BIOMARKERS FOR CARBON MONOXIDE AND BENZENE IN OUTDOOR AND INDOOR MICROENVIRONMENTS IN SOUTH WALES

KATRINA A HENDERSON

A THESIS SUBMITTED IN CANDITURE FOR THE DEGREE OF PhD

PhD 2008

CARDIFF UNIVERSITY, SCHOOL OF MEDICINE
DEPARTMENT OF PRIMARY CARE AND PUBLIC HEALTH

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed ..... (candidate)
Date .....11:8:08.....

STATEMENT 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD

Signed ..... (candidate)
Date .....11:8:08.....

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated.
Other sources are acknowledged by explicit references.

Signed ..... (candidate)
Date .....11:8:08.....

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed ..... (candidate)
Date .....11:8:08.....

STATEMENT 4 - BAR ON ACCESS APPROVED

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access** approved by the Graduate Development Committee.

Signed ..... (candidate)
Date .....

SUMMARY

A messenger role for carbon monoxide has been identified for the cardiovascular system and also possibly for neurological effects through activation of soluble guanylate cyclise (SGC) and consequent production of intracellular cyclic guanosine monophosphate (cGMP). Chronic exposure to carbon monoxide associated with indoor heating may affect this important mechanism. Therefore the differential effects upon cGMP in blood platelets for people residing in homes with different types of heating were investigated. A differential in ambient concentrations of benzene and nitrogen dioxide has also been hypothesized for residential areas of differing urbanicity in particular with respect to traffic flows. Therefore the differential in urinary biomarker of benzene exposure, s phenylmercaptuirc acid (sPMA) as well as in environmental concentrations of nitrogen dioxide was investigated in people residing in urban and less urban microenvironments.

Environmental concentrations of carbon monoxide indoors were measured in real-time over a period of one week and integrated measurements of benzene and nitrogen dioxide concentrations outdoors were obtained over a period of one month. cGMP was measured in the blood platelets of subjects and sPMA in their urine.

Environmental concentrations of carbon monoxide indoors were low but despite this in homes heated by liquid petroleum gas (LPG) the concentration of cGMP in subjects' blood platelets were twice those in subjects using other types of heating. Further, for the LPG group the difference between paired measurements for the winter and summer seasons were 91%.

Substantial differences between mean concentrations of benzene (37%) and nitrogen dioxide (65%) were observed between urban and less urban areas but this differential was not reflected by any difference in toxic uptake as measured by sPMA in urine.

Exposure to emissions from LPG heating substantially affected cGMP concentrations in blood platelets but this is very unlikely to be caused by the low levels of carbon monoxide measured. We hypothesize that Nitric oxide may be responsible for the differences observed in cGMP. Substantial differences in outdoor benzene and nitrogen dioxide concentrations exist within the city of Cardiff but sPMA is not sufficiently sensitive to be used as a biomarker of exposure.

ACKNOWLEDGEMENTS

I would like to thank Ian, whose enthusiastic supervision, guidance and motivation has ensured I've reached that light at the end of a very long tunnel.

I would like to express my sincere thanks to all those who have participated without whom this research would not have been possible. To Truda for helping to coordinate so many of the visits; to those who provided laboratory support for sample analysis, (Alan, Derek, Alun, and Paul, and the external laboratories); and to everyone who supported me throughout this research, your help was very much appreciated.

To my friends, especially Amanda for sharing your experience and for your advice and to Mum and Dad for continually encouraging me and believing in me, this is dedicated to you and to Aunty Tela.

Thanks to my colleagues within OSHEU for your continued pep talks and words of encouragement especially Rachel and to Mike for making me laugh. Thanks also to Dave for your invaluable IT assistance.

Lastly to Paul (and Thomas), thanks for your patience at the endless 'PhD clutter', for your willingness to proof read chapters, you have given me the confidence to get to the end of this.

TABLE OF CONTENTS

ABSTRACT

Methods

To investigate indoor carbon monoxide, households were identified using different types of fuel for heating and visited during the winter months when carbon monoxide was measured in real time within the main living area for the duration of one week. Participants were invited to provide a blood sample during the week the monitor was in place and also to donate a second blood sample during the summer months to allow seasonal comparison. Blood samples were analysed both for COHb (a biomarker of exposure) and for cyclic CMP (a biomarker of effect). A subset of smokers was also recruited for whom environmental measurements were made.

To investigate outdoor benzene, healthy adult males were recruited from residences in two different microenvironments of differing urbanicity with respect to traffic flow. Benzene and Nitrogen dioxide were measured for a period of one month. Residents provided a urine sample which was analysed for s-PMA (a biomarker of exposure to benzene).

Results

The mean concentrations of carbon monoxide were low for all non-smoking residences but were approximately 10 times higher in the homes of smokers. In non-smokers the mean concentration of cGMP in blood platelets was twice as high for the group using LPG as fuel for heating as for the group using gas. Further for the LPG group concentrations of

cGMP were approximately twice as high in the winter compared to the summer.

The mean difference in outdoor environmental concentrations between urban and less urban areas in Cardiff was 37% for benzene and 65% for Nitrogen dioxide. However this differential was not reflected in the concentration of s-PMA, a urinary biomarker of benzene exposure, between groups.

Conclusion

The substantial difference in cGMP concentration in blood platelets for the group using LPG is unlikely to be caused by carbon monoxide as the environmental levels measured were low for this group also. We hypothesize that Nitric oxide emitted by LPG heating may be the cause of the observed difference in cGMP.

There is a substantial difference in ambient concentrations of benzene and nitrogen dioxide between urban and less urban areas of Cardiff. Urinary concentration on sPMA however is not sufficiently sensitive as a biomarker of exposure at the relatively low concentration of environmental benzene.

AIMS AND OBJECTIVES

The aim of this thesis was to investigate if differential in indoor exposure to carbon monoxide or outdoor exposure to benzene leads to a differential in associated biomarkers.

Objectives related to this aim were:

1. To measure chronic residential exposure of the elderly to Carbon monoxide in homes using different fuels and to examine if chronic exposure affects the enzyme cyclic GMP in blood platelets.

2. To measure outdoor ambient benzene concentrations in residential areas of differing urbanicity, in particular in respect of traffic flow, and to investigate if differential exposure is reflected in a urinary biomarker of exposure. Ambient Nitrogen dioxide which is known to reflect traffic flow was also investigated.

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 INTRODUCTION

The quality of outdoor air in the UK has improved considerably over the last decade, but it still has a detrimental effect on human health. Further to this since people spend the majority of their time indoors, predominantly in the domestic setting, the significance to health of indoor sources of pollution should also be considered.

1.1 Microenvironments (indoor and outdoor) of exposure to Air Toxics

The exposure of the general population to air toxics has been the subject of many studies over recent decades. Much of the research has focused on the outdoor environment; however there has been an increasing awareness of the importance of the role of indoor air pollution on public health. This is of particularly significance as the general public spend an estimated 70-90% of their time indoors (Crump, 1995, Harrison, 1996, Lee, 1997) and one study (Lai, 2004) found that individuals spent only 4% outdoors. The determination of an individual's exposure to an air toxic is dependent on the duration of time that they spend in different microenvironments and this is of even more significance for susceptible groups such as the elderly and those with pre-existing disease who are likely to spend the majority of their time in the home. Indoor microenvironments contain in general, concentrations of the pollutant in lower concentrations than outdoors. However the presence of indoor sources can lead to increased indoor concentrations of certain pollutants,

depending on the level of ventilation. If there is a high ventilation rate then the accumulation of indoor pollutants is reduced although conversely higher penetration of outdoor pollutants is expected to the indoor environment. It cannot be assumed that the indoor air is 'cleaner' than the outdoor air. (Chan 2002).

The landmark Harvard Six Cities Study which studied outdoor pollution but which also provided a platform for the understanding of residential indoor pollution and its contribution to total personal exposure for a number of pollutants. The study used a combination of outdoor and microenvironmental monitoring and personal exposure assessment to characterise the contributions of various indoor sources to total personal exposure (Speizer 1980).

Some of the first measurements of the indoor environment to be made were in the 1960's. In 1965 Biersteker measured Nitrogen dioxide in the home finding high levels present in homes with gas fired combustion devices. The U.S EPA's Total Exposure Assessment Methodology (TEAM) study in the 1980's provided a model for the comprehensive assessment of the contributions of indoor and outdoor exposures to total personal exposure. This study concluded that indoor pollution sources were generally a far more significant contributor to total personal exposure for toxic volatile organic compounds than are emissions released by some industrial sources into the outdoor air. Further evidence was provided by

1.2 Sources of pollution (indoor and outdoors)

The main sources of domestic indoor pollution can be attributed to fumes generated from incomplete combustion associated with heating systems and cooking appliances. Alternatively to indoor pollution, the general public are also exposed to Air Toxics in the outdoor environment generated in the main by vehicular traffic.

Carbon monoxide (CO) – The main source of CO exposure is from incomplete combustion, primarily from road transport but also from domestic and industrial sources. Natural background levels of CO range between 0.009 and 0.2ppm (0.01 and 0.23mg/m^3) (WHO, 1994). In urban traffic environments the 8 hour Time Weighted Average (TWA) concentrations are generally higher, but generally lower than 17.5ppm (20mg/m^3) and CO concentrations are generally higher inside vehicles than those measured in outdoor air (EPAQS, 1994).

A significant number of UK homes that use gas for cooking have short term levels of CO (Ross 1996, 1997) that exceed the World Health Organisation (WHO) one-hour time-weighted average guideline of 26ppm (30mg/m^3) (WHO 1987). Outdoor Carbon monoxide levels can be determinants of indoor levels, but the main sources of CO in the home are fossil fuelled appliances. Other factors that can influence indoor levels include the presence of an attached garage, the proximity to a busy road and Environmental Tobacco Smoke (ETS) (IEH, CO, 1998).

Nitrogen dioxide (N_2O) – The main sources of N_2O in the UK are from road transport and the electricity supply industry. Nitrogen dioxide is often referred to within the group of oxides of Nitrogen (NOx) together with Nitric oxide (NO). Indoor concentrations of Nitrogen dioxide in the kitchens of homes with gas cookers average, over a year, around 15 ppb (29μg/m^3) and peak concentrations may be as high as almost 600 ppb (1146μg/m^3) over an hour. The outdoor concentration of Nitrogen dioxide is the main determinant of indoor concentrations in homes without gas cookers, the concentrations generally being somewhat lower indoors. In homes with such cookers, indoor levels are usually at or above outdoor levels, being higher in the winter months when homes are less well ventilated and more use is made of gas appliances. Thus people living in homes with gas cookers are exposed to more Nitrogen dioxide indoors than those in homes without Gas cookers (EPAQS, 1996).

Benzene (C_6 H_6) – The primary source of Benzene in the UK is from domestic and industrial combustion processes, from road transport and from cigarette smoke. Mean ambient levels of Benzene in rural areas are approximately 0.3ppb (1μg/m^3) and 1.5-6.2ppb (5-20μg/m^3) in urban areas. People living in cities are generally exposed to higher levels than those living in the countryside, estimations suggest an average air intake of 180-1,300μg/day (assuming a typical concentration range of 2.8-20ppb)(Wallace, 1995).In non-smokers the main routes of exposure are related to, residence in proximity to traffic, travel in vehicles; refuelling cars

and passive smoking which all contribute to cumulative exposure, whereas smokers receive an estimated 90% of their Benzene exposure from cigarette smoking (Lagorio, 1998). An average smoker (approximately 32 cigarettes a day) takes in about 1.8mg of Benzene per day; this is about 10 times the average daily intake of non-smokers (ATSDR, 1997). A study of 800 persons in eight areas in the US identified that global average personal exposure was about 4.6ppb (15µg/m^3) with indoor concentrations of the order of 3.1ppb (10µg/m^3) and outdoor concentrations 1.8ppb (6µg/m^3). No effect on personal exposure of living dose to major fixed sources of Benzene could be detected. A study looking at geographical distribution of Benzene in air in three fixed sites in Italy identified a direct relationship between population density and level of contamination, confirming the effects of automotive traffic and industrial activities on Benzene outdoor pollution. Levels were also higher during the winter months.

1.3 Pollutant toxicology, and health effects

Carbon monoxide

Carbon monoxide is rapidly absorbed by the lungs where it combines with haemoglobin resulting in the carboxyhaemoglobin concentration rising rapidly in the coronary and cerebral arteries. The affinity of human haemoglobin for Carbon monoxide is about 240 times greater than for oxygen, so CO significantly reduces the capacity of the blood to carry oxygen around the body. Carbon monoxide is only eliminated through the

lungs with the half life in the blood of sedentary adults being about 2-5 hours. (Meredith, Vane 1988). Displacement of oxygen from haemoglobin is the best known property of Carbon monoxide which poisons the body in many complex ways (Walker 1999, Weaver 1999, Miro 1998) including interfering with various enzymes, binding with myoglobin to form carboxymyoglobin which may disturb muscle metabolism, especially in the heart and producing oxidative damage to neurovascular epithelium. Carbon monoxide is a vasodilator (Marks 1991) and the regulation of vascular tone is through a mechanism by which CO activates Soluble Guanylyl Cyclase (SGC) (Maines 1997) in a similar way to that of nitric oxide. It involves binding and dislocation of its haem-iron to induce a conformational change and activation of SGC leads to elevated intracellular CGMP, which in turn lead to smooth muscle relaxation (Schmidt 1993). In addition, CO is able to bind to the heme moiety of nitric oxide synthetase and thereby inhibit NO production. Although the vasodilator potential of CO is significantly less than that of NO, the resistance of certain vascular beds, such as the hepatic circulation, is modulated by CO rather than NO (Suematsu 1996). Carbon monoxide poisoning effects many organs within the body, the brain is particularly affected, severe poisoning produces permanent pathological changes similar to the affects of asphyxia. The heart is at particular risk due to its high oxygen consumption and the very high affinity of myoglobin for Carbon monoxide, which is approximately 3 times higher than that of haemoglobin (Astrup 1972). Those with ischemic or coronary heart

disease are extremely vulnerable to Carbon monoxide exposure due to their inability to increase coronary perfusion, meaning that following exposure to Carbon monoxide they may suffer a myocardial infarction or even sudden death. The acute effects of Carbon monoxide are well documented. The level of risk to an individual maybe increased by the duration of the exposure, activity level, a high metabolic rate and their age – young children are particularly susceptible to the effects of CO. Table 1 shows the effects of acute exposure to CO: -

Duration of exposure			
229mg/m^3	1145mg/m^3	COHb %	Effects
2 hours	20 minutes	10	Exercise tolerance reduced
7 hours	45 minutes	20	Breathlessness on exertion,
-	75 minutes	30	headache
-	2 hours	40-50	Severe headache, weakness, dizziness, dimness of vision, disturbed judgement, nausea, vomiting, diarrhoea, fast pulse rate
-	5 hours	60-70	Confusion, collapse on exertion, coma, convulsions
			Coma, convulsions, slow pulse rate, low blood pressure, respiratory failure, death

Table 1.1 Acute effects of Carbon Monoxide Poisoning (Indoor Air Quality Handbook, 2001)

Repeated exposures to low levels or prolonged low exposure to CO may lead to long term damage, and are often misdiagnosed. Symptoms include headaches, tiredness, nausea and dizziness

In controlled human studies involving patients with documented coronary artery disease, mean post exposure COHb levels of 3% - 6% have been associated with a significant shortening in the time to onset of angina, with increased electrocardio graphic changes and with impaired left ventricular function during exercise (Allred 1989, Sheps 1997, Adams 1998) Carbon monoxide in the home is responsible for a considerable number of deaths each year as well as an unknown number of sub-lethal poisonings (Chief Medical Officer, WAG, 2007). Many incidents of acute poisoning are associated with the use of badly installed, poorly maintained or malfunctioning domestic combustion appliances or with the use of such appliances, in poorly vented rooms (Bailie 1999). About 50 people die from acute Carbon monoxide poisoning in the United Kingdom each year (DoH 1998) An epidemiological survey of non-intentional acute CO poisoning in the West Midlands between 1988 and 1994 demonstrated an annual rate of 1.1/100,000 (Wilson 1998). There was a strong seasonal variation linked to the use of domestic heaters. The elderly and very young were at greatest risk (The Carbon monoxide and Gas safety Society 1997). In the UK up to 250,000 gas appliances are condemned annually, and even if a small percentage of these are producing significant amounts of CO then a large number of people maybe experiencing chronic poisoning with non-specific manifestations (Turner 1999).

Nitrogen dioxide

The main effects of exposure to NO_2 are to the respiratory system causing damage to the lining of the smaller airways. Oxidant injury appears to be the main mechanism of action (Samet and Utell 1990). Animal studies show a reduction in the efficacy of the lung defence mechanisms, including effects on mucociliary clearance, particle removal by alveolar macrophages, and immunologic function (Dawson and Schenker 1979, Samet and Utell, 1990). As a result there maybe a reduced clearance of respiratory pathogens and greater susceptibility to bacterial infections. The evidence suggests that exposure to low concentrations over years is likely to cause lung damage but specifically with respect to increasing susceptibility to infection, concentration is more important than duration of exposure (WHO 1987b). Outdoor levels of Nitrogen dioxide are known to influence indoor levels but the most significant source of indoor NO_2 is from gas appliances (IEH, 1996), it has been suggested that short term peaks to high levels, such as those which may be experienced in the home, maybe important in determining health risks. Long term exposure may affect lung function and respiratory symptoms. It can also enhance the response to allergens in sensitive individuals. Levels experienced within the home could cause ill health including respiratory symptoms, susceptibility to respiratory infections, and possible impairment of lung function and have an adverse effect on some susceptible groups such as asthma sufferers. As there are no symptoms specifically attributable to low level NO_2 exposure the evidence for an effect must be obtained from

large epidemiological studies linking health effect to exposure. Since exposure to Nitrogen dioxide indoors is often an important contributor to the overall exposure of individuals, some studies have specifically investigated relationships between such exposure and health. It wasn't until the mid 1970's that a report carried out by Melia (1977) in the UK brought attention to the possible adverse health effects of indoor Nitrogen dioxide from gas cooking appliances. It found that children living in homes with gas stoves had a higher prevalence rate of respiratory symptoms and illnesses than those living in homes with electric stoves. Hasselblad et al 1992 carried out a meta analysis of 11 studies on children, they found an odds ratio of 1:2 for respiratory illness in children exposed to NO_2 with 95% confidence limits of 1.1 to 1.3, implying a 20% increase in the risk of respiratory illness corresponding to an increase of 16ppm ($30mg/m^3$) of Nitrogen dioxide. However other large studies have failed to replicate these results (Samet 1993, Farrow 1997). There are a lesser number of adult studies associated with NO_2 exposure. The use of gas cookers and other unvented gas appliances was associated with respiratory symptoms and impaired lung function in women but not men in a study by Jarvis (1996). Several studies have looked at the possible effects of low concentrations of NO_2 in persons with asthma although with conflicting results and no conclusive evidence.

Benzene

Benzene has been identified as a human genotoxic carcinogen; no absolute safe limit has been specified in ambient air. Long term exposure to Benzene may result in Leukaemia, although this is normally associated with occupational exposure (Agency for Toxic Substances and Disease Registry, 1997). Inhalation exposure accounts for more than 99% of Benzene exposure in humans. Human inhalation studies have exposed individuals to 50-100ppm (160-320µg/m^3) of Benzene and indicate that approximately 50% is absorbed of which 30% is retained from the inhaled dose with the rest being exhaled as unchanged Benzene.

Studies have shown an increased incidence of leukaemia in workers exposed to high levels of Benzene. Benzene is readily absorbed into the body when breathed in through the lungs, about half of it being retained. As it is more soluble in fat than in water, it is distributed in the body to fatty tissues including the brain and the bone marrow. In the absence of further exposure, Benzene is eliminated by chemical breakdown in the body or by metabolite excretion in the urine, 80% being eliminated within about two days (DoE 1994). Chronic exposure to Benzene can result in bone marrow depression expressed as leukopenia, anaemia and/or thrombocytopenia, leading to pancytopenia and aplastic anaemia. The main metabolic transformation of Benzene is oxidation by microsomal mixed function oxidases to Benzene epoxide, a highly reactive substance that can react with nucleic acids. The health effects of environmental exposures to low levels of Benzene have not yet been fully elucidated, due

in part to the incomplete knowledge of the pharmacokinetic effect of Benzene in humans (Synder, 1996, Smith, 1996, Bois, 1996) and mainly to difficulties in the assessment of exposures at or below the ppm level. The concentrations of airborne Benzene associated with an excess lifetime risk of Leukaemia are 1/10,000, 1/100,000 and 1/1000, 000 are, respectively 5.2ppb, 0.5ppb and 0.05ppb (17, 1.7 and 0.17μg/m^3) (WHO 2002).

1.4 Biomarkers as a tool for assessing environmental exposure

Human biomonitoring is defined as the acquisition of exposure and biological effect data through the analysis of cells, tissues, or body fluids (Suk, 1996). The biochemical or biological variable measured for the purpose of biomonitoring is designated as the biomarker. Biomarkers can be useful in confirming toxic exposures, estimating their effects and identifying persons most likely to be adversely affected from continued exposure. Biomarkers of exposure in isolation do not give an indication as to whether an exposure has produced a biologically significant result as the same dose in different individuals who maybe susceptible or resistant to a given exposure can have different results. Biomarkers of exposure which are most commonly used include measuring the level of the pollutant in the blood, urine or other body tissue. A biomarker of effect is a measurable cellular, physiologic or biochemical alteration within an organism caused by an interaction with a pollutant. Biomarkers are an accepted tool in identifying occupational exposures, and the use of

biomarkers in environmental epidemiological studies helps identify exposures and effects for environmental toxicants of significance to public health. The existence of a proven biomarker of CO (i.e. COHb) and the potential of biomarkers of its more systemic effects (e.g. SGC and cGMP) provide an epidemiological tool for investigating the potential effects upon health of environmental levels. Benzene epoxide can be hydrated by an epoxide hydratase and then condensed with glutathione to form mercapturic acid and measurement of urinary mercapturic acid (s-PMA) has been demonstrated to provide a reliable indicator of Benzene exposure.

1.5 Air Pollution standards

The UK Environment Act (1995) required the devolved administrations of Scotland, Wales and Northern Ireland to produce a National Air Quality Strategy containing standards, objectives and measures for improving ambient air quality. Since the adoption of the first Air Quality Strategy in 1997 air quality has generally improved in the UK, with an estimated reduction of more than 4,200 premature deaths and 3,500 hospital admissions per year.

Standards relating to the effects of different pollutants on human health are set following advice from scientific and medical evidence. The World Health Organisation (WHO) published air quality guidelines in 1987, and have published revisions approximately every 5 years since. In the UK,

the Expert Panel on Air Quality Standards (EPAQS) report on pollutants of national importance. Table 1.2 shows the standards in place within the UK for the pollutants of interest. These standards relate to both indoor and outdoor exposure. There is not an environmental standard in place for Nitrous oxide alone, there is an occupational standard but this far exceeds any levels that would be expected outside the workplace.

