), intermediate (OR 1.43, 95% CI 1.02 – 2.13) and late (OR 1.69, 95% CI 1.19 – 2.41) onset wheeze during early postnatal life [52]. A recent cohort study by Liu et al. [414] reported similar findings, linking maternal exposure to aerosol pesticides to an increase in non-infectious cough at 5 - 6 years of age (OR 2.27, 95% CI 1.11 – 4.67).
At present, the influence of increased VOC exposure on fetal lung development remains largely unknown. There has only been one longitudinal study that has explored the potential for increased VOC concentrations to affect lung development in later childhood, reporting that questionnaire-derived indoor VOC exposure was significantly related to lung function measures such as FEV$_1$, FVC and FEF$_{25-75}$ at 7.5 years of age [52].

1.5 Conclusion

Current evidence suggests that increased exposure to outdoor and indoor air pollutants can result in a wide range of adverse health outcomes in children and infants, although its long-term influence on the respiratory system remains unclear. Poor respiratory outcomes in children that could result from increased exposure to outdoor and indoor air pollutants may include increased respiratory symptoms, asthma incidence and prevalence, inflammation and decreased lung function.

Prenatal exposure to both indoor and outdoor pollutants have the potential to result in poorer respiratory health and lung function during infancy and childhood [50, 52, 87, 92]. As early life lung function is known to track through towards adulthood [53, 58, 183], this might have long-term implications on lung function in adulthood. Indoor pollutants are likely to present a significant contribution to overall pollutant exposure by pregnant women. To date the majority of research into maternal exposure to air pollutants during pregnancy has focussed on the outdoor environment [49, 50, 87, 92]. However, aside from the significant amount of time pregnant women spend in the domestic environment [189-191], factors such as the number and range of pollutant sources in the indoor environment [219-221] and decreased ventilation [223, 224] can result in higher pollutant exposure indoors.
Although it is possible that maternal exposure to pollutants may influence lung development during infancy, there is a lack of data in this area. Further research into the effects of maternal pollutant exposure on postnatal lung function development will improve our understanding of the impact of indoor pollutant exposure during pregnancy on lung development and long-term postnatal respiratory health.

This thesis aimed to investigate the effects maternal exposure to formaldehyde, nitrogen dioxide (NO$_2$) and volatile organic compounds (VOCs) on birth outcomes including birth length, weight and head circumference, as well as lung function indices such as tidal volume (V$_T$), respiratory rate (RR), ratio of inspiratory time over total breath time (Ti/T$_{tot}$), ratio of time to peak tidal expiratory flow to total expiratory time (tPTEF/tE), mean tidal inspiratory flow (V$_T$/Ti), fractional exhaled nitric oxide (FeNO), functional residual capacity (FRC) and lung clearance index (LCI) in early life. It is hypothesized that exposure to indoor air pollutants during the third trimester of pregnancy may influence fetal development, including that of the respiratory system.
Chapter 2
Materials and Methods


2.1 Introduction

This study was divided into three sections. The first explored the housing factors and indoor air pollutant levels. The second investigated the relationship between indoor air pollutant levels and birth outcomes. Finally, the third explored the association between indoor air pollutant levels and lung function during early infancy.

While the methodologies for specific investigations are discussed in relevant result chapters, this chapter describes the general methods used. More specifically, it includes details of recruitment and data collection protocols, as well as methods for quantifying indoor air pollutant levels and infant lung function testing.

2.2 Study design

The infant lung development study presented in this thesis was a sub-study of a large birth cohort study: the Peel Child Health Study. The larger study was a prospective birth cohort study that investigated the impact community, biological and environmental stressors had on maternal stress during the prenatal period and the subsequent impact on fetal and postnatal health outcomes. The Peel Child Health Study is a collaborative venture between Murdoch University, Curtin University, Edith Cowan University, the University of Western Australia, and the Telethon Institute for Child Health Research. Funding for the Peel Child Health Study was provided by the Australian Research Council (ARC LP0776722).

The infant lung development study focused on the effects of indoor air pollutants on prenatal lung development and respiratory health in early life. Subjects in the lung development study were recruited from the Peel Child Health study. The infant lung
development study was funded by a grant from the National Health and Medical Research Council (NHMRC 572616).

2.3 Subjects and recruitment methods

Recruitment for the Peel Child Health Study was coordinated by the study manager and a team of research staff by approaching general practitioners’ private practices, community support groups, local council offices, child health centres, as well as using print and radio advertisements. Participants from the Peel Child Health Study were given the option of being included in the infant lung development study, subject to certain specific criteria.

2.4 Inclusion and exclusion criteria

Inclusion criteria for the Peel Child Health Study included: less than 18 weeks pregnant at time of recruitment and subjects living in and around the peel region, as determined by postcode. Due to the nature of the infant lung development study, only non-smoking women were considered for inclusion. Exclusion criteria for this study at birth included stillbirths, prematurely births (defined as < 37 weeks gestation), having low birth weight (defined as < 2500g), or having major birth defects, as defined by the Western Australian Birth Defects Registry.

2.5 Ethics approval

Ethics approval for this was obtained from both the Princess Margaret hospital (Registration number 1611/EP) and Murdoch university (Project number 2007/238) HRECs. Written consent was obtained from study participants. Participants were coded with identification numbers in concordance with ethics requirements. Identifiable information was not distributed via non-secure electronic means. Information about
patients were kept within a database secured within Murdoch University (Peel Campus) and also stored as a hardcopy under lock and key in the staff research office.

2.6 Study region

The Peel region is located on the south-western coast of Australia, approximately 32° south and between 115° and 116° east. The region’s total population was 112,677 in September 2012, with approximately half this number in the workforce. It spans approximately 5,600 km² and is made up of five communities, namely Mandurah, Serpentine-Jarrahdale, Boddington, Murray and Waroona [417]. A map showing the Peel region, from which the participants are sourced from, is shown in Figure 2.1:

Figure 2.1: Map of Peel region [417]

The Peel region was selected for the main study for several reasons. At the time of study commencement, this region recorded an estimated population growth in the preceding years that qualified it as the fastest growing city in Western Australia [417]. As a developing area, this region also encompasses a significant proportion of young families.
with children under the age of 15. Furthermore, this region also contains both rural and urban areas, potentially allowing us to explore a range of pollutant exposure levels.

2.6.1 Climatic characteristics
The climate of the Peel region is similar to that of the Perth region [417]. Summers are marked by hot dry easterly winds, while winter usually brings consistent rainfall. During summer, temperatures average 29°C during summer, but can reach over 38°C. Winter temperatures range from a minimum of 9.4°C to a maximum of 17.5°C [417].

2.6.2 Population demographics
As of the middle of September 2012, the Peel region had approximately 112,000 residents [417]. The majority of residents are concentrated within the city of Mandurah (65.3%), with small percentages of residents living in the Shires of Serpentine-Jarrahdale (16.4%), Murray (13.0%), Waroona (3.2%) and Boddington (2.0%).

Population growth in the Peel region is higher than the state average. Between 2010 and 2011, Peel’s population increased by 4.4%, compared to the state’s average of 2.4% [418].

2.7 Protocol
Recruitment for the infant lung development study occurred between 2009 and 2012. Women were recruited prior to reaching 18 weeks gestation. A housing inventory questionnaire was provided at 18, 26 and 34 weeks for participants to complete.

Indoor air pollutant levels were measured at one time point only; 34 weeks gestation. This was considered a suitable time point for determining prenatal pollutant exposure.
for two reasons. Firstly, time-activity studies have demonstrated that pregnant women spend more time in the domestic environment with the progression of pregnancy [190]. Secondly, it was earlier demonstrated that pollutant exposure during the third trimester could influence lung function during early infancy [87]. Pollutants sampled included formaldehyde, NO$_2$ and VOCs, as these pollutants are known to be ubiquitous in the domestic indoor environment. Existing evidence have linked increased exposure to these pollutants to poor health outcomes, as described in section 1.4.5. The suite of chemicals constituting VOCs in this study was similar to that used by another recent study in Perth [419], as well as previous studies [420-424] for two main reasons. Firstly, VOCs in this suite have been shown to influence health outcomes, particularly the BTEX chemicals and terpenes. Secondly, these VOCs are more likely to be found in the indoor domestic environment [209, 348, 353, 419, 425]

Standard demographic variables such as gestational age, length, weight and head circumference were obtained at birth. Newborns meeting the inclusion criteria at birth subsequently underwent a lung assessment during early infancy, defined as between five and seven weeks of age. Tests performed included tidal breathing (TB), fractional exhaled nitric oxide (FeNO) and multiple breath wash out (MBW).

The range of tests in the infant lung development study provided information on respiratory effort and control of breathing, lung volumes, peripheral lung function, ventilation inhomogeneity and airway inflammation. A respiratory questionnaire was also administered on the day of the lung function assessment. The details are described below.
A respiratory symptom diary was also administered on a monthly basis for the first 12 months of life. Parents were asked to record any respiratory symptoms detected during this period.

The timeline in Figure 2.2 summarises the chronological order of events in this study:

**Figure 2.2: Timeline for data collection**

Weeks prior to birth represent weeks of pregnancy.

### 2.8 Techniques

#### 2.8.1 Collection of indoor air pollutant data

Homes of study participants were monitored for formaldehyde, NO$_2$, and volatile organic compounds (VOCs) during the third trimester of pregnancy (34 weeks). Passive diffusive sampling was used to monitor levels of formaldehyde, NO$_2$ and VOCs. This involved the direct exposure of sampling badges to air [426-430], followed by the trapping of gases of interest by chemical reaction or gaseous diffusion and adsorption [431].
In passive sampling, the gas sampling rate is controlled by the rate of diffusion of the compound through the air layer in the sampler, according to Fick’s law of diffusion, represented by the following equation:

\[
\text{Sampling rate (mL/min)} = D \times \frac{A}{L} \times 60
\]

Where A represents the cross sectional area (cm\(^2\)), L represents the diffusion path length (cm), and D represents the diffusion coefficient cm\(^2\)/s\(^{-1}\), which is unique to each compound.

The passive sampling methods have been validated for quantifying levels of formaldehyde [426, 427], NO\(_2\) [428] and VOCs [432], and have been used by numerous studies for monitoring formaldehyde [38, 234, 235, 262, 266, 274, 275, 359], NO\(_2\) [38, 39, 148, 213, 227, 234, 275, 288, 289, 318, 324, 337, 433] and VOC [33, 224, 262] levels. The main advantages associated with passive sampling compared to active sampling methods include being inexpensive [429-431, 434], compact [430, 434], and not requiring electrical power or constant calibration in the way that active samplers do [429, 431]. The main limitations associated with passive sampling are the lack of ability to monitor short term changes in analyte concentrations, sensitivity to extreme fluctuations of temperature and air movement [430], and the possibility of “back-diffusion” [427, 430], although this is more likely to only occur at high pollutant concentrations. Furthermore, the lower limit of detection for passive diffusion tubes is higher than that of active sampling requiring longer term monitoring [429].

Preparation and analysis of formaldehyde and NO\(_2\) sampling badges were conducted by trained research staff at the Children’s Clinical Research Facility (CCRF), Princess
Margaret Hospital. Tubes used for measuring VOC levels were prepared and analysed by the Chemistry Centre of Western Australia, a National Association of Testing Authorities (NATA) accredited chemical laboratory. The sampling protocol that was used is described below.

2.8.1.1 Formaldehyde

Formaldehyde levels were measured using the methods described by Levin et al. [426, 427], but modified by Dingle [435]. The modification involved the substitution of the stainless steel mesh used in the original study with a Teflon membrane filter as part of the cassette design, and was shown not to influence sampling rates [235]. Glass fibre filters in the badges (Gelman type A) were impregnated with 2-4 dinitrophenylhydrazone (DNPH), which acted as a derivatising agent to trap formaldehyde upon direct contact [426, 427].

2.8.1.1.1 Sampling protocol

Sampling was conducted at two sites of each participant’s home in the participant’s bedroom and the main lounge area of the homes for a 24-hour period. Research staff set-up and opened the samplers and recorded the starting time of exposure. Participants were instructed on how to end the exposure and record exposure end times. This sampling period was modified to seven days after November 2010. This was due to problems associated with relying on family members closing the badges and recording correct times; the 7-day time period meant opening and closing was conducted by the researcher. The change in protocol did not significantly influence formaldehyde levels (24-hour mean (SD) = 3.24 (2.58), 7-day mean (SD) = 3.99 (3.18), p = 0.06). Samples were stored in a sealed plastic bag prior to collection by research staff.
2.8.1.1.2 Formaldehyde badge preparation

Four mL of concentrated 2-4 dinitrophenylhydrazone (DNPH) was diluted with acetonitrile (ACN) and made up to 40 ml. 400 µl of 2 mol/L⁻¹ hydrochloric acid was added to form the 2-4 dinitrophenylhydrazone-acetonitrile (DNPH-ACN) solution. 310 µl of DNPH-ACN solution was pipetted drop-wise onto a piece of glass fibre filter paper (Gelman type A). Impregnated glass fibre filter papers were placed into a vacuum dryer to dry.

Dried glass fibre filters impregnated with DNPH/ACN were transferred into the middle column of standard three section cassette badges (Sensidyne 37 mm). The badges were stored in a cool environment and kept away from direct sun exposure. Figure 2.3 shows a schematic diagram of formaldehyde sample badge used in this study.

2.8.1.1.3 Trapping of formaldehyde

Sampling of formaldehyde commenced upon removal of the top section of the badge. Upon exposure, DNPH on the glass filter paper reacts with formaldehyde in the air, forming a stable hydrazone compound as represented by the reaction:

\[
\text{Formaldehyde} + \text{DNPH} = \text{Stable Hydrazone Compound} + \text{H}_2\text{O}
\]
The amount of formaldehyde in the sample could be accurately determined by the amount of hydrazone derivative in the sample [436].

2.8.1.1.4 Quantification of formaldehyde levels

Formaldehyde was analysed using high pressure liquid chromatography (HPLC) (Varian Proster Model 430), a time based assay involving the migration of analytes through liquid phases of a capillary system.

GF/A filters were transferred from the sampling badge into four ml tubes. Three ml of acetonitrile were then pipetted into the tubes. Tubes were left to stand for at least 60 minutes before removal of GF/A filters from the tubes. The solutions were then filtered through a 25 mm syringe filter (0.45 µm) into the HPLC vials. Vials were capped prior to HPLC analysis.

Formaldehyde stock solution was produced by dissolving 10.9 mg of DNPH-derivative formaldehyde (99%) into 10 ml of Acetonitrile (ACN), and subsequently diluting with the top standard. The components of each standard are displayed in Table 2.1.

Table 2.1: Formaldehyde concentrations for standard curves

<table>
<thead>
<tr>
<th>Standard Number</th>
<th>Amount of Acetonitrile (DNPH)</th>
<th>Amount of Formaldehyde Stock</th>
<th>Formaldehyde concentration (g/L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (top standard)</td>
<td>975µl</td>
<td>25µl</td>
<td>2.73E⁻²</td>
</tr>
<tr>
<td>2</td>
<td>990µl</td>
<td>10µl</td>
<td>1.09 E⁻²</td>
</tr>
<tr>
<td>3</td>
<td>996µl</td>
<td>4µl</td>
<td>4.36 E⁻³</td>
</tr>
<tr>
<td>4</td>
<td>950µl</td>
<td>50µl of standard 1</td>
<td>1.36E⁻⁶</td>
</tr>
<tr>
<td>5</td>
<td>980µl</td>
<td>20µl of standard 1</td>
<td>5.45E⁻⁷</td>
</tr>
</tbody>
</table>

Prior to HPLC analysis, each standard was filtered through a 25 mm syringe filter (0.45 µm) into the HPLC vials and capped.
The mobile phase used for HPLC was 70% methanol. This was prepared by the addition of 300 ml of ultra-high pure water (UHPW) (Milli-Q) to 700 ml of pure methanol. The needle wash solution was made up of 400 ml of UHPW, 50 ml of pure methanol and 45 ml of acetonitrile.

Following HPLC, the amount of hydrazone in each standard was used to produce a five-point calibration regression curve. Hydrazone levels of each sample were determined by interpolation from the regression equation of the standard curve. The detection limit for this analysis was 2.4 µg/m³. Formaldehyde levels were calculated by dividing its molecular weight (MW = 30) from the hydrazone compound (MW = 210).

2.8.1.2 Nitrogen dioxide (NO₂)

Nitrogen dioxide sampling was based on the method described by Standards Australia [437] and modified by Nitschke et al. [40]. This involved the use of cellulose filter papers impregnated with triethanolamine (TEA), which trapped NO₂ in the form of nitrile via chemical reaction upon exposure.

2.8.1.2.1 Sampling protocol

Sampling was conducted in the kitchen of each participant’s home in two ways. One sampler was exposed over a period of 24 hours in order to quantify average daily levels. This was also modified to a seven day period after November 2010. The change in protocol was made mainly due to the uncertainty that families were correctly closing the samplers and recording the closing times. It was also made as a longer sampling period increased the amount of analyte captured and improved the lower limit of detection, which was important in an area of low NO₂. The change in protocol did not significantly influence NO₂ levels (24-hour median = 0.4, 7-day median = 0.87, p = 50
0.102). A second sampler was exposed over a period of six-hours during the evening to try to capture peak hour exposure. Research staff set-up the samplers and recorded the starting time of exposure. Participants were instructed on how to end the exposure and record exposure end times. Samples were stored in a sealed plastic bag prior to collection by research staff.

2.8.1.2 NO₂ badge preparation

Stock solution of 20% (v/v) Triethanolamine (TEA) solution (Acros organics) was diluted to equal parts with acetone. 200 µl of the resulting solution was then pipetted onto a cellulose filter paper which was oven-dried prior to preparation (47 mm Whatman No 2, Whatman, Middlesex, UK). TEA-impregnated cellulose filters were then placed into a vacuum dryer to dry.

Preparation of NO₂ sampling badges was done following a method which conformed to Australian standards [437]. Firstly, dried cellulose-filter fibre sheets were inserted into plastic filter cartridges (No. M0025A0, Millipore Corp, Bedford, MA). A hydrophobic porex filter was then added to seal the edge of the badge case. This was made up of polytetrafluoroethylene (PTFE), with measurements of 47 mm in diameter, 0.8 mm thick, and had a pore size of 5 µm. Finally, a tight fitting lid was added over the petri dish. Badges were inserted into a leak-free plastic bag and stored in a cool environment. Figure 2.4 shows a schematic diagram of NO₂ sample badge used in this study.
2.8.1.2.3 Trapping of NO₂

Sampling of NO₂ commenced upon removal of the top section of the TEA-impregnated sampling badge. Upon exposure, TEA on the filter reacts with NO₂ in the air to form nitrite NO₂⁻. This was subsequently quantified by spectrophotometry.

2.8.1.2.4 Quantification of NO₂ levels

NO₂ levels were quantified using spectrophotometry (UV 1601, Shimadzu). The absorbance of individual NO₂ samples were then interpolated from a standard regression curve derived from NO₂ standards.

Prior to analysis, several preparatory steps had to be undertaken. As this was a colorimetric analysis, a colour forming solution (CFA) had to be prepared. A sulfanilic acid solution was prepared by dissolving 2.5 g of solid sulfanilic acid in 500 ml of UHPW. A magnetic stirrer was used in order to ensure full dissolution. A N-1-Naphthyl ethylenediamine dihydrochloride (NEDA) solution was prepared by dissolving 0.025 g of NEDA in 50 ml of UHPW. The CFA was then prepared using the NEDA solution, the sulfanilic acid solution, and 25 ml of orthophosphoric acid.

In order to prepare NO₂ standards, 0.1725g of solid sodium nitrite (NaNO₂) was dissolved in 100 ml of UHPW to form the NaNO₂ stock solution. 16 ml of the stock solution was made up to 50 ml with UHPW to form the primary standard, which
contained 0.552 g/L\(^{1}\) NaNO\(_2\). 100 µL of the primary standard was added to 10 ml of CFA to form the “top standard” for the colorimetric analysis. Subsequently, 1 in 2 serial dilutions were made with CFA to form standards two to seven. The components of each standard used for deriving the standard curve are displayed in Table 2.2.

Table 2.2: NO\(_2\) concentrations for standard curves

<table>
<thead>
<tr>
<th>Standard number</th>
<th>Concentration of NaNO(_3) *(µg/L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (top standard)</td>
<td>5.47 E(^{-3})</td>
</tr>
<tr>
<td>2</td>
<td>2.73 E(^{-3})</td>
</tr>
<tr>
<td>3</td>
<td>1.37 E(^{-3})</td>
</tr>
<tr>
<td>4</td>
<td>6.83 E(^{-4})</td>
</tr>
<tr>
<td>5</td>
<td>3.41 E(^{-4})</td>
</tr>
<tr>
<td>6</td>
<td>1.71 E(^{-5})</td>
</tr>
<tr>
<td>7</td>
<td>8.54 E(^{-5})</td>
</tr>
</tbody>
</table>

* NaNO\(_3\) = Sodium Nitrate

Standards were then pipetted into a 96 well plate and analysed by spectrophotometry.

Cellulose filters from air monitors were transferred into tubes containing 10 ml of CFA. Tubes were left to stand for at least 60 minutes for the colour forming reaction to take place. After removal of the cellulose filters from the sampling tubes, the supernatants were centrifuged at 3000 RPM (Eppendorf S424) for four minutes. 180 µl of each sample supernatant was pipetted into a column of a 96-well plate for analysis by spectrophotometry.

NO\(_2\) standards and samples were analysed by spectrophotometry using a UV-visible spectrophotometer (Shimadzu UV 1601, Kyoto, Japan) at a wavelength of 545 nm. The absorbance of each standard was fitted to derive a linear regression curve. NO\(_2\) levels in
each sample were obtained by interpolation from the standard regression curve, with a detection limit of 0.8 µg/m³.

2.8.1.3 Volatile Organic Compounds (VOCs)

The methods used for quantifying volatile organic compounds are outlined in US EPA method TO-17 [432]. Briefly, this involved the trapping of individual VOCs in a passive sampling tube (Perkin-Elmer, ATD Tenax TA 60/80), followed by analysis with a thermal desorption gas chromatography and mass spectroscopy (GC/MS). VOC sampling was conducted at the Chemistry Centre of Western Australia, a National Association of Testing Authorities (NATA) accredited chemistry laboratory.

2.8.1.3.1 Sampling protocol

Sampling was conducted at the main activity area of each participant’s home over a period of seven continuous days. Research staff set-up the tubes and recorded the starting time of exposure. Upon exposure, VOCs were trapped in the tubes by adsorption. At the end of the sampling period, research staff returned to the homes of participants, ended the exposure and recorded the ending time. Sampling tubes were stored in a tin and kept away from sunlight. VOCs were recovered by thermal desorption before analysis by GC/MS.

2.8.1.3.2 VOC tube preparation

VOC tube preparation was conducted in the Chemistry Centre of Western Australia in concordance with standards outlined in US EPA method TO-17 [432]. The tubes used for VOC sampling were stainless steel net cylinders (Perkin-Elmer, ATD Tenax TA 60/80), lined internally with Tenax with 3 by 8 µm mesh openings and a 4.8 mm diameter, packed with 350 mg of graphitised charcoal of size 35 - 50 mesh (Code 145).
2.8.1.3.3 Trapping of VOCs

Upon exposure, VOC compounds in the air passed through the Tenax tube columns and were trapped by adsorption. Sampling was conducted over a seven-day period.

2.8.1.3.4 Quantification of VOC levels

Sample analysis was performed by trained staff in the Chemistry Centre of Western Australia, a nationally accredited chemistry laboratory service.

Analysis of VOC samples involved several steps. Firstly, thermal desorption was employed to obtain VOC from the inner surface of the sampling tubes. Sampling tubes were transferred into the thermal desorption unit column (Perkin-Elmer Turbomatrix thermal desorber) for desorption at 370 °C for 15 minutes with helium at an estimated flow rate of 100 ml/min⁻¹.