Pollutant	Applies	Objective	Concentration measured as	Date to be achieved and maintained thereafter
Nitrogen dioxide	UK	200µg/m^3 not to be exceeded more than 10 times a year	1 hour mean	31 December 2005
	UK	40µg/m^3	Annual mean	31 December 2005
Benzene	UK	16.25µg/m^3	Running annual mean	31 December 2003
	England and Wales	5µg/m^3	Annual average	31 December 2010
	Scotland and Northern Ireland	3.25µg/m^3	Running annual mean	31 December 2010
Carbon monoxide	UK	10mg/m^3	Maximum daily running 8 hour mean	31 December 2003

Table 1.2 National Air Quality Objectives (AQS 2007).

1.6 Purpose of the research

One epidemiological study investigated chronic exposure to CO in the homes of elderly people using different fuels for heating. This aimed to explore if different environmental levels caused different biological effects as measured by cGMP in blood platelets.

A second epidemiological study investigated the environmental concentrations of NO_2, N_2O and Benzene in urban microenvironments of differing traffic flow. It also aimed to measure if microenvironment exposures determined personal uptake of Benzene and N_2O.

CHAPTER 2

Population exposure and effects from the primary pollutants CO, Benzene and Nitrogen dioxide

This chapter describes the main exposure sources from the primary pollutants Carbon monoxide, Benzene and Nitrogen dioxide. It also explores the health effects from the pollutants and where identified the biomarkers of exposure and effect.

2.1 Carbon Monoxide

2.1.1 Outdoor Carbon Monoxide

The main source of Carbon monoxide in the UK is road transport which in 1997 accounted for almost 75% of the UK total CO emissions (Chaitman, 2000). Annual emissions of Carbon monoxide have fallen 33% between 1990 and 1997, due partly to stricter environmental controls.

Natural background levels of CO, in rural areas, range between 0.009ppm and 0.2ppm (0.01 – 0.23mg/m^3), (WHO, 1994). Long term average outdoor levels of CO in the UK vary depending on the level of urbanisation and climatic conditions. In the UK Carbon monoxide is measured at 61 automatic monitoring stations. In Wales, in 1997-1998 the maximum hourly Carbon monoxide reading was 12ppm (14mg/m^3) and the largest annual mean was recorded in Cardiff at 0.78ppm (0.9mg/m^3) (Welsh Air Quality Forum, 2000).

Combustion engines are designed to operate efficiently when there is just enough air to oxidise the Carbon in the fuel. More Carbon monoxide is produced when the engine is cold, badly tuned or moving slowly; therefore levels of Carbon monoxide are more likely to be higher close to busy roads in urban areas. Roadside air quality is affected by changes in traffic density with time, vehicle type, vehicle composition, terrain and meteorological conditions.

2.1.2 Indoor Carbon Monoxide

In the home, exposure occurs from heating and cooking sources which have been badly installed, poorly maintained or sited in poorly ventilated rooms as well as from environmental tobacco smoke (ETS) (IEH, 1998).

Raw et al (2004) carried out a study for the Building Research Establishment (BRE) looking at exposure to air pollutants in English homes. A total of 876 homes were recruited through the Survey of English Housing (SHE) with monitoring being conducted over a full year to identify any seasonal differences. Carbon monoxide levels were monitored over 2 weeks in the kitchen and bedroom using Draeger colorimetric diffusion tubes (the manufacturer of the tubes had tested the tubes for measurement of low concentrations of CO over a period of up to 15 days and claimed an accuracy of ±50% for a single tube reading. Carbon monoxide results were obtained for 830 homes, the geometric mean levels recorded in the kitchen were 0.41ppm (0.47mg/m^3) with a range of 0.008 –

3.88ppm (0.01-4.45mg/m^3) and in the bedroom 0.34ppm (0.39mg/m^3) with a range of 0.008 – 3.4ppm (0.01-3.9mg/m^3). Season was also shown to have a significant effect on the CO levels, with higher levels in the autumn and winter than in the spring and summer, possibly due to greater use of fossil fuels during the colder months and less natural ventilation as shown in Table2.1:-

Kitchen CO	Spring	Summer	Autumn	Winter
Gas cooking including gas oven	0.61	0.51	0.88	1.06
Gas cooking, no gas oven	0.41	0.2	0.6	0.57
No fossil fuel cooking	0.2	0.14	0.44	0.39
All kitchens	0.35	0.27	0.59	0.62

Table 2.1 CO (mg/m^3) by season and cooking fuel (Raw, 2004).

The study also looked at the CO levels in relation to the area the homes were located. There was a significant difference between areas with CO levels being significantly lower in rural areas compared to suburban and central urban areas. Taking rural areas as a baseline, it was calculated that CO exposure in the home is almost doubled by living in a central urban area.

Ross et al (1996) carried out continuous monitoring of Carbon monoxide in fourteen homes in the UK, over a period of one week. Six of the homes used gas appliances only, 2 used electricity only and 6 used a

combination of gas and electric cooking appliances. Weekly average readings were calculated. Levels recorded in the kitchens of the homes using gas appliances were 0.3 – 2.4ppm (0.3 – 2.7mg/m^3). In the kitchens of the homes where gas cooking was not used the levels ranged between 0.7 – 0.8ppm (0.8 – 0.9mg/m^3). In the homes with gas cookers the levels recorded in the living room ranged between 0.2 – 2.2ppm (0.2 – 2.5mg/m^3). Whilst levels of indoor CO are generally low, badly installed or malfunctioning appliances can make dramatic increases to the measured levels. The study reported one home with a maximum 1-min average concentration of 59.8ppm (68.5mg/m^3) in the kitchen and 106ppm (121.4mg/m^3) in the living room and the levels were linked to a malfunctioning boiler.

Wiech et al (1995) looked at 40 households. Carbon monoxide monitoring was carried out using Draeger passive diffusion tubes. The reported results of the average indoor readings of CO were highest in the living room 0.21ppm (0.24mg/m^3) followed by the bedroom 0.18ppm (0.21mg/m^3). All levels were below 3ppm (3.4mg/m^3).

Alm et al (1999) looked at personal CO levels in 194 pre-school children in Helsinki. Children carried a personal monitor with them for 20-24 hours at a time, collecting exposure data for 1-4 days excluding weekends. Monitoring was carried out over a 24 -week period from autumn to spring. A total of 449 personal exposure measurements were made, although only

302 measurements (from 167 children) were of value due to problems with the monitoring equipment. Information was also collected on location of their home, cooking appliance, fireplace, ventilation and heating and the present smoking status of the parents. Half of the children lived in the suburban area and half lived downtown and who commuted by car or bus or walked. The mean level of the 1 hour and 8 hour exposure levels were 5.24 and 2.88ppm (6 and 3.3 mg/m^3). The WHO limits were exceeded for 2% of the children for the 1 hour exposure level and 4% for the 8 hour exposure limit. During November and December the 8 hour ambient air quality guideline was exceeded during three days in two inversion situations. The children who were taken to school by car or public transport had higher peak exposures than those who walked to school. A Gas stove at home, parents smoking and living in high- rise buildings all increased the children's CO exposure. Children who had fireplaces at home unexpectedly had lower averages than those homes without fireplaces, the author suggested that whilst these fireplaces were rarely used they were indicators of larger and more expensive dwellings in better environments. It was also reported that mechanically ventilated homes had lower levels than naturally ventilated homes, and there was little difference between home location (suburban vs. downtown).

Croxford et al (2005) as part of the UK Government Fuel Poverty programme monitored 56 homes for Carbon monoxide in 3 UK cities. The homes were selected as part for the fuel poverty programme so a higher

incidence of problem gas appliances emitting a higher than average amount of CO than the general population might be expected. To fulfil inclusion criteria, occupants of the homes were either on income support, over 60 years old or a single parent family and were eligible to receive central heating systems under the Warm Front programme. Battery powered data loggers with electrochemical sensors to detect CO were specifically built for the study. The loggers monitored every minute and were fixed to record fifteen minute average readings. The loggers were positioned at head height of a seated adult, within the main living area of the home. The monitoring period ranged from 1 week in 4 homes, 2 weeks for 2 homes and the remaining 50 homes were monitored for 4-5 weeks. Out of the 56 homes monitored 13 (23%) had Carbon monoxide levels that exceeded the WHO 8 hour guidelines for outdoor ambient air (8.6ppm (9.85mg/m^3)) at least once, of these 6 exceeded the WHO 1 hour level of 25ppm (28.64mg/m^3) and 3 exceeded the 30 minute guideline of 50ppm (57.8mg/m^3). As a result of the findings a CO gas safety expert was employed to investigate 10 of the 13 homes where levels had exceeded guidelines. The report showed high levels were due to old, poorly installed and poorly maintained gas fires and gas cookers.

Laquatra et al (2005) reported on indoor air quality in homes and child care facilities in New York State. The study looked at rural areas, counties were randomly selected based on a cluster analysis including housing characteristics, number of occupants and proportion of houses built prior

to 1979 and between 1980 and 1989. The total sample size was 328. A telephone survey was carried out to determine further characteristics and monitoring was carried out in 132 homes during the heating season 2000-2001. An additional 24 child care facilities were monitored. Carbon monoxide was measured with a Bacharach sample draw Carbon monoxide analyser for 10-15 minutes in the central living area of each household. Levels of CO monitored in the living room ranged from 0-9ppm (0 – 10.31mg/m^3) with a mean level of 0.39ppm (0.45mg/m^3). The results showed a significant and negative correlation between income and Carbon monoxide levels in the kitchen. Lower income households were more likely to have older cooking appliances that had not been maintained and poor ventilation was also identified.

Outdoor levels of CO can influence indoor levels through influx of outdoor air into the indoor environment. In regard of total exposure, indoor sources can account for a larger proportion than traffic. (EPAQS, 1994) although the limited data generated in the UK suggest that levels of indoor and outdoor CO are comparable (Harrison et al, 1988).

A Minnesota study found the presence of an integral garage increases the likelihood of Carbon monoxide exposure into the home from vehicle exhausts, the problem is exacerbated in the winter when engines are colder and run for longer to warm up. (Minnegasco, 1997).

Environmental Tobacco Smoke

Mannino et al (1997) looked at ETS exposure in the home, results showed that 89% of people in the US aged 6 and above had levels of cotinine, which is a marker for ETS in their blood greater than 5ng/dl. The percentage of American children exposed to ETS in the home has decreased from 62% in 1970 to 37% in 1991. The study used data from 41,638 adults 18 years and older from the 1991 National Health Interview Study.

Kirk et al (1988) carried out a study looking at environmental tobacco smoke in a range of smoking and non smoking environments in the UK. The study was carried out over 10 weeks, with 2912 samples being taken in the home (14%); at work (25%); at leisure (27%) and during travel (29%). Carbon monoxide was actively sampled over 20 minute periods with data being logged every 2 minutes: -

Sampling situation	Smoking environment	Non-smoking environment
Travel	3.3 (2.9)	3.1 (2.7)
Work	2.5 (2.8)	2.4 (2.1)
Home	2.6 (2.3)	2.1 (1.8)
Leisure	3.2 (2.8)	2.5 (2.2)

Table 2.2 Overall mean levels of Carbon Monoxide in mg/m^3 (ppm) for particular sampling situations. (Kirk et al, 1988)

There were no significant differences between smoking and non-smoking environments for any of the sampling situations ($p>0.05$), suggesting that

indoor levels of CO are generally determined by other sources, but in all cases, CO is higher in smoking environments.

Guerin et al 1992 looked at field studies of CO concentrations in smoking and non-smoking locations. Mean concentrations of CO in the air of offices where smoking was permitted ranged from 1.2 – 2.8ppm compared to offices where smoking was not permitted 1.2- 2.5ppm. In restaurants and cafeterias permitting smoking CO levels ranged from 1.2 – 9.9, compared with 0.5 – 7.1ppm where smoking was not permitted.

The BMA has been instrumental in the demand for the banning of smoking in the workplace and public areas, following the example set by the Irish Government. People in lower socioeconomic groups and workers in the hospitality industry are disproportionately exposed to other people's smoke and therefore the risk of lung cancer. (BMA 2004). Following the smoking ban in Ireland a survey carried out by the national quit line service has reported that 33% of smokers have given up smoking. (ASH) A full UK smoking ban in public places and work premises came into place on the 1st July 2007.

2.1.3 Standards

The World Health Organisation (WHO) has in place a one hour guideline for Carbon monoxide of 26ppm (30mg/m^3) and an 8-hour guideline of 8.73ppm (10mg/m^3) (WHO, 1987).

The environmental health criteria used for the recommendation for an exposure limit for the general population, were based on two groups: effects on non-smoking subjects with coronary artery disease exposed to Carbon monoxide while exercising and the potential effects on foetuses of non-smoking pregnant mothers exposed to ambient sources of Carbon monoxide. The principal cause of Carbon monoxide induced effect at low levels is thought to be increased carboxyhaemoglobin formation. After reviewing the large amount of evidence on the health effects of Carbon monoxide, the Expert Panel on Air Quality Standards (EPAQS) panel concluded that the people most susceptible to exposure to Carbon monoxide are those with angina and disease of the coronary arteries. The evidence suggests that the lowest level of carboxyhaemoglobin at which effects can be detected in such people lies between 3 and 4%. (EPAQS) have therefore concluded that ambient atmospheric concentrations of Carbon monoxide should be such that the concentration of carboxyhaemoglobin in the blood of people breathing that air over a prolonged period should not exceed 2.5%, thus allowing a safety margin. Exposure limits are therefore derived on the basis of carboxyhaemoglobin, with a recommendation that a carboxyhaemoglobin level of 2.5% should not be exceeded. It is possible to relate blood carboxyhaemoglobin levels to atmospheric concentrations of Carbon monoxide by use of mathematical formulae, this is a complex mathematical formula (Coburn-Forster-Kane (CFK) equation) (Bruce and Bruce, 2003). This relationship shows that carboxyhaemoglobin concentrations would be kept below 2.5%

when breathing the following concentrations of Carbon monoxide at maximum levels of activity:

Concentration of CO (in ppm)	Time (hours/minutes)
10	8
25	1
50	30
87	15

Table 2.3 EPAQS, 1994

The EPAQS recommended an Air Quality Standard for Carbon monoxide in the United Kingdom of 10 ppm; they recommended that a running 8-hour average be used for the Standard.

Kuller et al (1983) reviewed the epidemiological bases for the CO standards set by the US Environmental protection Agency (EPA). The ambient standard is based on the effects of elevated carboxyhaemoglobin levels on the cardiovascular system and behavioural responses. The ambient standard is set at an 8hour annual average of maximum of 9ppm (10.31mg/m^3), with a maximum 1 hour level of 35ppm (40.1mg/m^3).

2.1.4 Health Effects

2.1.4.1 Endogenous CO production, and carboxyhaemoglobin

Carbon Monoxide is endogenously formed from the catabolic degradation of heme. As red cells age they are removed from circulation and their heme is degraded. The body naturally produces small amounts of CO

when the enzyme haem oxygenase-1 (HO-1) breaks down haem. Heme degradation begins with oxidative cleavage and the methenyl bridge Carbon between porphyrin rings is released as CO. The major site of haem breakdown and therefore of production of endogenous CO is the liver. CO will form a resilient heme ligand and approximately 1% of hemoglobin's oxygen binding sites are blocked by CO from endogenous sources even in the absence of any air pollution. The COHb level from endogenous CO formation, on average amounts to 0.7% corresponding to approximately 4ppm (4.58mg/m^3) of CO in exhaled air (Coburn, 1979, Benowitz, 1983). Pregnant women also show a substantial increase in endogenous CO production related to an increase breakdown of red blood cells, this rapidly drops following delivery (Aubard 2000).

Inhaled Carbon monoxide binds to haemoglobin in red blood cells to form carboxyhaemoglobin, reducing the oxygen carrying capacity of haemoglobin and reducing the delivery of oxygen to the heart and other tissues (Chaitman, 2000). The affinity of haemoglobin for Carbon monoxide is 200–250 times that for oxygen (Stewart, 1976). Approximately 85% of the absorbed Carbon monoxide binds with haemoglobin to form carboxyhaemoglobin. The remaining 15% CO is distributed extravascularly. The concentration of COHb in blood correlates with the inhaled dose of Carbon monoxide that a person has breathed in and therefore is a useful biomarker of exposure. Carboxyhaemoglobin diffuses rapidly across the alveolar and capillary membrane and more

slowly across the placental membrane. Levels fluctuate in females during the menstrual cycle.

During an exposure to a fixed ambient concentration of Carbon monoxide, the carboxyhaemoglobin concentration increases rapidly at the onset of exposure and starts to level off after 3hours and approaches a steady state after 6-8 hours.

The most important variables determining the COHb level are Carbon monoxide concentration in inhaled air, duration of exposure, alveolar ventilation, health status and metabolic characteristics of the exposed individual. COHb can be used as a biomarker of exposure but is dependent on a number of assumptions including activity patterns, exposure duration and pre-existing susceptibility. It is also less precise as an indicator at low levels of CO, especially taking into consideration endogenous CO production

Blood carboxyhaemoglobin levels %	Observed health effects
2.5-4.0	Decreased short term maximal exercise duration in young healthy men
2.7-5.1	Decreased exercise duration due to increased chest pain (angina) in patients with ischaemic heart disease
2.0-20.0	Equivocal effects on visual perception, audition, motor and senorimotor performance, vigilance, and other measures of neurobehavioural performance
4.0-33.0	Decreased maximal oxygen consumption with short term strenuous exercise in young healthy men
20-30	Throbbing headache
30-50	Dizziness, nausea, weakness, collapse
Over 50	Unconsciousness and death

Table 2.4 Human health effects of exposure to Carbon monoxide (EPAQS, 1994)

On ending a period of exposure, the decline in COHb concentration depends on the rate of Carbon monoxide release from haem proteins, alveolar ventilation, oxygen concentration in inhaled air, duration of Carbon monoxide exposure, and the level of COHb saturation. The formation of COHb is a reversible process, but because of the tight binding of Carbon monoxide to haemoglobin, the elimination half-life while breathing room air is 2–6.5 hours depending on the initial COHb level. The elimination half-life of COHb is much longer in the fetus than in the pregnant mother. (WHO 2000)

Exposure situation	**COHB % non-smokers**
Measured values	
Urban background exposure	0.8
Point-duty police (after 3 hours in busy street)	1.9
Others on foot in busy street	1.2
Cyclists (city streets)	1.7
Motorists	1.8
Staff in parking garages	2.4
Staff in custom sheds, ferries	1.3

Table 2.5 Mean blood carboxyhaemoglobin levels in persons exposed to Carbon monoxide

Lambert et al (1988) looked at the application of end expired breath sampling to estimate COHb in community air pollution exposure assessments. Twenty-eight males (39 – 72 years old) with ischemic heart disease gave breath and blood samples. Breath samples were analysed using an IL282 CO-Oximeter. 112 samples were obtained, CO ranged from 2 – 13.3ppm (2.29 – 15.24mg/m^3)) in non-smokers and 6.1 – 36.7ppm (6.99 – 42.04mg/m^3) in smokers. COHb ranged from 0.4 – 3.2% in non-smokers and 2.5 – 6.7% in smokers.

2.1.4.2 Effects on Guanylate Cyclase and cyclic GMP production

It is only recently that the intracellular mechanisms for the actions of CO are beginning to be understood. It is known that CO binds to the iron of heme proteins and affects several intracellular signaling pathways, t has

been shown to interact with guanylate cyclase which generates cyclic guanosine monophosphate (cGMP) (Kapturczak-Hill 2005).

Cyclic Guanosine Monophosphate (cGMP) is a cyclic nucleotide derived from Guanosine Triphosphate (GTP). cGMP acts as a secondary messenger, most notably by activating intracellular protein kinases in response to the binding of membrane-impermeable peptide hormones to the external cell surface. cGMP is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. It also relaxes smooth muscle tissues and in blood vessels. Relaxation of vascular smooth muscles lead to vasodilation and increased blood flow. Hence, Carbon monoxide can be considered a vasodilator similarly to nitric oxide (Marks 1991) regulating vascular tone through a mechanism by which it activates Soluble Guanylyl Cyclase (SGC) (Maines 1997). This involves binding of its haem-iron to induce a conformational change and activation of SGC leads to elevated intracellular cGMP, which in turn leads to smooth muscle relaxation (Schmidt 1993). It should be noted that several conditions have been identified as leading to increased levels of cGMP including various types of premalignant disease, cardiovascular disease, pre-eclampsia, the luteal phase of the menstrual cycle and during and after normal pregnancy. The biokinetics of cGMP are also influenced by physiological factors and pharmacological agents. Endogenous CO has also been shown to stimulate cGMP production (Verma et al 1993). Snyder et al (1998) proposed that CO similarly to nitric oxide regulates

some of the body's functions including contraction of the intestines and emptying of the stomach.

The use of cGMP as a pathophysiological marker of CO poisoning requires detailed knowledge about its cellular biokinetics. Activation of guanylate cyclase is often due to the action of nitric oxide but can also be initiated by CO. In terms of CO poisoning binding of guanylate cyclase results in the increased production of cGMP. Cyclic GMP production results in excitatory neurotransmitter production and cerebral vasodilation, clinically the observed effects are fainting and neurologic injury. This has been demonstrated in an animal model of CO poisoning (Verma et al 1993).

Hernandez-Viadel et al (2004) found that chronic exposure of rats to a daily dose of 450-500ppm (515.5 – 572.8mg/m^3) of CO for 6 hours per day, 5 days per week over the period of one month ddn't change the content of cGMP in the cerebellum, whilst acute exposure of two groups of rats to 2400ppm (2749mg/m^3) for 1 hour and 2400ppm(2749mg/m^3) for 7 days to CO resulted in a decrease of cGMP content in the cerebellum and reduced activation of soluble guanylate cyclase by nitric oxide. Acute exposure effects were stronger at 7 days than after 24 hours exposure suggesting that this delayed impaired modulation of soluble guanylate cyclase by nitric oxide may contribute to delayed memory loss and cognitive impairment in humans exposed to Carbon monoxide.

Van Bel et al (2005) investigated whether Carbon monoxide mediated cGMP production was responsible for low blood pressure in neonatal respiratory distress syndrome. Infant RDS involves inflammatory processes causing an increased expression of inducible heme oxygenase with subsequent production of CO, the authors hypothesised that increased production of CO during RDS may be responsible for increased plasma levels of vasodilatroy cGMP. 52 infants were studied (31 with RDS, 21 without RDS), COHb and cGMP levels were determined at 0, 12,48,72 and 168 hour intervals. Infants with RDS had higher levels of cGMP compared to infants without ($p<0.001$) and higher levels of COHb ($p=0.0001$). Multiple linear regression analysis showed a statistically significant ($r=0.77$, $P<0.002$) correlation between cGMP and COHb, with an overall increase of 50nmol/l of plasma cGMP per 1% increase of COHb.

Flo et al (1995) looked at a number of variables affecting cGMP. The study looked at some aspects of the cGMP transport in human erthrocytes. VanUffelen et al (1996) found that CO caused a rapid and transient increase in the intracellular level of cGMP, and they suggested that CO acts as a biological signal in the immune system.

Some results indicate that cold exposure causes an increase in the release and metabolism of catecholamines. Prikryl et al (1982) looked at the effects of cold stress on cGMP in hardened and unhardened men.

Two groups of men were studied, hardened (trained athletes) and unhardened (non athletes). All subjects had baseline venous blood samples taken; they were then exposed for 1 minute in a swimming pool (water temperature 7°C and outdoor temperature of 5°C). Immediately after exposure a second venous blood sample was taken. cGMP increased only in the unhardened subjects.