Gas chromatography and mass spectrometry (GC/MS) were employed using a combined GC/MS system (Agilent 5973 MSD) with an atomic mass range from 35 to 300 amu. Samples were injected into the flow column, where ten scans were performed, recorded and exported as chromatograms outputs. At adequate quantities, individual VOCs appeared as visible peaks on the chromatograms. Concentrations of each compound were elucidated based on the following formula:

\[ C_x \text{ (ppbv)} = \frac{A_X C_{15}DF}{A_{15}RRF} \]

Where \( A_X \) refers to the area of the characteristic ion for the compound to be measured, \( A_{15} \) refers to the area of the characteristic ion for the specific internal standard,
RRF refers to the mean relative response factor from the initial calibration and
DF refers to the Dilution factor.

Chromatograms from individual samples were derived and analysed using MS Data Review (Varian Inc., V 6.5). Individual compounds from chromatograms were identified based on elution time. These were further verified by an automated mass spectra check from the National Institute of Standards and Technology (NIST) library, which was found within the program.

Individual VOC compounds were quantified by obtaining the area under the curve (AUC) from the chromatogram, where the AUC was proportional to compound concentrations. These were calculated using toluene-equivalents, where toluene was quantified by interpolation from a standard curve of known concentrations. Compounds which were not detected in sufficient quantities, deemed to be 0.5 ppb, were reported as below detectable levels (BDL).

2.8.2 Infant lung function (ILF) methods

A variety of lung function tests were performed in this study. All tests conducted in this study were based on standards for infant respiratory function testing by the European Respiratory Society and American Thoracic Society (ERS/ATS) [438, 439]. All tests conducted in this study were performed using an ultrasonic flowmeter (Exhalyser, Ecomedics, Duernnten, Switzerland). These tests provided information on the infant’s tidal breathing parameters (tidal breathing test), lung volumes and ventilation inhomogeneity (multiple breath washout) and lung inflammation (FeNO test).
Tidal breathing (TB) analysis provided data relating to lung volumes and flow. The parameters derived from tidal breathing were tidal volume ($V_T$) and respiratory rate (RR). $V_T$ represents the amount of air inhaled and exhaled by the infant per breath, while RR represents the number of breaths per minute. When considered together with other time-based variables, this allowed for the derivation of other volume and flow-related indices such as time to peak expiratory flow ($t_{PTEF}$), ratio of time to peak expiratory flow to total expiratory time ($t_{PTEF}/t_E$), mean tidal inspiratory flow ($V_T/t_i$) and ratio of inspiratory time to total breath time ($T_i/T_{tot}$).

The fractional exhaled nitric oxide (FeNO) test involved the measurement of nitric oxide levels during expiration. Several studies have suggested that FeNO could potentially act as a marker of inflammation in infants [87, 440-442]. The FeNO levels in infants have been shown to have good short term reproducibility and are unaffected by sedation or breastfeeding [440].

Multiple breath washout (MBW) involved the introduction of and washing out of sulphur hexafluoride ($SF_6$) into the lungs concurrently with a bias flow of standard medical air, as described originally by Schibler et al. [443] and modified by Latzin et al. [444]. A longer washout phase represents trapping of $SF_6$ in certain regions of the lungs, particularly in the peripheral regions and suggests an increased in ventilation inhomogeneity [445]. Indices obtained include functional residual capacity (FRC) and lung clearance index (LCI). FRC represents the volume of air left in the lungs at the end of a normal tidal breath, and was calculated by the following equation [443]:

$$FRC = \frac{V_{tracer}}{(C_{init} - C_{end})}$$
Where \( V_{\text{tracer}} \) represents the total volume of tracer gas exhaled till the end of the washout, \( C_{\text{init}} \) represents the tidal fraction of tracer gas and \( C_{\text{end}} \) represents the final fraction of tracer. LCI was calculated as the total cumulative expired volume needed to lower end-tidal SF\(_6\) to a set point of 1/40 (2.5%) divided by the FRC [443]. This provides an indication of gas mixing efficiency, with a higher LCI being indicative of ventilation inhomogeneity [443].

2.8.2.1 Protocol for infant lung function testing

Infant lung function tests were conducted in the Peel Community Health Centre in Mandurah. Set-up and calibration of equipment were completed prior to the arrival of study participants. Environmental conditions such as ambient temperature and barometric pressure were entered into the software, as recommended by ERS/ATS guidelines [438, 439]. The temperature was recorded via a standard digital thermometer (Testo, 608-H2) while the barometric pressure was obtained from the Bureau of Meteorology website [446].

The lengths of infants were measured using standard length (Seca 416, Hamburg) scales up to an accuracy of one millimetre. Standard weight scales (Nuweigh log 344, Queensland) were used to obtain infant weights, up to an accuracy of 10 grams. Details were recorded on an infant lung function testing data sheet (Appendix 1). A respiratory questionnaire was also provided to the parent in order to obtain information regarding environmental exposures during the first few weeks of life, as well as family history of hay fever, asthma and eczema. Infants were fed and attempts were made for infants to undertake quiet sleep.
Lung function testing was conducted on infants during quiet tidal sleep in the supine position with the head in the midline position, similar to that of other studies that performed infant lung function tests [87, 441, 442, 447]. In the event of infant awakening, the mask was removed in order for the infant to settle prior to subsequent attempts. Pulse oximetry was used to monitor haemoglobin saturation and heart rate of the infant throughout the lung function tests.

2.8.2.2 Technical aspects of the ultrasonic flowmeter (USFM)

The ultrasonic flowmeter (Exhalyser, Ecomedics, Duernten, Switzerland) was used for tidal breathing, multiple breath wash out and fractional exhaled nitric oxide tests. The fractional exhaled nitric oxide analyser (CLD 88 AM, Eco Medics AG) was connected to a sampling line attached to the USFM via a luer connector port. Infant lung function was collected using a face mask connected to the flow head (Timesco, TDM-MP-1500 Size 0, London). Medical air and SF₆ gas cylinders (BOC, Australia) were attached to the USFM. The USFM was also connected to a laptop which had the corresponding software for providing real-time visual outputs of lung function tests.

The spiroson control unit of the USFM was used to measure inspiratory and expiratory flow at a digitised sampling rate of 200 Hz. The transit time of a pulsed ultrasound travelling through the streaming medium was measured. As transit time is affected by gas velocity across the medium, the molar mass of the gas in the main stream of the USFM could be derived [443]. As molar mass is also directly proportional to the density of the medium [443], the density could be calculated using the following formula:

\[
\text{Density} = \frac{(\text{Molar Mass}) \times (\text{Pressure})}{(\text{Gas constant}) \times (\text{Temperature})}
\]
The volume-time trace was obtained by integrating the flow-time trace by applying the trapezoidal rule, which refers to the integration of a formula derived from the flow-time relationship between every two sampled time points, as previously described [438, 443].

2.8.2.3 Equipment calibration procedure

Equipment calibration was performed before each test to verify the accuracy and consistency of the equipment, and was based on ERS/ATS standards [438, 439]. Procedures included a volume calibration, a flow calibration and a fractional exhaled nitric oxide (FeNO) validation.

2.8.2.3.1 Volume calibration

Volume calibration was performed by calculating the average volume of 20 full pumps using a 100 ml calibration syringe (Hans Rudolph, 5510 series, Gambro AG, Hunebach, Switzerland) attached to the flow head via a luer connector. Volumes were accepted as accurate if average values were within the range of 100 ± 3 ml. Following that, a second calibration was performed in verification mode with ten pumps to verify the robustness of the initial calibration.

2.8.2.3.2 Flow calibration

The bias flow of the medical air was performed by occluding the bias-flow outlet in order to channel gas flows through the main chamber of the flow head. An acceptable flow rate was defined as 0.200 L/s⁻¹ ± 0.005 L/s⁻¹. Linear flow offset correction was performed to correct for technical drift. When necessary, flow levels were adjusted to acceptable levels by manually adjusting the flow control valve on the exhalyser unit. In a similar manner, verification of the SF₆ flow was performed by flushing it through the bias flow. The criterion for acceptable SF₆ flow was also 0.200 L/s⁻¹ ± 0.005 L/s⁻¹.
2.8.2.3 Nitric Oxide calibration

The fractional exhaled nitric oxide analyser (CLD 88 AM, Eco Medics AG) was used to perform nitric oxide calibration based on recommendations by the American Thoracic Society and European Respiratory Society (ATS/ERS) [448]. An initial sampling of room NO levels was conducted by exposing the FeNO sample line to room air for 10 - 15 seconds. The second step involved attaching the FeNO sampling line to the flow head with a bias flow of medical air at a rate of 0.200 L/s⁻¹ in order to provide a bias flow of NO free air for 10-15 seconds. Finally, the FeNO sampling line would be detached to quantify ambient NO levels. Calibration was accepted as successful if the bias flow phase of the calibration showed a maximum FeNO level of 0.5 ppb when measuring NO-free airflow.

2.8.2.4 Collection methods

2.8.2.4.1 Tidal breathing and Fractional Exhaled Nitric Oxide

Tidal breathing (TB) and fractional exhaled nitric oxide (FeNO) tests were conducted simultaneously and according to ERS/ATS guidelines [438, 439]. A size 0 face mask was placed over the infant’s nose and mouth with a leak-free seal for a period of 60 seconds to ensure a minimum of 30 breaths was obtained. A subsequent recording of longer duration, variable and up to 75 seconds was undertaken if 60 seconds of sampling did not provide an adequate number of breaths.

Recordings were made via the WBreath program (Version 3,28,0,0. ndd Medizintechnik, AG, Zurich, Switzerland), which controlled the USFM and gas cylinders. This program had internal automated algorithms allowing for the identification of individual breaths. The flow-volume loop of the infant’s tidal breathing
pattern was captured on-screen in real-time. Visual inspection of time-based traces of tidal breathing parameters allowed for the online assessment of data integrity.

2.8.2.4.2 Multiple breath washout

The collection of three acceptable readings of MBW was attempted, as recommended by ERS/ATS guidelines [449, 450]. The basic method for collection of MBW data was similar to that of the TB test. A size 0 face mask was placed over the infant’s nose and mouth with a leak-free seal. However, unlike the tidal breathing test, the gas density-time trace was displayed on-screen in real-time concurrently with the flow-time relationship.

Each recording commenced with the USFM detecting the first few tidal breaths before the introduction of 4% SF₆ gas concurrently with a bias flow of medical air. After SF₆ levels in inspired and expired gas were stable, as determined from the density trace, SF₆ gas flow was ceased, enabling SF₆ levels to decrease to baseline levels.

2.8.2.5 Acceptability criteria

2.8.2.5.1 Tidal breathing

Based on ERS/ATS guidelines [438, 439], several main criteria had to be met for a tidal breathing recording to be acceptable. Firstly, the recording needed to include a minimum of 30 continuous breaths with tidal volume of each breath falling within 20% of the average value. Secondly, the amount of leak in the overall recording should not exceed 2.5 ml/s⁻¹ after correcting for temperature and humidity adjustments. Finally, recordings should display similar peaks and troughs between breaths in both the volume and flow traces. Recordings with visible spontaneous deep inspirations (sighs) or unusual respiratory patterns are likely to indicate light sleep.
2.8.2.5.2 Fractional Exhaled Nitric Oxide

As FeNO tests were conducted concurrently with TB tests, the criteria for acceptability for FeNO tests included the TB acceptability criteria, as FeNO levels are flow-dependent [448]. Two additional criteria were provided. Firstly, the FeNO trace should be in-phase with the tidal breathing trace. A failure of the recording to meet this requirement indicates, in the majority of instances, a leak in the sampling line between the USFM and the FeNO unit. Secondly, FeNO levels during the inspiratory phase should be below one ppb, as it this represents NO-free air. A failure of the recording to meet this requirement is likely to be due to either improper calibration prior to testing or technical issues of the FeNO unit on day of testing.

2.8.2.5.3 Multiple breath washout

Attempts were made to obtain three acceptable recordings for this test, in concordance with recommendations by the ERS/ATS [449, 450]. However, data with only two acceptable recordings were accepted if LCI levels for each recording differed by less than 10%, which was deemed acceptable by ERS/ATS standards [449, 450].

The acceptability criteria for each recording included the main criteria described for tidal breathing analysis. However, sighs occurring during the wash-in phase were considered acceptable, except when they occurred within the segment of the recording used for analysis. This segment was defined as being from within five breaths prior to the wash-out phase to within ten breaths past the breath at which an estimated 1/40th (2.5%) of the maximum amount of SF₆ remained in the lungs.
2.8.2.6 Analysis

Analysis of all recordings was performed using WBreath (Version 3,28,0,0. ndd Medizintechnik, AG, Zurich, Switzerland). Prior to analysis, several settings were verified. Firstly, the environmental settings were manually altered in the program to ensure that the room temperature, pressure and humidity were accurate. The equation for the correction from atmospheric temperature and pressure (ATPS) to barometric temperature and pressure (BTPS) is described as follows:

\[
\frac{T_{\text{Body}}}{T_{\text{Ambient}}} = \frac{(P_{\text{Ambient}} - P_{\text{H2O}}(T_{\text{Ambient}},H_{\text{Ambient}}))}{(P_{\text{Ambient}} - P_{\text{H2O}}(T_{\text{Body}},H_{\text{Body}}))}
\]

Where T represents temperature, P represents pressure and H represents humidity levels.

Secondly, the infant’s body weight was entered into the program to account for the anatomical dead space. Thirdly, corrections had to be made to account for the dead space of the face mask. Details for corrections of body weight and dead space were included in the manufacturer’s software algorithm, and adhered to the linear range of 0 - 10 L/min\(^{-1}\) as recommended by current standards [438, 439, 444]. Finally, an assessment of the leak was checked using a function available within the software.

2.8.2.6.1 Tidal breathing

Analysis of tidal breathing was provided as a function of the WBreath program. This function provided comprehensive analysis of multiple variables derived from volumes, flows and times within the phases of each breath (Figure 2.5; left). The program also visually displayed the relationship between each primary variable for each breath (Figure 2.5; right). These were exported from the program as ASCII files.
While tidal breathing data was available for individual breaths, the average values of all breaths were used for analyses. This was accepted as values of an “average” breath, since it was earlier established that each breath varied by less than 20% variation from the average. Table 2.3 presents the variables that were used for subsequent analyses.

Table 2.3: Tidal breathing variables used for subsequent analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Scientific notation</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal volume</td>
<td>$V_T$</td>
<td>mL</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>RR</td>
<td>1/min</td>
</tr>
<tr>
<td>Ratio $t_I$ to $t_{tot}$</td>
<td>$T_i/T_{tot}$</td>
<td>%</td>
</tr>
<tr>
<td>Ratio $t_{PTEF}$ to $t_E$</td>
<td>$t_{PTEF}/t_E$</td>
<td>%</td>
</tr>
<tr>
<td>Mean tidal inspiratory flow</td>
<td>$V_{ti}$</td>
<td>mL/s</td>
</tr>
</tbody>
</table>

2.8.2.6.2 Fractional Exhaled Nitric Oxide

Analysis for FeNO tests were conducted after TB analysis as a separate function within the same program. Although the program provided several output variables, only end tidal FeNO levels were considered [441, 448]. Similar to TB analysis, the average value of the breaths in the entire recording was used for subsequent analyses. Outputs for FeNO values were exported as ASCII files.
2.8.2.6.3 Multiple breath washout

Prior to analysis, MBW recordings were analysed in a similar manner to TB tests in the WBreath program. The remainder of the analysis was performed as a continuation using other functions within the WBreath program.

As calculations for FRC and LCI only involved part of the recording, the range of analysis had to be manually selected. Firstly, the wash-out phase was identified from the molecular mass trace of the recording. The starting point of the analysis was identified as being five breaths prior to the wash-out phase.

In order to identify the end point, a preliminary analysis for MBW was undertaken. The breath table output allowed for the identification of the breath where the trace concentration of SF₆ falls below 2.5% of equilibrium. The end point was chosen as ten breaths past the breath at which SF₆ levels fall below 2.5% of equilibrium.

MBW analysis for the specified range was then conducted in the program. The output was exported as an ASCII file which included values of FRC (L) and LCI. FRC and LCI were averaged from acceptable recordings for use in subsequent analyses.

2.8.3 Respiratory questionnaire

As part of the study, a respiratory questionnaire was administered on the day of the infant lung function test (Appendix 2). This questionnaire provided information about the infant’s environmental conditions between birth and the day of the infant lung function test. Furthermore, additional information relating to the infant’s immunisation status, feeding habits, early-life hospitalisation history, exposure to cigarette smoke and allergens, and family history of asthma, hay fever and eczema were provided. The
format of this diary was modified from the International Study of Asthma and Allergies in Childhood (ISAAC) study [451].

2.8.4 Respiratory diary

Respiratory symptom diaries (Appendix 3) were sent monthly to homes of participants in order to explore the respiratory health of the infant during the first year of life. This involved recording present symptoms on the diary for each day of the month. Participants filled in symptom diaries for the first 12 months of the infant’s life. The format of this diary was modified from a previous study on the same age group [452].

2.8.5 Housing inventory questionnaire

A housing inventory questionnaire (Appendix 4) was administered at 18 weeks of pregnancy. It was completed again if the family moved before the 34 week sampling time. This included questions relating to house type, distance from main roadways, heating and cooking systems, renovations and use of household chemical products within the past 12 months. The content of the questionnaire was modified from Lebowitz et al. [453] to accommodate the aims of the infant lung development study.

Information regarding recent home renovations was also included in the housing questionnaire. Participants were asked whether the following renovation works had been carried out within 12 months prior to filling in the questionnaire: installation of new carpets, new linoleum, new wood floor or polish, painting of walls, and introduction of new furniture or new rooms in the household. Participants were also invited to provide information on areas of the house where such renovation works occurred. At 26 and 34 weeks subjects were asked if any changes had occurred to the home.
Usage of household chemicals was obtained using composite household chemical exposure (CHCE) scores. This scale was adopted and modified from the ALSPAC study [52]. Participants were asked about the usage frequency of eleven groups of household products: disinfectant, bleach, carpet cleaner, window cleaner, dry cleaning fluid, aerosols, turpentines/white spirits, air fresheners, paint stripper, paint/varnish and pesticides. Frequencies for usage of each product were recorded as being rarely, monthly, fortnightly, weekly, most days or daily. CHCE scores were calculated as a sum of reported usage frequencies of each category of household product. The scoring guide to each product based on frequencies of used were as follows: rarely (0), monthly (1), fortnightly (2), weekly (3), most days (4) or daily (5).

2.8.6 Sample size and power estimation

In concordance with the primary aim of this study, power estimations focused on the effect of prenatal exposure to indoor air pollutants on early life measures of lung function. As a previous study in Perth has shown that approximately 15% of homes have formaldehyde levels of the threshold associated with poorer respiratory health [235], sample size calculations were estimated based on comparisons of three exposure groups, with the top and bottom 15% as cut-offs for high and low exposure groups respectively.

Based on such a design, it was estimated that in order to demonstrate a 20% increase in ventilation inhomogeneity, tidal breathing data was required to be obtained from 246 infants, a 10% increase in LCI could be detected by obtaining 180 MBW samples, and 320 infants would provide sufficient power to detect a 25% increase in tidal FeNO.
2.8.7 Statistical methods

Statistical package for social sciences (SPSS) version 20.0.0 (IBM Corp, 2011) was used for all statistical analyses in this study. As the outcomes of most investigations were continuous variables, linear regression models were constructed where dependent variables were naturally or transformed to an approximately normal distribution.

When comparison of groups was the research question, students’ T-tests and one way analysis of variance (ANOVA) were used if the data set fulfilled the criteria. Where parametric tests could not be used, corresponding non-parametric tests were employed to compare two (Mann Whitney-U) or more (Kruskal-Wallis) groups. A p-value of < 0.05 was considered statistically significant. For the investigations on the infant lung development in this study, the detail of statistical tests used for each investigation has been described in the respective chapters.
Chapter 3
Factors influencing
Indoor Air Pollutant Levels
3.1 Introduction

The domestic environment is known to contain a wide variety of pollution sources. Major sources of pollutants in the indoor environment include emissions from building materials, varnishes, electrical appliances, aerosol sprays, cleaning products, as well as outdoor pollutants that have entered homes via open doors and windows [27, 29, 32, 221]. The levels of indoor pollution within a home are heavily influenced by a variety of climatic, housing, physical and behavioural factors, although indoor air pollutants (IAP) were referring to a variety of compounds - formaldehyde, nitrogen dioxide (NO\textsubscript{2}), and volatile organic compounds (VOCs), which are known to be ubiquitous [21, 30, 219, 349].

The aim of this chapter is to report on the concentrations of various IAPs, formaldehyde, nitrogen dioxide (NO\textsubscript{2}), and volatile organic compounds (VOCs), in the homes of pregnant women involved in a health study and explore factors that may influence IAP concentrations.

3.2 Methods

3.2.1 Subjects and protocol

Pregnant women were recruited prior to 18 weeks of gestation for a study on IAP and early life lung function. Only non-smokers were recruited in the infant lung development study. A housing inventory questionnaire was given to each participant at recruitment to provide information regarding physical characteristics of the home, local traffic conditions, as well as behavioural patterns such as the usage of household products and equipment that can emit indoor air pollutants such as formaldehyde, NO\textsubscript{2} and VOCs.
Sampling of indoor air pollutants were conducted in the homes of participants on one occasion only at 34 weeks of gestation. Passive sampling methodologies were used to measure indoor levels of formaldehyde, NO₂ and VOCs. The in-depth protocols for preparation and analysis of monitors can be found in Chapter Two. Participants were asked about environmental changes at 34 weeks of gestation. A new inventory was provided for participants that have moved into new dwellings.

Formaldehyde sampling was conducted over a period of 24 hours in both the bedroom and the lounge, although this was later changed to seven days, as described in Chapter Two. Sampling of NO₂ was conducted in the kitchen over both six and 24-hour periods to determine peak and daily averages respectively, although the latter was later changed to seven days, as described in Chapter Two. VOCs were sampled using Tenax tubes over a seven day period in the lounge or main activity area of the home.

3.2.2 Techniques

3.2.2.1 Housing questionnaires

The housing inventory questionnaire was administered at recruitment with an aim to provide greater insight into living conditions and behaviour within the domestic environment during pregnancy. Information regarding house type, distance from main roadways, heating and cooking systems, recent renovations and use of household chemical products were included in this questionnaire. Additional information relating to living conditions was also obtained from questionnaires which were administered to all participants in the broader Peel study at 18 and 34 weeks of gestation. Details from the questionnaires can be found in more detail in Chapter Two.
3.2.2.2 Geographic Information Systems (GIS)

Although participants were asked about traffic near their home, a more objective measure to determine the proximity of homes to roads was also used. This method involved a technique known as geocoding, which utilises data obtained from geographic information systems. This involved the identification of each participant’s home and major roads within a map of the region. Maps of the Peel region used for the program were obtained from Landgate, Western Australia. Roads were classified by Landgate based on the Western Australian Main Roads Act (1930).

A specialised computer program (ARC GIS version 10, Esri, California, 2010) was used to calculate the number of homes within each category of distance surrounding the perimeter of each main road.

3.2.2.3 Indoor air monitoring

Monitoring of indoor air levels was obtained using validated passive sampling techniques. Air monitors were impregnated with compounds that reacted with indoor air pollutants of interest when exposed. The samplers were analysed using techniques such as high pressure liquid chromatography (formaldehyde), gas chromatography (VOCs) and mass spectrometry (NO₂). The protocol on preparation of air monitors and analyses to quantify levels of formaldehyde, NO₂ and VOCs is described in more detail in Chapter Two.

3.2.2.4 Classification of individual residences

In order to classify individual residences as rural or urban, copies of the Peel region planning maps were obtained from the Western Australian Planning Commission. Regional land use maps were up-to-date as at 2011. The locations of each participant’s
primary residence were located and overlaid on the appropriate land-use maps, allowing for rural-urban classification.