Leppert et al (1995) looked at the effect of cold exposure on healthy women and women with Raynaud's phenomenon. The study investigated whether healthy women had the ability to increase cGMP during cold exposure whilst women with Raynaud's phenomenon do not. The healthy group consisted of 21 females, with the Raynaud's group consisting of 24 females. Baseline venous blood samples were taken; the subjects were then covered from chin to feet in a water-chilled blanket. A second blood sample was taken during cold exposure and then a third sample taken 20 minutes after termination of cold exposure. There was no significant difference between the groups for baseline cGMP levels. There were no significant changes in venous cGMP during the study for the Raynaud's group; however there was a significant increase in the cGMP levels in the healthy individuals. The data indicated that whole body cooling triggers an endothelial response resulting in an increase in cGMP, which prevents the contraction of vascular smooth muscle cells in healthy women.

2.1.4.3 Epidemiology

Time Series studies

Poloniecki et al (1997) tested for a significant association between outdoor air pollution within the preceding 24 hours and emergency hospital admissions for circulatory diseases that would be consistent with a causal effect of pollution on the previous day. Data was collected on O_3, NO_2, SO_2, CO and black smoke from a background monitoring site in London. The CO, NO2 and SO2 measurements were means of 24 hourly measurements. Temperature and humidity data was also collected from the meteorological office. 373,556 admissions were studied between 1st April 1987- 31st March 1994 (the daily median of admissions was 145). For diseases of the circulatory system, international classification of diseases 9th revision (ICD-9) 390-459 was used. Analyses were performed for the combined group and for the following subgroups: - acute myocardial infarction, angina pectoris, other ischaemic heart disease, arrhythmia, heart failure, cerebrovascular disease and all remaining codes. Single Poisson models were analysed for each of the eight groupings, and a pollutant was said to be significantly associated with admissions if $P<0.05$. For the single pollutant Poisson models, Carbon monoxide was significantly associated with combined circulatory diseases $P=0.004$ and acute myocardial infarction $P=0.001$. With the single pollutant partial models, the associations became undetectable for circulatory diseases for CO ($P=0.61$), although the association with myocardial infarction remained consistent ($P=0.0005$).

Ballester et al (2001) carried out a similar study looking at the short term association between cardiovascular hospital admissions and air pollution in Valencia, Spain between 1994-1996. Daily levels of air pollution and emergency admissions for cardiovascular diseases in two hospitals were examined controlling for the major confounding variables. The catchment population for the two hospitals was approximately 400,000 inhabitants. The number of daily admissions was obtained from the hospital databases and using the categories of ICD-9. Pollution data was obtained from the city air pollution monitoring network, The study found a clear association between CO and cardiovascular disease admissions; although as there was such a high correlation between the pollutants studied (CO, SO2 and black smoke) the study recognised that it would be difficult to assign the potential causal role to an isolated pollutant. But the association was significant during the summer months (RR for a 1mg/m3 increase in the daily CO concentration of 1.068; 95% CI: 1.007 to 1.133), which was not found during the rest of the year (RR 1mg/m^3 CO:0.990; 95% CI:0.959 to 1.022). The authors suggest that during the summer months, people modify there activities spending greater amounts of time outdoors and therefore exposure being more correlated to outdoor levels.

Gouveia et al (2000) carried out a time series study looking at the association between outdoor air pollution and mortality in Sao Paulo, Brazil. The main source of air pollution in Sao Paulo is from motor vehicle emissions. Daily environmental levels of SO_2, PM_{10}, CO, O_3 and NO_2 were

available from the Brazilian environment agency. During the study period, levels of air pollution were relatively high, with most pollutants exceeding the recommended guidelines. The study concentrated on children under 5 and the adults over 65years old. During the 3 year study, there were 151,756 non violent deaths of which 49% were the elderly and 10% children under 5. The study showed that cardiovascular deaths were significantly associated with PM_{10}, SO_2 and CO. For respiratory mortality in children under 5 positive associations were found for SO_2, CO and O_3, and the point estimates for these pollutants were higher than the ones found in the elderly. In the elderly a 3-4% increase in daily deaths for all causes and cardiovascular diseases was associated with increased daily averages of particulate matter and Sulphur dioxide from the 10^{th} to the 90^{th} percentile. Cardiovascular deaths were additionally associated with carbon monoxide (4% increase in daily deaths). For respiratory deaths, the increase in mortality was higher (6%). There was a significant trend of risk of death associated with age, with greater risk over 65 years of age. Overall the study showed that the effect of air pollution on all cause mortality was only statistically significant in people over the age of 65.

Mann et al (2000) also looked at how air pollution affected hospital admissions of persons with heart disease. The study covered a population of 1,515,776 in Southern California between 1988 and 1995. Hourly concentrations of the main pollutants were collected including CO, together with daily hospital admissions for Ischemic heart disease (IHD).

Over the 8 year study period there were 54,863 admissions for IHD. A 1ppm (1.15mg/m^3) increase in 8-hr average of CO was associated with a 3.6% increase in daily hospital admissions for CHF and a 2.99% change in those with a diagnosis of ARR. Analysis was also carried out for >60 years of age and a 1ppm (1.15mg/m^3) increase in 8-hr average of CO was associated with a 2.9% increase in daily hospital admissions for CHF. The study showed that concentrations of CO (and NO_2) below the US National Air Quality Standards were associated with admissions for IHD, the greatest effects were found for both pollutants and both for the same day and 2-day moving average concentrations.

Morris et al (1995) investigated the possibility that low levels of CO may have a significant health effect. The study used time series models to demonstrate an association between ambient CO and hospital admissions for congestive heart failure (CHF) among the elderly in the US.

A number of other studies have found consistent results with a synergistic effect of temperature and CO. Pantazopoulou et al (1995) looked at hospital admissions for cardiovascular disease and CO levels in Athens. The study found that emergency admissions in Athens for cardiac disease were associated with CO in the winter but not the summer. Poloniecki et al (1997) also looked at cardiac disease and CO levels in London. The study found that combined admissions for cardiovascular disease were

significantly associated with CO, particularly with myocardial infarction in the winter months.

2.1.4.4 Susceptible Groups

The fetus and newborn child are particularly susceptible to Carbon monoxide exposure. Fetal circulation is likely to have a higher carboxyhaemoglobin level than the maternal circulation as a result of differences in uptake and elimination of Carbon monoxide from fetal haemoglobin. As the fetus also has a lower oxygen tension in the blood than adults, any further drop in oxygen from carboxyhaemoglobin could be extremely serious. It is still unknown as to whether chronic exposure to Carbon monoxide at ambient levels can compromise the already marginal conditions in the fetus (WHO, 1999).

2.1.4.5 Carbon monoxide poisoning

Corgi (2007) reported on the number of Carbon monoxide incidents during the period January 2006 to April 2007 within the UK. A total of 102 accidental poisoning incidents were reported resulting in 50 fatalities and causing 218 injuries which were attributable to fossil fuel appliances. The overall figures for injuries are likely to be higher due to undiagnosed cases. The elderly (60+) accounted for 22 of the fatalities and 32 of the injuries. 53% of the incidents reported in 2006 were in the winter months (November to February) and there were 24 incidents in January and February 2007. The Health Protection Agency annual report 2006/2007

reported that of the 57,474 telephone enquiries to the National Poisons information service, 237 (0.4%) related to concerned suspected exposure to Carbon monoxide. No deaths were reported to the service during this period (HPA 2007).

The Institute for Environment and Health (IEH) report (2001) reviewed studies and associations between short term and long term exposures to CO in the home and associated acute and chronic health effects. The report highlighted that CO symptoms were similar to other ailments and that it was highly likely that missed or misdiagnosis of CO intoxication is occurring. Other possible health effects that were identified including atherosclerosis promotion, changes in immune function and altered neuropsychological and neurotransmission functions. The report concluded that in some homes CO levels routinely occur that may cause chronic health effects particularly among sensitive groups and that there was significant cause for concern to increase awareness of symptomatology among health care professionals.

Gajdos et al (1991) carried out a 3 year study to investigate the incidence, mortality and causes of CO intoxication in France. 735 cases were reported that were linked to 291 events. The average incidence was 17.5 per 100,000. The main mechanisms of poisoning were defective device, poor ventilation, or poor evacuation of combustion gases.

Carbon monoxide poisoning is a leading cause of accidental poisoning deaths in America, resulting in over 500 unintentional deaths a year (Parmet, 2002). In addition, the US Consumer Product Safety Commission estimates that 8,000 to15, 000 people each year are examined or treated in hospitals for non-fire related CO poisoning.

2.1.5 Methods of environmental measurement of Carbon monoxide

To enable low cost accurate monitoring of Carbon monoxide, a number of studies have emplbyed the use of small portable data loggers utilising the technology of electrochemical fuel cells. The sensing mechanism relies upon the oxidation of the analyte gas at the sensing electrode which becomes anodic due to the accumulation of electrons. These may be applied to one end of a resistor connected to an inert counter electrode to complete the circuit and the output is the voltage developed across the load resistor. Croxford et al 2005 reported on the development of the ICOM monitoring device which monitors real time values of CO every minute and stores an average reading every 15 minutes, with a resolution of 0.1ppm (0.11mg/m^3) and a maximum range of 0-500ppm (0 – 572.8mg/m^3). Experiments were carried out to look at the accuracy at low and high concentrations. Even at low concentrations the monitors were found to be accurate to ±0.2ppm (0.23mg/m^3).

In summary, the literature shows that susceptible groups are particularly at risk from chronic low levels of CO. The elderly are at an increased risk of

heart failure from ambient CO levels, it is suggested that a 1ppm increase in CO maybe increasing the level of fatalities, not from hypoxic effects but from more subtle chronic and toxic mechanism. The use of biomarkers of effect such as cGMP may help to identify those at greater risk.

2.2 Benzene

Benzene (C_6H_6) is a constituent of petrol and is released into the outdoor environment through automobile exhausts, refuelling and through industrial emissions and into the indoor environment through environmental tobacco smoke (ETS).

Benzene is not persistent in the environment, it has an atmospheric residence time of a few days and undergoes a number of degradation and transformation processes in air, water and soil. Benzene is ubiquitous in ambient air and most of the general population is exposed via inhalation over long periods.

In the UK the main atmospheric source of Benzene is the combustion of petrol. Published urban inventories for 2003 estimated that road traffic emissions accounted for 90% of Benzene concentrations at urban background locations. It is estimated using projected figures that by 2010 approximately 500 UK major road links (excluding motorways) could still exceed the running annual mean target of 10ppb (3.25µg/m^3) with a maximum predicted concentration of 13ppb (4.2µg/m^3) in comparison to over 11,000 road links exceeding the target in 1999.

In 2004, the estimated emission total from the road transport sector for Benzene was 24%. In 2000, the EU maximum Benzene content in

petrol was reduced to 1% by volume and the current average Benzene content in petrol sold in the UK is 0.7% by volume. Benzene is also produced during the combustion process from aromatics in the petrol. Benzene emissions have been steadily decreasing since 1990. The Benzene content of petrol was substantially decreased between 1999 and 2004, resulting in a corresponding decrease in emissions and due to the introduction since 1991 of cars equipped with catalytic converters. Emissions from industrial sectors are also falling, but the impact is relatively small compared with the changes in the emissions from transport. (NAEIreport, 2004).

2.2.1 Outdoor Benzene

Over the last twenty years there has been a substantial decline in Benzene concentrations, with annual mean concentration in urban areas being recorded as below 5ppb (16.25μg/m^3). (Air Quality Strategy, 1999).

The first long term time series measurements of Benzene in the UK were made in the 1970's using spot measurements in rural locations, annual average levels ranged between 0.34 – 0.82ppb (1.11μg/m^3 - 2.63μg/m^3). Figures recorded in 1996 were 0.37ppb (1.2μg/m^3) and 0.33ppb (1.07μg/m^3) in 1998 at a monitoring site in Harwell, Oxfordshire. The national automatic hydroCarbon network provide

hourly data on Benzene across 14 sites in the UK. In 1998 maximum running annual mean concentrations ranged between 0.66ppb (2.11µg/m^3) in Edinburgh to 1.82ppb (5.82µg/m^3) in Southampton. A maximum figure of 4ppb (13.2µg/m^3) was recorded at a kerbside in London.

Site	Year	Annual mean µg/m^3	(ppb)	Max RAM* µg/m^3	(ppb)	Data capture %
Cardiff East	1994	4.88	1.5	4.94	1.52	94
(urban	1995	3.97	1.22	4.91	1.51	86
background)	1996	3.9	1.2	4.45	1.37	93
	1997	3.71	1.14	4.06	1.25	77
	1998	3.19	0.98	3.93	1.21	95
Edinburgh	1994	2.28	0.7	2.7	0.83	91
(urban	1995	2.37	0.73	2.37	0.73	90
background)	1996	2.28	0.7	2.6	0.8	95
	1997	2.24	0.69	2.37	0.73	62
	1998	1.92	0.59	2.11	0.65	94
London	1994	5.79	1.78	6.08	1.87	91
(Roadside)	1995	5.49	1.69	5.79	1.78	91
	1996	6.05	1.86	6.57	2.02	95
	1997	5.69	1.75	6.14	1.89	92
	1998	3.9	1.2	5.69	1.75	87

*Max RAM – Maximum running annual mean recorded during the year.

Table 2.6 Annual UK Benzene concentration 1994-1998µg/m^3 (ppb) National Air Quality Strategy 2000

An analysis of trends was undertaken for the 6 national automatic hydroCarbon sites with more than 5 years uninterrupted data with a minimum of 50% data capture in a year. The three sites showing a

statistically significant correlation between concentration and year are shown below: -

Site	Site type	Statistic	Years	Slope µg/m^3	Estimates of 95% CI	
					Lowest Slope	Highest Slope
Birmingham east	Urban background	Annual mean	1994-1998	-0.13	-0.49	0.03
		Running annual mean	1994-1998	-0.10	-0.29	0.16
Belfast south	Urban background	Running annual mean	1994-1998	-0.32	-0.84	-0.03
Cardiff East	Urban background	Annual mean	1994-1998	-0.32	-0.91	-0.06
		Running annual mean	1994-1998	-0.32	-0.45	-0.03

Table 2.7 Long term trends in annual mean and running mean Benzene concentrations. (Air Quality Strategy 1999)

Gonzalez-Flesca et al 2002 showed that concentrations taken at petrol stations are dependent on traffic density, recovery systems operating and location.

In studies that have simultaneously measured ambient, indoor and personal levels, the ambient concentrations have been lower (Cocheo et al 2000; Bertoni et al 2002).

2.2.2 Indoor Benzene

Sources of Benzene in the indoor environment include environmental tobacco smoke, combustion processes and emissions from Benzene

containing cleaning solvents. There may also be an infiltration of Benzene from the ambient air generated from traffic emissions (WHO, 2006).

Kim et al (2001) investigated Benzene concentrations in a wide range of urban microenvironments including homes. The sampling was carried out within a 3km radius of central Birmingham, 12 homes were sampled (6 smokers and 6 non-smokers). Sampling was conducted between November 1999 and February 2000 using adsorbent tubes operated at a flowrate of 40ml/minute. Analysis was carried out using a thermal desorber interfaced with a gas chromatograph and mass selective detector. A total of 64 samples were collected, with a mean concentration of 4.3ppb (13.9µg/m^3) in the homes monitored.

Environmental Tobacco Smoke

The contribution of smoking to Benzene exposure is substantial. It has been estimated that the adsorbed dose of Benzene from smoking one cigarette is 40ng (Travis 1990), Wallace et al (1987) estimated in their study of exposures to Benzene from active and passive smoking that exposure to Benzene maybe increased for the approximately 60% of children and non-smokers living in homes with smokers.

2.2.3 Standards

The Air Quality (Wales) Regulations 2000 set the air quality objectives for Wales and prescribe the periods within which they must be achieved. Figures from the Welsh air quality forum recorded that the maximum annual 1-hourly reading was recorded in Cardiff with a figure of 11ppb (35μg/m^3), although the running annual average for Cardiff was >5ppb (>16μg/m^3)(April 1998-March 1999).

The Regulations introduced a second air quality objective for Benzene of 5 micrograms per cubic metre or less, when expressed as an annual mean, to be achieved by 31 December 2010. The WHO is currently looking at proposing an even lower guidance levels in air of 0.9ppb (3ug/m^3) for Benzene.

Pollutant	**Objective**	**Measured as**	**To be achieved by**
Benzene All Authorities	16.25μg/m^3	Running Annual mean	31 December 2003
Benzene All Authorities in England and Wales only	5μg/m^3	Annual mean	31 December 2010

Table 2.8 Summary of objectives of the National Air Quality Strategy

Through the Air Quality Strategy the UK Government has identified that it should be the objective of national policy to reduce

concentrations of Benzene in air to as low as reasonably practicable, so that they represent an exceedingly small risk to human health. The long term aim is to reduce concentrations of Benzene to 1ppb (3.2μg/m^3). Further reductions will be achieved through the Auto Oil programme by reducing the emissions for vehicles, emissions have already been reduced by the reduction of Benzene content in petrol to 1% from January 2000. The UK Government also propose to put greater controls on emissions from petrol station forecourts during the process of storage and distribution.

Gilli et al (1996) studied Benzene exposure in outdoor and indoor air and personal exposure at three locations in North West Italy. Outdoor concentrations were measured in three locations of varying urbanisation in 1991, prior to anticipated changes from leaded to unleaded in gasoline and then again in 1994. They also looked at the impact of meteorological factors. The study also looked at the relationship between outdoor and indoor Benzene pollution and personal exposure of residents in Turin. Lastly they also investigated the influence of environmental tobacco smoke on the indoor Benzene levels measured. Sampling was undertaken for 10 consecutive 24 hour periods per month from January to December for the urban site and during March, May, July, October and December for the suburban site. At the rural site samples were taken in January and December for a consecutive week. Personal samples were also

measured using a passive sampler equipped with a sorbent capsule containing granular activated Carbon (GAC). Indoor measurements were taken in apartments, with outdoor levels being taken from the balcony or window. Personal samplers were attached to the lapel with records being kept of all the confined environments visited during the day. In addition eighty eight 14 year olds wore personal samplers for 24 hours to identify the impact of environmental tobacco smoke from their own habits, as well as from parents and cohabitants.

The results show a direct relationship between the level of urbanisation and the level of Benzene pollution. There is also a higher level of Benzene pollution during the winter months. The two annual averages measured in Turin, 6.85ppb (21.8μg/m^3) in 1991 and 6.62ppb (21.1μg/m^3)in 1994, do not indicate a difference in Benzene pollution. During the day personal Benzene contamination is higher than the indoor and outdoor levels.

The results showed significant correlations between Benzene, Carbon monoxide and Nitrogen dioxide confirming a similar origin and atmospheric fate. The data observed from the 88 14 year olds showed that exposure to ETS increased indoor Benzene contamination and personal exposure. They conclude that human exposure to Benzene is due principally to indoor air contamination

and depends on lifestyle, smoking and home habits including the level of ventilation in the home. They also recognised that due to the low levels of Benzene in the air, only Benzene biomarkers of exposure will allow studies to truly look at the environmental and public health concerns.

Whilst it is not possible to estimate personal exposure based on environmental concentrations, the analysis of micro-environments has provided data on the relative importance of different activities to personal Benzene exposure. In 1997, a study in California calculated that for non-smoking adults, ambient air concentrations contributed 48% to average Benzene exposure, followed by ETS (23%) and in-vehicle (16%), residential exposure from attached garage with parked vehicle (9%) and direct petrol vapour (4%). For smokers, however active smoking made the major contribution (85%) followed by ETS (8%), in-vehicle exposure (1%), direct petrol vapour (0.7%) and residential exposure from attached garage with parked vehicle (0.4%) (Fruin et al 2001).

2.2.4 Health Effects

Exposure to Benzene occurs via inhalation, ingestion and dermal contact. Of most concern is the long term inhalation exposure.

It is not always relevant to extrapolate studies of high Benzene exposures to the general population; it is therefore more useful to look at occupational exposures to lower Benzene concentrations when trying to compare with the rest of the population. Lynge et al 1997 looked at the incidence of cancers in service station workers exposed to petrol vapour containing around 0.2 – 0.3ppb (0.5 - 1mg/m^3) Benzene in Scandinavia, exact concentrations and exposures were unknown. The incidence of kidney cancer (SIR=1.3, 95%CI=1.0-1.7), lung cancer (SIR 1.3, 95%CI=1.1-1.4) and nasal cancer (SIR=3.5, 95%CI=1.8-6.1) all were higher than the national average.

Hayes et al 1997, carried out a study In China of 74,828 Benzene exposed workers and 35,805 unexposed workers between 1972 and 1987 with the aim to investigating the relationship between the extent of Benzene exposure and the level of risk. The results showed that for workers exposed to Benzene at average levels of less than 10ppm the relative risk for all hematologic neoplasms was 2.2 (95%CI=1.1-4.2) and for acute nonlymphocytic leukaemia plus related myelodysplastic syndromes the relative risk was 3.2 (95%CI=1.0-10.1). The authors suggest that Benzene exposure is associated with a spectrum of hematologic neoplasms and related disorders in humans, the risks for these conditions are elevated in

average Benzene exposures of less than 10ppm (32mg/m^3) and show a tendency to rise with increasing levels of exposure.

There is sufficient evidence that chronic exposure to Benzene can give rise to leukaemia and IARC have classified Benzene as group 1, sufficient evidence of carcinogenicity in humans (IARC, 1990).

There is no threshold value below which there is no danger for human health from Benzene and a Unit risk excess of $6x10^{-6}$ per ug/m^3 is specified by WHO (1996): which means that if one million people are exposed to 1 ug/m^3 for a lifetime, 6 are expected to suffer leukaemia at some point in their lives. However the concentrations with which associations with an increased risk of cancer have been observed are in the region of 101ppm (325mg/m^3) and above, which are several orders of magnitude above ambient concentrations (WHO 2000a; IARC, 1987; EPAQS, 1994b).

Harrison et al 1999 studied the incidence of childhood cancer in relation to proximity to main roads and petrol stations in the West Midlands between 1990 and 1994. Data for Children between the ages of 0-15years diagnosed with cancers (ICD-9) were analysed for proximity to main roads and petrol stations separately and both together. Odds ratios were calculated with solid tumours as a control and incidence ratios with population density as a control. Where

solid tumours were used as the control, the odds ratio was 1.61 (95% confidence interval 0.90-2.87) and 1.99 (95% CI 0.73-5.43) for those living within 100m of a main road or petrol station. The general population was used as a control, the estimated incidence ratio for leukaemia was 1.16 (95% CI 0.74-1.72) and 1.48 (95% CI 0.65-2.93) for residence within 100m of a main road or petrol station. The results suggested a small increase in the risk of childhood leukaemia, but not solid tumours for those living within 100m to a main road or petrol station, although the increase was not statistically significant.

Reynolds et al 2002 studied children living in California between 1988 and 1994 and the associated risks of developing cancers in neighbourhoods with over 320,700 vehicle miles travelled per day per square mile compared to those with below 33,290 vehicle miles travelled per day per square mile. The results were 1.08 (95%CI=0.98-1.20) for all cancers, 1.15 (0.97-1.37) for leukaemia and 1.14 (95%CI=0.90-1.45) for gliomas.