3.2.3 Statistical analysis

Statistical analyses were run using SPSS version 20.0.0 (IBM Corp, 2011). As the distribution of each indoor air pollutant in this study varied, a range of statistical analyses were used to explore the effects of individual factors of interest on indoor air pollutants.

With investigations relating to the seasonal impact on pollutants, seasons were classified with spring being from September to November, summer being from December to February, autumn being from March to May, and winter being from June to August. Indoor air pollutant levels were measured at one time point only.

3.2.3.1 Comparison of rural and urban environments

Comparisons of housing types and indoor pollution levels were made between rural and urban dwellings. Factors which were investigated included materials of outer walls, garage type, dwelling type, number of bedrooms, age of homes, recent renovations, distances from homes to main roads, presence of unflued gas heaters and stoves, and concentrations of measured pollutants; formaldehyde, NO2 and VOCs. Comparisons were made using chi-squared tests (for categorical data) and Mann-Whitney U- or Students T-tests (for continuous data).

3.2.3.2 Formaldehyde

Formaldehyde levels were transformed to their natural logarithm to achieve an approximately normal distribution. Transformed data were used for all analyses. Half
the detection limit (1.2 µg/m³) was assumed for samples that were below detection limits.

The correlation of the levels of formaldehyde in the bedroom and lounge was examined using Pearson’s correlations. Comparisons between formaldehyde levels across seasons were conducted using ANOVA.

The influence of home age on formaldehyde levels was examined. Age of homes was obtained as a continuous variable from questionnaire data. As this variable could not be transformed, age of homes were categorised into three groups: <5 years, 5-15 years, and >15 years and ANOVA was employed to investigate the association of home age with formaldehyde levels.

‘Recent home renovations’ was treated as a bivariate (yes/no) variable and students’ T-test was used to examine its influence on formaldehyde levels. Similarly, differences between formaldehyde levels in rural and urban homes were also compared using a students’ T-test.

3.2.3.3 NO₂

NO₂ levels could not be transformed to a normal distribution. Therefore, non-parametric tests were used for all investigations. Half the detection limit, as determined by time-weighted averages (0.4 µg/m³ for 24-hour exposures, 4.9 µg/m³ for peak exposures) was assumed for samples that were below detection limits.

A Spearman’s test was employed to examine the correlation between peak (six hour sampling) and daily (24-hour, later changed to seven-day sampling) NO₂ levels.
The effect of season on NO₂ exposures was investigated using the Kruskal-Wallis test. A Mann-Whitney U test was constructed to compare differences between winter NO₂ levels and NO₂ levels for the rest of the year.

The influence of proximity to roads on 24-hour NO₂ levels was examined using Mann-Whitney U tests. Distances between homes and nearest main roads were recorded as an ordinal variable and categorised as follows: 0 – 49 m, 50 – 99 m, 100 – 249 m, 250 – 499 m, 500 – 999 m and > 1000 m. Two analyses were used using 50 m and 250 m cut-off values. The first analysis compared the 0 - 49m exposure group (<50 m) with a pooled variable comprising of the rest of the data (>50 m). The second analysis involved the comparison of two groups, with one group (< 250 m) comprising of a pooling of the categories 0 - 49 m, 50 - 99 m, and 100 - 249 m, and the other (> 250 m) comprising of the categories 250 - 499 m, 500 - 999 m and >1000 m.

The impact of having gas-emitting sources such as unvented gas stoves and unflued gas heaters on indoor NO₂ levels was investigated. The presence of unvented gas stoves and unflued gas heaters were independently recorded as dichotomous variables (yes/no). The effects of unflued gas heater use on indoor NO₂ only included samples taken during winter, when heaters were actively used. Mann-Whitney U tests were conducted to investigate the relationship between each variable (cooking and heating) and indoor NO₂ levels.

The effect of total gas-emitting sources on indoor NO₂ levels was also explored. Participants were categorised into three groups – no usage of unvented gas cookers or unflued gas heaters, usage of either unvented gas cookers or unflued gas heaters, or
usage of both unvented gas cookers and unflued gas heaters. Krustal-Wallis tests were used to examine the relationship between each category and indoor NO₂ levels.

Comparisons between levels of NO₂ in rural and urban areas were conducted using Mann-Whitney U tests.

### 3.2.3.4 VOC

VOC levels could not be transformed to obtain a normal distribution. Therefore, non-parametric tests were used for all investigations. VOCs that were below detection limit were assigned half the detection limit (0.25 ppb). Total VOC levels (TVOC) were obtained by summing up the levels of each reported VOC in the suite. Levels of BTEX were obtained by summing up levels of benzene, toluene, ethylbenzene, mp-xylene and o-xylene.

In order to determine the influence of the BTEX compounds on overall TVOC levels, correlations between BTEX and TVOC levels were investigated using a Spearman’s test.

The impact of household chemical exposures on indoor TVOC and BTEX levels, as well as individual VOCs of interest (toluene, mp-xylene, o-xylene, α-pinene and limonene) was explored. The selection of these VOCs of interest was due to their association with poor health outcomes, as described in section 1.5.3. CHCE scores were categorised into quartiles. Kruskal-Wallis tests were then conducted in order to compare the quartiles with each dependent variable. Furthermore, a Mann-Whitney U test was performed in order to compare differences in levels between the lowest and highest quartile. Due to the heavily skewed distribution of the data, results of each quartile were
presented as arithmetic means. Furthermore, the percentage of samples which were below detectable levels was also reported.

The effect of recent home renovations (binary variable) on TVOC, BTEX and individual VOCs which were most often found in measurable concentrations (toluene, mp-xylene, o-xylene, α-pinene and limonene) was also explored. Differences between VOC levels in rural and urban households were explored using Mann-Whitney U tests.

3.3 Results

3.3.1 Descriptive statistics

Indoor air pollution was monitored in the homes of 305 pregnant women who initially agreed to participate in this infant lung health study, although not every participant provided every bit of information for the study. Reasons for this included damage or loss of air monitors, participants being unable to open and close samplers in the right manner, and uncertainty regarding opening and closing times of samplers. The numbers of participants that provided data for each indoor air pollutant is found in Table 3.2. The study population consisted of mostly urban dwellings (86.4%). 93% of all participants lived in individual homes, slightly higher than the estimated 85.5% of the entire region [418]. 93% of the homes in this study had brick outer walls, with the remainder having timber, asbestos, fibrous cement sheets or stone outer walls. In addition, a large percentage of residences in this study had fitted carpet (89%) or timber (87%) floor coverings in some parts of the home, with lower numbers having linoleum (57%) and concrete/stone (40%). A large proportion of homes also had an enclosed but attached garage (59%), with less homes having open (25%), non-attached (9%) and no (7%) garage. Home age was collected from 265 households. Mean age of homes in this study was 11.94 (SD 12.27) years, with the distribution skewed to the right. The breakdown
of home age, recent renovation, distances from homes to nearest main roads, as well as unflued gas heater and unvented gas stove use based on rural-urban classification is presented (Table 3.1).

Most of the homes in this region were individual houses with brick outer walls. Most of the dwellings in this population were built less than 30 years ago, and are situated more than 250 metres from the main road. Urban homes were more likely to have attached garages, had more bedrooms and were more varied in dwelling types compared to rural homes. Participants living in urban homes were also more likely to use unflued gas heaters as a heating source.

Several differences were found between distributions of housing factors of rural and urban dwellings. Despite the majority of study dwellings having brick outer walls in both categories, differences in outer wall materials were present for rural and urban dwellings \( (p = 0.002) \). Urban dwellings were shown to have a higher proportion of attached closed garages and a lower proportion of unattached garages compared to rural homes \( (p = 0.004) \). Significant differences in distributions were also detected in dwelling types \( (p = 0.005) \), although a majority of rural (92.68 %) and urban (93.17 %) participants lived in individual houses. Urban dwellings also appeared to have, on average, more bedrooms compared to rural homes \( (p < 0.001) \). The presence of unflued gas heaters were also significantly higher in urban (43.44 %) compared to rural (22.50 %) homes \( (p = 0.012) \).
Table 3.1: Housing information based on rural-urban classification

<table>
<thead>
<tr>
<th></th>
<th>Rural n (%)</th>
<th>Urban n (%)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Material of outer walls</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brick</td>
<td>32 (80.00)</td>
<td>231 (94.67)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Timber/ Asbestos/ Fibrous cement sheets</td>
<td>6 (15.00)</td>
<td>11 (4.51)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (5.00)</td>
<td>2 (0.82)</td>
<td></td>
</tr>
<tr>
<td><strong>Garage type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7 (17.50)</td>
<td>14 (5.69)</td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>10 (25.00)</td>
<td>66 (26.83)</td>
<td></td>
</tr>
<tr>
<td>Closed (attached to home)</td>
<td>17 (42.50)</td>
<td>153 (62.20)</td>
<td></td>
</tr>
<tr>
<td>Closed (not attached to home)</td>
<td>6 (15.00)</td>
<td>13 (5.28)</td>
<td></td>
</tr>
<tr>
<td><strong>Type of dwelling</strong></td>
<td></td>
<td></td>
<td>0.005*</td>
</tr>
<tr>
<td>Individual house</td>
<td>38 (92.68)</td>
<td>232 (93.17)</td>
<td></td>
</tr>
<tr>
<td>Semi-detached/ townhouse</td>
<td>0 (0)</td>
<td>6 (2.41)</td>
<td></td>
</tr>
<tr>
<td>Apartment</td>
<td>0 (0)</td>
<td>10 (4.02)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>3 (7.32)</td>
<td>1 (0.40)</td>
<td></td>
</tr>
<tr>
<td><strong>Number of bedrooms</strong></td>
<td></td>
<td></td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>1</td>
<td>1 (2.44)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9 (22.00)</td>
<td>7 (2.86)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7 (17.07)</td>
<td>61 (24.90)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22 (53.66)</td>
<td>162 (66.12)</td>
<td></td>
</tr>
<tr>
<td>5+</td>
<td>2 (4.88)</td>
<td>15 (6.12)</td>
<td></td>
</tr>
<tr>
<td><strong>Home age (years)</strong></td>
<td></td>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td>0-5</td>
<td>20 (55.56)</td>
<td>81 (34.91)</td>
<td></td>
</tr>
<tr>
<td>5-15</td>
<td>8 (22.22)</td>
<td>69 (29.74)</td>
<td></td>
</tr>
<tr>
<td>15+</td>
<td>8 (22.22)</td>
<td>82 (35.34)</td>
<td></td>
</tr>
<tr>
<td><strong>Recent renovation</strong></td>
<td></td>
<td></td>
<td>0.519</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (25.00)</td>
<td>75 (30.12)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>30 (75.00)</td>
<td>174 (69.88)</td>
<td></td>
</tr>
<tr>
<td><strong>Distance from home to main road</strong></td>
<td></td>
<td></td>
<td>0.140</td>
</tr>
<tr>
<td>0 - 49m</td>
<td>0 (0)</td>
<td>9 (3.45)</td>
<td></td>
</tr>
<tr>
<td>50 - 99m</td>
<td>0 (0)</td>
<td>15 (5.75)</td>
<td></td>
</tr>
<tr>
<td>100 - 249m</td>
<td>2 (4.88)</td>
<td>37 (14.18)</td>
<td></td>
</tr>
<tr>
<td>250 - 499m</td>
<td>15 (36.59)</td>
<td>80 (30.65)</td>
<td></td>
</tr>
<tr>
<td>500 - 999m</td>
<td>15 (36.59)</td>
<td>84 (32.18)</td>
<td></td>
</tr>
<tr>
<td>&gt; 1000m</td>
<td>9 (21.95)</td>
<td>36 (13.79)</td>
<td></td>
</tr>
<tr>
<td><strong>Unflued gas heater usage</strong></td>
<td></td>
<td></td>
<td>0.012*</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (22.50)</td>
<td>106 (43.44)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>31 (77.50)</td>
<td>138 (56.56)</td>
<td></td>
</tr>
<tr>
<td><strong>Unvented gas stove usage</strong></td>
<td></td>
<td></td>
<td>0.817</td>
</tr>
<tr>
<td>Yes</td>
<td>4 (10.00)</td>
<td>22 (88.71)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>36 (90.00)</td>
<td>226 (91.13)</td>
<td></td>
</tr>
</tbody>
</table>

n = number of homes;
* p-value < 0.05 indicates significant differences between categories
3.3.2 Levels of individual indoor pollutants in homes

The levels of indoor air pollutants found in this study are displayed in Table 3.2. Geometric mean of formaldehyde levels were 0.749 and 0.679 µg/m³ for the bedroom and lounge respectively. The median level of 24-hour NO₂ exposure was 0.73 µg/m³. Over 83% of peak NO₂ levels were below detectable limits. There were changes in the protocol for 24-hour NO₂ monitoring, but these did not result in significant changes, (24-hour median = 0.4, 7-day median = 0.87, p = 0.102). Median levels of indoor TVOC and BTEX levels were 14.50 and 3.00 ppb respectively.

Table 3.2 shows the number of samples collected for each indoor air pollutant, as well as the levels, range, and proportion of samples that were below detection limits. Formaldehyde levels were reported as geometric means, while medians were used for NO₂ and VOC levels. A large proportion of NO₂ samples were below detectable levels (Table 3.2).

Formaldehyde samples were collected from 276 (bedroom) and 272 (lounge) households, 24 hour NO₂ samples were obtained from 298 households, while six-hourly (peak) NO₂ levels were obtained from 188 households. VOC samples were collected from 298 households.
Table 3.2: Levels of indoor air pollutants in the Peel study

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Geometric Mean</th>
<th>Median</th>
<th>Range</th>
<th>BDL n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formaldehyde (µg/m³)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedroom (n = 276)</td>
<td>0.749</td>
<td>2.65</td>
<td>BDL – 21.74</td>
<td>57 (20.65)</td>
</tr>
<tr>
<td>Lounge (n = 272)</td>
<td>0.679</td>
<td>2.31</td>
<td>BDL – 23.91</td>
<td>71 (26.10)</td>
</tr>
<tr>
<td><strong>NO₂ (µg/m³)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Six-hour (n = 188)</td>
<td>N/A</td>
<td>BDL</td>
<td>BDL – 69.36</td>
<td>157 (83.51)</td>
</tr>
<tr>
<td>24-hour (n = 298)</td>
<td>N/A</td>
<td>0.73</td>
<td>BDL – 70.57</td>
<td>139 (46.64)</td>
</tr>
<tr>
<td><strong>VOC (ppb)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVOC (n = 289)</td>
<td>N/A</td>
<td>14.50</td>
<td>BDL – 638.70</td>
<td>24 (8.28)</td>
</tr>
<tr>
<td>BTEX (n = 289)</td>
<td>N/A</td>
<td>3.00</td>
<td>BDL – 145.00</td>
<td>56 (19.38)</td>
</tr>
</tbody>
</table>

n = number of samples in each category  
BDL = below detection limits  
NO₂ = Nitrogen Dioxide, VOC = volatile organic compounds

3.3.3 Factors influencing indoor formaldehyde levels

A moderate correlation was found between bedroom and lounge formaldehyde levels (R² = 0.461, p <0.01).

Formaldehyde levels across different seasons are displayed in Table 3.3. No significant differences between seasons were found.

Table 3.3: Distribution of formaldehyde levels across seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>Bedroom (µg/m³)</th>
<th>Lounge (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Geometric mean</td>
<td>n Geometric mean</td>
</tr>
<tr>
<td>Spring</td>
<td>65 0.578</td>
<td>65 0.540</td>
</tr>
<tr>
<td>Summer</td>
<td>90 0.767</td>
<td>90 0.651</td>
</tr>
<tr>
<td>Autumn</td>
<td>72 0.851</td>
<td>68 0.726</td>
</tr>
<tr>
<td>Winter</td>
<td>48 0.821</td>
<td>47 0.878</td>
</tr>
</tbody>
</table>

n = number of samples in each season  
Age of homes was not associated with domestic formaldehyde levels in either the bedroom or the lounge (Table 3.4).
Table 3.4: Comparison of home age and domestic formaldehyde levels

<table>
<thead>
<tr>
<th>Model</th>
<th>Bedroom (µg/m³)</th>
<th>Lounge (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Geometric Mean</td>
</tr>
<tr>
<td>Home age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>85</td>
<td>0.811</td>
</tr>
<tr>
<td>5-15 years</td>
<td>88</td>
<td>0.701</td>
</tr>
<tr>
<td>&gt;15 years</td>
<td>67</td>
<td>0.820</td>
</tr>
</tbody>
</table>

n = number of samples for each exposure

292 participants provided data on recent home renovations, with 266 and 262 participants also having measured levels of bedroom and lounge formaldehyde respectively. No associations were found between recent home renovation and bedroom or lounge formaldehyde levels (Table 3.5).

Table 3.5: Comparison between formaldehyde levels in homes with and without home renovations

<table>
<thead>
<tr>
<th>Model</th>
<th>n</th>
<th>Geometric Mean (µg/m³)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde (bedroom)</td>
<td>No renovation</td>
<td>188</td>
<td>0.724</td>
</tr>
<tr>
<td></td>
<td>Recent renovation</td>
<td>78</td>
<td>0.775</td>
</tr>
<tr>
<td>Formaldehyde (lounge)</td>
<td>No renovation</td>
<td>186</td>
<td>0.727</td>
</tr>
<tr>
<td></td>
<td>Recent renovation</td>
<td>76</td>
<td>0.544</td>
</tr>
</tbody>
</table>

n = number of samples for each exposure

There were no differences in either bedroom (p = 0.458) or lounge (p = 0.785) formaldehyde levels between rural (n = 37) and urban (n = 238) dwellings.

3.3.4 Factors influencing indoor NO₂ levels

There was a weak, but significant, correlation between six-hour and 24-hour NO₂ levels ($R^2 = 0.234$, $p < 0.01$).
The distribution of NO$_2$ levels across seasons are displayed in Table 3.6. There was a seasonal effect on indoor NO$_2$ levels with 24-hour NO$_2$ exposure ($p < 0.001$), although this was not found with peak NO$_2$ exposures ($p = 0.388$). Similarly, higher levels of NO$_2$ were found in winter compared to the rest of the year for 24-hour ($p < 0.001$) but not peak ($p = 0.198$) exposures.

### Table 3.6: Distribution of NO$_2$ levels across seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>NO$_2$ (24-hour)</th>
<th>NO$_2$ (peak)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Median (µg/m$^3$)</td>
<td>n</td>
</tr>
<tr>
<td>Spring</td>
<td>74</td>
<td>0.934</td>
</tr>
<tr>
<td>Summer</td>
<td>100</td>
<td>0.623</td>
</tr>
<tr>
<td>Autumn</td>
<td>72</td>
<td>BDL</td>
</tr>
<tr>
<td>Winter</td>
<td>50</td>
<td>3.868</td>
</tr>
</tbody>
</table>

n = number of samples for each exposure  
BDL = below detection limit  
NO$_2$ = Nitrogen Dioxide

Distances from homes to main roads were not found to be associated with 24-hour or peak NO$_2$ levels in homes using either 50 m or 250 m cut-off values (Table 3.7).

### Table 3.7: Associations between indoor NO$_2$ and distance between homes and nearest main roadway

| Model | 24-hour | | | Peak | | |
|-------|---------|----------|----------|----------|----------|
|       | n       | Median (µg/m$^3$) | Difference (p-value) | n       | Median (µg/m$^3$) | Difference (p-value) |
|       |         |           |          |       |           |          |
| Geocoding: 50 m cut-off | | | | | | |
| <50 m | 9       | 1.77     | 0.330    | 5    | BDL       | 0.316    |
| >50 m | 288     | 0.69     |          | 182  | BDL       |          |
| Geocoding: 250 m cut-off | | | | | | |
| <250 m | 59     | 0.81     | 0.772    | 34   | BDL       | 0.774    |
| >250 m | 238    | 0.67     |          | 153  | BDL       |          |

n = number of samples for each exposure  
BDL = below detection limit

The numbers of participants with data for usage of unvented gas stoves and NO$_2$ measurements were 283 and 175 for 24-hour and peak NO$_2$ samples, respectively. Data
on unflued gas heater usage was obtained from 298 participants, although only 45 and 24 participants had data on 24-hour and peak NO\textsubscript{2} levels respectively during winter periods.

The effect of unflued gas heater usage on peak NO\textsubscript{2} exposure during winter approached significance (p = 0.056) although the differences observed for 24-hour exposure were not significant (Table 3.8). No associations were found between indoor usage of unvented gas stoves and 24-hour or peak NO\textsubscript{2} levels (Table 3.8). Furthermore, no significant differences were observed between numbers of gas emitting appliances for 24-hour (p = 0.340) or peak (p = 0.147) NO\textsubscript{2} levels.

Table 3.8: Associations between NO\textsubscript{2} levels and unvented gas source exposure

<table>
<thead>
<tr>
<th>Model</th>
<th>Group</th>
<th>n</th>
<th>Median (µg/m\textsuperscript{3})</th>
<th>Maximum (µg/m\textsuperscript{3})</th>
<th>Difference (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unflued gas heater usage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>during the winter period</td>
<td>24 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>7.81</td>
<td>49.54</td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>3.35</td>
<td>23.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>BDL</td>
<td>43.79</td>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>BDL</td>
<td>8.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas stove usage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>BDL</td>
<td>49.54</td>
<td></td>
<td>0.568</td>
</tr>
<tr>
<td>No</td>
<td>257</td>
<td>0.70</td>
<td>70.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>BDL</td>
<td>42.98</td>
<td></td>
<td>0.559</td>
</tr>
<tr>
<td>No</td>
<td>161</td>
<td>BDL</td>
<td>69.36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number of samples for each exposure
BDL = below detection limit
NO\textsubscript{2} = Nitrogen Dioxide

There were no differences in 24-hour NO\textsubscript{2} levels (p = 0.252) between rural (n = 40) and urban (n = 257) dwellings. Similarly, no differences in peak NO\textsubscript{2} levels (p = 0.535) were observed between rural (n = 27) and urban (n = 160) dwellings.
3.3.5 Factors influencing indoor VOC levels

A moderate to strong correlation was found between TVOC and BTEX levels ($R^2 = 0.56, p < 0.01$). This was expected as the BTEX compounds were the compounds measured most consistently in homes and, therefore, constituted the majority of the TVOC in most homes. Significant associations were found between CHCE scores and indoor levels of TVOC, BTEX, Toluene and o-xylene, although none were found with levels of Mp-xylene, $\alpha$-pinene and limonene (Table 3.9). The highest quartile of exposure had significantly higher TVOC levels compared to the lowest quartile of CHCE scores ($p = 0.027$). No significant differences were found for BTEX or individual VOC levels.

Table 3.9: Associations between CHCE scores and VOCs of interest

<table>
<thead>
<tr>
<th>CHCE score</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>Overall</th>
<th>Q1 vs Q4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (ppb)</td>
<td>BDL (%)</td>
<td>Mean (ppb)</td>
<td>BDL (%)</td>
<td>Mean (ppb)</td>
<td>BDL (%)</td>
</tr>
<tr>
<td>TVOC</td>
<td>31.89</td>
<td>NA</td>
<td>19.78</td>
<td>0</td>
<td>27.45</td>
<td>NA</td>
</tr>
<tr>
<td>BTEX</td>
<td>5.81</td>
<td>NA</td>
<td>6.06</td>
<td>0</td>
<td>6.05</td>
<td>NA</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.15</td>
<td>50.67</td>
<td>2.58</td>
<td>64.29</td>
<td>2.26</td>
<td>48.48</td>
</tr>
<tr>
<td>mp-xylene</td>
<td>2.00</td>
<td>29.73</td>
<td>1.94</td>
<td>37.31</td>
<td>2.22</td>
<td>26.15</td>
</tr>
<tr>
<td>O-xylene</td>
<td>0.68</td>
<td>75.68</td>
<td>0.68</td>
<td>83.82</td>
<td>0.57</td>
<td>89.06</td>
</tr>
<tr>
<td>$\alpha$-pinene</td>
<td>1.12</td>
<td>68.00</td>
<td>0.78</td>
<td>71.43</td>
<td>1.33</td>
<td>65.15</td>
</tr>
<tr>
<td>Limonene</td>
<td>2.01</td>
<td>54.67</td>
<td>2.34</td>
<td>52.86</td>
<td>2.15</td>
<td>65.15</td>
</tr>
</tbody>
</table>

BDL = below detection limit
* p-value <0.05 indicates significant differences between categories
CHCE = composite household chemical exposure, VOC = volatile organic compounds, TVOC = total volatile organic compounds, BTEX = benzene, toluene, ethylbenzene and xylene

278 participants provided data on recent home renovations and VOCs. No differences in distributions were observed in comparisons of any volatile organic compounds between participants with (n = 83) and without (n = 195) recent renovations (Table 3.10).
Table 3.10: Associations between recent renovation and VOCs of interest

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recent renovations (median, ppb)</th>
<th>No recent renovations (median, ppb)</th>
<th>Difference (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVOC</td>
<td>14.6</td>
<td>14</td>
<td>0.354</td>
</tr>
<tr>
<td>BTEX</td>
<td>3.8</td>
<td>3</td>
<td>0.327</td>
</tr>
<tr>
<td>Toluene</td>
<td>1</td>
<td>BDL</td>
<td>0.907</td>
</tr>
<tr>
<td>mp-xylene</td>
<td>2</td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td>o-xylene</td>
<td>BDL</td>
<td>BDL</td>
<td>0.284</td>
</tr>
<tr>
<td>α-pinene</td>
<td>BDL</td>
<td>BDL</td>
<td>0.839</td>
</tr>
<tr>
<td>Limonene</td>
<td>1</td>
<td>BDL</td>
<td>0.344</td>
</tr>
</tbody>
</table>

BDL = below detection limit
VOC = volatile organic compounds, TVOC = total volatile organic compounds, BTEX = benzene, toluene, ethylbenzene and xylene

There were no differences in TVOC levels (p = 0.266) between rural (n = 39) and urban (n = 249) dwellings. Similarly, no differences in BTEX (p = 0.744) were observed between rural (n = 39) and urban (n = 249) dwellings.

3.4 Discussion

The levels of each indoor air pollutant sampled in this region were generally low. The factors influencing levels of indoor air pollutants will be discussed individually.

3.4.1 Formaldehyde levels and their influences

Geometric mean formaldehyde levels in this study were 0.749 µg/m³ (bedroom) and 0.679 µg/m³ (lounge). This was substantially lower than that of other studies, as well as the WHO recommendation of no more than 0.1 mg/m³ (80 ppb) over a 30 minute period in the indoor setting for the prevention of sensory irritation. Several studies from Europe and North America have reported levels such as 17 ppb (20.4 µg/m³) [226], 26 ppb (31.2 µg/m³) [35], 11.8 µg/m³ [206] and 33 ppb (39.7 µg/m³) [211]. Other West Australian studies have also reported higher levels of indoor formaldehyde compared to this study [235, 274, 419]. For example, Rumchev et al. [274] reported mean levels of
30.2 and 27.5 μg/m³ of formaldehyde in bedrooms and lounges respectively across 192 households. Similarly, Dingle and Franklin [235] reported daily geometric mean levels of 22.8 ppb (27.4 μg/m³) across 185 households when sampled over three days. A recent study conducted in Melbourne reported mean formaldehyde levels of 11 ppb (13.2 μg/m³) in winter/spring, and 13.5 ppb (16.2 μg/m³) in summer/autumn [228]. Two studies from New South Wales, however, reported low formaldehyde values comparable to this study, with the authors attributing such low levels to regional climatic factors, seasonal variation, population selection biases, and higher detection limits from the passive sampling methodology [236, 454]. The reasons for low formaldehyde concentrations in this study are not fully known but may include reduced use of materials with higher formaldehyde emissions, seasonal variations and ventilation practices, and changes in building practices [419].

The moderate correlation between bedroom and lounge formaldehyde levels agreed with other studies [455-458], including three Perth-based studies [235, 274, 419] that have reported similar levels between rooms. Other studies, however, have reported different levels between rooms [458-461].

In this study, no significant differences between indoor formaldehyde levels were detected across different seasons (Table 3.2). While this finding was similar to that of a few studies, [226, 234, 458], other data from Australia and other developed countries have reported significantly higher formaldehyde levels in warmer compared to cooler seasons [228, 235, 459, 462]. It was likely that the increased formaldehyde levels during summer in those studies were due to both increased off-gassing during periods of higher temperature and relative humidity, as well as poor ventilation in homes [228, 462]. Despite that, it was possible that some households respond to warm weather by opening
windows instead of using indoor air-conditioning systems. This has the effect of increasing ventilation, thereby resulting in reduced levels of indoor formaldehyde [228, 236, 462-465]. Furthermore, the overall low levels found in this study might result in difficulties with detecting seasonal differences.

There was no effect of home age on indoor formaldehyde levels in this study (Table 3.3). While this result was consistent with some studies [226, 234, 290, 455, 466], others have reported a significantly inverse relationship between home age and indoor formaldehyde levels [228, 235, 236, 459, 462, 467], including one study in Perth [235]. There is evidence to suggest that building materials in newer homes may emit a large amount of formaldehyde particularly in its first year, before stabilising with time [359]. Emission rates from building and painting materials after the first year are likely to have stabilised and ceased to influence indoor formaldehyde levels [359]. Home age in this study varied greatly, although the distribution was skewed to the right. 35.4% of homes were less than five years of age, with approximately one-third of those homes (10.6%) being built less than one year from time of sampling. Again the lack of an association between house age and formaldehyde in this study may be due to ventilation practices of the residents.

Another factor that might have influenced these findings could be the shift towards lower formaldehyde emitting products in the past few decades [468]. This followed the rapid reduction in usage of formaldehyde-emitting urea-formaldehyde-foam insulation (UFFI) leading up to the 1970s and 1980s, as well as further emphasis on building codes [468]. Although not discussed explicitly, this could also have accounted for the low levels of formaldehyde in studies in New South Wales [236, 454], and might also explain the low formaldehyde levels in this study, which had a large proportion of
homes (72.1%) aged less than 15 years. This was in agreement with research conducted by the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) which showed that reduction of formaldehyde levels in known formaldehyde emission sources in the home are due to changes in resin technology, improved manufacturing controls for product emission, and reduced usage of products such as pressed wood, which are known to have high formaldehyde emission levels [468].

Recent renovations were not found to influence formaldehyde levels in this study. These findings were consistent with published studies from New South Wales [236] and Victoria [228]. However, other studies have reported associations between domestic formaldehyde levels and recent renovation works such as painting, varnishing, having new furniture, glued wood products and particle boards in the home [211, 234, 274, 290, 461, 467]. These findings could be attributable to the low levels found throughout the study region.

Overall, the findings of this study seem to contradict some evidence which points to significant impacts of season, home age and recent renovations on indoor formaldehyde levels [211, 228, 234, 235].

3.4.2 NO\textsubscript{2} levels and their influences

NO\textsubscript{2} levels in this study were also generally low compared to other known studies in the developed world, as well as the WHO indoor air quality guideline level of 200 μg/ m\textsuperscript{3} hourly, or an annual average of 40 μg/ m\textsuperscript{3}. Such studies have reported mean levels of NO\textsubscript{2} such as 8.3 μg/m\textsuperscript{3} in Canada [234], 6.7 ppb (3.48 μg/m\textsuperscript{3}) in Sweden [469], 8.6 ppb (4.47 μg/m\textsuperscript{3}) in the United States [323], 18 μg/m\textsuperscript{3} in Spain [288] and 8.6 μg/m\textsuperscript{3} in Denmark [38]. Australian studies have also reported levels similar to that of European
and North American studies, with mean NO₂ concentrations such as 16.2 μg/m³ in Perth (geometric mean) [289], 22.6 μg/m³ in Brisbane (geometric mean) [470], 11.6 μg/m³ in the La Trobe valley in Victoria (median) [315] and between 6.8 and 10 μg/m³ during the warmer and cooler months respectively in Melbourne [228].

The finding of a higher peak NO₂ level compared to 24-hourly NO₂ measurements was in agreement with that of other studies in Perth [289] and the Netherlands [212]. This result reflected the intended aim of demonstrating that the use of gas heater and stove use resulted in higher NO₂ levels. However, this was not confirmed by specific information regarding the presence of gas appliances during the sampling period. The weak correlations between 24-hour and peak NO₂ levels were also similar to that from a previous study in Perth [289], as well as an earlier study in the Netherlands [212]. Franklin et al. [289] attributed the lack of a strong correlation between peak and 24-hour NO₂ primarily to the differences in cooking periods and ventilation practices between households. This was supported by a recent study by the CSIRO [228], which also reported daily variations of peak unflued combustion activities.

Seasonal effects were found to influence NO₂ levels in this study, with 24-hour NO₂ higher in winter compared to spring and summer (Table 3.5). This finding was consistent with that of several other studies in the developed world, including those conducted in Australia [228, 275, 288, 290, 318, 467, 471]. Colder temperatures in winter might result in an increased usage of NO₂-emitting gas heaters. This factor, coupled with decreased ventilation, may contribute to a build-up of NO₂ in the indoor environment [228]. Farrar et al. [318] demonstrated in a Perth study that unflued gas heater usage was the major contributor to increased indoor NO₂ levels during winter. Consistent with that finding, this study showed that peak indoor NO₂ levels were higher
in winter in homes with an unflued gas heater but not those without, although the relationship was not significance \( p = 0.056 \).

Unlike 24-hour NO\(_2\) exposures, peak NO\(_2\) levels were not influenced by seasonal effects in our study (Table 3.5). This was likely to be due to the low levels of peak NO\(_2\) found in this study, including the large proportion of samples that were below detectable levels (Table 3.1). Although participants recorded the opening and closing time of the sampling badges, the sampling period might not have coincided with the period of peak unflued gas exposures.

Current knowledge regarding the impact of unflued gas heater usage on domestic NO\(_2\) levels have been inconsistent, with some studies reporting a strong influence \([236, 290, 318]\) and others generally reporting moderate or weak influence \([323, 324, 433, 467]\). This uncertainty might arise from an overreliance of the presence of unflued gas heaters as an indicator of its usage. Other factors which might also influence NO\(_2\) emission levels by gas heaters in households include frequency and duration of use, the size of the room, maintenance of appliances and distance from exposure sources \([290, 316, 318]\).

In this study, differences in NO\(_2\) levels between households which used unvented gas stoves and those which did not were not significant. While this finding was similar to those of several Australian studies \([289, 290, 318]\), studies from other developed countries have reported higher NO\(_2\) levels with increased gas stove use \([212, 227, 234, 236, 288, 323, 324, 462, 467, 470, 472-475]\). Farrar et al. \([318]\) conducted a study in Perth and found similar results to the Peel study. The authors suggested that cooking duration might have been insufficient to influence NO\(_2\) levels. Furthermore, other
factors that could have influenced results in this study were kitchen sizes and venting. While the previous Perth study conducted by Farrar et al. [318] reported that 75% of participants used gas stoves, no information was available on whether they were vented. In the Peel study, the number and proportion of participants which reported use of unvented gas stoves was small. This could have been an additional contributing factor to the results found.

The distance between homes and the closest main roadway did not influence indoor NO2 levels in this study. This was inconsistent with other studies, which have suggested that homes which were nearer to main roadways tend to have higher levels of indoor NO2, especially during the summer months [223, 228, 433]. The finding from this study could be due to main roads in the urban regions of Peel not being heavily trafficked relative to many European, Asian and North American cities where other studies have been conducted [476]. This was consequently reflected in the lower levels of NO2 around the Peel region (10.6 µg/m³) [477] compared to urban centres in Canada (22.9 – 56.4 µg/m³) [478], the Netherlands (30.6 – 47.8 µg/m³) [138] and Italy (40 – 50 µg/m³) [144].

The inverse relationship between distance between closest main roadway and levels of indoor NO2 found in other studies could be attributed to the movement of traffic-emitted NO2 from the outdoor environment into homes through open doors and windows during the summer period [228]. Two studies have supported these findings, showing that higher rates of open doors and windows, as well as greater air exchange rates were found in subpopulations where distance from major main roads significantly influenced indoor NO2 levels [223, 228]. It was possible that the use of distance between homes and main roadways was not a suitable proxy for overall traffic levels in the Peel study
population. The classification of main roads included a variety of factors in addition to traffic volume considerations. Furthermore, traffic volumes around individual homes were difficult to ascertain due to a lack of reliable data.

### 3.4.3 VOC levels and their influences

Median levels of TVOC obtained from this study were comparable to four other studies, including two Australian studies, one of which was from Perth [419, 479-481], although comparisons to other studies remain a difficult task due to wide variations in constituent VOCs and study methodologies between studies. In some studies, the definition of TVOC included only ten or less constituent VOCs [33, 361], while in others, over 20 constituent VOCs were included [208, 229, 397, 419, 480]. The suite used in this study comprised of 32 chemicals which were known to be commonly found in households [27, 29, 221]. The rationale for selecting individual VOCs for further investigation is outlined in section 3.3.2.

The low BTEX levels observed in this study supported the findings of a previous study which reported that Australian cities have low BTEX levels compared to major cities in Europe and the United States, with Perth having one of the lowest levels in the country [481]. More recently, Maisey et al. [419] also found similarly low levels of BTEX in Perth. Possible reasons for the low levels found in Perth include city size, climatic conditions, lower traffic density and lower proportion of benzene in petroleum compared to that of other states [481]. These findings were in line with trends acknowledged in a recent review [26], which reported decreasing domestic BTEX levels over the past few decades in the developed world.
In this study, low levels of α-pinene and limonene were found. This was in contrast to several studies which have found that terpene levels, particularly that of α-pinene and limonene had a significant influence on overall TVOC levels [208, 353, 359, 371, 419, 479, 482]. As α-pinene and limonene are primarily known to be emitted from aerosols such as deodorants and air fresheners, the frequency and intensity of usage will influence emission levels [29, 353]. It is likely that usage of such aerosols is sporadic and might only last for short periods [29, 353]. Furthermore, these compounds are also known to be highly reactive and are therefore unlikely to remain airborne in its natural state for a long period [219, 221, 352]. If measured in larger rooms, dilution of aerosol chemicals could take place rapidly during periods of usage [483-485]. It may be possible that the low levels of α-pinene and limonene in our study was due to the distance between the source point of aerosol usage and our sampling tubes. Furthermore, sampling was also conducted over a continuous seven-day period instead of during peak periods of active chemical usage. Studies which have utilised shorter sampling periods, but have focused on periods of chemical usage have reported higher indoor VOC levels, especially terpenes, compared to the Peel study [353, 419, 483, 484].

A moderate to high correlation was observed between BTEX and TVOC levels in our study ($R^2 = 0.56$, $p < 0.01$), which was expected as the BTEX compounds were the main contributors to TVOC levels in domestic environments. This has also been found in numerous other studies [208, 230, 275, 285, 358, 359, 361, 371, 397, 479, 482, 486-488].
This study demonstrated a significant association between questionnaire data and TVOC levels, although this was less reliable for BTEX and other specific VOCs. This finding reflected the nature of household chemicals containing a wide variety of VOCs rather than a few specific compounds. The CHCE scale for this study was slightly modified from in the Avon Longitudinal Study of Parents and Children (ALSPAC) study, which reported significant associations between prenatal chemical exposure levels and wheezing, as well as non-respiratory health symptoms during early life [52, 91, 360].

Recent renovations, defined as renovation works within one year prior to air monitoring, was shown not to have an influence on indoor VOC levels in this study. This finding was in contrast to several studies which have reported that recent renovation works such as painting, using thinner and having new carpets resulted in increased TVOC or BTEX levels [262, 361, 419, 467, 489]. Another recent study from Perth also showed that homes with current renovations or renovations six months prior to sampling resulted in an increase in TVOC and BTEX levels [419].

The period of time taken for indoor VOC levels to stabilise after renovation works is dependent on various factors such as ventilation and home size, even though this period is unclear, and may vary between two weeks and six months [490, 491]. Furthermore, ventilation rates may have the potential to significantly influence indoor VOC levels in a similar manner to its effect on indoor formaldehyde levels.

3.4.4 Differences between urban and rural dwellings

In the context of the Peel study, the significant differences found for factors such as materials of outer walls, garage types, dwelling types and numbers of bedroom did not result in differences between indoor air pollutant levels in rural and urban populations.
There were also no significant differences in the distribution of home ages and recent renovation between rural and urban dwellings – factors which were described as significantly contributing to different levels of indoor pollutants in the abovementioned studies. Furthermore, there was no evidence that traffic exposure levels between rural and urban dwellings differed significantly in the Peel study, where dwellings were likely to be more than 250 m from main roads, even in urban areas.

In this study, overall levels of indoor air pollutants between participants living in rural and urban areas did not differ significantly (Table 3.9). This finding was in contrast to that of several studies, which have reported higher levels of indoor air pollutants such as NO₂ and VOCs in urban centres compared to rural regions [139, 236, 275, 467, 474, 492]. However, this result should be treated with caution due to the small sample size for rural homes in this study. The most commonly cited reason for higher levels of NO₂ in urban dwellings relate to increased ventilation in conjunction with increased outdoor pollutants from traffic emissions [474, 492]. An alternative explanation which included other indoor air pollutants was proposed in Hulin et al. [275], which suggested that pollutant levels in rural dwellings may be lowered by leakage due to increased indoor-outdoor air circulation resulting from older home age. Contrary to these findings, one study found that formaldehyde and VOC levels were higher in rural compared to urban areas [215]. This was attributed to the higher prevalence of recent renovations in rural households in their study population. Other factors that might have contributed to the lack of significant differences in the Peel study include the low traffic density, limited amount of industry, and low population density in the Peel region [417, 418, 476], compared to that of other studies which included larger cities in Japan [139], France [275], and England [467], that were likely to have more industry and higher population densities.
3.5 Conclusion

While existing studies purport that levels of formaldehyde, NO$_2$ and VOC within the domestic setting are influenced by a number of climatic, household and behavioural factors, this study only confirms some of those findings. Significant determinants of indoor NO$_2$ levels include season and unflued gas heater use during winter periods. Household chemical use was also associated with levels of TVOC, BTEX and several individual VOCs.

It was not one of the primary aims of this study to focus on contributors to indoor air pollution levels. Therefore, only limited information was obtained on housing factors. More detailed questionnaires could also have provided a better understanding of behavioural aspects such as opening and shutting of doors and windows, duration of renovation and usage of cooking and heating sources.
Chapter 4
Impact of Maternal Exposure to Indoor Air Pollution on Birth Outcomes
4.1 Introduction

Fetal development is known to be influenced by a complex interplay between genetic and environmental factors during the prenatal period [493-496]. Events occurring during the prenatal period has the potential to affect long-term postnatal health outcomes [374, 495-499], including respiratory-related outcomes [75, 76, 82, 83, 90, 183-185, 500-506].

Exposure to toxicants during pregnancy has the potential to affect fetal development. For example, maternal smoking can reduce fetal growth, resulting in smaller birth sizes [68, 500, 507]. A large number studies have also linked fetal exposure to several traffic-related air pollutants with newborns that were shorter, lighter, or had reduced head circumference [49, 109-111, 494, 507-517], although this remains inconsistent, with some studies reporting no associations [112, 113, 518].

In addition to single-pollutant exposures, several studies have shown that pollution exposure during pregnancy can affect birth outcomes in models where the effect of individual pollutants included adjustments for the effects of other pollutants by their inclusion in the analysis [28, 175, 519]. These “multiple linear regression models” are commonly used in epidemiological studies to disentangle health effects that have been brought about by individual pollutants where exposure to multiple common pollutants occurred [28, 520-522].

At present, the relationship between indoor air pollutant exposure during pregnancy and birth outcomes are unclear. Existing studies, however, have suggested that increased exposure to outdoor sources of formaldehyde and NO₂, two important indoor pollutants, may result in reduced birth length, weight and head circumference [286, 508, 510, 511].
While the mechanisms behind such associations are not well understood, it was postulated that increased pollutant exposure during pregnancy may result in an inflammatory response, increasing the viscosity of maternal blood, subsequently leading to reduced umbilical and placental blood flow [511, 517]. Additionally, it is also thought that toxic effects arising from pollutant exposure may lead to reduced oxygen transport to the fetus, thereby influencing fetal development [508, 510, 511, 514].

The main aim of this chapter was to explore the effects of indoor levels of formaldehyde, NO$_2$ and VOCs during pregnancy on various birth outcomes such as birth length, weight and head circumference using both single and multiple linear regression approaches. In addition, this chapter also investigated whether presence of unflued gas heaters, traffic pollutant exposure and indoor chemical usage, all as proxies of indoor pollution exposure, could also influence birth outcomes.

4.2 Methods

4.2.1 Subjects and protocol

305 pregnant women were recruited into this study prior to 18 weeks of gestation. A housing inventory questionnaire administered prior to 18 weeks of pregnancy provided information regarding usage of unflued gas heaters and chemical exposures during pregnancy. Sampling of indoor air pollutants were conducted in the homes of participants on one occasion only at 34 weeks of gestation. Passive sampling methodologies were used to measure indoor levels of formaldehyde, NO$_2$ and VOCs. The in-depth protocols for preparation and analysis of monitors can be found in Chapter Two.
Formaldehyde sampling was conducted over a period of 24 hours in both the bedroom and the lounge, although this was later changed to seven days, as described in Chapter Two. Sampling of NO₂ was conducted in the kitchen over six and 24-hour periods to determine peak and daily averages respectively, although the latter was modified to seven days for part of the study. VOCs were sampled using Tenax tubes over a seven day period in the lounge or main activity area of the home.

At birth, hospital staff completed a notification of case attended (NOCA) form as required by the Department of Health. Information on details such as gestational age, birth weight, birth length and head circumference of the newborn was included. Ethics was obtained to permit access to these forms in this study.

4.2.2 Techniques

4.2.2.1 Housing questionnaires

The housing inventory questionnaire provided information regarding the home environment during pregnancy. Participants were asked about their primary heating systems in order to ascertain usage of unflued gas heaters. In addition, the behavioural and temporal usage of household chemicals was captured using composite household chemical exposure (CHCE) scores. This was provided in more detail in Chapter Three.

4.2.2.2 GIS modelling

Distance from home to main roads was used as a proxy for traffic pollutant exposure. This was obtained by geocoding, a method which uses data obtained from geographic information systems (GIS). The distance from homes to the closest main road was calculated from maps obtained from landgate (Western Australia) using a specialised
computer software package (ARC GIS version 10, Esri, California, 2010). Main roads were classified by landgate based on the Western Australian Main Roads Act (1930).

4.2.2.3 Indoor air monitoring

Monitoring of indoor air levels was obtained using passive sampling techniques. This involved the preparation of air monitors impregnated with compounds that react with indoor air pollutants of interest when exposed to room air. Subsequently, levels of indoor air pollutants can be quantified by techniques such as high pressure liquid chromatography (HPLC), gas chromatography and mass spectrometry (GC/MS). The detailed protocol on preparation of air monitors and analyses to quantify levels of formaldehyde, NO₂ and VOCs can be found in Chapter Two. The calculation for overall TVOC, as well as BTEX levels can be found in Chapter Three.

4.2.3 Statistical analysis

Statistical analyses were run using SPSS version 20.0.0 (IBM Corp, 2011).