Lee et al 2002 examined haematological changes in children. Subjects aged 8-11 years were recruited from an exposed area (petrochemical estate region) and an unexposed area (suburban region). Estimates of Benzene exposure were imprecise as measures of atmospheric concentrations recorded previously were not consistent. Ambient air Benzene concentrations in the exposed

region were between 1.06 and 53 ppb (3.38 - 169μg/m^3)and in the unexposed region 0.08 and 6 ppb (0.25 - 19μg/m^3). Total white blood cell, red cell and platelet counts were lower in children living in the exposed region.

2.2.5 Methods of environmental measurement of Benzene

The most accepted method for the measurement of Benzene in air is passive absorption using ATD tubes for BTEX compounds (Benzene, Toluene, EthylBenzene, Xylene).The ATD tubes are then analysed by thermal desorption-gas chromatography-mass spectrometry.

There are a number of sorbents that can be used for the sampling of Benzene (Porous polymers, Carbon molecular sieves and specially-constructed Carbons). Chromosorb 106 (Perkin Elmer Ltd) has been identified as an appropriate sorbent for diffusive sampling (Muir et al 2001). Diffusive uptake rates for Benzene on C106 have been characterised fully for atmospheric sampling, the estimated diffusive uptake rate for Benzene using a Perkin Elmer diffusive sampler packed with C106 is estimated as 1.47ng ppm^{-1} min^{-1} (Brown 1998).

The Perkin-Elmer ATD stainless-steel tube fitted with the diffusion cap is the most widely used thermal desorption tube for diffusive monitoring. This system is the standard for such regulatory bodies as the UK HSE (Health and Safety Executive) for occupational hygiene applications. In diffusive monitoring, one end only of the tube is

exposed to the vapours to be sampled, which flow as shown through a special cap which has a controlled orifice. Since the dimensions of the cap, and the position of the packing, are manufactured to very close tolerances each tube performs like every other, thus ensuring uniformity of sampling.

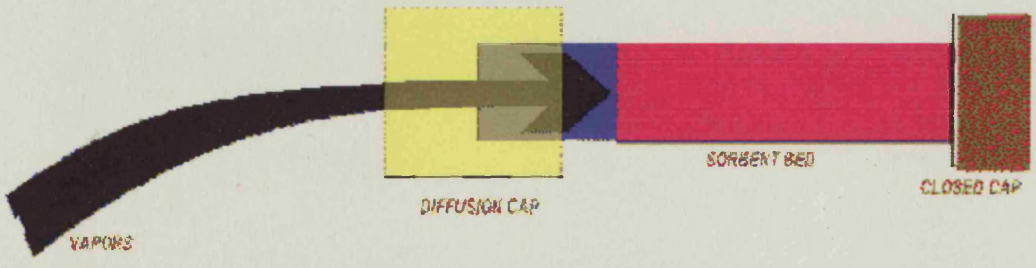


Figure 2.1 Schematic of a Diffusive monitoring tube

The sorbent that is exposed to the external vapours establishes the uptake rate for the system. Diffusive monitors are filled with a single sorbent, that must be strong enough to collect sufficient sample from the given atmosphere, and yet not so strong that it is difficult to desorb reversibly.

2.2.6 Techniques of Biological Monitoring

Methods exist for measuring Benzene in breath (Gruenke et al 1986). The methods are sensitive and accurate for determining exposure levels of Benzene at which health effects have been observed to occur as well as for background levels in the general population. Methods for determination of Benzene in blood are sensitive and with

the application of GC/MS techniques for the analysis of blood samples, rapid, cost effective clinical screening has been developed (Volatile Organic Screening Test) demonstrating levels down to 0.1ppb. (Antonoine et al 1986), tissues (Bechtold et al 1988).

S-Phenyl Mercapturic acid (s-PMA) and trans-trans-muconic acid are minor metabolites of Benzene and urinary measurement of these are used for the assessment of occupational exposure to Benzene.

Biomarkers of Exposure

Benzene is primarily metabolised by the liver and it is thought that the metabolism of Benzene by hepatic cytochrome P-450 may play an important role in the bioactivation and therefore the toxicity of Benzene. Following inhalation exposure some Benzene is excreted again via exhalation, some is excreted unchanged in the urine and some is metabolised. From this information biomarkers of exposure have been developed, mainly from occupational exposure in chemical workers and outdoor urban workers. (Hotz et al 1997, Fustinoni et al 1995; Brugnone et al 1998; Crebelli et al 2001; Gobba et al 1997). The method for measuring Benzene in body fluids and tissues is gas chromatography in conjunction with either mass spectrometry, photoionisation detection or flame ionisation detection.

Boogaard et al (1996) investigated the suitability of S-Phenyl Mercapturic acid and trans-trans-muconic acid as biomarkers for exposure to low concentrations of Benzene. The two biomarkers were measured in 434 urine samples collected from 188 petrochemical workers and 52 control workers. Exposure to 1ppm Benzene led to a mean concentration in end of shift samples of 21 mol s-PMA and 1.5 mmol tt-MA per mol creatinine. Of an inhaled dose of Benzene, on average 0.11% (range 0.05-0.26%) was excreted as s-PMA with an apparent half life of 9.1 hours (standard error 0.7) and 3.9% (range 1.9-7.3%)as tt-MA with a half life of 5 hours (SE 0.5). Due to its longer elimination half-life, s-PMA proved a more reliable biomarker than tt-MA for Benzene exposures during 12 hour shifts. They concluded that s-PMA was superior to tt-MA as a biomarker for low level Benzene exposures as it is more specific, enabling reliable determination of Benzene exposures down to 0.3ppm.

The use of muconic acid as a biomarker of Benzene exposure in the general population has been problematic in studies of adults and children. The ingestion of sorbic acid also produces urinary moconic acid (ATSDR 1997; Yu and Weisel, 1996; Amodio-Cocchieri et al 2001; Barbieri et al 2002). In the study described the urinary concentrations of t,t-MA were not able to distinguish subjects with or without an occupational exposure to Benzene.

Matthews et al (2001) investigated the feasibility of using urinary phenyl mercapturic acid (s-PMA) as a biomarker for environmental Benzene exposure in South Wales. The study looked at two groups of residents, one from a high density traffic area, and one from a semi rural area. Each participant completed a questionnaire giving details on potential sources of Benzene exposure. Each subject also supplied a urine sample, environmental monitoring of Benzene was undertaken at the same time as the biomonitoring. The mean ratio in the low Benzene area (21 participants) was 0.633, in the high Benzene area (38 participants) was 0.966 the p value was 0.019. The results showed that the participants living in a high Benzene area had a significantly higher ratio of umol/mol PMA to creatinine than those living in a low Benzene area.

Fustinoni et al (1995) measured blood Benzene concentrations in policemen working outdoor shifts (exposed) and indoors (unexposed). The 14 indoor non-smoking workers had similar pre and post shift measurements (253ng/l and 264ng/l) which did not differ significantly to the outdoor subjects. The study did observe that the pre shift levels in smokers (358ng/l) were significantly higher than in non-smokers (256ng/l). The mean blood Benzene levels in 243 nonoccupationally exposed subjects (165ng/l) was significantly lower than that measured post-shift in 167 workers exposed in petrol stations or refineries (mean=420ng/l). Benzene levels in smokers

were significantly higher than in non-smokers (264 – 123ng/l) for non occupationally exposed subjects (Brugnone et al 1998). Perbellini et al (1988) selected hospital staff as a non occupationally exposed group and recorded a mean blood Benzene level of 269ng/l with a mean alveolar air level of 14ng/l. Similar workers had a mean alveolar concentration of 21.8ng/l in a study by Brugnone et al (1989) and a mean blood concentration of 332ng/l. Blood Benzene measured in 155 healthy adult rural subjects was significantly lower (200ng/l) than that measured in urban subjects (296ng/l). The rural subjects were farmers and unlikely to be occupationally exposed. Urban workers measured included several occupational groups with potential exposure to Benzene including chemical workers. The mean level amongst white collar urban workers was 258ng/l. Further analysis revealed that blood Benzene levels were directly proportional to the number of cigarettes smoked (Brugnone et al 1992). Breath concentrations of Benzene were similar in housewives living near petrol stations (mean=10.5ng/l) to those living further away (11.2ng/l) (Jo and Moon 1999).

In summary, no level of benzene is safe. The largest exposure source of Benzene to the general public in the UK is from traffic fumes and there is a direct relationship between the level of urbanisation and the increased level of atmospheric benzene, there is also an increased exposure level during the winter months. To

enable estimations of health effects on the general population, biomarkers of exposure and effect need to be used to identify those potentially at higher risk, s-PMA is suggested as the most appropriate biomarker of effect for urinary Benzene.

2.3 Nitrogen dioxide

2.3.1 Outdoor Nitrogen Dioxide

All combustion processes in air produce oxides of Nitrogen. Nitrogen dioxide (N02), Nitric oxide (N0) and Nitrous oxide (N20) are together referred to as NOX. (DETR 2000). In the UK, the highest source of Nitrogen oxides is from Road transport (50%), followed by 20% from the electricity supply industry and 17% from the industrial and commercial sectors (The Air Quality Strategy, 1999). Nitrogen dioxide is produced with nitric oxide in large quantities by motor vehicles and is a good marker for vehicle generated air pollution (COMEAP, 1998).

Nitrogen dioxide concentrations are monitored using automatic instruments in 83 national network sites across the UK.

In the 1997 UK National Air Quality Strategy there were two objectives for Nitrogen dioxide, an annual mean objective of 21ppb (40µg/m^3) and an hourly mean of 106ppb (200µg/m^3)– both to be

achieved by the end of 2005, the AQS 2007 maintained these limit values. The EU has also set limits within the Air Quality Framework Directive, limit and guide values have been set for the continuous monitoring of Nitrogen dioxide. The Limit Value for NO_2 is 106ppm (200µg/m^3) for the 98th percentile of hourly average NO_2 concentrations over a calendar year, (measured using a continuously monitoring NOx analyser).

Organisation	Guideline Standard	Description	Level Units (µg/m^3)
Department of Environment, Transport and the Regions	Peak hourly average concentration in a 24 hour period	Very Good	<286
		Good	286-572
		Poor	574-763
		Very Poor	>=765
World Health Organisation	1-hour mean	Health guideline	200
	Annual average	Health guideline	40
European Union	98%ile of hourly means	Limit value	200
	98%ile of hourly means	Guide value	135
	98%ile of hourly means	Guide value	50
Expert Panel on Air Quality Standards	1 hourly mean	Health Guideline	286
Department of Environment, Transport and Regions	Annual mean	UK National Air Quality Strategy Objective	40
	Hourly mean (maximum 18 exceedences)		200

Table 2.9 Air Quality Standards and Guidelines for continuous monitoring of Nitrogen dioxide.

In 2005 the UK Air Quality hourly objective for NO_2 was exceeded at 4% of the monitoring stations (four sites), including Marylebone road in London which had 853 exceedences. The annual objective was

exceeded at 29% of the monitoring stations (27 sites, 11 of which were non-roadside sites) (AQS, 2007).

The annual objective is expected to be met at all background locations across the UK by 2010, although it is not expected to be met at roadside locations under baseline conditions by 2020. The percentage of total major road length exceeding the objective is expected to reduce from 53% in 2003 to 9% in 2020.

The mean of all the 92 monitoring sites where NO_2 is measured was 18ppm (34µg/m^3) in 2005. Between 1993 and 2002 the 13 longest running sites have shown a decrease of 3.1% per year at urban monitoring sites (AQS, 2007).

Levels of Nitrogen dioxide vary widely due to a continuous background level being present with additional exposure sources adding to the levels found. Levels of Nitrogen dioxide can also be very dependent on the weather and more specifically the wind speed, in London an approximate halving of NOx was observed with a doubling of wind speed from 5 to 10m/s.

Maximum hourly concentrations of Nitrogen dioxide in outdoor air do not usually exceed a maximum of 299ppb (564 µg/m^3) however levels of up to 478ppb (900 µg/m^3) have been recorded near to busy

roads. Annual mean outdoor Nitrogen dioxide concentrations in urban areas are in the range 11 – 48ppb (20-90μg/m^3) (IEH, 1996).

The ADMS modelling indicated that exceedence of both the annual mean and 1997 Strategy hourly NO_2 objectives are currently widespread throughout both Birmingham and Coventry when emissions data was modelled using 1994, 1995 and 1996 meteorological data. The forecast for 2005 suggests that the situation will have improved significantly for NO_2. This is due in a large part to the substantial reduction in vehicle emissions brought about by the Auto Oil vehicle emission and fuel quality standards which will have cleaned up emissions from the vehicle stock substantially by that time.

The London case study (Beevers and Carslaw 1998) used the high resolution mapping technique developed by Stedman (Stedman 1998) to produce 'background' concentrations throughout the whole London area. Annual average NO_2 concentrations were estimated for each 2466 1x1 km grid squares in London. These background levels were combined with modelled predictions of roadside concentrations using the dispersion element of the Dutch CAR International model, and information from the London Transportation Study model (LTS) on traffic composition, to produce annual average NO_2 concentrations at the roadside. The London case study suggests that by 2005, up to 48% of road links assessed by the model would

exceed the annual mean NO_2 objective. The modelling considered the impact that NOx emission reductions would have on concentrations. It was found that with a reduction of traffic of 40% and a 15% increase in vehicle speed (on a 2005 base); around 16% of road links would still exceed the annual mean NO_2 objective.

A number of studies (Hutchinson et al 1996, Buckingham et al 1997, Buckingham et al 1998) have indicated that road traffic emissions dominate Nitrogen dioxide emissions in urban areas and estimate that traffic contributes 90% of NOx at urban locations.

The control of public exposure is extremely important. The approach adopted in the Strategy is to apply the objectives where members of the public are likely to be exposed over the averaging time of the objective. This includes roadsides in the case of annual averages, but only where there is housing, schools, hospitals etc. along the road, and only then at the building facade. Nitrogen dioxide concentrations decrease significantly with distance away from the immediate vicinity of the road. In central London levels of Nitrogen dioxide reach background levels at about 20 to 30m from the middle of the road (QUARG 1993). If housing is located away from the immediate roadside, Nitrogen dioxide levels will be significantly lower than the roadside levels predicted by the models.

2.3.2 Indoor Nitrogen dioxide

The main indoor sources of Nitrogen oxides are from sources of combustion. The greatest risk of indoor pollution occurring is when combustion sources are not vented or where the venting system is blocked or not working effectively.

The indoor environment contributes more to personal exposure than outdoor exposure due to the time spent indoors. High levels of Nitrogen dioxide have been shown in British homes (Goldstein 1979 and Melia 1982) to be associated with gas pilot lights, gas fires, paraffin heaters and the use of gas cookers for drying clothes and heating. NO_2 was measured for one week in the winter outside and inside the homes of children aged 6-7 years living and attending primary schools in a defined 4 square km area in Middlesbrough, UK. Outdoor levels of NO_2 measured at 75 points within the area ranged from 14-24 ppb (26 - 45$\mu g/m^3$) weekly average. Measurements were also made in 428 kitchens with gas cookers, range 5-317 ppb (9 - 84$\mu g/m^3$), mean 112.2 ppb (211$\mu g/m^3$), and in 87 kitchens with electric cookers, range 6-188 ppb (11 - 354$\mu g/m^3$), mean 18.0 ppb (34$\mu g/m^3$). In a random sub sample of homes the range of NO_2 levels in 107 children's bedrooms in homes where gas was used for cooking was 4-169 ppb (7 - 318$\mu g/m^3$), mean 30.5 ppb (57$\mu g/m^3$). In 18 bedrooms in electric cooking homes the range was 3-37 ppb (6 - 69$\mu g/m^3$), mean 13.9 ppb (26$\mu g/m^3$).

Raw et al (1992) looked at Nitrogen dioxide exposure during the summer in homes in the Manchester area of the UK. Following an initial visit, 72 homes were selected (60 with natural gas cooking only and 12 with electric cooking only) and passive sampling diffusion tubes (Palmes tubes) were placed in the living room, bedroom, and kitchen indoors and then a further tube was placed outside the home. Personal exposure was also carried out by placing tubes on two of the occupants (one who spent the most time in the home and the one who spent the most time away from the home); this was carried out over a two week period. Data was also collected using questionnaires and diaries. As would be expected, outdoor concentration was related to area with inner city and suburban being higher than rural (mean level for inner city 13ppb (25.3µg/m^3); suburban 13ppb (23.9µg/m^3) and rural 8ppb (14.3µg/m^3). The study observed that the main influences of Nitrogen dioxide in the home relate to the use of gas cooking and the numbers of people in the household. The results showed that levels were higher in the inner city and suburban areas than the rural areas monitored although neither area or outdoor levels affect indoor or personal exposures. Approximately 70-75% of the personal exposure was calculated as indoor exposure to Nitrogen dioxide.

A British study by Coward and Raw (1996) looked at Nitrogen dioxide levels in 174 houses, the kitchen, bedrooms and living room were

monitored. Levels were highest in the kitchen and lowest in the bedroom, with outside levels being a major influence on the levels. The main source was from cooking with tobacco smoke and heating only having a negligible effect. The size of the household i.e. number of occupants was also identified as a factor in the greater levels of Nitrogen dioxide measured.

Speizer et al 1980 studied 8120 children aged 6-9 years old in 6 American cities looking at the effects of respiratory disease and pulmonary function associated with NO_2 exposure from cooking and heating fuel. A multivariate analysis adjusted for parental smoking showed that the type of cooker had a significant association with respiratory disease before the age of two. The measures of pulmonary function, forced vital capacity (FVC) and forced expiratory volume (FEV) were reported to be significantly lower among children whose homes had gas cookers as a pose to those with electric cookers. The levels recorded by a British study were averaged out over a year with results of 15ppb (28.1$\mu g/m^3$) in the kitchen where gas was used and 8ppb (14.9$\mu g/m^3$) where electricity was the fuel source (Berry 1996).

Harrison et al (2002) investigated the relationship between personal exposure monitoring of a range of pollutants including Nitrogen dioxide and static measurements for healthy individuals and

susceptible groups. Eleven healthy adults and 18 susceptible individuals (6 schoolchildren, 6 elderly subjects and 6 with pre-existing disease (2 with chronic obstructive pulmonary disease, 2 with left ventricular failure and 2 with severe asthma)) were recruited. The results showed overall good correlation confirming a close relation between personal exposure and the associated microenvironment.

Lee et al (2002) investigated levels of Nitrogen dioxide and Nitrous acid concentrations in residential areas. Average Nitrogen dioxide indoor and outdoor levels were 28 and 20.1ppb (53 and 38μg/m^3). Yang et al (2003) carried out multiple measurements of NO_2 to characterize indoor air quality in Brisbane and Seoul. Daily indoor and outdoor NO_2 measurements were carried out in 30 houses over 30 days in Brisbane and in 40 houses over 21 days in Seoul. The results for Brisbane indoor air were 2.7 – 33ppb (5.1 – 61.9μg/m^3) mean 12ppb (22.6μg/m^3), and outdoor air 4 – 33ppb (7.8 – 61.6μg/m^3) mean 15ppb (29.3μg/m^3). The results for Seoul indoor air were 10 – 59ppb (18.5 – 111.6μg/m^3) mean 31ppb (58.9μg/m^3), and outdoor air 15 – 74.5ppb (27.5 – 140.4μg/m^3) mean 38ppb (71.0μg/m^3).

Mosqueron et al (2002) investigated personal exposure of Paris office workers to Nitrogen dioxide. Sixty two administrative workers

(53 female and 9 male, age range 23-61), all non-smoking wore passive samplers for 48 hours. Average personal exposure 23ppb (43.6μg/m^3) was higher than in-home concentration 17.5ppb (33.1μg/m^3) but lower than ambient levels during the same period 32ppb (60.1μg/m^3). The results showed that on average personal exposure was not significantly different from occupational exposure, but was significantly higher than in-home concentration ($p<0.001$) and lower than background outdoor concentration ($p<0.0001$).

2.3.3 Health effects

When inhaled, NO_2 reacts with the moist linings of the respiratory passages to form nitric and Nitrous acids and the production of acids within the respiratory system damages sensitive tissues.

Studies have identified respiratory system causing damage to the lining of the smaller airways. Oxidant injury has been identified as the major mechanism of action (Samet and Utell 1990).

When humans are exposed to 998ppb (1880μg/m^3) there is virtually no health effect, however exposure to healthy humans at rest or during light exercise for less than 2 hours at levels of 2495ppb (4700μg/m^3) resulted in pronounced decrements in pulmonary function.

2.3.3.1 Epidemiology

Epidemiological studies that have looked at the effects of the mixture of air pollutants commonly found in outdoor ambient air have tended not to show that Nitrogen dioxide contributes much to the overall effects Studies involving exposure to Nitrogen dioxide of both healthy volunteers and those suffering from respiratory diseases have not consistently revealed effects at ambient conditions. Long term exposure may affect lung function and respiratory symptoms. It can also enhance the response to allergens in sensitive individuals. Levels experienced within the home could cause ill health including respiratory symptoms, susceptibility to respiratory infections, and possible impairment of lung function and have an adverse effect on some susceptible groups such as asthma sufferers. As there are no symptoms specifically attributable to low level NO_2 exposure the evidence for an effect must be obtained from large epidemiological studies linking health effect to exposure. (COMEAP, 1998).

2.3.3.2 Susceptible groups

Those that may be more susceptible include children and those suffering with conditions such as asthma.

A review of experimental studies showed that 300ppb (560 $\mu g/m^3$) is the lowest observed level that has been reported as to affect the

pulmonary function of asthmatics with intermittent exercise and without a bronchoconstrictor (WHO 1987). Patients with chronic bronchitis don't appear to be more responsive to Nitrogen dioxide than are healthy subjects. Concentrations above 2000ppb (3760 $\mu g/m^3$) raise the airway resistance of normal subjects, while lower levels have had effects in some but not all studies.

A meta analysis of 11 studies looking at children exposed to low concentrations of Nitrogen dioxide. The results showed an odds ratio of 1.2 for respiratory illness in children exposed to Nitrogen dioxide with 95% confidence limits of 1.1 to 1.3, implying a 20% increase risk of respiratory illness corresponding to an increase of 16ppb ($30\mu g/m^3$) of Nitrogen dioxide exposure (Hasselblad, 1992). Other large studies have not been consistent with these findings (Samet 1993 and Farrow 1997).

2.3.4 Methods of environmental measurement of Nitrogen dioxide

Plaisance et al (2002) investigated the performance and application of a passive sampling method for the determination of NO_2 in ambient air. Palmes tubes were used to measure NO_2 and the tubes were placed 1.5 metres from the ground for an exposure period of 14 days. The levels were determined with analysis by ion chromatography. Eight batches of tubes were exposed at each site,

6 exposed and two blanks. Measurements were carried out at 4 monitoring locations over a period of 10 months. Accuracy of the passive sampling method was determined by comparison with a chemiluminescent analyser. A high degree of correlation was found between the passive sampler and the chemiluminescent analyser. The study also looked at the use of a protective shelter for the tubes, previous studies had concluded that the diffusion efficiency was affected by wind-induced turbulence (Campbell, 1994, Hargreaves 1989). The protective device was in the form of a cylinder with two inlets/outlets allowing a good circulation of air without turbulence around the tubes. The study collected 145 measurements over an area of 300km^2 with 1 tube per km^2 in urban areas and 1 tube per 4 km^2 in surburban and rural areas, all measurements were at least 50 metres from significant sources of air pollution. The detection limit for NO_2 over the two week period was estimated at 1.22ppb (2.3$\mu g/m^3$). The background NO_2 pollution was 21 – 24ppb (40-46$\mu g/m^3$) in urban areas. The average NO_2 level measured for all sites was 22.7ppb (42.8$\mu g/m^3$) and the average level measured by the chemiluminescent analyser in the urban areas was 23ppb (43$\mu g/m^3$) indicating a good comparison between the monitoring techniques.