Birth outcomes were reported as z-scores based on recently revised birth centile charts from the WHO [523]. These charts reported the mean and standard deviations of newborns based on gender and gestational age. The WHO birth data included 9443 weights, 985 lengths and 1841 head circumferences. Z-scores for participants in the Peel study were calculated using the following formula:

\[ Z\text{-score} = \frac{O - E}{SD} \]

where:

\( O \) = Participant’s score

\( E \) = Mean score from WHO birth centile charts, based on gender and gestational age

\( SD \) = Residual standard deviation from WHO birth centile charts
4.2.3.1 Effects of season on birth outcomes

As the previous chapter showed differences in 24-hour NO\textsubscript{2} levels during different seasons, an investigation on the potential influence of season on fetal development was explored. ANOVA was used to explore the effect of season of birth on birth outcomes. Season of birth was classified with spring being from September to November, summer being from December to February, autumn being from March to May, and winter being from June to August.

4.2.3.2 Effect of unflued gas heater presence on birth outcomes

The association between unflued gas heater presence and birth outcomes were also explored. As presence of unflued gas heaters was recorded as a dichotomous variable (yes/no), students’ T-tests were used to examine its effect on each birth outcome.

4.2.3.3 Effect of indoor chemical usage on birth outcomes

The effect of indoor chemical usage on birth outcomes was examined, with CHCE scores as the independent variable. A univariate linear regression model was constructed to investigate its relationship with each birth outcome. A multivariate linear regression model which adjusts for the effects of gender and gestational age was also constructed.

4.2.3.4 Effect of traffic pollutant exposure on birth outcomes

The distance between participants’ home and nearest main road was classified as an ordinal variable in the following categories of distance: 0 – 49 m, 50 – 99 m, 100 – 249 m, 250 – 499 m, 500 – 999 m and > 1000 m. The relationship between traffic pollutant exposure and birth outcomes was explored using ANOVA to compare differences
between groups, as well as univariate linear regression with distance categories as the independent variable. Z-scores were used for individual birth outcomes.

4.2.3.5 The influence of indoor air pollutant levels on birth outcomes

The impact of formaldehyde, NO₂ and VOCs on physical development during the fetal period were investigated using single and multiple linear regression models. Formaldehyde levels in the bedroom and lounge, levels of 24-hour and peak NO₂, as well as TVOC and BTEX were log-transformed to approximate a normal distribution of the standard residuals in the linear regression analyses. In addition, “average” formaldehyde levels, obtained by averaging the bedroom and lounge levels of each participant, was also obtained and log-transformed prior to linear regression.

The relationship between each pollutant and birth outcome was investigated using univariate linear regression models with z-scores for individual birth outcomes as the dependent variable. Thereafter, a multiple linear regression model was constructed with average formaldehyde, NO₂ and TVOC as independent variables and birth outcome z-scores as the dependent variable. In addition, the multiple linear regression model also adjusted for proxies of traffic pollutant exposure.
4.3 Results

4.3.1 Descriptive statistics

The distribution for gestational age, birth length, birth weight and head circumference were approximately normal and presented in Table 4.1.

CHCE scores were obtained from 290 participants and were normally distributed. Participants recorded a mean score of 17.17 (SD 6.89), with scores ranging from 0 to 34.

The number of participants with indoor air pollution data and birth outcomes data for birth length, birth weight and birth head circumference were 232, 233, and 229 respectively.

Table 4.1: Distributions of birth outcomes in the Peel cohort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (z-score)</th>
<th>Range (z-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (days)</td>
<td>259</td>
<td>272.90 (7.34)</td>
<td>252 - 290</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Birth Length (cm)</td>
<td>265</td>
<td>51.33 (2.50)</td>
<td>44 - 59</td>
<td>0.78</td>
<td>-3.24 – 3.96</td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>266</td>
<td>3513.47 (436.08)</td>
<td>2835 - 4925</td>
<td>0.47</td>
<td>-1.95 – 3.3</td>
</tr>
<tr>
<td>Head Circumference (cm)</td>
<td>261</td>
<td>34.86 (1.45)</td>
<td>30 - 40</td>
<td>0.65</td>
<td>-2.5 – 4.02</td>
</tr>
</tbody>
</table>

n = number of infants with birth data in each category
SD = residual standard deviation

A high proportion of participants (92.1%) in this study lived over 100 m away from the nearest main road, with the majority (77.3%) living between 250 and 1000 m from the main road. The numbers of participants for each category of distance, as well as the number of participants with both traffic exposure and birth outcome data are presented in Table 4.2.
Table 4.2: Numbers of participants with GIS and birth outcome data

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>n</th>
<th>Birth Length</th>
<th>Birth Weight</th>
<th>Birth Head Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 49</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>50 - 99</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>100 - 249</td>
<td>40</td>
<td>28</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>250 - 499</td>
<td>95</td>
<td>82</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>500 - 999</td>
<td>100</td>
<td>84</td>
<td>86</td>
<td>82</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>45</td>
<td>40</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>304</td>
<td>257</td>
<td>258</td>
<td>254</td>
</tr>
</tbody>
</table>

n = number of participants with GIS and birth outcome data
GIS = geographic information systems

4.3.2 Effect of season on birth outcomes

Seasonal data was available for 303 participants, out of which 66, 98, 87, and 52 infants were born during spring, summer, autumn, and winter respectively. No significant differences for birth length (p = 0.815), birth weight (p = 0.512), or head circumference (p = 0.606) between infants born during different seasons.

4.3.3 Effect of unflued gas heater presence on birth outcomes

Out of the participants who provided birth outcome data, 107 reported having unflued gas heaters in their homes while 144 did not. No significant differences were detected for birth length (p = 0.861), birth weight (p = 0.717), or head circumference (p = 0.888) between the groups.

4.3.4 Effect of chemical usage on birth outcomes

Out of the 290 participants providing CHCE scores in this study, birth outcome data was also available for 256 (birth length), 257 (birth weight) and 252 (birth head circumference) participants. No associations were found between CHCE scores and birth length (p = 0.135), birth weight (p = 0.164) and birth head circumference (p = 0.207).
4.3.5 Effect of proximity to roads on birth outcomes

No significant associations were found between different exposure levels in ANOVA for birth length (p = 0.388), birth weight (p = 0.483), or head circumference (p = 0.628). This was consistent with findings from linear regression which also revealed no association with birth length (p = 0.298), birth weight (p = 0.636), or head circumference (p = 0.829).

4.3.6 Effect of indoor air pollutants on birth outcomes

Several associations were found with the single-pollutant models. Bedroom formaldehyde was inversely associated with head circumference (p = 0.025) (Table 4.3; Figure 4.1). This was also seen with lounge formaldehyde levels which approached significance (p = 0.062) (Table 4.4), as well as the averaged formaldehyde levels (p = 0.051) (Table 4.5). An inverse relationship between bedroom formaldehyde levels and birth weight also approached significance (p = 0.055) (Table 4.3). No associations were found between formaldehyde or NO₂ levels and birth outcomes (Table 4.4; Table 4.5; Table 4.6; Table 4.7). Positive associations were found between birth length and both TVOC (p = 0.036) (Table 4.8; Figure 4.2) and BTEX (p = 0.017; Figure 4.3) (Table 4.9) levels.

Table 4.3: Associations between bedroom formaldehyde levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>246</td>
<td>-0.021</td>
<td>0.753</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>247</td>
<td>-0.124</td>
<td>0.055</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>243</td>
<td>-0.146</td>
<td>0.025*</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)
* p-value <0.05 indicates significant findings
Figure 4.1: Association between bedroom formaldehyde levels and head circumference at birth

![Figure 4.1 Scatterplot showing the association between formaldehyde levels in the bedroom on the (lnFormBR) and head circumference at birth (BHCZ). Closed circles represent each participant.](image)

Table 4.4: Associations between lounge formaldehyde levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>242</td>
<td>-0.017</td>
<td>0.797</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>243</td>
<td>-0.087</td>
<td>0.185</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>239</td>
<td>-0.123</td>
<td>0.062</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)

Table 4.5: Associations between averaged formaldehyde levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>242</td>
<td>-0.017</td>
<td>0.795</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>243</td>
<td>-0.106</td>
<td>0.105</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>239</td>
<td>-0.128</td>
<td>0.051</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)
Table 4.6: Associations between 24-hour NO\textsubscript{2} levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>197</td>
<td>-0.036</td>
<td>0.619</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>199</td>
<td>0.051</td>
<td>0.483</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>194</td>
<td>-0.007</td>
<td>0.922</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)
NO\textsubscript{2} = Nitrogen Dioxide

Table 4.7: Associations between peak NO\textsubscript{2} levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>173</td>
<td>0.063</td>
<td>0.418</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>172</td>
<td>0.062</td>
<td>0.428</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>172</td>
<td>0.076</td>
<td>0.329</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)
NO\textsubscript{2} = Nitrogen Dioxide

Table 4.8: Associations between TVOC levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>252</td>
<td>0.134</td>
<td>0.036*</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>253</td>
<td>0.009</td>
<td>0.893</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>248</td>
<td>-0.008</td>
<td>0.905</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)
TVOC = total volatile organic compounds
* p-value <0.05 indicates significant findings

Table 4.9: Associations between BTEX levels and birth outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Length</td>
<td>252</td>
<td>0.153</td>
<td>0.017*</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>253</td>
<td>0.009</td>
<td>0.884</td>
</tr>
<tr>
<td>Head Circumference</td>
<td>248</td>
<td>0.013</td>
<td>0.842</td>
</tr>
</tbody>
</table>

n = number of infants with indoor air pollutant and birth data (z-scores)
BTEX = benzene, toluene, ethylbenzene and xylene
* p-value <0.05 indicates significant findings
Figure 4.2: Association between TVOC levels and birth length

![Figure 4.2 Scatterplot showing the association between total volatile organic compound (lnTVOC) and length at birth (BLZ). Closed circles represent each participant.](image)

Figure 4.3: Association between BTEX levels and birth length

![Figure 4.3 Scatterplot showing the association between total levels of benzene, toluene, ethylbenzene and xylene (lnBTEX) and length at birth (BLZ). Closed circles represent each participant.](image)

The multiple linear regression models showed that TVOC levels were significantly associated with birth length ($p = 0.032$), even after adjustment for other indoor pollutants (Table 4.10). The inverse relationship between formaldehyde and head circumference also remained significant ($p = 0.025$).
Table 4.10: Effect of individual IAP on birth outcomes in a multiple linear regression model (adjusted for GIS distance to main road)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Birth length</th>
<th>Birth weight</th>
<th>Head circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standardised</td>
<td>p-Value</td>
<td>Standardised</td>
</tr>
<tr>
<td></td>
<td>β-coefficient</td>
<td></td>
<td>β-coefficient</td>
</tr>
<tr>
<td>Formaldehyde (averaged)</td>
<td>-0.039</td>
<td>0.576</td>
<td>-0.129</td>
</tr>
<tr>
<td>NO₂</td>
<td>-0.066</td>
<td>0.326</td>
<td>0.050</td>
</tr>
<tr>
<td>TVOC</td>
<td>0.151</td>
<td></td>
<td>0.063</td>
</tr>
<tr>
<td>GIS</td>
<td>0.009</td>
<td>0.890</td>
<td>0.049</td>
</tr>
</tbody>
</table>

* p-value <0.05 indicates significant findings, presented as z-scores

NO₂ = Nitrogen Dioxide, TVOC = total volatile organic compounds
GIS = geographic information systems

4.4 Discussion

To our knowledge, this was the first study exploring the effects of maternal exposure to indoor air pollutants on birth outcomes. Our findings suggested a possible relationship between indoor levels of formaldehyde during pregnancy and the likelihood of having a reduced head circumference at birth. Conversely, a positive linear relationship was found with levels of TVOC, as well as BTEX (separately), and birth length.

Formaldehyde levels in the bedroom were inversely associated with head circumference at birth for both the univariate and multivariate analyses. The association between lounge formaldehyde levels and head circumference at birth also approached significance. Several studies which have reported that brain development is of utmost importance during the fetal growth period, even occurring at the expense of fetal weight gain, although these were not conducted specifically for air pollutant exposures [524-527]. Although evidence relating maternal formaldehyde exposure and fetal birth outcomes remain scarce, a study by Maroziene and Grazuleviciene [286] reported that outdoor formaldehyde levels were inversely associated with birth weight. While that study used outdoor formaldehyde levels as a marker for residential exposure, levels in
that study were also very low (3.14 (SD 2.36) µg/m³) and comparable to that of the Peel cohort.

Despite significant findings, the relationship between prenatal formaldehyde exposure and birth outcomes remains uncertain. A detailed review by Collins et al. [528] reported that distant site effects for low formaldehyde exposure was unlikely, given its ready detoxification into formate shortly after inhalation. More recently, a meta-analysis by Duong et al. [529] suggested potential mechanisms that may underlie the effect of formaldehyde on reproductive outcomes. These may include genotoxicity, oxidative stress, disruption of the protein, enzyme and hormonal activity and DNA methylation. However, in addition to the assumption of high formaldehyde exposure levels, these proposed mechanisms are hypothetical and have not been adequately validated [529]. Furthermore, the concentration of formaldehyde that has been shown to contribute to adverse health effects would be around the range of 50 - 100 ppb [35, 262, 274, 276, 530], far above levels found in this study.

No associations were found between 24-hour or peak NO₂ exposure and any measure of birth outcome in this study. Several studies have explored the impact of fetal exposure to outdoor NO₂ levels and physical parameters at birth, although findings have been inconsistent [111, 112, 286, 508, 510-512, 514, 515, 518]. It was likely that NO₂ levels in these studies reflect a component of vehicular exhaust, and may not be the key component. In the indoor environment however, NO₂ is known to be the primary pollutant from gas appliances.

The lack of finding in this study was most likely due to the low NO₂ levels in this study, especially given that a large number of samples were below detection limits. Factors
which were associated with NO\textsubscript{2} levels such as season and presence of unflued gas heaters were also not found to be linked to birth outcomes.

Levels of TVOC and BTEX were positively associated with birth length in this study, although no associations with birth weight or head circumference were found. The relationship between VOC levels and birth length was shown in the multiple linear regression model to remain significant even after adjusting for levels of other pollutants. No association, however, was observed between CHCE scores and birth outcomes.

To date, there have only been a small number of studies on the effect of VOC exposures on birth outcomes. Aguilera et al. [531] and Forand et al. [532] showed that BTEX and trichloroethylene (TCE) exposure, respectively, during pregnancy were associated with reduced birth weight while Estarlich et al. [510] found no associations with exposure to benzene. Unlike measures of birth weight, length at birth had not received as much interest in studies investigating VOC exposures and birth outcomes. A few studies, however, have found that increased maternal exposure to other pollutants were associated with reduced birth length [49, 508, 510]. At present, possible causal mechanisms linking VOC levels to increased birth length remains unknown. This finding from this study was anomalous, and does not support the commonly accepted understanding that environmental toxicants typically hinder fetal growth, instead of stimulating it.

While this finding may be anomalous, there could be a biologically plausible explanation for such a finding. It is possible that certain VOCs may be able to stimulate fetal development indirectly by mechanisms such as the triggering of growth factors or altering of epigenetic factors. Although regulation of fetal growth involves multiple
factors, it is believed that insulin-like growth factors (IGF) and their associated binding proteins (IGFBP) have a significant influence on fetal growth. Current evidence suggests that factors such as IGFBP-2 and IGFBP-1 are associated with decreased birth lengths, while others such as acid-labile subunit (ALS), leptin, IGF-1 and IGFBP-3 are linked to increased birth length [533-535]. The potential for these factors to impact upon fetal development is known to be influenced particularly by genetics and environmental acidity levels [536-538]. As such, if certain VOCs are able to influence these factors via mechanisms that may stimulate genetic mutations or alter acidity levels, increased fetal growth might result, accounting for the anomalous finding in this study. Currently, however, the potential for these mechanisms to occur remains speculative at best as this area of research remains largely unexplored.

There was also no seasonal effect on birth outcomes in this study. This finding agreed with one other study [539], although findings from other studies, including a study from Perth, reported associations between warmer seasons and reduced birth weight [540-542], or peak birth weights during spring and autumn [543]. Although the reasons for these findings remain unclear, these results show that seasonal or temperature differences do not significantly contribute to differential development during the fetal period. Similar findings were also found by McGrath et al. [543], which reported less pronounced seasonal differences in birth weight in Australian cities further from the equator compared to areas in Australia with warm, subtropical climates.

A unique feature of this study was the use of multiple-pollutant models to explore the health effects of prenatal exposure to individual indoor air pollutants, which has not been used in indoor air pollutant studies. Such models include several pollutants in the analysis, as opposed to focusing on the health effects of one pollutant. There has been
recent interest in considering a framework of multiple pollutant systems by bodies such as the United States’ Environmental Protection Agency (EPA) and the Health Effects Institute (HEI) [544, 545]. Furthermore, independent effects of individual pollutants can be difficult to establish due to several factors, such as the relationship dynamics between different pollutants, common sources, and the influence of environmental conditions [545]. Although the use of this statistical method remains relatively uncommon, several recent studies have employed similar multiple linear regression models to explore the impact of outdoor pollutants on lung function outcomes [28, 175, 519].

4.5 Conclusion

Overall, the findings of this study supported that of some existing studies. Results of this study provided some evidence that even at low levels, differences in indoor air pollution levels may have a weak but significant influence on fetal development. These findings, however, could have occurred by chance, as current understanding of potential pathways by which indoor air pollution may affect birth outcomes remain inadequate. Further research into this area is required to attain a better understanding of how indoor pollutant exposure may influence fetal development.
Chapter 5

Impact of Maternal Exposure to Indoor Air Pollution on Early Life Respiratory Function
5.1 Introduction

Growth during the fetal period has been shown to play a critical role in childhood growth and development [374, 497, 498]. Poor health outcomes in early life have also been shown to be associated with a wide range of chronic diseases in adult life [187, 495]. As lung development commences prenatally, fetal development can be crucial in determining long-term lung function outcomes [546]. The influence of early life lung function on long-term respiratory health has been demonstrated in several studies [53, 58-61].

A number of studies have shown that prenatal exposure to outdoor air pollutants [50, 87, 92] and household chemicals [52] can contribute to poorer lung function and respiratory outcomes throughout infancy and childhood. The impact of household chemical exposure during pregnancy and lung function in later childhood has been explored in one study [52]. This study found a weak but significant relationship between household chemical use during pregnancy and reduced FEV1 in children at age of 8 years [52]. Overall, however, the relationship between exposure to household pollutants and lung function and development relationship remains unclear. This is an area of ongoing concern, as people living in urban environments spend the majority of their time, on average, in an indoor environment [24, 31, 32, 189, 219-221]. Pregnant women spend the majority of their time indoors and this proportion increases as pregnancy progresses [189, 190]. Therefore, the indoor environment is where exposure to air pollutants will predominantly occur. It is unknown if exposure to indoor pollutants during this time can influence fetal development.

The main aim of this chapter was to explore the effects of indoor levels of formaldehyde, NO2 and VOCs during the third trimester of pregnancy on lung function.
outcomes at six weeks of age using both objective and subjective (questionnaire) exposure measures. In addition, the impact of prenatal exposures to traffic pollutants on lung development during infancy was also explored.

5.2 Methods

5.2.1 Subjects and protocol

305 pregnant women were recruited into this study prior to 18 weeks of gestation. A housing inventory questionnaire administered at recruitment provided information regarding chemical exposures during pregnancy. Sampling of indoor air pollutants were conducted in the homes of participants on one occasion only at 34 weeks of gestation. Passive sampling methodologies were used to measure indoor levels of formaldehyde, NO₂ and VOCs. The in-depth protocols for preparation and analysis of monitors can be found in Chapter Two.

Formaldehyde sampling was conducted over a period of 24 hours in both the bedroom and the lounge, although this was later changed to seven days, as described in Chapter Two. Sampling of NO₂ was conducted in the kitchen over six and 24-hour periods to determine peak and daily averages respectively, although the latter was later changed to seven days, as previously described. VOCs were sampled using Tenax tubes over a seven day period in the lounge or main activity area of the home.

At the age of six weeks, lung function tests were conducted in the Peel Community Health Centre. Tests conducted included tidal breathing (TB), multiple breath washout (MBW) and fractional exhaled nitric oxide (FeNO). At the time of testing a questionnaire was completed by the mother, providing information about family history
of asthma, allergies as well as feeding habits and respiratory symptoms between birth and six weeks of age.

5.2.2 Techniques

The techniques used for this study has been described in greater detail in previous chapters. Chapter Two included the detailed protocol on preparation of air monitors and analyses to quantify levels of formaldehyde, NO₂ and VOCs. This included the calculation for overall TVOC, as well as BTEX levels. Similarly, details regarding infant lung function tests can be found in Chapter Two. The methods for CHCE calculation can be found in more detail in Chapter Three. This section only provides a brief description of the following techniques involved in this study.

5.2.2.1 Housing questionnaires

The housing inventory questionnaire, completed at recruitment, provided information regarding the home environment during pregnancy. Participants were asked about the frequency of household chemical use. CHCE scores were then calculated for each participant to determine overall chemical exposure during pregnancy.

5.2.2.2 GIS

The approach for obtaining traffic pollutant exposure was the same as Chapter Four. The distance from home to main roads was used as a proxy for traffic pollutant exposure. Distances were calculated using geocoding from maps obtained from landgate (Western Australia) with a specialised computer software package (ARC GIS version 10, Esri, California, 2010). Main roads were classified by landgate based on the Western Australian Main Roads Act (1930).
The distance between participants’ home and nearest main road was classified as an ordinal variable with the following distance categories: 0 – 49 m, 50 – 99 m, 100 – 249 m, 250 – 499 m, 500 – 999 m and > 1000 m.

5.2.2.3 Indoor air monitoring
Monitoring of indoor air levels was obtained using passive sampling techniques. This involved the preparation of air monitors impregnated with compounds that react with indoor air pollutants of interest when exposed to room air.

5.2.2.4 Infant lung function
Infant lung function testing was conducted using the Exhalyser set-up (Ecomedics, Duernten, Switzerland) with an ultrasonic flowmeter. A size 0 facemask (Timesco, TDM-MP-1500, London) was placed over the infant’s nose and mouth with a leak-free seal and connected to the flow head with a continuous bias flow of medical air. Tidal breathing and fractional exhaled nitric oxide tests were run simultaneously over a minimum of thirty breaths. This was followed by the multiple breath washout test. The primary outcomes of interest for the tidal breathing measurements were \( V_T \), RR, \( Ti/T_{tot} \), tPTEF/tE and \( V_T/Ti \), fractional exhaled nitric oxide tests were used to obtain FeNO and NO output and multiple breath washout tests were used to obtain FRC and LCI.

5.2.3 Statistical analysis
Lung function outcomes are reported as z-scores based on regression equations reported from a cohort of healthy infants of a similar age group and using the same equipment and techniques [442] and as shown in Table 5.1. This was to adjust for possible
influence of demographics known to affect infant lung function. Z-scores were calculated using the formula:

\[ Z\text{-score} = \frac{O - E}{SD} \]

where:

\( O \) = Participant’s lung function result
\( E \) = Expected lung function derived from regression equations
\( SD \) = Residual standard deviation of the regression equations

Table 5.1: Regression equation for expected values of lung function in infants

<table>
<thead>
<tr>
<th>Factor</th>
<th>Equation</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT (Males)</td>
<td>20.302 + 3.040*study weight (kg)</td>
<td>1.73</td>
</tr>
<tr>
<td>VT (Females)</td>
<td>-59.464 + 1.088<em>gestational age (weeks) + 0.857</em>birth length (cm)</td>
<td>2.64</td>
</tr>
<tr>
<td>RR (Males)</td>
<td>30.569 + 3.040*study weight (kg)</td>
<td>1.81</td>
</tr>
<tr>
<td>RR (Females)</td>
<td>223.938 – 2.456<em>gestational age (weeks) – 1.410</em>birth length (cm) + 7.924*caesarean section (no = 0, yes = 1)</td>
<td>5.77</td>
</tr>
<tr>
<td>VT/Ti (Males)</td>
<td>84.953 + 11.868<em>study weight (kg) – 1.488</em>study length (cm)</td>
<td>5.29</td>
</tr>
<tr>
<td>VT/Ti (Females)</td>
<td>52.070 + 6.827*maternal asthma (no = 0, yes = 1)</td>
<td>1.99</td>
</tr>
<tr>
<td>FeNO</td>
<td>1.923 + 1.171 + 0.447<em>study length (cm) – 0.009</em>minute ventilation (ml/min^-1)</td>
<td>2.50</td>
</tr>
<tr>
<td>FRC</td>
<td>-10.529 + 6.185<em>study weight (kg) + 1.735</em>birth length (cm)</td>
<td>5.90</td>
</tr>
</tbody>
</table>

SD = residual standard deviation, \( VT \) = tidal volume, \( RR \) = respiratory rate, \( VT/Ti \) = mean tidal expiratory flow, \( FeNO \) = fractional exhaled nitric oxide, \( FRC \) = functional residual capacity

Raw scores of tPTEF/tE, Ti/Ttot and LCI were used for analyses rather than z scores. Fuchs et al. [442] reported that tPTEF/tE and LCI were not significantly influenced by relevant demographic or other risk factors. The effect of demographics and risk factors on Ti/Ttot was not investigated in the study by Fuchs et al. and therefore Ti/Ttot was assessed as absolute numbers.

5.2.3.1 Effect of traffic pollutant exposure on infant lung function

The relationship between traffic pollutant exposure and infant lung function was explored using univariate linear regression.
5.2.3.2 The influence of indoor air pollution on infant lung function

The levels of indoor air pollutants (formaldehyde, NO₂ and VOCs) were log-transformed to have an approximately normal distribution of the standard residuals in linear regression analyses. The influence of fetal exposure to indoor air pollution on early life lung function was explored using both single and multiple linear regression models. Univariate linear regression models were constructed to explore the effects of each indoor pollutant on each lung function variable of interest in the single-pollutant models, in the same manner described and used in Chapter Four.

In addition to the influence of individual pollutants, multiple linear regression models were constructed. Multiple linear regression models involved the use of linear regression which included formaldehyde, NO₂ and VOCs as independent variables for each outcome of interest. As two measures of formaldehyde were provided, levels of bedroom and lounge formaldehyde were averaged in the same manner described and used in Chapter Four. Only 24-hour NO₂ levels were used for the multiple linear regression model. The analysis also adjusted for the distance from home to main roads, which was included in the model as an ordinal variable. Statistical analyses were run using SPSS version 20.0.0 (IBM Corp, 2011).

5.3 Results

5.3.1 Descriptive statistics

A total of 305 participants were recruited for this study. After subsequent exclusions (presented in Figure 5.1), 239 appointments for infant lung function testing were scheduled, of which 189 participants attended on the day of testing. Despite reminders being sent to all families prior to the ILF appointment, 50 participants did not show up on the day of their test. Research staff attempted to contact participants that were absent
on the day of testing. While the reasons for the absences were unknown in some cases, other commonly reported reasons include not remembering about the appointment on the day of test, having inadequate rest on the night prior to day of testing, and illness of infant. This was unlikely, however, to have an impact on the results, as no significant differences in indoor air pollutant levels, child’s sex and mode of delivery were detected between participants with and without lung function data (Table 5.2). The numbers for each infant lung function test collected, accepted and subsequently used for analysis are presented in Figure 5.1.

Figure 5.1: Flowchart of infant lung function collection
Table 5.2: Differences between participants with and without lung function tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participants with ILF</th>
<th>Participants without ILF</th>
<th>( P )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Formaldehyde (Bedroom) (µg/m³)</td>
<td>108</td>
<td>4.12</td>
<td>3.81</td>
</tr>
<tr>
<td>Formaldehyde (Lounge) (µg/m³)</td>
<td>108</td>
<td>4.31</td>
<td>3.93</td>
</tr>
<tr>
<td>NO₂ (24-hours) (µg/m³)</td>
<td>111</td>
<td>4.73</td>
<td>8.98</td>
</tr>
<tr>
<td>NO₂ (peak) (µg/m³)</td>
<td>75</td>
<td>9.08</td>
<td>11.52</td>
</tr>
<tr>
<td>TVOC (ppb)</td>
<td>107</td>
<td>27.16</td>
<td>39.84</td>
</tr>
<tr>
<td>BTEX (ppb)</td>
<td>107</td>
<td>6.66</td>
<td>8.65</td>
</tr>
<tr>
<td>CHCE scores</td>
<td>107</td>
<td>17.47</td>
<td>7.23</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal delivery</td>
<td>47</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Forceps/Suction</td>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caesarian section</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n = number of participants, SD = residual standard deviation, NO₂ = Nitrogen Dioxide, TVOC = total volatile organic compounds, BTEX = benzene, toluene, ethylbenzene and xylene, CHCE = composite household chemical exposure.

Data collection success rates for each test were primarily influenced by the ability of the infant to reach a state of uninterrupted quiet sleep. The success rate for MBW tests were also lower (39.2%), as it was the most time-consuming and technically challenging test. Furthermore, consent rates for MBW were lower than that of other tests as this test involved the use of SF₆ and despite being informed of the low risks of this test, some participants were unwilling to give consent for this test. Reasons for subsequent exclusion of some data files included excessive leak, defined as more than as more than 2.5 ml/s⁻¹ (n = 72), inconsistent breathing patterns during rapid eye movement (REM) sleep (n = 125), spontaneous sighs (n = 139), insufficient data for multiple breath
washout (n = 17) and technical issues with the equipment or software package (n = 28) during data collection.

Distributions for indoor air pollutants, CHCE scores and potential confounders have been described in Chapters Three and Four. Lung function outcomes displayed either a normal or attained a near-normal distribution following transformation to their natural logarithms.

The distribution of distance between homes and main roads has been described in Chapter Four. The numbers of participants with both GIS and infant lung function data are as presented in Table 5.3.

Table 5.3: Numbers of participants with GIS and lung function data

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>TB n</th>
<th>FeNO n</th>
<th>MBW n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 49</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>50 - 99</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>100 - 249</td>
<td>11</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>250 - 499</td>
<td>40</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>500 - 999</td>
<td>31</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>21</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>68</td>
<td>44</td>
</tr>
</tbody>
</table>

n = number of participants
GIS = geographic information systems
TB = tidal breathing test, FeNO = fractional exhaled nitric oxide test, MBW = multiple breath washout test

As this study involved several indoor air pollutants and lung function tests with varying success rates, numbers of participants which provided information for each exposure and outcome variable are presented in Table 5.4.
Table 5.4: Numbers of participants with pollutant and lung function data

<table>
<thead>
<tr>
<th>Pollutant/Measure</th>
<th>TB n</th>
<th>FeNO n</th>
<th>MBW n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde (Bedroom)</td>
<td>109</td>
<td>67</td>
<td>41</td>
</tr>
<tr>
<td>Formaldehyde (Lounge)</td>
<td>109</td>
<td>67</td>
<td>41</td>
</tr>
<tr>
<td>NO₂ (24-hours)</td>
<td>111</td>
<td>67</td>
<td>43</td>
</tr>
<tr>
<td>NO₂ (peak)</td>
<td>78</td>
<td>64</td>
<td>23</td>
</tr>
<tr>
<td>TVOC</td>
<td>108</td>
<td>65</td>
<td>41</td>
</tr>
<tr>
<td>BTEX</td>
<td>108</td>
<td>65</td>
<td>41</td>
</tr>
<tr>
<td>CHCE scores</td>
<td>107</td>
<td>64</td>
<td>41</td>
</tr>
</tbody>
</table>

n = number of participants, NO₂ = Nitrogen Dioxide, TVOC = total volatile organic compounds, BTEX = benzene, toluene, ethylbenzene and xylene, CHCE = composite household chemical exposure
TB = tidal breathing test, FeNO = fractional exhaled nitric oxide test, MBW = multiple breath washout test

5.3.2 Effects of proximity to roads on infant lung function

Univariate linear regression revealed a positive relationship between proximity to roads, and RR (Table 5.5). No other associations were found between maternal traffic exposure and infant lung function tests.

Table 5.5: Effects of proximity to main roads on infant lung function

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vₜ</td>
<td>-0.055</td>
<td>0.573</td>
</tr>
<tr>
<td>RR</td>
<td>0.190</td>
<td>0.046*</td>
</tr>
<tr>
<td>Ti/Tₜtot</td>
<td>0.006</td>
<td>0.948</td>
</tr>
<tr>
<td>tPTEF/tE</td>
<td>0.058</td>
<td>0.542</td>
</tr>
<tr>
<td>Vₜ/ti</td>
<td>-0.043</td>
<td>0.651</td>
</tr>
<tr>
<td>FeNO</td>
<td>0.100</td>
<td>0.416</td>
</tr>
<tr>
<td>FRC</td>
<td>-0.120</td>
<td>0.450</td>
</tr>
<tr>
<td>LCI</td>
<td>0.143</td>
<td>0.356</td>
</tr>
</tbody>
</table>

* p-value <0.05 indicates significant findings
Vₜ = tidal volume (z-score), RR = respiratory rate (z-score), Ti/Tₜtot = ratio of inspiratory time to total breath time, tPTEF/tE = ratio of total time to peak tidal expiratory flow to total expiratory time, Vₜ/ti = mean tidal expiratory flow (z-score), FeNO = fractional exhaled nitric oxide (z-score), FRC = functional residual capacity (z-score), LCI = lung clearance index
5.3.3 Associations between indoor air pollutant exposure and infant lung function

A statistically significant inverse univariate relationship was observed between bedroom formaldehyde levels and tPTEF/tE (p = 0.032) (Table 5.6; Figure 5.2). Peak NO₂ levels were negatively correlated with FRC (p = 0.05; Figure 5.3) (Table 5.7). Positive associations were found between CHCE scores and FRC (p = 0.048) as well as LCI (p = 0.028) (Table 5.9; Figure 5.4; Figure 5.5). The multiple linear regression model only displayed one significant finding, which was between formaldehyde levels and Ti/Ttot (p = 0.019) (Table 5.10).

Table 5.6: Association between prenatal domestic formaldehyde levels and lung function indices during early infancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bedroom</td>
<td></td>
<td>Lounge</td>
<td></td>
<td>Averaged</td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>0.085</td>
<td>0.387</td>
<td>0.061</td>
<td>0.534</td>
<td>0.077</td>
<td>0.430</td>
</tr>
<tr>
<td>RR</td>
<td>-0.140</td>
<td>0.151</td>
<td>-0.053</td>
<td>0.586</td>
<td>-0.019</td>
<td>0.363</td>
</tr>
<tr>
<td>Ti/Ttot</td>
<td>-0.11</td>
<td>0.255</td>
<td>0.038</td>
<td>0.695</td>
<td>-0.017</td>
<td>0.859</td>
</tr>
<tr>
<td>tPTEF/tE</td>
<td>-0.205</td>
<td>0.032*</td>
<td>-0.139</td>
<td>0.149</td>
<td>-0.174</td>
<td>0.070</td>
</tr>
<tr>
<td>VT/ti</td>
<td>-0.003</td>
<td>0.977</td>
<td>0.022</td>
<td>0.823</td>
<td>0.015</td>
<td>0.879</td>
</tr>
<tr>
<td>FeNO</td>
<td>0.039</td>
<td>0.756</td>
<td>0.04</td>
<td>0.746</td>
<td>0.041</td>
<td>0.740</td>
</tr>
<tr>
<td>FRC</td>
<td>-0.092</td>
<td>0.576</td>
<td>-0.181</td>
<td>0.271</td>
<td>-0.144</td>
<td>0.380</td>
</tr>
<tr>
<td>LCI</td>
<td>-0.117</td>
<td>0.465</td>
<td>0.116</td>
<td>0.470</td>
<td>0.049</td>
<td>0.762</td>
</tr>
</tbody>
</table>

* p-value < 0.05 indicates significant findings

VT = tidal volume (z-score), RR = respiratory rate (z-score), Ti/Ttot = ratio of inspiratory time to total breath time, tPTEF/tE = ratio of total time to peak tidal expiratory flow to total expiratory time, VT/Ti = mean tidal expiratory flow (z-score), FeNO = fractional exhaled nitric oxide (z-score), FRC = functional residual capacity (z-score), LCI = lung clearance index
Figure 5.2: Association between bedroom formaldehyde levels and tPTEF/tE

![Figure 5.2 Scatterplot showing the association between formaldehyde levels in the bedroom (lnFormBR) and ratio of total time to peak tidal expiratory flow to total expiratory time during early infancy (tPTEFtE). Closed circles represent each participant.](image)

Table 5.7: Association between prenatal NO$_2$ levels and lung function indices during early infancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised $\beta$-coefficient</th>
<th>p-Value</th>
<th>Standardised $\beta$-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model: 24-hour</td>
<td></td>
<td></td>
<td>Model: Peak</td>
<td></td>
</tr>
<tr>
<td>$V_T$</td>
<td>-0.086</td>
<td>0.378</td>
<td>0.097</td>
<td>0.406</td>
</tr>
<tr>
<td>RR</td>
<td>-0.159</td>
<td>0.100</td>
<td>-0.208</td>
<td>0.070</td>
</tr>
<tr>
<td>Ti/T$_{tot}$</td>
<td>-0.087</td>
<td>0.364</td>
<td>-0.068</td>
<td>0.555</td>
</tr>
<tr>
<td>tPTEF/tE</td>
<td>-0.067</td>
<td>0.487</td>
<td>-0.080</td>
<td>0.485</td>
</tr>
<tr>
<td>$V_T/i$</td>
<td>-0.150</td>
<td>0.119</td>
<td>-0.040</td>
<td>0.728</td>
</tr>
<tr>
<td>FeNO</td>
<td>-0.066</td>
<td>0.593</td>
<td>-0.097</td>
<td>0.447</td>
</tr>
<tr>
<td>FRC</td>
<td>0.273</td>
<td>0.084</td>
<td>-0.413</td>
<td>0.050*</td>
</tr>
<tr>
<td>LCI</td>
<td>0.178</td>
<td>0.253</td>
<td>0.393</td>
<td>0.064</td>
</tr>
</tbody>
</table>

* p-value $\leq$0.05 indicates near-significant findings

NO$_2$ = Nitrogen Dioxide

$V_T$ = tidal volume (z-score), RR = respiratory rate (z-score), Ti/T$_{tot}$ = ratio of inspiratory time to total breath time, tPTEF/tE = ratio of total time to peak tidal expiratory flow to total expiratory time, $V_T/i$ = mean tidal expiratory flow (z-score), FeNO = fractional exhaled nitric oxide (z-score), FRC = functional residual capacity (z-score), LCI = lung clearance index
Figure 5.3: Association between peak NO$_2$ levels and FRC

Figure 5.3 Scatterplot showing the association between peak nitrogen dioxide levels (lnNO$_2$peak) and functional residual capacity during early infancy (FRC$_z$). Closed circles represent each participant.

Table 5.8: Association between prenatal domestic VOC levels and lung function indices during early infancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised $\beta$-coefficient</th>
<th>p-Value</th>
<th>Standardised $\beta$-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model: TVOC</td>
<td>BTEX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_T$</td>
<td>-0.031</td>
<td>0.756</td>
<td>-0.051</td>
<td>0.605</td>
</tr>
<tr>
<td>RR</td>
<td>-0.013</td>
<td>0.894</td>
<td>0.103</td>
<td>0.291</td>
</tr>
<tr>
<td>Ti/T$_{tot}$</td>
<td>0.010</td>
<td>0.919</td>
<td>0.058</td>
<td>0.552</td>
</tr>
<tr>
<td>tPTEF/tE</td>
<td>-0.093</td>
<td>0.341</td>
<td>-0.060</td>
<td>0.535</td>
</tr>
<tr>
<td>$V_T/ti$</td>
<td>0.109</td>
<td>0.263</td>
<td>0.091</td>
<td>0.351</td>
</tr>
<tr>
<td>FeNO</td>
<td>-0.145</td>
<td>0.248</td>
<td>-0.198</td>
<td>0.114</td>
</tr>
<tr>
<td>FRC</td>
<td>-0.087</td>
<td>0.594</td>
<td>0.063</td>
<td>0.701</td>
</tr>
<tr>
<td>LCI</td>
<td>-0.160</td>
<td>0.316</td>
<td>-0.225</td>
<td>0.157</td>
</tr>
</tbody>
</table>

VOC = volatile organic compounds, TVOC = total volatile organic compounds, BTEX = benzene, toluene, ethylbenzene and xylene
$V_T$ = tidal volume (z-score), RR = respiratory rate (z-score), Ti/T$_{tot}$ = ratio of inspiratory time to total breath time, tPTEF/tE = ratio of total time to peak tidal expiratory flow to total expiratory time, $V_T/ti$ = mean tidal expiratory flow (z-score), FeNO = fractional exhaled nitric oxide (z-score), FRC = functional residual capacity (z-score), LCI = lung clearance index
Table 5.9: Association between prenatal CHCE scores and lung function indices during early infancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_T$</td>
<td>-0.023</td>
<td>0.814</td>
</tr>
<tr>
<td>RR</td>
<td>0.012</td>
<td>0.903</td>
</tr>
<tr>
<td>$T_i/T_{tot}$</td>
<td>0.066</td>
<td>0.496</td>
</tr>
<tr>
<td>$tPTEF/tE$</td>
<td>0.012</td>
<td>0.901</td>
</tr>
<tr>
<td>$V_T/t_i$</td>
<td>-0.063</td>
<td>0.519</td>
</tr>
<tr>
<td>FeNO</td>
<td>0.091</td>
<td>0.475</td>
</tr>
<tr>
<td>FRC</td>
<td>0.319</td>
<td>0.048*</td>
</tr>
<tr>
<td>LCI</td>
<td>0.344</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

* p-value <0.05 indicates significant findings

CHCE = composite household chemical exposure

$V_T$ = tidal volume (z-score), RR = respiratory rate (z-score), $T_i/T_{tot}$ = ratio of inspiratory time to total breath time, $tPTEF/tE$ = ratio of total time to peak tidal expiratory flow to total expiratory time, $V_T/t_i$ = mean tidal expiratory flow (z-score), FeNO = fractional exhaled nitric oxide (z-score), FRC = functional residual capacity (z-score), LCI = lung clearance index

Figure 5.4: Association between CHCE scores and FRC

Figure 5.4 Scatterplot showing the association between composite chemical household exposure scores in the bedroom (CHCE) and functional residual capacity during early infancy (FRC_z). Closed circles represent each participant.
Figure 5.5: Association between CHCE scores and LCI

Figure 5.5 Scatterplot showing the association between chemical household exposure scores (CHCE) and lung clearance index during early infancy (LnLCI). Closed circles represent each participant.

Table 5.10: Effect of individual IAP on lung function in a multiple linear regression model (adjusted for GIS distance to main road)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
<th>Variable</th>
<th>Standardised β-coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_T$</td>
<td></td>
<td></td>
<td>RR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>0.123</td>
<td>0.264</td>
<td>Formaldehyde</td>
<td>0.058</td>
<td>0.590</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>0.038</td>
<td>0.722</td>
<td>NO$_2$</td>
<td>0.029</td>
<td>0.785</td>
</tr>
<tr>
<td>VOC</td>
<td>0.014</td>
<td>0.896</td>
<td>VOC</td>
<td>0.039</td>
<td>0.721</td>
</tr>
<tr>
<td>GIS</td>
<td>-0.055</td>
<td>0.572</td>
<td>GIS</td>
<td>0.193</td>
<td>0.063</td>
</tr>
<tr>
<td>$\frac{T_i}{T_{tot}}$</td>
<td>-0.252</td>
<td>0.019*</td>
<td>$\frac{T_i}{T_{tot}}$</td>
<td>0.063</td>
<td>0.564</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>0.000</td>
<td>0.997</td>
<td>NO$_2$</td>
<td>-0.025</td>
<td>0.812</td>
</tr>
<tr>
<td>VOC</td>
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<td>0.747</td>
<td>VOC</td>
<td>-0.061</td>
<td>0.578</td>
</tr>
<tr>
<td>GIS</td>
<td>-0.031</td>
<td>0.757</td>
<td>GIS</td>
<td>0.072</td>
<td>0.484</td>
</tr>
<tr>
<td>$V_T/\tau_i$</td>
<td>0.160</td>
<td>0.143</td>
<td>RR</td>
<td>-0.042</td>
<td>0.769</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>0.004</td>
<td>0.973</td>
<td>NO$_2$</td>
<td>0.137</td>
<td>0.317</td>
</tr>
<tr>
<td>VOC</td>
<td>-0.027</td>
<td>0.807</td>
<td>VOC</td>
<td>-0.054</td>
<td>0.700</td>
</tr>
<tr>
<td>GIS</td>
<td>-0.017</td>
<td>0.873</td>
<td>GIS</td>
<td>0.138</td>
<td>0.299</td>
</tr>
<tr>
<td>FRC</td>
<td></td>
<td></td>
<td>FeNO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>-0.146</td>
<td>0.401</td>
<td>Formaldehyde</td>
<td>0.146</td>
<td>0.407</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>-0.284</td>
<td>0.130</td>
<td>NO$_2$</td>
<td>-0.051</td>
<td>0.782</td>
</tr>
<tr>
<td>VOC</td>
<td>-0.110</td>
<td>0.563</td>
<td>VOC</td>
<td>0.269</td>
<td>0.159</td>
</tr>
<tr>
<td>GIS</td>
<td>0.000</td>
<td>0.999</td>
<td>GIS</td>
<td>0.098</td>
<td>0.568</td>
</tr>
</tbody>
</table>

* p-value <0.05 indicates significant findings

NO$_2$ = Nitrogen Dioxide, VOC = volatile organic compounds, GIS = geographic information systems
5.4 Discussion

To our knowledge, this is the first study to explore the effects of prenatal exposure to indoor air pollutant levels and respiratory outcomes, particularly lung function during early infancy. Similar studies have focused on the effects of maternal exposure to outdoor air pollutants [48, 50, 51] or indoor air pollution from chemical use [52, 91] on early life respiratory outcomes and lung function. Overall, few associations were found between pollutant exposure and lung function. The findings from this study suggest that indoor air pollutant exposure may have an effect on early life lung function, even at the low exposure levels documented, although the results need to be treated with caution.

The mechanisms of action by which prenatal exposure to indoor air pollutants produce changes in lung development has not been investigated before. While formaldehyde, NO₂ and VOCs have distinctive chemical properties, their effect on lung health may result from similar general mechanisms; inflammation and oxidative stress [252, 261, 376, 377, 379, 381, 382, 547-549]. Numerous in-vitro studies have linked exposure to excessively high levels of formaldehyde, NO₂ and VOCs with increased oxidative stress and inflammation in cells [376-380, 482] and mouse models [256, 259, 306, 307, 381-384]. The type of inflammation, however, is known to differ; formaldehyde and VOC exposures have been associated with eosinophilic inflammation [262, 263, 305, 381] while NO₂ exposure is associated with neutrophilic inflammation [295, 298, 307, 311, 550]. Apart from inflammation, exposure to high levels of formaldehyde, NO₂ and VOCs have also been linked to structural, alveolar and cellular damage in lung tissue, as well as airflow obstruction and reduced respiratory rates [304, 306, 551, 552].
In this study, a significant inverse relationship between bedroom formaldehyde levels and tPTEF/tE was found in the univariate analysis. While the current understanding behind what tPTEF/tE may reflect physiologically is still unclear, previous studies have linked maternal exposure to tobacco smoke to reduced tPTEF/tE during early infancy [68-70]. Although it remains unclear, the manner by which formaldehyde exposure could result in lower tPTEF/tE might be explained by a cascade of events occurring at the molecular level.