In summary, Nitrogen dioxide is a good environmental marker of traffic pollution. Susceptible groups including children and asthmatics are particularly effected by increased levels of nitrogen dioxide.

CHAPTER 3 METHODS

This chapter describes the selection of homes and individuals in order to achieve the objectives listed on page xv above. The objective was to test the hypothesis that in homes using different types of fuel for heating, the levels of cyclic guanosine monophosphate in blood platelets would differ as a consequence of differing CO exposure. Therefore the method proposed was to identify and select homes with heating types that were likely to differ with respect to emissions e.g. electricity or gas heating. Further, in order to restrict natural variability in the proposed biomarker of effect the age range of subjects was restricted and also confined to the elderly as this group is likely to spend a greater time indoors.

Another objective was to test the hypothesis that residents living in homes of differing urbanicity with respect to traffic flow would have differing levels of a biomarker of uptake of benzene in their urine. Therefore the method proposed was to identify and select residences in proximity and at distance from heavy traffic flow and to sample urine from residents and measure benzene concentrations in outdoor air. Since Nitrogen dioxide is a known marker of motor vehicle pollution, this was also sampled simultaneously at residences both outdoor and indoor.

3.1 Sample selection and recruitment

The main steps in both studies were:

- Selection of the areas to be included
- Selection of the individuals to be included
- Recruitment of individuals
- Measurement of environmental concentrations of pollutants
- Measurement of biomarkers

3.1.1 Sample selection and recruitment for the indoor environment

The study aimed to recruit 100 households to the study. The majority of households in the Gas and solid fuel heating type were recruited from Neath, Port Talbot and the surrounding Valleys.

Figure 3.1 Sample area for Neath and Port Talbot

A further area was identified as a static caravan residential site at Culverhouse Cross on the outskirts of Cardiff to allow sampling of a different type of household with LPG as the main fuel source.

Figure 3.2 Sample area for the Static Caravan residential site

Selection criteria (non smoking study)

- 55-75 years of age
- Permanent resident of the sampled house
- Non-smoker in the past 5 years
- reside in a non-smoking household
- No history of cardiovascular disease

  No history of coronary obstructive pulmonary disease
- Not taking any nitrate medication

The study aimed to investigate the effects of low levels of CO on the retirement population, therefore participants were recruited in the age range 55-75 years of age. Participants were also required to be non-smokers, and have been non-smokers for the previous 5 years and live in a smoke free home. To limit variation in the biomarker of effect (i.e. cGMP) from factors other than CO, as well as limiting the age range individuals with cardiovascular and pulmonary disease were excluded. Individuals who were on nitrate medication were excluded as it would have affected the cGMP measurements, a full list of the excluded medication can be seen in Appendix 1.

Prior to the recruitment process, ethical approval was sought from the Bro Morgannwg Ethics committee. As part of the granting of the approval it was stipulated that a detailed explanation was provided to the individuals taking part and that a consent form was completed, ensuring that the individual understood the implications and had an opportunity to ask questions. Latterly as the study moved to Cardiff, Ethics approval was sought from the Bro Taf Ethics committee.

Recruitment was carried out in three phrases. The first phase of recruitment delivered self addressed postcards to postcodes selected in the Neath Port Talbot Borough as part of the Housing and Neighbourhood and Health project (HANAH) requesting details on heating and cooking fuel type. The second phase of recruitment was

obtained from addresses of solid fuel households from an existing database held by the Cardiff University School of Architecture. The third phase of recruitment was made up of a sample of fixed mobile homes on a private residential site in the Culverhouse Cross area of Cardiff. Recruitment for residential mobile homes was carried out by generating postcodes from the Royal Mail base for the identified site.

Invitations to participants in the study (Appendix 1) were sent to households and responders were then followed up with a telephone call to further explain the research and to arrange a suitable time to visit.

Households with a range of different heating sources were sampled, including Gas, Solid fuel, Liquid Petroleum Gas, Oil and Electricity. Both private and rented accommodation, were sampled.
This study chose to monitor within the main living room as it was perceived that this would be the room in the house where the occupants would spend the majority of their time, when at home. A full description of household layout and ventilation characteristics was beyond the scope of the study. Generally it is accepted that the greater potential source of CO in the home would be during the winter months when greater use is made of heating systems, therefore environmental monitoring of Carbon monoxide was carried out during the months of October to April.

The study concentrated on non-smokers homes, as it is widely recognised that smokers have a higher level of COHb traceable within their blood due to exposure to tobacco smoke. As the study progressed it was decided to sample an additional group of smoker's households to more clearly assess the differences between the two groups.

Smokers were identified from the previous recruitment processes where households had been rejected because of a smoker within the household. No biological monitoring was carried out in this group as it was recognised that the greater source of carbon monoxide in this group would be received from the cigarette smoke rather than the potential heating exposure.

3.1.2 Sample selection and recruitment for the outdoor environment

Households were selected on busy and non-busy traffic routes in Cardiff, allowing a comparison of exposed 'busy' traffic routes and a control group of suburban 'non-busy traffic routes'.

The study population consisted of healthy non smoking males in the age range 50-70 years. The aim was to recruit 150 males to be split 75 into the exposed area and 75 to the unexposed area. The main steps in the exposure study were: -

For the two groups the inclusion criteria are shown in Table 3.1: -

Control group	**Exposed Group**
Minor road / cul-de-sac	Major A or B road
Low traffic flow	High traffic flow
At least 750metres from exposed road	To reside within 20 metres of urban road with high traffic volume
Sufficient number of houses	Sufficient number of houses

Table 3.1 Criteria for Exposed and Control groups

The Local authority Transport Department provided information to help identify suitable monitoring areas in Cardiff, although there was limited data available on traffic flows. It was also important to ensure there were adequate households on the roads identified as having high or low volumes of traffic flow, local knowledge was a factor in identifying suitable sampling locations.

Road	AM	PM	Daily	Road	AM	PM	Daily
The Philog	1107	1271	2378	Ty Glas Road	1330	1394	2724
Mackintosh place	731	619	1350	Fidlas Road	1910	1984	3894
Cowbridge road west	2557	2274	4831	Pendwyallt Road	1014	1098	2112
Ninian Road	579	900	1479	Sloper Road	1118	1205	2323
Llandaff road	973	970	1943	North Road	2350	2541	4891
Bridge road	1127	1402	2529	Cowbridge road east	1206	1032	2238
Moorland road	1115	572	1687	Heol Hir North	207	125	332
Planet Street	398	426	824	Cherry Orchard Road	727	446	1173

Table 3.2 Traffic flow data provided by Cardiff City council

The following table highlights extra roads that were identified and used in the study: -

Control group		Exposed group	
Nant y Drope	Crosswells Way	[1]The Philog	Pendwyalt Road
Dennison Way	Everswell Road	[2]Cowbridge Road West	Cowbridge Road East
Lon y Ffin	Margarites Way	Fidlas Road	Ty Glas Road
Penmark Green	Clos y Cwarra	[2]Western Avenue	Mackintosh Place
Deepfield Close	Ninian Road	Cathedral Road	Llandaff Road
Deepwood close		Heol Hir	Moorland Road

1. Air Quality Management Area – The Philog (pollutant declared Nitrogen dioxide)
2. Air Quality Management Area – Cardiff West (pollutant declared Nitrogen dioxide)

Table 3.3 Roads identified for the exposed and Control groups

The data supplied in Table 3.2 was used to identify the exposed and unexposed roads, although at the time of the study traffic flow data

was not routinely collected in Cardiff. Additional areas were chosen on the basis of local knowledge of high traffic volumes in residential areas. Exposed roads typically were routes into the city centre or routes to the motorway. Unexposed roads were typically residential areas including housing estates where the main traffic flow was the local residents.

Subject criteria:

- Male
- 50-70 years of age
- Permanent resident of the sampled house
- Non-smoker in the past 5 years
- reside in a non-smoking household
- No occupational exposure to chemicals / traffic

To further reduce variability only males in the age range 50-70 years were recruited. Participants were also required to be non-smokers, and have been non-smokers for the previous 5 years and live in a smoke free home, this was essential due to the plan to carry out urinary Benzene measurements. In support of this theme, participants were not accepted if they were frequently exposed to chemicals and worked in the transport or engineering professions.

Ethical approval

Prior to the recruitment process, ethical approval was sought from the Bro Taf Ethics committee. As part of the granting of the approval it was stipulated that a detailed explanation was provided to the individuals taking part and that a consent form was completed, ensuring that the individual understood the implications and had an opportunity to ask questions.

After selecting the roads of interest the Royal Mail address finder was used to select residential addresses. These addresses were then used to form an access database from which invitations to participate could be sent. To ensure that the method of recruitment was effective, letters were sent to 3 streets from the exposed group to start with (Mackintosh Place, Ninian Road and Ty-Glas Road). Invitations to participants in the study were sent to households including a brief outline of the study and asking a series of questions, those who were interested in taking part in the study were requested to complete the yes / no and return to the department in a prepaid envelope (see appendix). Responders were then followed up with a telephone call to further explain the research and to arrange a suitable time to visit. During this initial visit the researchers informed the individuals of the study. A first batch of approximately 500 letters was sent to residences of exposed streets (Mackintosh Place and Ninian Road) to try and gauge the potential response. The

information sent included a detailed information sheet providing full information about the study and what would be required of participants. Recruitment was a continuous process carried out over a three year period.

3.2 Environmental monitoring

3.2.1 Environmental monitoring for the indoor environment

Environmental concentrations of CO in the main living room were measured and data-logged every 5 minutes over a period of 7 days in non-smoking households and 2 days in smoker's households, less time was spent monitoring in the smoking households as the aim of this monitoring was to obtain a snapshot of the environmental levels occurring in these homes. Carbon monoxide levels were monitored in the winter months only due to the higher incidence of central heating systems being on for long periods of time and reduced natural ventilation from windows being open. Monitoring took place between March and April and September and November 2002, February and March 2003 and for the smoking households January and March 2004. An electrochemical sensor using mains electricity supply and data logging, was attached to a tripod (see figure 3.3) and located within the living room. Previous studies have investigated CO levels in other areas of the home, notably the kitchen and bedrooms. This study chose to monitor within the main living room as it was perceived that this would be the room in the house where the

occupants would spend the majority of their time, when at home. Following a discussion with the residents explaining the objectives of the study and allowing sufficient time to ensure checks could be made that the unit was operating effectively, the logger was activated before leaving the household. At the end of the monitoring period the data was downloaded to a laptop computer for analysis.

During the initial visit the householders were asked a series of questions relating to the main fuel type in the home used for heating and cooking; the duration that the heating was on during the winter months and the time spent in the main living room of the home during the winter months.

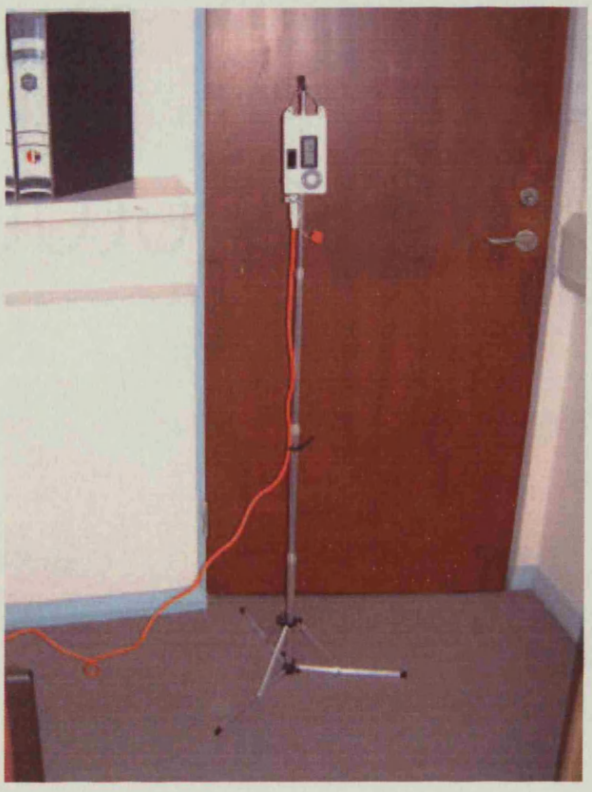


Figure 3.3 Environmental Carbon monoxide monitor

Environmental Carbon Monoxide monitor

The availability of a small real-time, electrochemical Carbon monoxide (CO) detector utilising state-of-the-art gel electrolyte and membrane diffusion technology enabled the construction of the monitors used in the study.

The new generation of sensors used exhibit long-term stability with sub-part per million (ppm) sensitivity. Electrochemical sensors have several advantages and a few disadvantages over alternative methods of detecting gases. Electrochemical sensors allow for

continuous monitoring of a wide range of gas types, with high sensitivity, and rapid response times.

The instrument is comprised of two main sections, the sensor and the supporting electronics. The role of the sensor is to provide an electrical output, which reflects the concentration of the gas to be detected. The gas diffuses into the senor where it is oxidised in the cell by a catalytic reaction, a current is generated in the cell by loss and gain of electrons, the resulting current can be measured in an external circuit and is directly proportional to the concentration of carbon monoxide.

Carbon Monoxide

$CO + H2O == > CO_2 + 2H^+ + 2e$

Four environmental Carbon monoxide monitors were built to an in house specification using a commercially available electrochemical sensor.

The particular device employed incorporated a dedicated 4-20 mA loop-powered transmitter enabling direct connection to a suitable digital display and data-logging module.

The monitors were constructed in a 152 mm x 82 mm x 50 mm die-cast enclosures (BIM 5000, IP65, RS 244-8634) fitted with a screwed lid. A City Technology Type T3E/F, 0-50 ppm CiTicel CO sensor/4-

20 mA loop powered transmitter was mounted at one end of the lid. A Lascar 3½ digit EL-1-12 bit, 8k, data-logging, current/voltage display module (RS 289-0467) was also mounted in the lid assembly close to the sensor module. A Lascar Panel-IR, infrared data communications interface was mounted adjacent to and parallel with the display module.

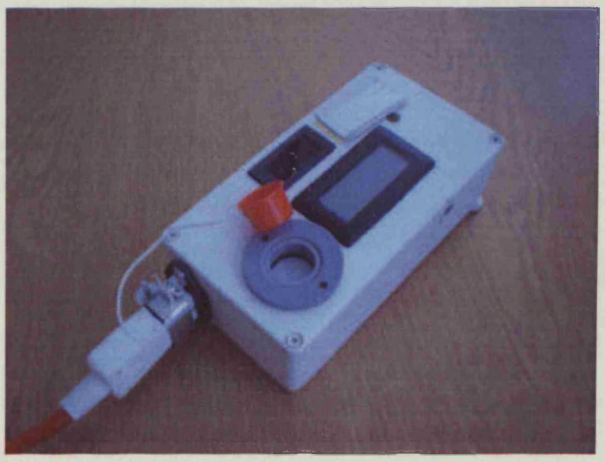


Figure 3.4 The purpose built Carbon monoxide monitor

Power for the monitor was derived from a standard domestic, 240 Volt single phase, 50 Hz mains supply. The mains voltage was transformed to low voltage by a Traco TMS 05124 Power Supply Unit (PSU) (RS 338-2620) that supplied 24 volts d.c. to the CO sensor/4-20 mA loop- powered transmitter module. The latter device provided 3.6 volts D.C. to the data-logger and infrared data communications interface.

Electrochemical sensors produce a current that is proportional to the concentration of the target gas in contact with the sensing electrode. The galvanic type used in this design produced a current of approximately 100 nanoamps per ppm of CO. The sensing mechanism relies upon the oxidation of the analyte gas (CO) at the sensing electrode that becomes anodic due to the accumulation of electrons. These may be applied to one end of a load resistor connected to an inert counter electrode to complete the circuit, the output being the voltage developed across the load. In this arrangement no external power is required. Alternatively for greater sensitivity the sensor may be operated in current follower mode using an operational amplifier (Op-Amp) to convert the current flow into a voltage output. The incorporation of a third electrode producing a known e.m.f at constant temperature with respect to the counter electrode provides a means of accurately referencing cell output at known gas concentration, regardless of counter electrode potential.

The current signal produced in sensing a concentration of greater than 0.5 ppm of CO is amplified and converted into a proportional current having a minimum value of 4 mA and maximum of 20 mA. In measuring D.C. voltage signals major errors can occur due to variations in resistance of wires and connectors in the transmission line. The use of a defined current (mA) value to represent a given gas concentration signal overcomes the problem by eliminating the

resistance dependence of the signal as would occur if a voltage signal were used. At the LCD digital display/data-logger interface an analogue to digital converter produced a 12 bit binary number corresponding to any instantaneous current value appearing at the input. Each digitised value was stored for later access in a non-volatile 8k memory area. Set up and control of the data-acquisition and handling parameters was managed by Lascar EasyLog EL-WIN Control Software (RS 206-2347) provided with the Lascar EL-HL, hand held, infra red controller (RS 324-5666) or the RS232-IR infra-red EL - Link – IR serial port transceiver (RS 307-6347).

As part of the risk assessment carried out for the fieldwork, it was deemed advisable to operate the monitor from a Residual Current Circuit Breaker (RCCB) and an electrical surge suppresser. A plug tester was also used to check the integrity of the plug sockets prior to 'plugging in' the monitors.

Calibration of environmental Carbon Monoxide monitor

The monitors were calibrated against British Oxygen Company (BOC) certified gas standards obtained from BOC, Special Gases division. Nominal concentrations of 2.5 ppm and 5.0 ppm carbon monoxide made up in Nitrogen balance gas were used. Other values were prepared from a 1000 ppm mixture by serial dilution with clean

air in a 50 millilitre (ml) glass syringe. The gas sensor cell was provided with a ported face cap with a nitrile 'O' seal with which a small void or "flow-cell" was created in proximity to the sensor membrane. The dead volume of the void was about 1 or 2 ml thus 50 ml of a challenge concentration of 50 ppm of CO was more than sufficient to produce a stable signal at or near the maximum of the device measuring range. Standard CO mixtures were supplied in size AZ cylinders of 1.2 litres capacity fitted with BS15 valve outlets and HP1500B, GG-BS15 (low standards) and GG-BS4 (high standard) high purity, two stage pressure regulators. The analytical values of BOC Certified Standard mixtures were accurate to +/- 5% and were traceable to UK National Standards.

Gas standards were delivered to the sensor flow cell via 4mm inside diameter. x 6mm outside diameter. PTFE tubing. Approximate flow rates were measured with a bubble flow meter at the cell outlet. A typical gas flow rate was in the range of 150 – 200 ml per minute with provision for adequate ambient oxygen concentration to prevent sensor oxygen depletion. The date, start-time, sampling duration, zero and span settings of the data-logger/display module were set by commands generated by the EL-WIN software package.

3.2.2 Environmental monitoring for the outdoor environment

Environmental Benzene, Nitrogen dioxide, Nitrous oxide monitoring was undertaken using commercially available pre-prepared passive diffusion sampling tubes. The tubes were placed on the external wall of the home.

To monitor the atmospheric Benzene commercially available BTEX tubes (Benzene, Toluene, and Xylene) were used. For atmospheric Nitrogen dioxide pre-coated tubes were used and for Nitrous oxide, Hewlett Packard (HP) tubes packed with molecular sieve 5a were used. The tubes were placed in a suitable external location to each household (mainly drainpipes) approximately 1 metre from the ground, at the front of the property and away from any garage. The tubes were left in place for the period of one month. The tubes were then collected sealed and sent for analysis by an external laboratory. Monitoring was carried out between August and October 2003 and January and April 2004.

In addition to the outdoor environmental monitoring, indoor Nitrous oxide measurements were carried out in a sub set of homes. The Molecular sieve 5a tubes were located within the main living room of the home, this area was chosen as theoretically the occupants of the house would spend the majority of time at home in this area.

Figure 3.5 Passive Nitrogen dioxide, Nitrous oxide and BTEX tubes in place

During the fieldwork, the repeatability of the results was not tested. This was due to limitations with available resources both time and financial. Information was requested from the commercial suppliers on the quality control and assurance tests in place for their products. The BTEX, Nitrogen dioxide diffusion tubes and Nitrous oxide ATD tubes were known to be robust as they are routinely used to gather environmental information.

Analysis of Environmental Benzene

Standard preparation and sample measurement was carried out according to the Harwell Scientific 'in house' method HS/GWI/3015.

A summary of the measurement technique is as follows, : -

1. The target analytes were collected on an adsorbent contained within steel diffusion tubes. The tubes were

then returned to the laboratory from the field sealed with brass end fittings.

2. Each tube was uncapped and placed in a sequence composed of calibration standards and exposed sample tubes in the automatic thermal desorption (ATD) instrument.

3. The tubes were then taken in turn by the instrument and sealed within a carrier gas stream. A leak test was performed to ensure the tube was sealed correctly and it was then heated to pre-selected temperature for a pre-selected time to thermally desorb the volatile species.

4. The desorbed compounds were then concentrated in a low thermal mass cold trap within the ATD prior to transfer through a heated line to the gas chromatograph. The cold trap was heated very rapidly to ensure that the sample was transferred to the gas chromatograph in a tight band, compatible with capillary GC columns.

5. The Benzene, toluene, ethylBenzene, m- and p-xylene and 0-xylene were separated in time by the GC before introduction to the mass selective detector where the responses obtained were compared with those from the standard tubes. The MSD was used in scan mode to enable confirmation of the identity of the eluted compounds.

The limit of detection (LOD) was based on the sampling times given. The overall uncertainty on those results significantly above the LOD, have been calculated to be 19% for Benzene.

Analysis of Environmental Nitrogen dioxide

Commercially available passive tubes were purchased for the monitoring (Harwell Scientifics). The samples were analysed in accordance with Harwell Scientifics standard operating procedure HS/GWI/1015 issue 8.

The tubes were prepared by spiking acetone: triethanolamine (50:50) onto the grids prior to the tubes being assembled. The tubes were desorbed with distilled water and the extract analysed using a segmented flow autoanalyser with ultraviolet detection. The analysis of diffusion tube samples to determine the amount of Nitrogen dioxide present on the tube was within the scope of the laboratory's UKAS schedule. In the WASP intercomparison scheme for comparing spiked Nitrogen Dioxide diffusion tubes, Harwell Scientifics is currently ranked as a Category Good laboratory.

The limit of detection (LOD) was based on the sampling times given. The overall uncertainty on those results significantly above the LOD, have been calculated to be 0.03ug for Nitrogen dioxide (Harwell Scientifics).

Analysis of Environmental Nitrous oxide

Commercially available Perkin-Elmer ATD stainless steel tubes were used for the study. The steel tube was packed with molecular sieve 5a with a diffusive cap covering the exposed end.