Increased formaldehyde exposure has been shown to result in increased oxidative stress, inflammation, and subsequently airway wall thickening in fetal lungs in animal [553] and human tissue studies [52, 554]. Excessive thickening of the airway wall may also drive compensatory mechanisms in order to sustain optimal respiration, one of which is post inspiratory activity of the diaphragm [555]. This compensatory mechanism is necessary in order to stabilise lung volume during the expiratory phase of respiration [555]. Post-inspiratory activity of the diaphragm may result in an increased expiratory time after peak inspiratory flow, subsequently reducing tPTEF/tE. This can be supported by other studies which have found that pathologies such as flow limitation, airway obstruction, asthma and respiratory tract illness are likely to result in reduced tPTEF/tE in both adults and infants [54, 556-560].

The cascade of events described above assumes the direct exposure of formaldehyde to fetal lung development tissue. Animal studies have demonstrated that formaldehyde from maternal systemic blood has the capability of entering fetal tissue by crossing the placenta [561, 562]. Furthermore, Katakura et al. [563] also reported that formaldehyde has a tendency to accumulate in placental and fetal tissue after crossing over from the maternal circulation. Despite its biological plausibility, the probability of this process
occurring remains uncertain, as formaldehyde levels in this study are lower than that of animal or occupational studies which have reported significant findings. Existing studies which have demonstrated the presence of formaldehyde in placental tissue are often based on exposure levels higher than that of non-occupational human exposure. A review by Collins et al. [528] concluded that at low exposure levels, maternally inhaled formaldehyde is likely to be rapidly converted to formate in the lungs, which is not known to cause toxic effects.

Alternatively, the effect of maternal formaldehyde exposure on tPTEF/tE could have resulted from indirect effects. Several studies in mice have linked increased maternal formaldehyde exposure to impaired placental structure [259, 564]. While the specific mechanisms have not been elucidated, this could involve the interaction between formaldehyde and membrane proteins, which can limit the activation of receptors for placental growth factors [152]. Reduced placental growth would subsequently result in a reduced oxygen supply to the fetus, which has been shown to result in smaller or stiffer airways, and altered control of breathing [66, 565]. Overall, the potential for formaldehyde to influence indices such as tPTEF/tE remains poorly understood and needs to be investigated further.

The multiple linear regression model demonstrated an inverse relationship between formaldehyde levels and Ti/T_{tot}, but not tPTEF/tE. While the effect of pollutant exposures on Ti/T_{tot} remains unknown, this result was consistent with the study of Hathlol et al. [555], which reported lower Ti/T_{tot} with altered lung development from premature birth. It was likely that low Ti/T_{tot} ratios were mainly driven by increased expiratory time, which was shown in infants to be a result of factors such as post-inspiratory diaphragmatic activity, increased upper airway resistance, and airway
obstruction [555, 560, 566-568]. Other studies, however, have reported a decreased expiratory period associated with preterm birth [569, 570]. This was likely to be driven primarily by an increased respiratory rate in response to reduced lung growth. However, this relationship remains unclear, as the univariate models did not demonstrate any relationship between any exposure of formaldehyde and Ti/T_{tot}.

Peak NO$_2$ levels were found to be significantly associated with reduced FRC levels in the univariate analysis. As a measure of lung volume, FRC has been described as an indicator of lung development [571], although the majority of multiple breath washout studies to date have placed more emphasis on other associated indices such as lung clearance index and moment ratios [445, 449, 450, 572-574]. Current knowledge recognises that FRC is primarily determined by the forces of inward and outward elastic recoil of the lungs and the chest wall [575, 576], and correlates with developmental indices such as birth and postnatal weight and length and gestational and postnatal age [48, 74, 442, 571, 575]. Given the lack of evidence between NO$_2$ exposure and any measure of birth outcome, indices of lung volume (V$_T$) or respiratory control (RR), as well as the low numbers for this association (n = 23), this finding was likely to have occurred by chance.

CHCE scores were found to be positively associated with both LCI and FRC, although this was not found with measures of VOCs. Existing evidence suggests that VOCs often found in common household chemicals, are capable of causing eosinophilic inflammation [371, 376-380]. Increased inflammation in the lungs may subsequently result in uneven gas distribution in different region of the lungs, often described as ventilation inhomogeneity (VI), which is reflected by high LCI scores [449, 573]. Proietti et al. [152] reported that maternally-introduced pollutants have the potential
interact with membrane proteins to limit receptor activation for placental growth. The resulting reduction on placental size could subsequently limit maternal oxygen supply to the fetus. Maternal hypoxia can therefore result in increased FRC during infancy, as recently demonstrated by Llapur et al. [577]. However, just like with formaldehyde exposure, such a finding has to be interpreted cautiously. It is uncertain whether VOCs inhaled from household chemicals are able to remain in the maternal blood for a sufficient period of time to diffuse through the placenta. The pharmacokinetics of each VOC is unique, with some having a short half-life in blood, while others remain stable for longer periods [216, 578]. Despite the uncertainty, results from this study relate well to findings from the ALSPAC study, which have shown that the use of household chemical questionnaires can influence wheeze outcomes in early life, as well as lung function later in childhood [52, 91].

A positive association between the distance from homes to main roads and early life respiratory rate was found in this study, although this relationship was not significant when adjusted for indoor pollutant exposures. This result, however, also has to be interpreted cautiously, especially given the very small number of homes that were found within 250 m of the main road. Latzin et al. [48], however, reported findings that were in contrast to this study. The authors found that fetal exposure to particulate matter was positively associated with minute ventilation during infancy, with the relationship likely to be driven by respiratory rate.

Overall, however, there were very few significant findings from the multiple linear regression models. In environments where pollutant levels are high, the overall effect of pollutants on health outcomes could be non-additive [579]. Other processes that can complicate the effect of multiple pollutants on health outcomes include processes of
synergism, antagonism, inhibition, potentiation or masking [579]. These processes could also be relevant to indoor air pollution, where some pollutants, typically involving ozone, are part of the formation pathway of others [233, 357, 521, 580].

5.5 Conclusion

The overall findings from this study, as well as similar studies focusing on outdoor air pollutants [49, 87] suggest the possibility that fetal exposure to pollutants, even at low exposure levels, may influence fetal lung development. However, the results need to be interpreted cautiously due to the small sample size and inconsistent findings. Possible adverse health effects arising from indoor air pollutant exposure may include decreased lung volume, ventilation inhomogeneity, airway thickening and airway obstruction, some of which are likely to have occurred by inflammation brought about by oxidative stress. It remains unclear whether these effects are likely to be due to direct or indirect pollutant exposures, or what the underlying mechanisms might be. Further research into this area with a focus on mechanisms of influence is required for a better understanding of how indoor pollutant exposure may affect fetal lung development.
Chapter 6
General Discussion and Conclusion
6.1 Introduction

Several studies have explored the relationship between maternal pollutant exposure and fetal development [49, 109-113, 494, 507-518] including that of the respiratory system [50, 52, 87, 92]. However, the current understanding of such relationships remains uncertain due to the paucity of data in this area of research. There is, however, good evidence that in-utero exposure to environmental factors such as smoking [67-81] and outdoor air pollution [50, 52, 67, 72-75, 87-89] are linked to poor respiratory outcomes in early, and possibly later [82, 83, 86, 546, 581, 582], postnatal life. Despite the potential importance of the indoor environment for exposure to air pollutants, there has been little attention paid to exploring the effects of indoor air pollution on fetal development. Furthermore, the mechanisms that could underlie the developmental effects of pollutant exposure during pregnancy remain largely unknown.

This thesis aimed to specifically explore the relationship between fetal exposure to indoor air pollutants and early life lung function and birth outcomes. This was done as part of a cohort study, where pollutant exposure data was collected by both indoor air monitoring and questionnaire data during the third trimester of pregnancy and analysed with both anthropometric measures at birth and lung function tests during early infancy.

Overall, this thesis demonstrated that increased exposure to specific pollutants has the potential to adversely influence fetal growth. However, the results need to be treated with caution due to small numbers and a lack of consistency in the findings. The evidence, therefore, is insufficient to draw definite conclusions on the nature of these relationships. This chapter presents a summary of the key findings, the strengths and limitations of this study, the contribution to current knowledge and recommendations for future research.
6.2 Summary and interpretation of key findings

6.2.1 IAP levels in study homes

Levels of IAP were measured in 305 houses across rural and urban areas in the Peel region. Levels of indoor air pollutants observed in the Peel region were low. A comparison of indoor air pollutant levels between this study and several other studies are presented in section 3.4. While there are no specific guidelines for domestic indoor air formaldehyde, NO₂ and VOC levels, findings from this study displayed levels far below general recommendations for indoor air pollutant exposure by the World Health Organisation [94, 199] and Health Canada [583]. The World Health Organisation (WHO) has recommended formaldehyde levels of no more than 80 ppb over a 30 minute period in the indoor setting, and NO₂ levels of no more than 200 µg/ m³ hourly, or an annual average of 40 µg/ m³ as a general guide for indoor exposures [199]. There are no Australian standards for indoor levels of formaldehyde, NO₂ and VOC for the domestic setting.

A high proportion of NO₂ and VOC samples were below detectable levels (BDL). Reasons for the low levels could be climatic factors, high ventilation, and methodological considerations, as discussed in Chapter Three. Other factors include variations in building materials, sizes of homes, traffic densities, and behavioural factors. Useful information that could assist with clearer understanding of the various influences on indoor air pollutant levels include additional details on home sizes, indoor temperature, humidity levels and ventilation rates. Data on ventilation rates, in particular, could have been useful as this was known to interact with other variables that may influence indoor air pollution levels.
As indoor air pollutant levels in this study were low, it was difficult to compare the results of this study to that of other studies which have explored the impact of housing or environmental factors on indoor levels. Despite the low levels, there were several findings which were consistent with previous studies.

6.2.1.1 Formaldehyde levels

In this study, formaldehyde levels were not affected by seasons, age of homes, recent renovation, or rural-urban classification. The relationship between home age and formaldehyde levels was expected, as this study population did not contain a high proportion of homes that were less than five years of age (35.4%), with the understanding that formaldehyde levels are known to stabilise at low levels past the first two years in new homes [359]. Similarly, building codes from the 1970s which were designed to minimise formaldehyde emissions [468] could have also contributed to the low levels found in this study. This could also have accounted for the lack of differences between homes based on their status of having undergone recent renovations works.

6.2.1.2 NO₂ levels

In this study, indoor NO₂ levels were influenced by seasonal differences and usage of unflued gas heaters. It was likely that the finding of higher daily NO₂ averages in winter compared to other seasons in this study were influenced by unflued gas heater use, consistent with findings from other cities in the developed world [275, 290, 318, 467, 471]. No differences were found between participants with or without unvented gas stoves at home, which could be due to differences between cooking periods in homes and increased ventilation in kitchens [228, 289].
6.2.1.3 VOC levels

VOC levels in this study were found to be associated with CHCE scores. These included TVOC, BTEX, toluene, and o-xylene. The CHCE scores obtained in this study were also significantly associated with levels of total volatile organic compounds (TVOC). This finding demonstrated that the suite of compounds selected to measure TVOC was an adequate representation of VOCs commonly found in household chemicals. VOC levels in this study were not affected by recent renovations, with the reason likely to be similar to that of the relationship between formaldehyde levels and recent renovations.

Although the factors which may contribute to indoor air pollutant levels were explored, this study was not designed to specifically investigate such relationships. There were limitations on the questionnaire due to the needs of the Peel Child Health Study. Therefore, only the minimum amount of house questionnaire data was obtained. The main outcomes of this study were related to the respiratory health outcomes arising from indoor air pollutant exposure.

6.2.2 IAP levels and early life health outcomes

This was the first study that measured IAP levels in order to explore birth outcomes and early life lung function arising from maternal exposure to indoor air pollutants. Previous studies of the effects of prenatal pollutant exposure on respiratory outcomes and fetal development have overwhelmingly focused on outdoor pollutants [28, 49, 50, 52, 87, 92, 109-113, 175, 494, 507-519].
Overall, results from this study suggest that maternal exposure to air pollutants even at low exposure levels, could influence some aspects of fetal development, although such effects might not apply specifically to the development of the respiratory system.

A recent review by Proietti et al. [152] reported that based on in-vitro and animal studies, small amounts of inhaled ultrafine particles could be transported to organs, although it is unclear whether this is likely to occur in humans. The reviewers speculated its possibility based on current knowledge of toxicokinetics, even though the toxicodynamics are complicated, and are likely to involve the immune reaction, pH, oxidant levels, and the potential for particles to influence aspects of the metabolism [152]. Other reviews have also reported a considerable heterogeneity that exists between studies in this area of research [494, 584]. The following sections discuss the findings of this study in the wider context of known evidence based on individual pollutants, as well as its wider implications on fetal development.

6.2.2.1 Formaldehyde exposure

In this study, there was an association between increased exposure to formaldehyde during the later stages of pregnancy and reduced fetal development, including that of the lungs. Although the amount of research in this area remains scarce, reviews describing the relationship between formaldehyde exposure and health outcomes have been mixed. Collins et al. [528] concluded that maternally inhaled formaldehyde was unlikely to result in any long-term effects given its quick conversion to formate, On the other hand, a more recent review by Duong et al. [529] suggested that inhaled formaldehyde can potentially influence fetal development by indirect effects such as genotoxicity, oxidative stress, protein, enzyme, and hormonal disruption, as well as DNA methylation, although critical levels remain unknown. Despite the uncertainty arising
from the paucity of data in this area of research, the significant findings from this study raise the possibility that maternal exposure to formaldehyde, even at low levels, may affect some aspects of fetal development.

One of the most significant findings in this study was the relationship between bedroom formaldehyde levels and reduced head circumference at birth ($p = 0.025$). This finding remained significant even after adjusting for other pollutants ($p = 0.025$). The inverse relationship between lounge formaldehyde levels and head circumference at birth was also found to approach significance ($p = 0.062$). Apart from head circumference, the relationship between increased bedroom formaldehyde levels during pregnancy and reduced birth weight also approached significance ($p = 0.055$). Findings from this study were in agreement with another study by Maroziene et al. [286], which reported that even at low levels formaldehyde exposure during pregnancy was associated with lower birth weights. It remains uncertain whether these findings reflect a true relationship between the influence of formaldehyde and birth outcomes, especially as levels of exposure found in this study were well below levels recognised by the WHO and IARC as likely to cause health effects [195, 199, 468, 585].

Maternal formaldehyde exposure was also linked to poorer tPTEF/tE in the univariate model and Ti/T$_{tot}$ in the multivariate model. Although the potential mechanisms underlying such associations remain unknown, tissue and animal studies have provided evidence that with toxic exposures such as long-term exposure to tobacco smoke, increased oxidative stress plays a key role in affecting such outcomes [52, 553, 554], with the pathways described in further detail in Chapter Five. Further research is required to elucidate possible mechanisms of action on how fetal formaldehyde levels can influence lung development.
6.2.2.2 NO\textsubscript{2} exposure

The current evidence holds that the relationship between fetal NO\textsubscript{2} exposure to birth outcomes remain unclear, although most current studies have focused on outdoor NO\textsubscript{2} levels [111, 112, 286, 508, 510-512, 514, 515, 518]. A threshold level for health effects from NO\textsubscript{2} has previously been suggested by Hesterberg et al. [335], although this was not specific for effects arising from fetal exposure. It remains difficult to draw conclusions regarding the potential for a threshold effects given the paucity of data in this area. Furthermore, the health effects of NO\textsubscript{2} exposure are not thought to be systemic [294, 306, 309, 311, 586], especially given its highly reactive nature and low solubility [297, 335, 587].

In this study, there were no associations between maternal exposure to NO\textsubscript{2} and fetal development. There was also no association between the presence of unflued gas heaters, which is known to be a major source of indoor NO\textsubscript{2} during winter in Perth [318], and birth outcomes in this study. It was likely that health effects may not exist at such low pollutant levels. In the context of the wider literature, some studies have linked increased traffic pollutant exposure and outdoor NO\textsubscript{2} levels to reduced fetal growth [49, 109-111, 494, 507-517] and early life lung function [50, 87, 92]. It is, however, difficult to compare the results of this study to the available evidence, given that those studies were mostly focused on traffic derived NO\textsubscript{2} levels with levels that were demonstrably higher than that of this study [508, 588]. Furthermore, NO\textsubscript{2} levels obtained from outdoor measurements may be an indicator of a complex mixture of pollutants associated with traffic exhaust levels [589-591], whereas indoor NO\textsubscript{2} levels mostly reflects emission levels from indoor gas appliances [212, 227, 234, 236, 288, 290, 318, 324, 462, 467] and may not reflect as complex a mixture of chemicals and particles.
While no associations between indoor NO2 levels and birth outcomes were found, there were some associations between increased NO2 exposures during pregnancy to specific measures of early life lung function. Peak indoor NO2 levels was found to be associated with reduced functional residual capacity (FRC) in the single pollutant model (p = 0.05), although it was not significant after controlling for other pollutants. However, given the low numbers explored (n = 23), the results need to be treated cautiously.

There was no association found between maternal NO2 exposure and FeNO levels in this study. This was in direct contrast with a study by Latzin et al. [87], which, using the same lung function tests, showed that exposures to similarly low levels of outdoor NO2 (mean 15.8 µg/m3, range 11.8 – 19.6 µg/m3) resulted in increased levels of FeNO during early infancy. The lack of finding in this study may also be due to the small sample size (n = 23). Alternatively, it was also possible that threshold levels exist, but they could be higher than the levels found in this study. Furthermore, this might also be a reflection of the differences between what indoor and outdoor NO2 represents, as mentioned above.

The findings from this study suggest that maternal exposure to low indoor NO2 levels during the third trimester does not affect fetal development. Further studies are required to determine if there are any effects from higher exposure levels that have been reported in other indoor air studies as well as any potential threshold effects.

6.2.2.3 VOC exposure

Current knowledge on the potential for maternal VOC exposure to influence fetal development also remains unclear. The few studies in this area have focused on specific VOC chemicals and findings have been mixed [510, 532, 592]. In this study, there were several significant associations between increased maternal exposures to TVOC and
various birth and infant lung function outcomes, although the nature of the relationship remains unclear.

Contrary to the accepted notion that increased pollutant exposure could adversely affect fetal development, increased maternal TVOC exposure was shown to result in marginal increases in birth length, even after adjustment for other pollutants. This finding is contrary to what would be expected if VOCs, at the levels measured in this study, truly had a biological effect on fetal development. While there is no evidence linking fetal VOC exposure to poorer fetal development, exposure to other pollutants during this period of development usually result in impaired birth outcomes [50, 111, 507, 508, 510-515, 531, 593].

The findings are difficult to explain and could be purely chance, as could the findings discussed above. However, it is possible, but not probable, that it could be due to growth factor or epigenetic regulation by VOC compounds, as described in detail Chapter Four. There could also be other contributing factors to such a finding, such as socioeconomic status and parental size. However, this area of research remains relatively unexplored.

While no significant relationships between VOC levels and lung function were found, CHCE scores in this study were positively associated with both functional residual capacity (FRC) and lung clearance index (LCI). There is some evidence which have shown that increased chemical exposure can stimulate eosinophilic inflammation in lung tissue, resulting in uneven gas distribution in the lungs, as reflected in the increased LCI score [262, 263, 305, 381]. This was discussed in greater detail in Chapter Five.
The results in this study supported that of the ALSPAC study [52], which showed that increased CHCE scores during pregnancy were linked to poorer lung function measured using spirometry in eight year old children. Decrements in indices associated with lower FRC and LCI in early life have also been shown to result in poorer lung function in childhood and later life [53, 55, 58]. As with other indoor air pollutants, potential pathways describing the mechanistic effects of VOCs on lung development have not been demonstrated in human studies. There are several reasons that could explain differences in findings between CHCE and TVOC, despite the weak relationship between these variables. Firstly, TVOC represents only part of the suite of total exposure arising from household chemicals. Secondly, intermittent use of household products may not be picked up by the seven-day sampling period in this study. Finally, usage of household chemicals situated far from VOC monitors may not be detected.

Overall, this study has reported an anomalous finding of increased birth length resulting from increased exposure to TVOC and BTEX. Although in-vitro and animal studies provide a platform for a potential explanation of the mechanistic processes involved, more studies in this area of research should be undertaken in order to obtain a better understanding of such relationships.

### 6.3 Strengths and limitations of this study

This study had several strengths and limitations. The main issues this section addresses relate to its longitudinal design, the passive sampling methodology, inclusion and exclusion criteria, the use of questionnaire data, the timing of indoor air sampling, timing of lung function tests and the sample size.
6.3.1 Strengths

A strength of this study was its longitudinal design and the short time period between exposure and outcome. This included air monitoring during the routine third trimester home visit, which had been shown by a similar study to be the time point when associations between maternal exposure to traffic pollutants and early life lung function outcomes are strongest [87]. Furthermore, the third trimester of pregnancy represents the period of alveolar growth, which corresponds with an increase in lung volume [66, 594], and is therefore likely to be influential in the development of the distal regions of the lungs. Secondly, the longitudinal nature of this study and the inclusion of questionnaires during pregnancy reduced the potential recall bias which could be an issue in other study designs.

The passive sampling methodology used in this study had advantages and disadvantages. One advantage of this method is its widespread use and validation by various studies that have investigated long-term pollutant exposures, allowing for comparisons with existing studies [430, 431, 595-597]. Apart from methodological advantages, passive sampling methods is also known to be energy-efficient, easy to conduct and economical [422, 424, 429, 434]. Potential disadvantages of passive sampling include difficulties with monitoring short term analyte concentration changes, sensitivity to temperature and air movement fluctuations [430], and a higher detection limit compared to active sampling [429]. Apart from detection limits, the disadvantages of this technique were unlikely to affect the outcomes of interest in this study, as the indoor sites used for sampling were unlikely to have large fluctuations in temperature or air flow. Furthermore, passive samplers have been found to be reliable over the normal range of temperature and relative humidity expected in indoor environments [420, 422, 423, 425-429]. Overall, passive sampling was the most suitable option for indoor air
pollutant sampling based on the study design, even though recent data have questioned its suitability for monitoring very low pollutant levels, particularly that of VOCs [419].

The stringent inclusion and exclusion criteria applied for the infant lung development study were applied to increase the chance of observing effects of the environmental exposures of interest. Smokers were not included in this study, as several studies have shown strong associations between maternal smoking and fetal development, including poor lung function brought about by direct exposure to numerous toxicant chemicals in cigarette smoke [70, 516, 598]. However, other factors, such as dietary intake of vitamins, other pollutants and genetics, were not able to be assessed. The inclusion and exclusion criteria also applied at birth. Most notably, premature infants were excluded, as it is accepted that their lung development has been shown to differ from healthy term babies [48, 533, 569, 571, 599, 600].

A further strength of this study was the collection of infant lung function data at six weeks of age. There is some evidence to suggest that lung function during infancy can potentially be predictive of later life lung function [53, 58-61] and respiratory health [53-57]. As lung function during early life is influenced by fetal lung development, collection of early life lung function data may bridge the gap into understanding how prenatal environmental exposures can ultimately influence long-term respiratory health, as demonstrated in studies that have focused on smoking [82-86]. Furthermore, a review by Stern et al. [601] reported the usage and standardisation of guidelines have made it easier for non-invasive lung function testing in infants. In addition, Fuchs et al. [442] recently published normative data for lung function in this age group, including prediction equations for lung function which adjust for potentially predictive factors such as size and maternal asthma. The availability for such data derived from a larger
population allowed for the comparison of lung function in this study with that of a larger study population. The lung function methods and equipment used in this study were identical to the equipment used for the development of the reference data and were also used in a similar study on maternal exposure to outdoor pollutants and lung function at six weeks of age [87].

6.3.2 Weaknesses

The use of a single indoor air monitoring period (one week) as a proxy for maternal pollutant exposure throughout pregnancy was a potential limitation, as a single sampling point may not take into account total pollutant exposure (indoors and outdoors) across the whole of pregnancy. However, this was done based on the design with the primary outcome of this research in mind. Furthermore, it would be expensive and logistically difficult to monitor continuously in a home over the entire period of gestation for a large number of subjects. This is a limitation for most indoor air studies, as continuous long-term monitoring similar to that used in outdoor settings of most urban areas, are generally not feasible. Sampling during several points of pregnancy may provide a clearer indication of how pollutant exposure during different time points of pregnancy may influence fetal development. A possible alternative would be the use of personal monitoring devices for longer time periods throughout pregnancy, as was done by Jedrychowski et al. [49]. However, the use of personal monitoring devices might be an inconvenience to participants and therefore result in a reduced compliance rate. Repeat sampling protocols can also result in high costs. Furthermore, studies have shown that domestic levels of indoor pollutants are often a major predictor of personal exposure [190, 207-213], and that pregnant women spend a large proportion of their time in indoor environments, particularly in the domestic setting [189, 190].
There are also other general limitations of indoor air exposure assessment. One limit of modern environmental science is the difficulty of understanding the amount of human contact with toxicants after release into the indoor environment [212, 602]. As such, there is a need to combine source exposure assessments with other elements of investigative research such as toxicology and dose response relationships, which may be often expensive to conduct [602]. While it is possible to better understand personal exposure to pollutants by the use of biomarkers such as sampling from bloods, such methods are known to be invasive, and could also be difficult to interpret generally, given that some inhaled indoor pollutants are rapidly metabolised [216, 528], while others may accumulate and have long-term effects [602]. Another potential limitation of using early life lung function is its inclusion of environmental effects between birth and the time of measurement.

The time point when lung function data is determined to be an adequate reflection of prenatal environmental exposures with minimal influence from early postnatal life is also an issue that had to be considered. Existing studies have measured lung function between five and seven weeks as a suitable proxy for prenatal exposures, and reference equations for lung function in this age range has been established [180, 442].

The design of this study also had some limitations. Due to the recruitment protocol for the Peel Child Health Study, self-selection bias could have been a potential issue in this study. For example, the proportion of smokers in the Peel Child Health Study was much lower than population rates for this region. While this did not influence the infant lung development study due to the exclusion of smokers, a bias might have occurred in the overall recruitment as smoking may be associated with socioeconomic status. There is, however, no evidence of a relationship between socioeconomic status and domestic air
pollution in Australia. Furthermore, the nature of prospective cohort studies resulted in some inevitable loss to follow up, as discussed in Chapter Five. Despite these limitations, a prospective cohort study was the most desirable study design for such an investigation, particularly as the research had an emphasis on using objective measures for exposure and for outcome variables of interest.

This study focused on three important indoor air pollutants or, in the case of VOCs, one class of indoor air pollutants. These were chosen because of their ubiquitous nature in the indoor environment and their links to poor health outcomes [1, 94, 199] However, there are many other indoor air pollutants, including carbon dioxide (CO₂), CO, hydrogen chloride (HCl), nitrous acid (NHO₂), nitric acid vapour (HNO₃), chlorinated solvents, chlorinated pesticides, and phthalate esters that were not assessed in this study. The costs, and logistics, of monitoring a large array of pollutants are high. Potential health effects of these pollutants cannot be discounted. Furthermore, outdoor air pollutants have been shown to affect maternal and child health [49, 50, 87, 603]. Data was not available for any outdoor air pollutants in the region of the study. Prior to the commencement of the study there was an understanding that the state environment department would be placing a monitoring station in the region. This would have been used to determine maternal exposure to outdoor pollutants such as particulate matter and NO₂, which have been shown to affect lung function in children [161, 173, 334, 518]. Unfortunately, a monitoring station was not established. Despite the unavailability of data regarding outdoor air pollutant levels, this study used questionnaire data to as a proxy for possible traffic exposure, as described in section 5.3.2. Traffic is an important contributor to outdoor air pollution, although not the only one.
Finally, the sample size was considerably less than originally calculated to adequately power this study and this could have influenced some of the results, especially for the main outcomes (lung function). There were several reasons why the targeted sample size was not reached. These included insufficient numbers recruited for the main Peel study; which had a direct effect on recruiting for the current study, drop outs that occurred between collection of indoor air pollutant and infant lung function data, inability to collect data due to infants not falling asleep for a sufficient duration, and collection of poor or unsuitable data. There was also a significant number of absences on the day of infant lung function testing (n = 50), of which the reasons were unclear despite later attempts to contact the participants. However, this was unlikely to have impacted upon the findings of this study, as indoor air pollutant levels and other variables between participants who were present and absent on day of testing did not differ significantly.

Consequently, the low sample size resulted in a requirement to modify the statistical design and methods used. Furthermore, differences in participant numbers between exposure groups could have influenced the results of the statistical analyses in Chapter Three.

6.4 Contribution to current knowledge and implications for future research

To our knowledge, this study was the first to explore the effect of maternal exposure to domestic air pollutants on birth outcomes and early life lung function. The main findings demonstrate that exposures to indoor air pollutant levels during the third trimester of pregnancy has the potential to adversely affect birth outcomes and lung
growth during the gestational period, although this remain uncertain as the data remains inconsistent.

More research is required in areas with higher IAP levels in order to better understand how indoor air pollution may affect lung development during the prenatal period. Future studies could focus on the long-term respiratory health implications of prenatal exposure to indoor air pollution. An increased understanding of the threshold effects and dose-response relationships arising from indoor air pollutant exposure should be considered. Finally, further research could focus on a better understanding of the possible mechanisms by which maternal pollutant exposures could influence growth and development of fetal tissues.
REFERENCES


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296. Balmes JR, Erle DJ, Solomon C, Christian DL, Chen LL, Welch BS, Kleinman MT, Dunham E: The Effects of Serial-day Exposure to Nitrogen Dioxide on


310. Pathmanathan S, Krishna MT, Blomberg A, Helleday R, Kelly FJ, Sandstrom T, Holgate ST, Wilson SJ, Frew AJ: Repeated daily exposure to 2 ppm nitrogen dioxide stimu...


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506. Li YF, Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Rappaport EB, Peters JM: Effects of in utero and environmental tobacco smoke exposure on


564. Monfared AL: **Histomorphological and ultrastructural changes of the placenta in mice exposed to formaldehyde.** *Toxicol Ind Health* 2012.


## Peel Health Study
### Infant Lung Function Testing

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<thead>
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<th>Name:</th>
<th>Study ID:</th>
<th>Study Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.O.B:</td>
<td>Gender:</td>
<td>Time Arrived:</td>
</tr>
<tr>
<td>Length (cm):</td>
<td>Weight(kg):</td>
<td>Time Left:</td>
</tr>
</tbody>
</table>

### Study Details

<table>
<thead>
<tr>
<th>MBW Mask Size:</th>
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<th>FOT Mask Size:</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature:</td>
<td>°C</td>
<td>Case Temperature:</td>
<td>°C</td>
</tr>
<tr>
<td>Relative Humidity:</td>
<td>%</td>
<td>Pressure:</td>
<td>hPa</td>
</tr>
</tbody>
</table>

Additional Information:

### Equipment Calibration

#### Ultrasonic Flowmeter:

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
<th>Recording Value(s)</th>
<th>File Reference</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Syringe Calibration:</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>Verify Syringe Vol:</td>
<td>L</td>
<td>L</td>
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<td></td>
<td>Bias Flow:</td>
<td>L/sec</td>
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<td></td>
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#### FOT Machine:

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<tbody>
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<tr>
<td>Baseline Calibration</td>
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<tr>
<td>After Test</td>
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<tr>
<td>Syringe Calibration:</td>
<td></td>
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<tr>
<td>Baseline Calibration</td>
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</tbody>
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## Data Recording Sheet

### Tidal Breathing and eNO

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<tr>
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<th>File Reference</th>
<th>Comments</th>
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<tr>
<td><strong>COMPLETED:</strong></td>
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### Multiple Breath Washout

<table>
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<th>Wash Out</th>
<th>Comments</th>
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<td><strong>COMPLETED:</strong></td>
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</table>

### Forced Oscillation Technique

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<th>Time:</th>
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<th>Comments</th>
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<tr>
<td><strong>COMPLETED:</strong></td>
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<td>□</td>
</tr>
</tbody>
</table>
# QUESTIONNAIRE
AT 6 SIX WEEK INFANT LUNG FUNCTION TESTING FOR PEEL STUDY

## A. ABOUT YOUR BABY AND THE BIRTH

<table>
<thead>
<tr>
<th>A1. What is this baby’s date of birth?</th>
<th>.....day</th>
<th>.....month</th>
<th>.....year</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>A2. What is this baby’s sex?</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A3. Was the labour with this baby induced?</th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A4. Which of the following would describe this baby’s birth?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal delivery</td>
</tr>
<tr>
<td>Forceps or suction delivery</td>
</tr>
<tr>
<td>Caesarean section</td>
</tr>
<tr>
<td>Elective</td>
</tr>
<tr>
<td>Emergency</td>
</tr>
<tr>
<td>Not sure</td>
</tr>
<tr>
<td>Reason?</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Don’t know</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A5. What was this baby’s birth weight?</th>
<th>.......... grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○ don’t know</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A6. What was this baby’s length at birth?</th>
<th>.......... cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○ don’t know</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A7. What was this baby’s head circumference at birth?</th>
<th>.......... cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○ don’t know</td>
</tr>
</tbody>
</table>
A8. After how many weeks of pregnancy was this baby born?  

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>A9. Did this baby have to go into a Neonatal Intensive Care Unit or Special Care Nursery after he/she was born?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10. Did this baby need any help with his/her breathing from a ventilator?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11. For about how many days in total?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A12. Was this baby a single birth, or a twin, triplet or more?  

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triplet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than a triplet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A13. Were there any other issues associated with the pregnancy or delivery not previously mentioned?  

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

B. FEEDING AND DIET  

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>
| B1. Was this baby ever breastfed? (Include colostrum in the first few days after birth) |     |    | Go to B6
### B2. Is this baby still being breastfed?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>⃝</td>
<td>⃝</td>
</tr>
</tbody>
</table>

### B3. How old was this baby when he/she completely stopped being breastfed? (Include expressed breast milk)

<table>
<thead>
<tr>
<th>Description</th>
<th>Days</th>
<th>Weeks Specify</th>
<th>Months Specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Still having breast milk</td>
<td>⃝</td>
<td>⃝</td>
<td></td>
</tr>
<tr>
<td>Weeks</td>
<td>⃝</td>
<td>Please specify</td>
<td></td>
</tr>
<tr>
<td>Months</td>
<td>⃝</td>
<td>Please specify</td>
<td></td>
</tr>
</tbody>
</table>

### B4. How old was this baby when he/she first had any milk OR food other than breast milk? (Include colostrum as breast milk)

<table>
<thead>
<tr>
<th>Description</th>
<th>Days</th>
<th>Weeks Specify</th>
<th>Months Specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hasn’t had anything else yet</td>
<td>⃝</td>
<td>⃝</td>
<td></td>
</tr>
<tr>
<td>Weeks</td>
<td>⃝</td>
<td>Please specify</td>
<td></td>
</tr>
<tr>
<td>Months</td>
<td>⃝</td>
<td>Please specify</td>
<td></td>
</tr>
</tbody>
</table>

### B5. How old was this baby when he/she was first given infant formula or other non-breast milk regularly? (Regularly = more than twice a week)

<table>
<thead>
<tr>
<th>Description</th>
<th>Days</th>
<th>Weeks Specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hasn’t had formula regularly</td>
<td>⃝</td>
<td>⃝</td>
</tr>
<tr>
<td>Weeks</td>
<td>⃝</td>
<td>Please specify</td>
</tr>
</tbody>
</table>

### B6. Are you having any problems feeding this baby at present? (If more than one, please mark main problem)

<table>
<thead>
<tr>
<th>Description</th>
<th>Yes, breastfeeding</th>
<th>Yes, starting solids</th>
<th>Please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>No problems</td>
<td>⃝</td>
<td>⃝</td>
<td></td>
</tr>
<tr>
<td>Yes, weaning</td>
<td>⃝</td>
<td>⃝</td>
<td></td>
</tr>
<tr>
<td>Yes, other</td>
<td>⃝</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### C. GENERAL RESPIRATORY HEALTH

<table>
<thead>
<tr>
<th>Description</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1. Has your child had a persistent runny nose since birth?</td>
<td>⃝</td>
<td>⃝</td>
</tr>
<tr>
<td>(persistent = present more often than not since birth)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C2. Has your child had a persistent cough since birth? (persistent = present more often than not since birth)  
<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

C3. If yes to question C2, when does your child usually cough?  
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>On awakening</td>
<td>○</td>
<td>At night-time</td>
</tr>
<tr>
<td>During the day</td>
<td>○</td>
<td>At any other time</td>
</tr>
</tbody>
</table>

C4. Has your child ever had any of the following health problems **diagnosed by a health professional**?  
<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
<th>AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Upper respiratory tract infection</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
<tr>
<td>(b) Bronchiolitis</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
<tr>
<td>(c) Bronchitis</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
<tr>
<td>(d) Croup</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
<tr>
<td>(e) Whooping cough</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
<tr>
<td>(f) Pneumonia</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
<tr>
<td>(g) Gastro-oesophageal reflux</td>
<td>○</td>
<td>○</td>
<td>……… weeks</td>
</tr>
</tbody>
</table>

C5. Has your child ever been hospitalised for one of these conditions?  
<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>C6. Is your child taking any regular medications for any of the above?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td>(a) Please list the medications and relevant condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C7. Has your child ever had a wheezy or whistling sound in the chest?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>C8. When has he/she had this?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>With a cold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most days or nights</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**D: ABOUT YOUR BABY – GENERAL HEALTH**

<table>
<thead>
<tr>
<th>D1. In general, how would you say your baby’s health is?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D2. Has your child been admitted to hospital at any time since original discharge home not asked about previously?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Date of admission</td>
<td>Reason for admission</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------------------------------------------</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

200
D3. Has your baby suffered from any medical or other health condition since birth not previously mentioned?

Please list

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D4. Does your baby currently need or use medicine prescribed by a doctor?

Please list

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D5. Please indicate which vaccinations your child has had, if any

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
<th>UNSURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) Diphtheria/Tetanus/Pertussis (DTP)

(b) Measles/Mumps/Rubella (MMR)

(c) Hepatitis B (may be part of DTP)

(d) Haemophilus Influenza. B (Hib)

(e) Polio (OPV)

(f) Chickenpox (Varicella)

(g) Meningitis (MenC)

(h) Others (please indicate)

(i) Up to date

(j) Date of last vaccination
### E. ECZEMA

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1. Has your child ever had an itchy rash which was coming and going in the past six weeks?</td>
<td>○</td>
<td>○ GO TO SECTION F</td>
</tr>
<tr>
<td>E2. Has this itchy rash at any time affected any of the following places: The folds of the elbows, behind the knees, ankles, under the buttocks or around the neck, ears or eyes?</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>E3. At what age did this rash first occur?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>E4. Has your child had this itchy rash in the last week?</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>E5. Has the rash cleared completely any time during the past six weeks?</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>E6. In the past 6 weeks, how often, on average, has your child been kept awake by this itchy rash?</td>
<td>Never woken: ○</td>
<td>One or more nights per week: ○</td>
</tr>
<tr>
<td></td>
<td>Less than one night per week: ○</td>
<td></td>
</tr>
<tr>
<td>E7. If the rash has cleared, what age was your child when it cleared?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>E8. Has your child ever had eczema diagnosed by a doctor?</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
**F. FAMILY HISTORY**

<table>
<thead>
<tr>
<th>(a) Hayfever</th>
<th>Mother</th>
<th>Father</th>
<th>Sibling 1</th>
<th>Sibling 2</th>
<th>Sibling 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

| (b) Asthma    | ○      | ○      | ○         | ○         | ○         |

| (c) Eczema    | ○      | ○      | ○         | ○         | ○         |

**F1. Is there a history of any of the following in your family?**
*(LEAVE BLANK IF NO HISTORY)*

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2. If the Mother has had asthma was this diagnosed by a doctor?</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3. If yes what age was asthma first diagnosed?</td>
<td>……….. years</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4. If the asthma has stopped what age did it stop?</td>
<td>……….. years</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5. If the Father has had asthma was this diagnosed by a doctor?</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>F6. If yes what age was asthma first diagnosed?</td>
<td>……….. years</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>F7. If the asthma has stopped what age did it stop?</td>
<td>……….. years</td>
<td></td>
</tr>
</tbody>
</table>

**G. RESPIRATORY IRRITANTS**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1. Does anyone smoke in the family?</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2. Does anyone smoke inside the home?</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
G3. Does your family own any pets? (please indicate where they spend their time)

<table>
<thead>
<tr>
<th>Type</th>
<th>YES</th>
<th>NO</th>
<th>Indoor/outdoor/both</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Dog</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>(b) Cat</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>(c) Bird</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>(d) Rabbit</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>(e) Guinea-Pig</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>(f) Other (please specify)</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>

Study date: __________
# APPENDIX 1: 12-month Respiratory Diary

**Month:** ______________________  **Code:** _____________  **DOB of child:** ________

| DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|-----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Fever > 38c** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Runny/snuffy nose** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Cough** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Sneezing** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Eye symptoms** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **itchy/watery/sore** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Diarrhoea** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Vomiting** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Diagnosis** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Dr/Hospital** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Breastfed** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Bottle fed** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Solids** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Medications for baby** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Vaccinations** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Maternal Health** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Medications for mum** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Our Children, Our Families, Our Place

Housing Inventory

Murdoch University
Telethon Institute for Child Health Research
The University of Western Australia
Curtin University of Technology
Edith Cowan University

Completion Instructions

Please use a black or blue pen to complete the questionnaire

<table>
<thead>
<tr>
<th>Please print clearly where required</th>
<th>.................................</th>
</tr>
</thead>
<tbody>
<tr>
<td>or</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Please make marks that fill the circle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please shade the circle completely</td>
</tr>
<tr>
<td>Please do not use crosses</td>
</tr>
<tr>
<td>Please do not use ticks</td>
</tr>
</tbody>
</table>

PART A: ABOUT YOUR HOUSE – HEATING – COOLING – COOKING – PESTS - PETS
**A1. What is the main building material of the outer walls?**

<table>
<thead>
<tr>
<th>Material</th>
<th>〇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brick</td>
<td>〇</td>
</tr>
<tr>
<td>Timber/asbestos/fibro</td>
<td>〇</td>
</tr>
<tr>
<td>Stone</td>
<td>〇</td>
</tr>
<tr>
<td>Other (please specify)</td>
<td></td>
</tr>
</tbody>
</table>

**A2. What kinds of floor coverings do you have in your home? (Please mark all that apply)**

<table>
<thead>
<tr>
<th>Floor Covering</th>
<th>Living room</th>
<th>Parent's bedroom</th>
<th>Infant's bedroom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fitted carpet</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>Timber</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>Linoleum</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>Concrete/slate/stone/tiles</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>Other (please specify)</td>
<td></td>
<td>〇</td>
<td>〇</td>
</tr>
</tbody>
</table>

**A3. During the last 12 months, have you undertaken any renovations / refurbishments to your home?**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No Go to A5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>〇</td>
<td>〇</td>
</tr>
</tbody>
</table>

**A4. If yes, what type of renovation / refurbishment in what room(s)? (Please mark all that apply)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Main living room</th>
<th>Parent's bedroom</th>
<th>Infant's bedroom</th>
<th>Kitchen</th>
<th>Bathroom</th>
<th>Whole house</th>
</tr>
</thead>
<tbody>
<tr>
<td>New carpet</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>New linoleum</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>New wood floor or polish</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>Walls painted</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>New furniture</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>New rooms</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
<tr>
<td>Other (Please specify)</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
<td>〇</td>
</tr>
</tbody>
</table>
A5. What sort of heater(s) do you use in your home? (Please number them in order of frequency of use, e.g. if gas is your main source of heating, but you also have an open fireplace, put a 1 next to gas and a 2 next to open fireplace)

<table>
<thead>
<tr>
<th>Electric</th>
<th>Gas</th>
<th>Reverse cycle air-conditioning</th>
<th>Oil</th>
<th>Open fireplace</th>
<th>Pot belly stove / closed slow combustion</th>
<th>Open fireplace</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>None</td>
<td></td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

Other (Please specify) ........................................................................................................................................

<table>
<thead>
<tr>
<th>Flued</th>
<th>Unflued</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A6. If you have a gas heater is it flued or unflued? (Unflued gas heaters have no flue or chimney and are often portable. Flued heaters are fixed and the fumes are vented directly outdoors or through a chimney) No gas heater, go to A7.

- No gas heater

A7. What is the main type of fuel you use for cooking?

<table>
<thead>
<tr>
<th>Electric</th>
<th>Gas – vented (with flue, chimney or extractor fan)</th>
<th>Gas – unvented (no flue, chimney or extractor fan)</th>
<th>Other (please specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A8. Do you have air-conditioning?

- No

A9. How often is your home treated professionally for pests (e.g. termites, spiders)?

<table>
<thead>
<tr>
<th>Never</th>
<th>Every 2 to 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Once a year</td>
</tr>
<tr>
<td></td>
<td>More than 12 months ago</td>
</tr>
</tbody>
</table>

A10. When was your home last treated professionally for pests (e.g. termites, spiders)?

<table>
<thead>
<tr>
<th>Don’t know</th>
<th>6 to 12 months ago</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within the last 6 months</td>
</tr>
</tbody>
</table>
A11. Have you noticed any signs of dampness in any of the following rooms (this includes visible damp spots and/or mould on the floor, walls or ceiling)?

<table>
<thead>
<tr>
<th>Room</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitchen</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Bathroom</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Living room</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Parent's bedroom</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Infant's bedroom</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

A12. How often are the following products used in the home?

<table>
<thead>
<tr>
<th>Product</th>
<th>Daily</th>
<th>Most days</th>
<th>Weekly</th>
<th>Fortnightly</th>
<th>Monthly</th>
<th>Rarely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insect sprays</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Disinfectants</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Carpet cleaner</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Window cleaner</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Spray cleaners (e.g. Mr Muscle)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Bleach</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Paints or varnish</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Air fresheners</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Deodorants</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Hair sprays</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
### A13. Do you have any pets?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Go to A14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

#### How many pets are there? (Please write number in space provided)

<table>
<thead>
<tr>
<th></th>
<th>Inside</th>
<th>Outside</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (please specify)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### A14. Does your infant have any regular contact with pets elsewhere (e.g. relatives, neighbours, friends)?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Go to A15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

#### Which pets are there?

<table>
<thead>
<tr>
<th>pets</th>
<th>Dogs</th>
<th>Cats</th>
<th>Birds</th>
<th>Rodents</th>
<th>Fish</th>
<th>Reptiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>☒</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Birds</td>
<td>☐</td>
<td>☐</td>
<td>☒</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Fish</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☒</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Other (please specify) ………………………………………………………………………………………………………………………………

### A15. Is your garage …?

<table>
<thead>
<tr>
<th></th>
<th>No garage</th>
<th>Enclosed and attached to the house</th>
<th>Open (e.g. carport) and attached to the house</th>
<th>Not attached to the house</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

### A16. How close is your home to the nearest main highway?

<table>
<thead>
<tr>
<th></th>
<th>Less than 50 metres</th>
<th>50 to 100 metres</th>
<th>100 to 300 metres</th>
<th>More than 300 metres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

### A17. How would you describe the road traffic around your house?

<table>
<thead>
<tr>
<th></th>
<th>Quite</th>
<th>Moderate</th>
<th>Busy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

### A18. How close is your home to any industrial plant?
PART B: ABOUT THE QUESTIONNAIRE

B1. Please indicate the date you completed this questionnaire: .........../........../........

B2. Approximately how long did it take you to complete this questionnaire? ............ Hours ........... Minutes

B3. Please write below any comments concerning this questionnaire, the research or anything else you would like to tell us about.

...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................

Thank you very much, we appreciate the time you have spent completing this questionnaire

Please keep the completed questionnaire in a safe place until one of our research assistants visits you at home. She will go through it with you and clarify any questions you have had difficulty answering