At the end of the monitoring period the passive sampling tube was sealed and it was sent for analysis by thermal desorption and Gas Chromatography (Llandough Hospital, Toxicology Laboratory). Environmental sampling tubes were thermally desorbed at 165°C in forward flush direction for 3 minutes with a flow rate of 30 ml min^{-1} helium using a Perkin-Elmer ATD-400. Analysis was performed using a Perkin-Elmer Autosystem XL gas chromatograph. A 60m x 0.32 mm Gas Pro column was used with inlet pressure of 22 psi helium. The oven programme was: 10 minutes isothermal at 150°C, 250°C for 2 minutes post run and Nitrous oxide elutes at 7.2 minutes. Detection was by electron-capture at 340°C. For calibration, a static 2% gas mixture in air was prepared by syringe addition of 20 ml Nitrous oxide to an IL calibrated glass flask. Clean Molecular Sieve 5A tubes were fitted to a spare ¼ inch GC port and purged with helium. 0.01 – 10 ml amounts of the diluted Nitrous oxide mixture were injected into the calibration tubes.

The limit of detection (LOD) was calculated to be 1ng for Nitrous oxide.

3.3 Biological monitoring

3.3.1 Biomarkers associated with the indoor environment

In addition to the study of trace levels of Carbon monoxide in the indoor environment, biomarkers of exposure and effect were also investigated. Carboxyhaemoglobin (COHb) is the most accepted biomarker of exposure to Carbon monoxide, with levels of in excess of 5% causing medical concern. A blood sample was taken from the participants during the week that the environmental monitor was within the home to check for COHb levels. In addition to COHb, a further blood sample was taken to measure for cyclic Guanosine Monophosphate (cGMP). Participants were then requested to attend for a second blood sample during the summer months to assess if any seasonal differences in the blood levels measured could be identified.

On each separate occasion during the seven-day period that the environmental monitor was in place, the residents were requested to donate a blood sample. Initially it had been intended to take the samples within the participant's homes to reduce the effects of the time away from the exposed area of the home. However it was not possible to get a qualified phlebotomist to attend each of the households, therefore the participants were requested to attend the phlebotomy clinic at the local hospital. Blood samples were taken

and analysed for carboxyhaemoglobin (COHb) and cyclic Guanosine Monophosphate (cGMP).

Carboxyhaemoglobin (COHb)

Blood samples for COHb were collected into lithium heparin vacutainers. All samples were marked with the unique identification number allocated by the study, the vacutainers were then stored in the pathology laboratory refrigerator. Where practicable COHb samples were collected by the researcher on a weekly basis and returned to the Medical Biochemistry laboratory at the University Hospital of Wales (UHW), where the samples were analysed. Latterly the samples were from participants in the Cardiff area who attended the UHW direct and therefore samples were analysed on the same day.

The analysis method employed at UHW for the detection of COHb was the Optical system using the ABL625 series analyser. The optical system was based on a 128-wavelength spectrophotometer with a measuring range of 478-672nm. The spectrometer was connected via an optical fiber to a combined hemolyzer and measuring chamber. The method used was visible absorption spectroscopy (Cardiff and Vale NHS Trust, 2005)

Cyclic Guanosine Monophosphate (cGMP) Measurements

The analysis of the cGMP was carried out by the Department of Pharmacology within the Welsh Heart Research Institute, University of Wales College of Medicine (UWCM).

Where practicable cGMP samples were collected on a weekly basis and returned to the Pharmacology department, UWCM, where the samples were analysed. Aliquots of blood samples were centrifuged before being frozen for subsequent analysis of cyclic GMP by radioimmunoassay. Latterly the samples were from subjects in the Cardiff area who attended the UHW direct. The samples were taken directly from the phlebotomy clinic to the Haematology department where they were spun down and frozen prior to analysis.

The vaccutainers were supplemented with Zaprinast (10microM) that is a cGMP phospodiesterase inhibitor. Zaprinast prevents the breakdown of cGMP. They were therefore added so that a 'snap shot' could be obtained of the cGMP present in the platlets at the time of sampling.

Platelet pellets were resuspended in 1ml of ice cold 65% (v/v) ethanol to extract the cGMP from the cells. Following centrifugation at 3000rpm for 10 minutes at 4°C the resulting supernatant was removed and evaporated to dryness. The pellet of cell debris was dissolved in 1ml of 1M sodium hydroxide solution and assayed for

protein content using a commercially available kit (Biorad). The dried sample was resuspended in the appropriate assay buffer and the cGMP content measured by a commercially available radioimmunoassay kit (Amersham Biosciences). The cGMP content of the sample was then normalised to the total pellet protein concentration.

Data Analysis

An initial contacts database was set up using Microsoft Access. The data collected from the environmental monitoring was downloaded using the Lascar data reader and initially downloaded onto a laptop computer, using the 'Elwin' software. The data was then converted to Microsoft Excel to enable graphs to be produced for each of the monitoring sessions allowing the peaks and troughs to be identified; basic statistics were then calculated looking at the peak and mean levels recorded.

Information gathered from the questioning the participants was recorded within the SPSS spreadsheet, the key data recorded included the fuel type used for heating and cooking, the duration the heating was on over a 24 hour period during the winter months and an approximate time that the occupants spent in the main living room during a 24 hour period.

For the main analysis all environmental and blood measurements were recorded in a Microsoft Excel database. In addition to the results obtained, information was included on I.D., age, sex, date of measurements, and fuel type. Statistical analysis was carried out following inputting the data into a SPSS database.

3.3.2 Biological monitoring associated with outdoor environment

Biological monitoring of Benzene and Nitrous oxide was also undertaken. Two urinary samples were collected from the participants at the time of the interview. Samples were collected into a universal container for the Benzene and a sterile glass bottle supplied by the laboratory carrying out the analysis for Nitrous oxide. Once collected, the Benzene samples were delivered to the laboratory undertaking the analysis; the Nitrous oxide urinary samples were frozen prior to analysis by the toxicology laboratory at Llandough hospital.

Urinary Benzene

Benzene can enter the body by respiratory inhalation, ingestion and through absorption through the skin. A proportion of the inhaled benzene is excreted unchanged, the remaining proportion is metabolised by the microsomal cytochrome P-450 monoxgenase system into benzene epoxide. Benzene epoxide is metabolised in three different ways and excreted as s-PMA, t,t muconic acid and Phenols. S-Phenylmercapturic Acid (s-PMA) is a breakdown product of Benzene which can be measured in the urine of exposed individuals and derives only from Benzene. Urinary creatinine is measured at the same time as the PMA, as the concentration of urine produced by an individual varies significantly during the day,

due to normal physiological changes that affect the excretion or retention of water. This affects the concentration of PMA in urine regardless of the amount of PMA excreted by the kidneys. Creatinine is the breakdown product of normal metabolism which is eliminated from the body via the kidneys at a constant rate irrespective of excretion or retention of water. In order to interpret a urinary PMA result by relating it to reference ranges, the urinary creatinine concentration is measured on each sample in addition to the PMA concentration. By relating the PMA to creatinine as a direct ratio, the effect of fluctuating water excretion can be excluded. Urine samples were collected by the researchers. The samples were returned to UWCM and frozen prior to analysis.

Analysis of s-PMA

Benzene is rapidly converted to PMA in the body, however the elimination half-life of PMA is approximately 9 hours. Background PMA / creatinine ratios for non-occupationally exposed individuals who are non-smokers vary from 0-1.8umolmol (mean 0.8 umol/mol). For non-occupationally exposed smokers the mean PMA/Creatinine ratio is 1.7 umolmol. An exposure to airborne Benzene of 1ppm (8 hour Time Weighted Average) would result in a urinary PMA/creatinine ratio of approximately 21 umolmol. The method used for the measurement of PMA was developed and performed by AB Biomonitoring (Ball J), this can be seen in Appendix 2.

Analysis of Urinary Nitrous oxide

Urine was collected in a glass sample bottle with a screw cap. Sterile receptacles were provided for convenience of urine collection with subsequent decanting into the sample bottle. Once the sample had been collected, it was taken to the laboratory where it was either analysed immediately or stored in a freezer prior to analysis. Determination of Nitrous oxide concentration in urine was by headspace analysis and gas chromatography and followed the procedure published by Sonander (1983).

The limit of detection (LOD) was calculated to be 10ng/ml for urinary Nitrous oxide.

Data Analysis

An initial contacts database was set up using Microsoft access. The data collected from the external analysis of the environmental and biological monitoring was entered into a Microsoft excel database. The data was exported into a Statistical software package SPSS to carry out more detailed analysis. For the main analysis all environmental and blood measurements were recorded in a Microsoft Excel database. In addition to the results obtained, information was included on I.D., age, sex, date of measurements, and fuel type. Following inputting the data into a SPSS database statistical analysis was carried out including basic summary statistics,

boxplots, ANOVA and Tukey tests to establish significance between the different fuel groups, and Mann Whitney U test to test whether there was a significant difference between the medians in the smoking and the non-smoking groups. In addition for the biological measurements, The Wilcoxon matched pairs signed ranks test was used to test if there was a difference between the winter and summer measurements. The size effect was also calculated between winter and summer measurements

CHAPTER 4 RESULTS

This chapter describes the results of the monitoring undertaken to investigate the differences in environmental concentrations of Carbon monoxide for indoor environments by heating type and of differences of benzene and nitrogen dioxide for outdoor environments by degree of urbanicity. It also reports the results of the measurements of a biomarker of effect of exposure for CO and of a biomarker of exposure for benzene.

4.1 Sample selection and recruitment for the indoor environment

Sample selection and recruitment for CO Study

Non smoking households

Table 4.1 details the responses obtained from the HANAH letters sent to householders. Of the 140 letters, there were 98 responses, 77 of which were eligible responses. However when these persons were contacted to take part only 47 agreed to participate in the study. The second batch of 533 letters was sent to households with solid fuel which were selected from a database held by the Cardiff University, School of Architecture. A very low response rate was achieved within this group with only 86 responses received, 45 of which were eligible and 17 agreed to take part. The third batch of 219 letters was sent to the residents of static mobile homes in the Culverhouse Cross area of Cardiff. A low response rate was achieved with only 37 responses, of which 19 were eligible and 14

agreed to take part in the study. Recruitment was a continuous process carried out over a three year period.

The response rate of the study was calculated by the number of people who participated in the study divided by the number of eligible individuals responding. The response rates were 58% for the HANAH mailings, 11% for the Solid fuel group and 74% for the Static homes. Nearly all of the Hannah households and static homes that agreed to take part were visited during the study. Only five of 17 homes using solid fuel where residents had agreed to participate were recruited. This was due partly to the fact that these homes were further away from the University which resulted in increased problems arranging suitable appointments and also partly as a consequence of a higher number of cancelled appointments from participants.

Total number	HANAH Homes	Solid fuel Homes	Static Homes	Totals
Letters sent out	140	533	219	892
Response received	98	86	37	221
Eligible response received	77	45	19	141
Households who agreed to take part	47	17	14	78
Households who actually took part	45	5	14	64

Table 4.1 Recruitment of non-smokers from the three main residential settings identified

A total of 64 non smoking households were visited of both private and rented accommodation, and sampled for environmental Carbon monoxide. The mean age of the non-smoking residents was 67 years (range 53-82 years), 17 non smokers were currently in work and 7 non-smoking households had an integral garage.

Smoking households

The households approached included sixty households with a smoker and 23 of these households agreed to participate and were visited and sampled for environmental Carbon monoxide. The households were located across the whole sample area including Neath, Port Talbot and Culverhouse Cross. No biological measurements were taken from this group. The mean age of the smokers was 61 (range 35-80 years), 6 smokers were currently in work and 2 smoking households had an integral garage.

4.1.1 Sample selection and recruitment for the outdoor environment

Recruitment was carried out as part of a sister study looking at other aspects of traffic pollution. The results of the recruitment are shown in table 4.16. A total of 4043 letters were sent to households in the trafficked areas of Cardiff. From this mail out 250 responses were received, with an eligible response (male, age range and medical status) of 90 and of these 70 agreed to participate. A total of 4276 letters were sent to households in the sub-urban areas. On this

occasion 189 responses were received, with an eligible response of 68, of these 51 agreed to participate. Of the 121 households eligible responding, 72 were contacted by telephone of whom 70 agreed to take part and were monitored, 55 in exposed areas and 15 in unexposed areas. The response rate of the study was calculated as the number of people who participated in the study divided by the number of eligible individuals contacted. The response rates were 61% and 22% for the exposed and unexposed groups respectively. It was not possible to monitor all the households who agreed to take part due to budget constraints

Total number (n)	**Urban area (exposed)**	**Suburban area (unexposed)**	**Totals**
Letters sent out	4043	4276	8319
Response received	250	189	439
Eligible response received	90	68	158
Respondents who agreed to take part	70	51	121
Respondents who actually took part	**55**	**15**	**70**

Table 4.2 Recruitment from the two microenvironments

Table 4.3 details the roads monitored in the two microenvironments (urban and suburban) and the number of households monitored in each road

Unexposed Roads (Number of households)	Exposed Roads (Number of households)
Nant y Drope (1)	[1]The Philog (5)
Dennison Way (1)	[1]Manor Way
Lon y Ffin (2)	Ty Glas Road (6)
Penmark Green (2)	Fidlas Road (7)
Deepfield Close (1)	[2]Western Avenue (5)
Deepwood close (3)	Cathedral Road (4)
Clos y Cwarra (1)	Heol Hir (1)
Margarites Way (2)	Moorland Road (5)
Everswell Road (1)	Ninian Road (2)
Crosswells Way (1)	Llandaff Road (3)
	Mackintosh Place (1)
	Pendwyalt Road (5)
	Cowbridge Road East (2)
	[2]Cowbridge Road West (2)
Total (15)	**Total (55)**

1. Air Quality Management Area – The Philog (pollutant declared Nitrogen dioxide)
2. Air Quality Management Area – Cardiff West (pollutant declared Nitrogen dioxide)

Table 4.3 Numbers of households sampled in the roads identified for the two microenvironments (exposed and control groups)

Environmental Nitrogen dioxide and Benzene were monitored outside, for a period of 1 month for a total of 70 households. Monitoring was carried out between August and October 2003 and between January and April 2004. The mean monitoring period was 790 hours (range 647-965 hours)

4.2 Environmental monitoring

4.2.1 Environmental monitoring for the indoor environment

This section reports the ambient levels of Carbon monoxide monitored over a period of one week in the main living area of homes.

Fuel type (Heating)	Number of households	Number of residents	Environmental Grand mean (ppm) (SD)	COHb winter mean level (%) (SD)	COHb Summer mean level (%) (SD)	cGMP Winter Mean level (fmol/mg protein) (SD)	cGMP Summer Mean level (fmol/mg protein) (SD)
Gas	37	59	0.23	0.19 (0.47)	0.22 (0.75)	266 (131.9)	224 (100.5)
Coal	7	11	0.3	0.4 (1.13)	0 (0)	322 (176.5)	325 (232.7)
Electricity	4	5	0.33	0.05 (0.1)	0 (0)	148 (26.5)	344 (203)
LPG	13	21	0.49	0.11 (0.22)	-	544 (506.3)	284 (131.9)
Other	3	3	0.2	0.05 (0.12)	0.2 (0)	176 (50.5)	195 (9.9)

Table 4.4 Summary of descriptive results – All carbon Monoxide non-smoking results

Non-Smoking Households

Environmental Carbon monoxide was monitored during the winter months (October – April) in 2003-2004. The CO monitor was placed in the living room of 64 households for 7 days. Complete environmental monitoring results were not available for all households visited; data was not available for 21 non-smoking households, this was due to a combination of technical problems, power cuts and also participants switching the equipment off in error.

The graphs produced from the environmental monitoring in each household displayed peaks and troughs of carbon monoxide over the duration of the monitoring period. Whilst detailed diaries were not requested from the households as to the time that cooking and central heating were in use, the patterns would suggest that the peak levels recorded on the graphs coincided with central heating systems coming on and with cooking taking place. In general, the exposure versus time graphs produced from the environmental monitoring sessions showed three different types of temporal pattern. Pattern 1 (Figure 4.1) had no clearly defined peaks. In the example, the peak level recorded was 1.2ppm (1.37mg/m^3), with a mean level of 0.7ppm (0.8mg/m^3).

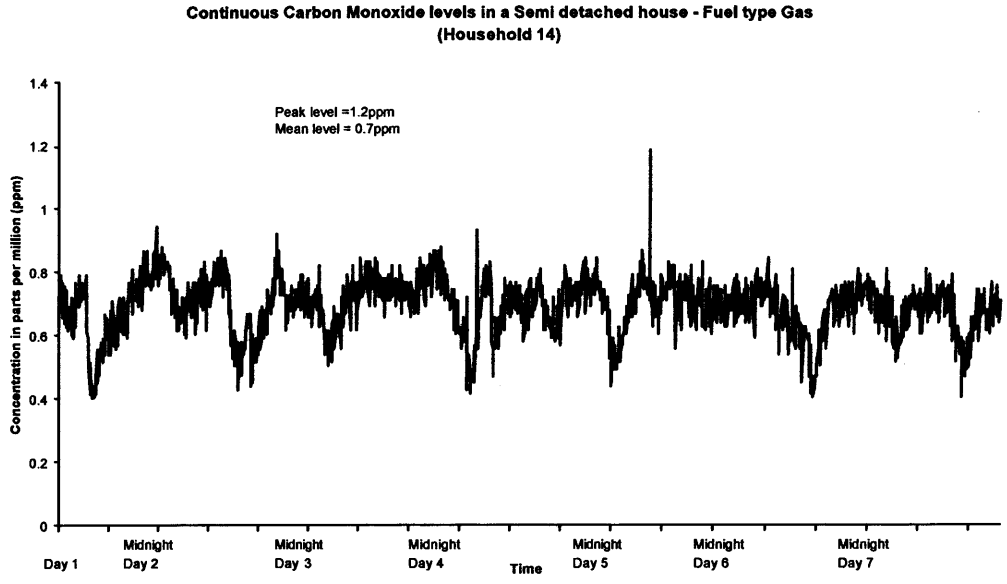


Figure 4.1 Pattern '1' 'no defined peaks'

Pattern 2 (Figure 4.2) displayed broad peaks exhibiting concentration fluctuations within a single peak and some evidence of periodicity of peaks over the whole monitoring period. In the example shown, the peak level recorded was 5ppm (5.73mg/m^3), with a mean level of 1.2ppm (1.37mg/m^3).

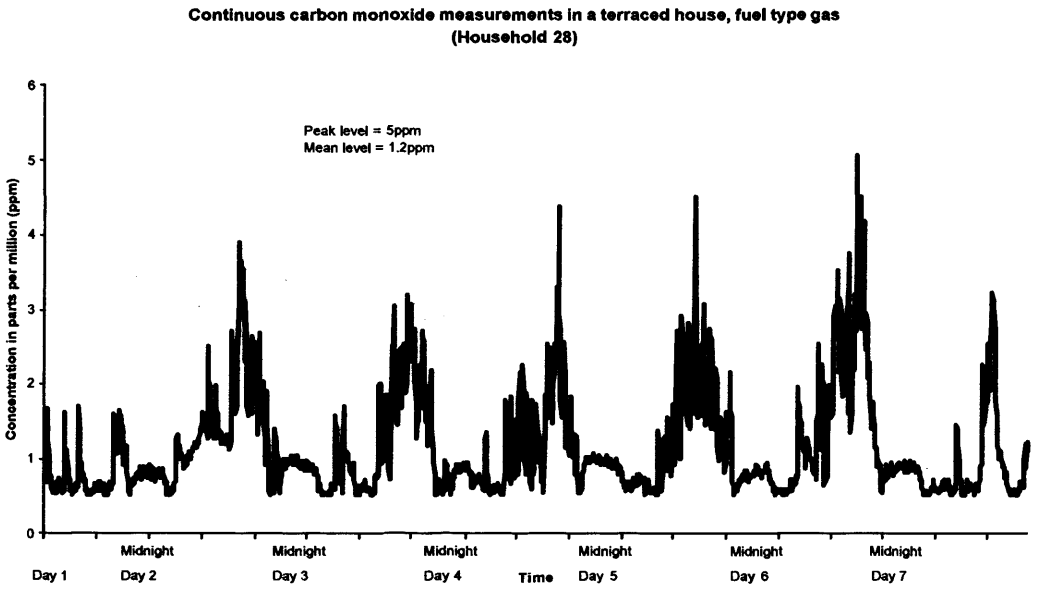


Figure 4.2 Pattern '2' 'broad peaks'

Pattern 3 (Figure 4.3) displayed narrow peaks, which often formed a regular pattern over each 24 hour period. In the example shown, the peak level recorded was 8ppm (9.16mg/m^3), with a mean level of 0.6ppm (0.69mg/m^3).

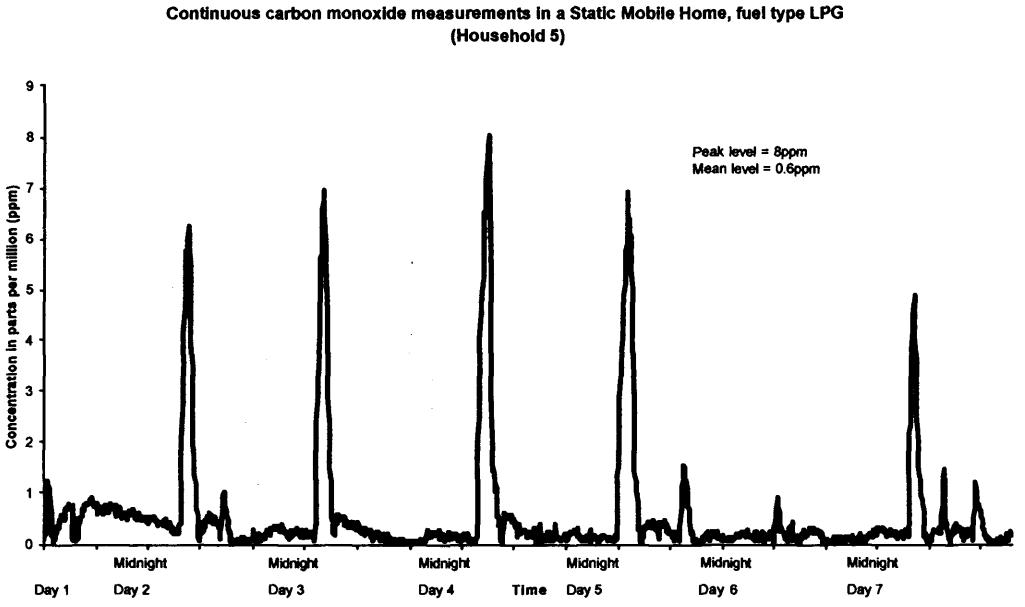


Figure 4.3 Pattern '3' narrow peaks

Whilst information was not collected on the floor area of the households or the level of ventilation within the households it is accepted that this may have influenced the level of CO building up within the homes, for example differences between the Traditional brick built households and the Static mobile homes that were measured.

In the non-smoking households, the grand mean environmental concentration was 0.22 parts per million (ppm) (0.25mg/m^3) with a grand range of 0 - 0.98ppm (0 - 1.12mg/m^3). In the households monitored the highest peak CO level (recorded as the highest Carbon monoxide level during the monitoring period) ranged from 0 - 22ppm (0 – 25.2mg/m^3) (mean = 3.46ppm (3.96mg/m^3)). The

exposure versus time graphs from each of the households monitored can be seen in Appendix 4.

Household heating Fuel analysis

The heating (and cooking) fuel type used by the household was identified on the initial visit, this was potentially the largest factor affecting the levels of Carbon monoxide produced in the home. The data was therefore analysed by the different household fuel types sampled (Gas, Solid fuel, Liquid Petroleum Gas (LPG), Electricity and Other (Oil and Wood)). The data collected on fuel type relating to length of time the heating was left on, reflected that on average, the heating was on in the living room area for 15 hours a day (maximum 24 hours, minimum 4 hours).The LPG homes tended to have their heating on for the duration of the day. Figure 4.4 shows the number of hours per day the heating was on in each of the five fuel groups: -

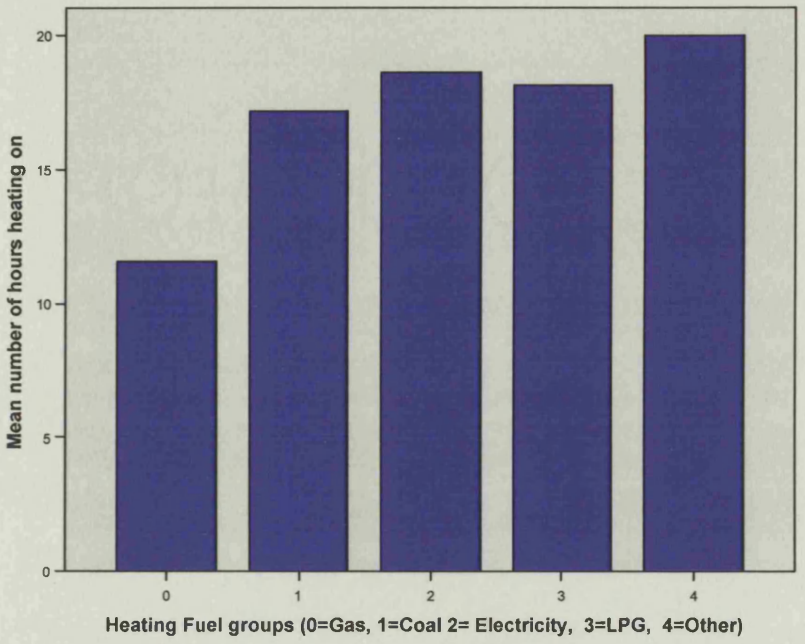


Figure 4.4 Mean number of hours the heating was on in each household for each of the five different fuel groups

The reported time spent in the living room was on average 9 hours (maximum 20 hours, minimum 2 hours). As shown in Figure 4.5, this was particularly high for the occupants of the LPG homes, this cannot be explained. The time reported for duration of heating left on, and for time spent in the living room was the same for both the non-smoking group and the smoking group.

Figure 4.5 shows the number of hours that each of the participants spent in the main living room of the house during an average 24 hour period: -

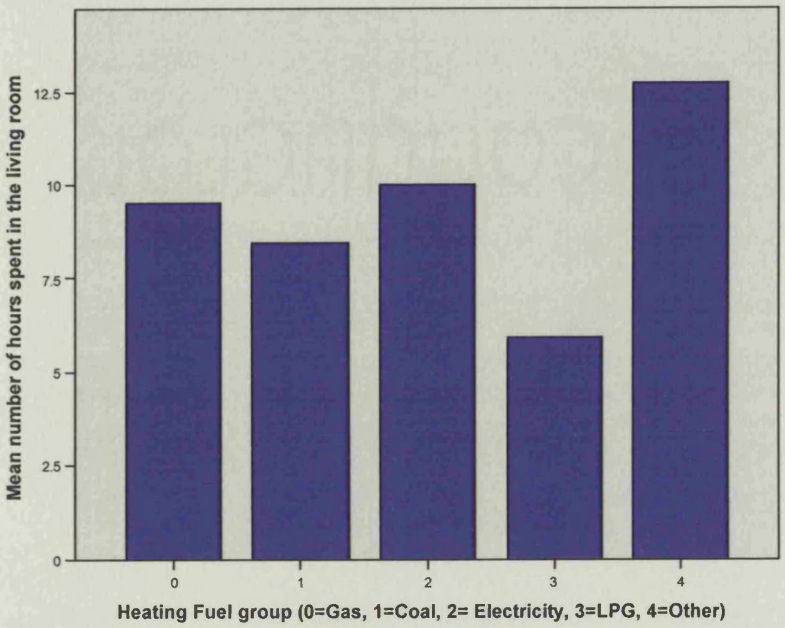


Figure 4.5 Mean number of hours spent in the living room between the five different fuel groups (non-smoking)

The LPG fuel group had the highest incidences of the heating being left on for 24 hours. As the LPG homes had the greatest duration of heating on during a 24 hour period, a bar chart (Figure 4.6) was produced to view the number of hours the heating was on and the number of hours that the participants spent in the main living area in a 24 hour period in the LPG group only (All the results from the LPG households are included in the Chart): -

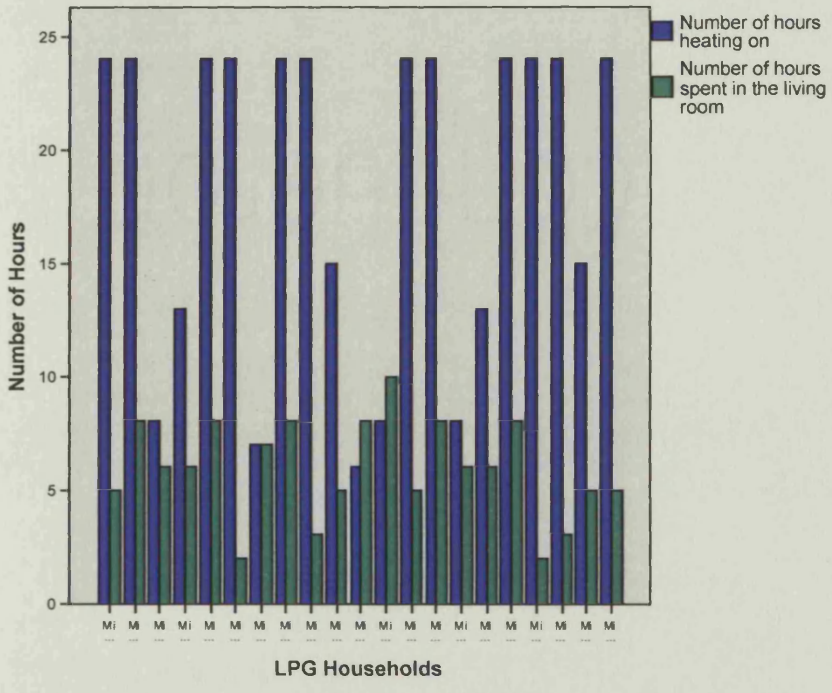


Figure 4.6 Bar chart representing the number of hours the heating was on and the number of hours the participants spent in the living room (LPG households only)

Table 4.5 represents the different fuel types monitored in the households and the analysis of the environmental levels undertaken:

-

Fuel type (Heating)	Number of households	Number of residents	Number of Households with environmental results	Highest mean[1] CO levels (ppm)	Grand mean[2]	Range of mean[3] CO levels (ppm)	Highest peak[4] CO level(ppm)	Range of Highest peak[5] CO level (ppm)
Gas	37	59	21	0.88	0.23	0.001-0.88	17.0	0.031-17
Coal	7	11	5	0.65	0.3	0.003-0.65	6.2	0.085-6.2
Electricity	4	5	3	0.46	0.33	0.22-0.46	12.0	1.6-12
LPG	13	21	12	0.98	0.49	0.16-0.98	22.0	0.75-22
Other	3	3	3	0.24	0.19	0.12-0.55	4.0	0.55-4

1. Highest mean – mean of all the highest peak CO values recorded in each fuel type
2. Grand mean – the mean of means
3. Range of mean – the range of the Grand mean
4. Highest peak – highest single peak CO level recorded in each fuel type
5. Range of highest peak – the range of the highest peaks obtained in each of the households monitored in each fuel type.

Table 4.5 Carbon monoxide concentrations by heating fuel type – non-smoking households (in parts per million)

Table 4.5 shows the environmental Carbon monoxide levels recorded. The information presented includes data on the highest peak levels for each of the heating fuel types, the highest mean levels and the range of levels observed. The results show that throughout the sampling, the levels of environmental Carbon monoxide recorded were very low, the grand mean of all fuel types was 0.31ppm (0.36mg/m^3). On analysis by fuel type, the highest Carbon monoxide levels were recorded in households using LPG. All the LPG locations were static homes with a small floor area. Concentrations did not differ markedly between the fuel groups in the non-smoking households. For the households sampled the mean environmental concentration over the total period monitored ranged from <0.1 to 1 ppm (<0.11 – 1.15mg/m^3). The highest single peaks recorded in each of the individual households during the measurement period ranged from 4ppm to 22 ppm (4.58 – 25.2mg/m^3).

Further analysis was carried out using boxplots to examine the data within each of the five heating fuel types (Figure 4.7). In these figures, the box represents the portion of the distribution falling between the 25^{th} and 75^{th} percentiles (the lower and upper quartiles). The line across the middle of the box represents the median and the whiskers extend to the largest and smallest values not considered outliers or extreme values. An outlier is a value more than 1.5 box

lengths above or below the box and will be flagged up by SPSS, usually with a circle, with a number which identifies the outlying value. An extreme value is a value more than 3 box lengths above or below the box and again SPSS will flag these values with asterisks along with a number which identifies the outlying value.

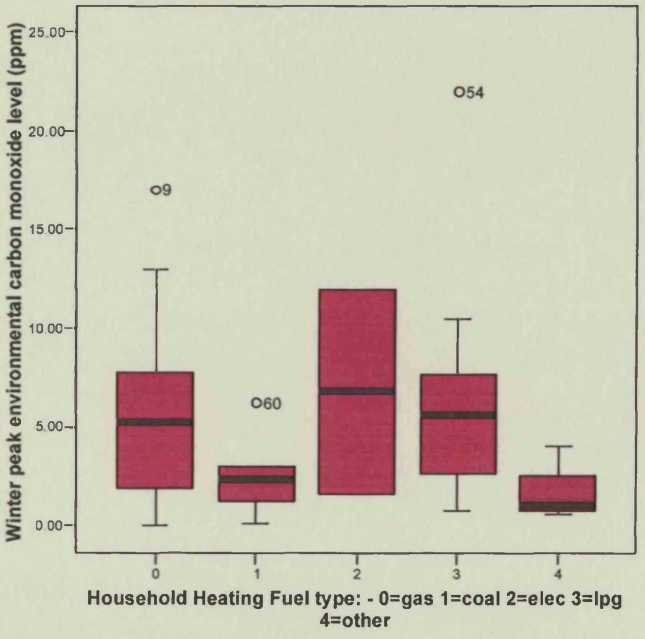


Figure 4.7 Winter environmental peak Carbon monoxide level against heating fuel type in non-smoking households

In the boxplot above (Figure 4.7), it can be seen that the data for the winter environmental peak values is skewed for the coal and other fuel groups, this maybe due to the small number of households in these groups. It also identifies outliers in the gas group (17ppm) (19.48mg/m^3), coal (6.2ppm) (7.1mg/m^3) and LPG (22ppm) (25.2mg/m^3). Further analysis was carried out using the Kruskal-Wallis test. This is a non-parametric test used to decide whether *k*

independent samples are from different populations. This analysis showed that there is not a statistical difference between the winter peak environmental Carbon monoxide levels measured and the 5 different heating fuel types sampled (p=0.78).

The same analysis was then carried out for winter mean environmental levels of CO (Figure 4.8). The data is skewed for the gas, coal, LPG and other fuel groups. An outlier in the gas group, this relates to a household where the mean winter Carbon monoxide level was recorded as 0.88ppm (1.01mg/m^3).

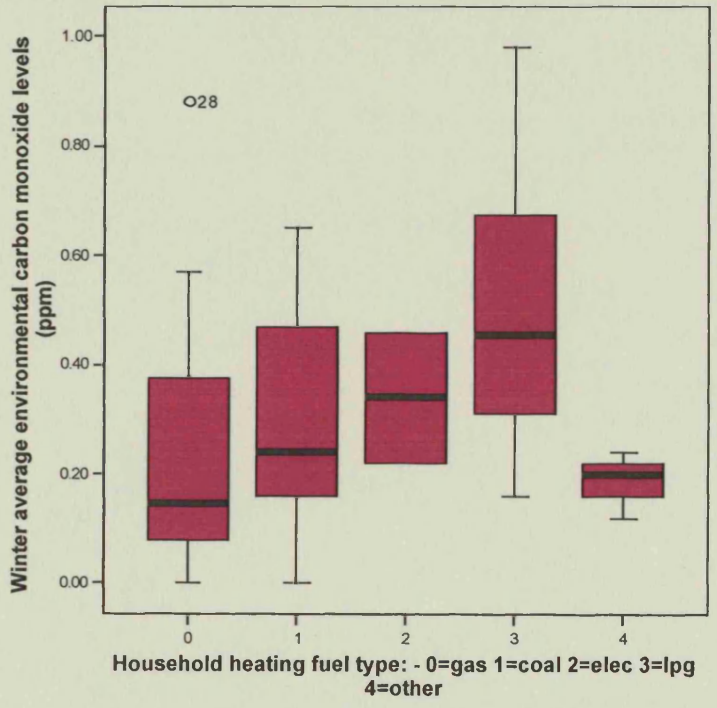


Figure 4.8 Winter environmental mean Carbon monoxide level against heating fuel type in non-smoking households

An ANOVA test linked to a Tukey test was carried out to test for the differences between winter mean environmental levels for the 5 different fuel groups.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.194	4	.298	5.968	.000
Within Groups	3.000	60	.050		
Total	4.194	64			

Table 4.6 Results of the ANOVA test for the differences between winter mean environmental levels in the 5 fuel groups in non-smoking households

The results of the ANOVA (table 4.6) show that there is a statistically significant difference between winter mean environmental levels and the five different fuel groups. Following the results from the ANOVA test, a multiple comparisons test linked to a Tukey test between each of the five different fuel types and the associated winter mean environmental levels was carried out, this is shown in Table 4.7: -

(I) 0=Gas 1=Coal 2=Electricity 3=LPG 4=Oil	(J) 0=Gas 1=Coal 2=Electricity 3=LPG 4=Oil	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	-.11326	.08868	.706	-.3627	.1361
	2	-.08251	.13521	.973	-.4628	.2978
	3	-.30451(*)	.06413	.000	-.4849	-.1241
	4	.03083	.13521	.999	-.3494	.4111
1	0	.11326	.08868	.706	-.1361	.3627
	2	.03075	.15139	1.000	-.3950	.4565
	3	-.19125	.09355	.258	-.4543	.0718
	4	.14408	.15139	.875	-.2817	.5699
2	0	.08251	.13521	.973	-.2978	.4628
	1	-.03075	.15139	1.000	-.4565	.3950
	3	-.22200	.13845	.501	-.6114	.1674
	4	.11333	.18258	.971	-.4002	.6268
3	0	.30451(*)	.06413	.000	.1241	.4849
	1	.19125	.09355	.258	-.0718	.4543
	2	.22200	.13845	.501	-.1674	.6114
	4	.33533	.13845	.123	-.0540	.7247
4	0	-.03083	.13521	.999	-.4111	.3494
	1	-.14408	.15139	.875	-.5699	.2817
	2	-.11333	.18258	.971	-.6268	.4002
	3	-.33533	.13845	.123	-.7247	.0540

- The mean difference is significant at the .05 level.

Table 4.7 Results of the Multiple Comparisons Tukey HSD test for the differences between winter mean environmental levels in the 5 fuel groups in non-smoking households

The Tukey test calculates multiple comparisons to identify which fuel groups are statistically significant. The results in table 4.7 show that the only groups where the means differ for winter mean environmental levels are the Gas and LPG groups which are statistically significant (p=0.001).

From the environmental monitoring data available, an analysis was carried out to establish if at any time during the monitoring periods,

the levels recorded had risen above the WHO 8 hour standard of 8.6ppm (11.46mg/m^3). Table 4.8 details the households that exceeded the limit, which fuel groups they belonged to and also details the length of time that each of the households was above the limit.

Household Identifier	Heating Fuel type	Peak level CO (ppm)	Time above 8.6ppm
45	Electricity	12	5 minutes (16.01)
Static 13	LPG	10.5	5 minutes (19.19)
Static 4	LPG	22	35 minutes (12.17-12.47) 15 minutes (11.17-11.27) 25 minutes (12.32-12.52) 30 minutes (11.57-12.22)
9	Gas	17	1 hour 25 minutes (19.06-20.26)
15	Gas	10.5	15 minutes (16.23-16.33)
11	Gas	13	1 hour 35 minutes (10.48-12.18)
20	Gas	11	25 minutes (11.20-11.45)

Table 4.8 Duration of CO peaks above 10ppm in non-smoking households

Table 4.8 shows that of the 7 households where peak levels rose above the WHO limit of 10ppm over an 8 hour period, 4 households

were heated by gas, 2 by LPG and one by Electricity. The highest peak recorded was 22ppm (25.2mg/m^3) in a LPG heated static home, this household reported 4 occasions where the level rose above 10ppm (11.46mg/m^3). The longest period recorded above the 10ppm (11.46mg/m^3) limit was 1 hour 35 minutes in a gas heated household.

Smoking households

Environmental monitoring results were not available for 3 smoking households visited; this was due to the monitors being switched off in error by the participants.

Table 4.9 details the number of households recruited in each of the fuel groups and the basic statistics associated with the environmental monitoring carried out. In the smoking subset, participants were only available from three of the fuel groups (Gas, Coal and LPG). The grand mean environmental concentration was 2.5ppm (2.86mg/m^3), with a range of 0.2-21ppm (0.23 – 24.06mg/m^3). The peak level ranged from 1.8-53.6ppm (2.06 – 61.4mg/m^3) (mean = 11.4ppm) (13.06mg/m^3).

Fuel type (Heating)	Number of households	Number of residents	Number of Households with environmental results	Highest mean[1] CO levels (ppm)	Grand mean[2]	Range of mean[3] CO levels (ppm)	Highest peak[4] CO level(ppm)	Range of Highest peak[5] CO level (ppm)
Gas	14	12	12	10.7	1.8	0.3-10.7	45.7	1.4-45.7
Coal	4	5	3	1.9	0.5	0.2-0.7	1.9	1.9-1.9
LPG	5	6	5	17.4	6.1	1-21	53.6	5.1-53.6

1. Highest mean – mean of the highest peak CO levels followed by the Grand mean – the mean of means in brackets
2. Grand mean – the mean of means
3. Range of mean – the range of the Grand mean
4. Highest peak – highest single peak CO level recorded in each fuel type, followed by the grand mean peak in brackets
5. Range of highest peak – the range of the highest peaks obtained in each of the households monitored in each fuel type.

Table 4.9 Carbon monoxide concentrations by heating fuel type (in parts per million), Smoking households

In smoking households, the mean environmental concentration over the total period monitored ranged from 0.1ppm to 21 ppm (0.11 – 24.06mg/m^3) and the highest measurement recorded in an individual household ranged from 1.9ppm to 53.6 ppm (2.18 – 61.4mg/m^3). Similar to the non-smoking households, the highest Carbon monoxide levels recorded were in LPG heated homes.

A boxplot was drawn to show the relationship between the winter mean environmental levels and the different fuel groups (Figure 4.9).

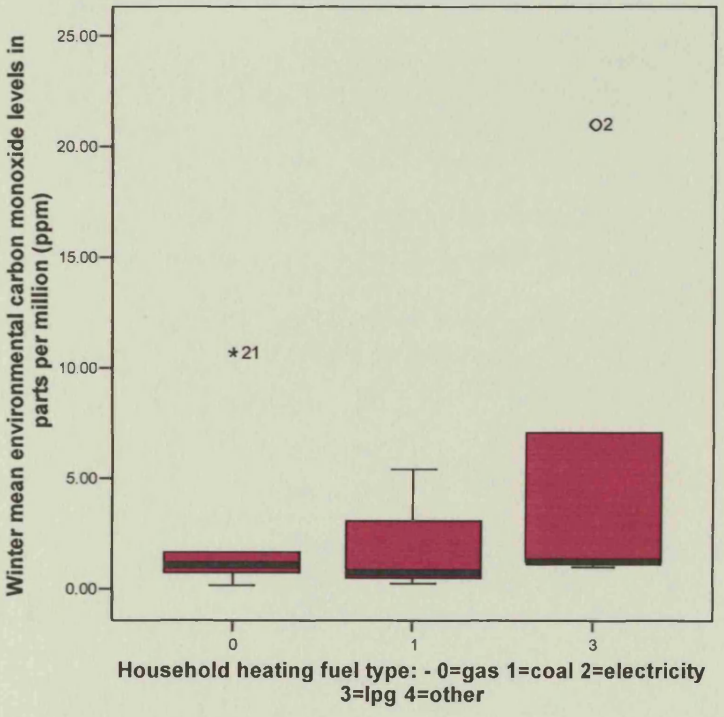


Figure 4.9 Boxplot winter environmental mean Carbon monoxide level against heating fuel type in smoking households

An ANOVA test showed that there was no statistically significant difference between winter mean environmental CO levels in the different fuel groups; p=0.378.

None of the smoking households monitored exceeded the WHO limit of 26ppm (30mg/mg^3) over 1 hour. With Regards to the WHO limit of 8.6ppm (10mg/m^3) over 8 hours, 4 households had peaks above 8.6ppm, these are shown in table 4.10, together with details of which fuel group the households were in and the duration of time that they were above the WHO limit: -

Household Identifier	Fuel	Peak (ppm)	Time above 8.6ppm
13	Gas	19.86	1 hour 40 minutes (17.18-18.53)
12	Gas	11.37	5 minutes (14.29)
8	Gas	14	30 minutes (13.52-14.17)
2	LPG	53.58	5.1.04 1 hour 55 minutes (16.44-18.09) 6 hours 25 minutes (20.49-03.19) 6.1.04 3 hours (7.09-10.04)

Table 4.10 Smoking Households with CO peaks above the WHO 8 hour limit of 10ppm

Table 4.10 shows that of the 4 households where peak levels exceeded the WHO limit of 8.6ppm over an 8 hour period, 3 households were heated by gas, and 1 by LPG. The highest peak

recorded was 53.58ppm (61.4mg/m^3) in a LPG heated static home, this household reported 3 occasions where the peak level exceeded the WHO limit. This household also recorded the longest period above the 8.6ppm limit at 6 hour 25 minutes.

Whilst it maybe expected to observe a difference between Carbon monoxide levels in a smoking and a non-smoking home, a boxplot was drawn to establish whether the winter peak environmental monitoring data collected confirmed this expectation. The results can be seen in Figure 4.10: -

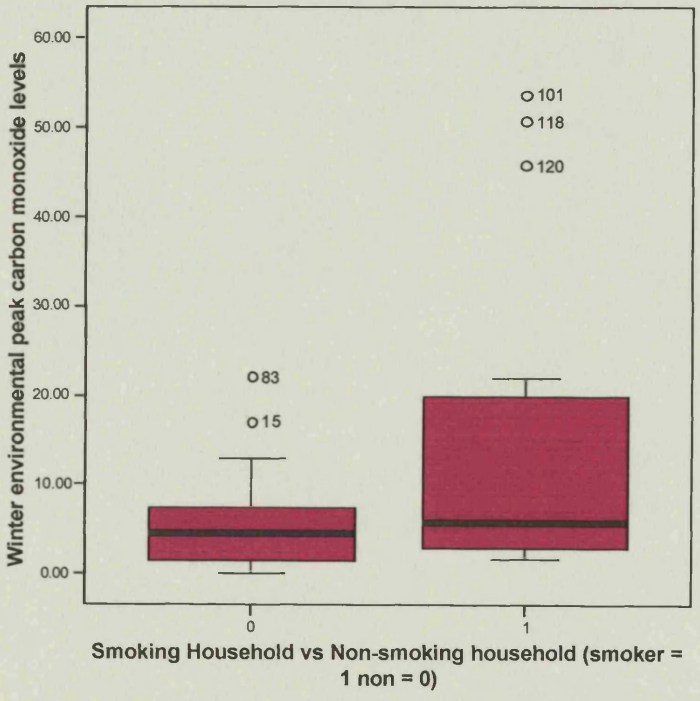


Figure 4.10 Differences between smoking and non-smoking households for winter Peak Carbon monoxide levels

There was a 31% difference in median values between the smoking (5.9ppm) (6.76mg/m^3) and non-smoking (4.5ppm) (5.16mg/m^3)groups.

The next step was to test if there was a statistically significant difference between the smoking and non smoking households for peak environmental levels monitored. Statistical analysis was carried out using a Mann Whitney U test. This is the non parametric alternative to the t-test for independent samples but instead of comparing the means of the two groups, the Mann Whitney U test compares the medians. Non parametric tests can be used in place of their parametric counterparts and are particularly useful for dealing with data with extreme outlying values rather than having to exclude them from the analysis altogether.

The difference between smokers versus non-smokers for peak CO levels were not statistically significant (p=0.23) although this may have been due to the low numbers who participated in the smoking group.

The analysis was then repeated for the winter mean environmental levels monitored. Figure 4.11 shows the boxplot drawn for winter mean environmental levels in smokers and non smokers' households: -

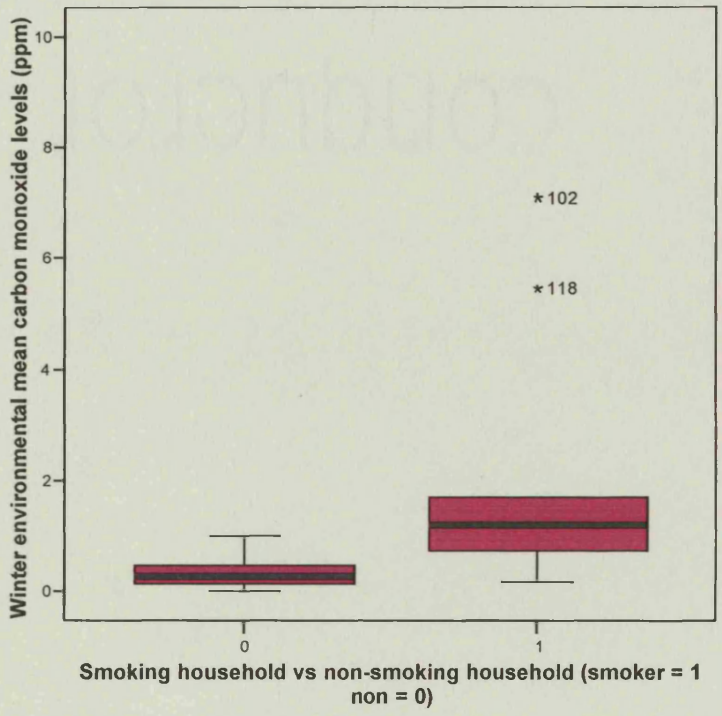


Figure 4.11 Differences between smoking and non-smoking households for winter mean Carbon monoxide levels

There was a 300% difference in winter mean CO levels between the smoking (1.2ppm) (1.37mg/m^3) and non-smoking (0.3ppm) (0.34mg/m^3)groups, a Mann Whitney U test was carried out, and this was statistically significant $p<0.001$.

4.2.2 Environmental monitoring for the outdoor environment

This study measured and compared the environmental levels of Benzene, Nitrogen dioxide and Nitrous oxide experienced at households in two different microenvironments of vehicular traffic.

The study also investigated the relationships between environmental levels and biomarkers of exposure for Benzene and Nitrous oxide.

Table 4.11 Details the descriptive results of all the samples:-

	Number of samples	Mean level	Range	Standard deviation
Benzene	66	0.25ppb	0.1-0.57ppb	0.9
s-PMA	52	1.75umol/mol	0.2-7.7umol/mol	1.19
Nitrogen dioxide	68	15.4ppb	7.5-24ppb	4.3
Nitrous oxide (inside)	21	15.67ppb	0-152ppb	40.5
Nitrous oxide (outside)	25	260ppb	0-1233ppb	383.7
Urinary Nitrous oxide	17	183	0-1863	515.1

Table 4.11 All results Traffic study

Table 4.12 And 4.13 Present the descriptive results for the highly trafficked and lowly trafficked areas:-

	Number of samples	Mean level	Range	Standard deviation
Benzene	52	0.26ppb (0.83 ug/m^3)	0.12-0.57 (0.38-1.81 ug/m^3)	0.95
s-PMA	40	1.89	0.2-7.7	1.28
Nitrogen dioxide	54	16.75 (31.9 mg/m^3)	14.3-46 (0-2219 mg/m^3)	3.7
Nitrous oxide (inside)	12	14.75 (26.5 mg/m^3)	0-116 (0-208.8 mg/m^3)	33.3
Nitrous oxide – outside	16	318 (572 mg/m^3)	0-1233 (0-2219 mg/m^3)	383.5
Urinary Nitrous oxide	12	163.5	0-1863.4	535.9

Table 4.12 High Trafficked areas only

	Number of samples	Mean level	Range	Standard deviation
Benzene	14	0.19 (0.61 ug/m^3)	0.1-0.31 (0.32-0.99 ug/m^3)	0.7
s-PMA	12	1.32	0.3-3.4	0.76
Nitrogen dioxide	14	10.16 (19.4 mg/m^3)	7.6-12.3 (14.5-23.5 mg/m^3)	1.4
Nitrous oxide (inside)	9	16.89 (30.4 mg/m^3)	0-152 (0-273.6 mg/m^3)	50.6
Nitrous oxide (outside)	9	158 (284 mg/m^3)	0-1162 (0-2092 mg/m^3)	384
Urinary Nitrous oxide	5	232.5	0-1160	518.7

Table 4.13 Low trafficked areas only

Environmental levels of Benzene

	Number of Samples	Mean level ppb	Range ppb	Standard deviation
Benzene	66	0.25 (0.79 ug/m^3)	0.1-0.57ppb (0-1.81 ug/m^3)	0.9
Benzene (control group)	14	0.19 (0.61 ug/m^3)	0.1-0.31 (0.32-0.99 ug/m^3)	0.7
Benzene (exposed group)	52	0.26ppb (0.83 ug/m^3)	0.12-0.57 (0.38-1.81 ug/m^3)	0.95

Table 4.14 Environmental Benzene results

A total of 66 samples were collected for Benzene, with a mean of 0.25ppb (0.79mg/m^3) and range of 0.1-0.57ppb (0 – 1.81mg/m^3). In the exposed group there were 52 samples with a mean of 0.26 (0.83mg/m^3) and range of 0.12-0.57ppb (0.38 – 1.81mg/m^3) and in the control group there were 14 samples, with a mean of 0.19ppb (0.61mg/m^3) and range of 0.1-0.31ppb (0.32 – 0.99mg/m^3).

Analysis of the normality of the data was carried out and showed that the Benzene in the exposed and control areas were normally distributed therefore a parametric t-test was chosen to test the environmental variables against the two microenvironments, the results are shown in table 4.15: -

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Benzene	21.187	65	.000	.24667	.2234	.2699

Table 4.15 Results of t - test for Benzene data in exposed and control areas

The results of the t-test (Table 4.15) show that the mean levels for Benzene are statistically significantly different between the exposed and control areas ($p<0.001$).

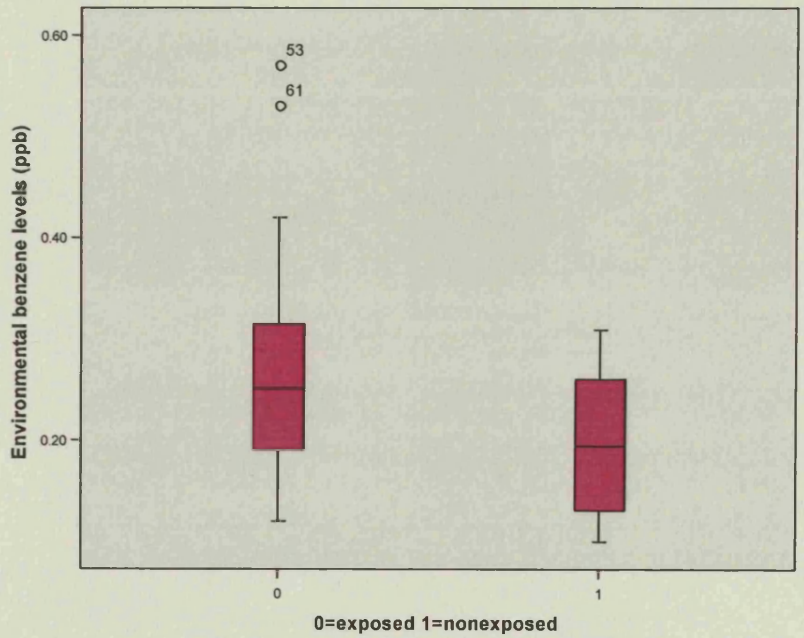


Figure 4.12 Environmental Benzene in exposed and control groups

The boxplot in figure 4.12 shows the Benzene levels monitored in the exposed and control microenvironments. In the exposed area there are two outliers, 53, which is in Moorland Road and 61, which is in Western Avenue. The mean level of Benzene in the exposed group was 0.3, with a mean level of 0.2 in the control group; the difference translates to an effect size of 50%.

Nitrogen dioxide and Nitrous oxide measurements

	Number of Samples	Mean level ppb	Range ppb	Standard deviation
Nitrogen dioxide	68	15.39 (29.4 mg/m^3)	7.5-24.1 (14.3-46 mg/m^3)	4.3
Nitrogen dioxide (control group)	14	10.16 (19.4 mg/m^3)	7.6-12.3 (14.5-23.5 mg/m^3)	1.4
Nitrogen dioxide (exposed group)	54	16.75 (31.9 mg/m^3)	14.3-46 (0-2219 mg/m^3)	3.7

Table 4.16 Environmental Nitrogen dioxide results

A total of 68 samples were collected for Nitrogen dioxide, with a mean of 15.39ppb (29.4mg/m^3) and range of 7.5-24.1ppb (14.3 - 46mg/m^3). In the exposed group there were 54 samples with a mean of 16.75ppb (31.9mg/m^3) and a range of (14.3 - 46mg/m^3) and in the control group there were 14 sample, with a mean of 10.16ppb (19.4mg/m^3) and a range of 7.6-12.3ppb (14.5 – 23.5mg/m^3).

Analysis of the normality of the data showed that the Nitrogen dioxide in the exposed and control areas was normally distributed therefore a parametric t-test was chosen to test the environmental variables against the samples taken in a non-exposed traffic pollution area and an exposed traffic pollution area, the results are shown in table 4.17:

-

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Nitrogen dioxide	29.555	67	.000	15.3911 8	14.351 7	16.430 6

Table 4.17 Results of the t - test for the Nitrogen dioxide levels in the exposed and control areas

The results of the t-test shown in table 4.17 show that the mean levels for Nitrogen dioxide are statistically significantly different between the exposed and control areas ($p<0.001$).

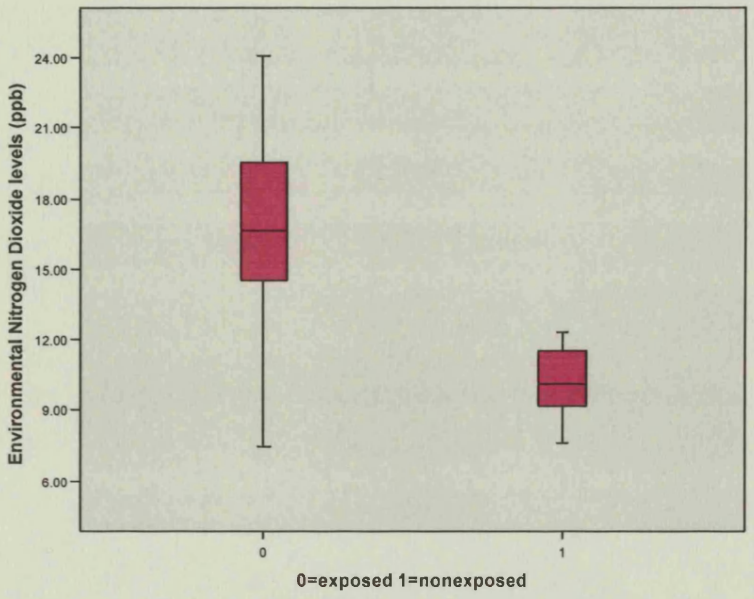


Figure 4.13 Boxplot illustrating the difference between environmental Nitrogen dioxide exposure in exposed and non-exposed areas

Figure 4.13 shows the Nitrogen dioxide levels monitored in the exposed and control areas. The mean level of Nitrogen dioxide in the exposed group was 16.7, with a mean level of 10.2 in the control group; the difference translates to an effect size of 64%. To test whether there is a statistically significant difference between the exposed and control areas, an ANOVA test was carried out, the results are shown in table 4.18: -

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	482.966	1	482.966	42.356	.000
Within Groups	752.569	66	11.403		
Total	1235.535	67			

Table 4.18 ANOVA test testing the significance of Nitrogen dioxide within the exposed group and the control group

The results of the ANOVA shown in table 4.18, show that there is a statistically significant difference between the Nitrogen dioxide monitored in the exposed and control microenvironments ($p<0.001$).

Within the funds available it was also decided to investigate a small subgroup of households where Nitrous oxide measurements were sampled outside and inside the home. Nitrous oxide was monitored in 25 households, both outside and inside, it was not possible to retrieve Nitrous oxide tubes from inside 4 of the households, despite numerous attempted contacts, due to no response.

	Number of Samples	Mean level ppb	Range ppb	Standard deviation
Nitrous oxide - outside	25	260 (469 mg/m^3)	0-1233 (0-2219 mg/m^3)	383.7
Nitrous oxide – outside (control group)	9	158 (284 mg/m^3)	0-1162 (0-2092 mg/m^3)	384
Nitrous oxide – outside (exposed group)	16	318 (572 mg/m^3)	0-1233 (0-2219 mg/m^3)	383.5
Nitrous oxide - inside	21	15.67 (28.2 mg/m^3)	0-152 (0-273.6 mg/m^3)	40.5
Nitrous oxide – inside (control group)	9	16.89 (30.4 mg/m^3)	0-152 (0-273.6 mg/m^3)	50.6
Nitrous oxide – inside (exposed group)	12	14.75 (26.5 mg/m^3)	0-116 (0-208.8 mg/m^3)	33.3

Table 4.19 Environmental Nitrous oxide results

The Nitrous oxide results were not normally distributed between the exposed and the non-exposed and also between the inside and outside measurements, therefore a non-parametric test was chosen. The Mann Whitney test was chosen to test the environmental variables against the samples taken in a non-exposed traffic pollution area and an exposed traffic pollution area.

Nitrous oxide (Outside)

A total of 25 samples were collected for Nitrous oxide outside of households, with a mean of 260.44ppb (469mg/m^3) and a range of 0-1233ppb (0 - 2219mg/m^3). In the exposed group there were 16 samples with a mean of 318ppb (572mg/m^3) and a range of 0-1233ppb (0 - 2219mg/m^3) and in the control group there were 9 samples, with a mean of 158.11ppb (284mg/m^3) and a range of 0-1162ppb (0 - 2092mg/m^3).

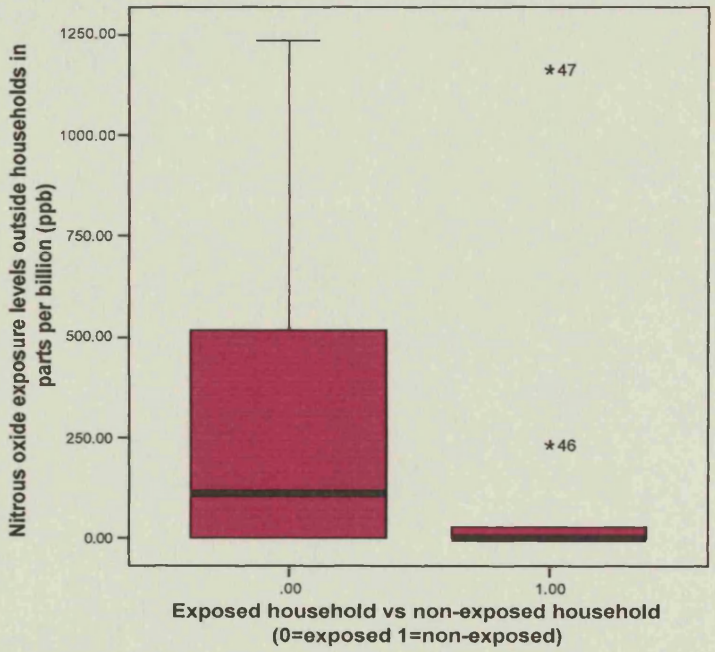


Figure 4.14 environmental Nitrous oxide exposures (outside households) in exposed and non-exposed areas

The boxplot in figure 4.14 shows the Nitrous oxide measurements (outside) in the two microenvironments, with levels appearing to be higher in the exposed area but a Mann Whitney test showed that

there is no statistical significance between the Nitrous oxide measurements in the exposed roads and the measurements in the control roads (p=0.098) this may be due to the low numbers of households that took part in the Nitrous oxide monitoring.

Nitrous oxide (Inside)

A total of 21 samples were collected for Nitrous oxide inside households, with a mean of 15.67ppb (28.2mg/m^3) and a range of 0-152ppb (0 – 273.6mg/m^3). In the exposed group there were 12 samples with a mean of 14.75ppb (26.5mg/m^3) and a range of 0-116ppb (0 – 208.8mg/m^3) and in the control group there were 9 samples, with a mean of 16.89ppb (30.4mg/m^3) and a range of 0-152ppb (0 – 273.6mg/m^3).

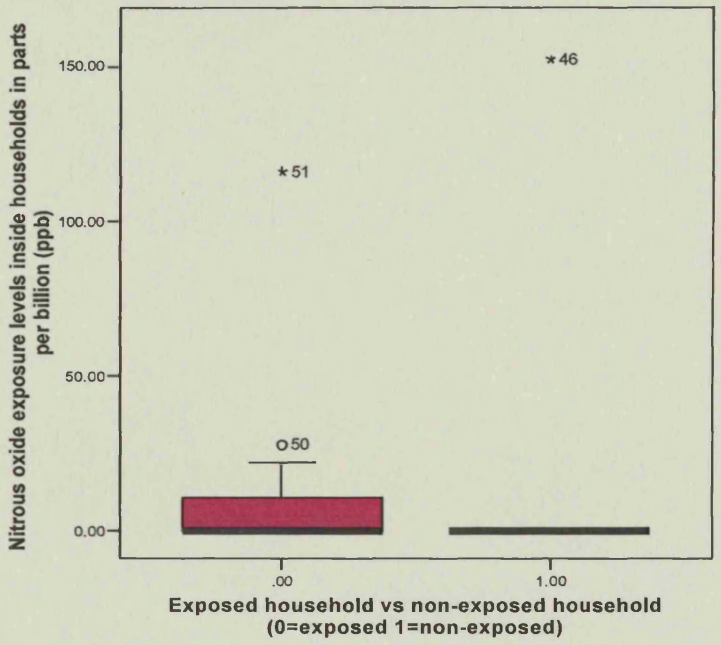


Figure 4.15 Environmental Nitrous oxide exposure (inside households) in exposed and non-exposed areas

Figure 4.15 shows the inside Nitrous oxide measurements in the two microenvironments monitored. Two outliers were observed in the exposed area; both 50 and 51 were in Manor Way. There was one outlier within the control area this was 46 which was in Denison way. The median level of Nitrous oxide (inside) in the exposed group and the control group was 0.

To observe whether there was a relationship between outside and inside measurements taken for Nitrous oxide a scatterplot was drawn, this is shown in figure 4.16: -

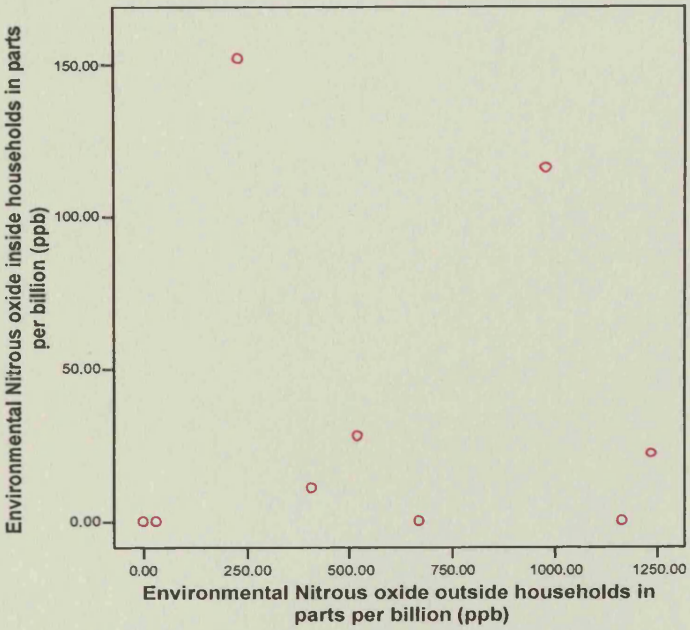


Figure 4.16 Scatter plot illustrating the distribution of environmental Nitrous oxide levels, outside household's against inside households

The scatter plot shown in figure 4.16 shows that there is a statistically significant correlation between the exposed and control groups (p=0.004, r=0.603).

			Nitrous oxide inside	Nitrous oxide outside
Spearman's rho	Nitrous oxide inside	Correlation Coefficient	1.000	.603(**)
		Sig. (2-tailed)	.	.004
		N	21	21
	Nitrous oxide outside	Correlation Coefficient	.603(**)	1.000
		Sig. (2-tailed)	.004	.
		N	21	25

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

Table 4.20 Results of Spearman Rank-Nitrous oxide inside versus outside

The reduced number of plots illustrated on the scatter plot can be explained by the large number of equal results obtained (i.e. 17 Inside and 11 outside results were 0ppb).

To further look at the data relating to the exposed areas only, a boxplot was drawn to observe the relationship between Nitrous oxide levels inside and Nitrous oxide levels outside, this is shown in figure 4.17: -

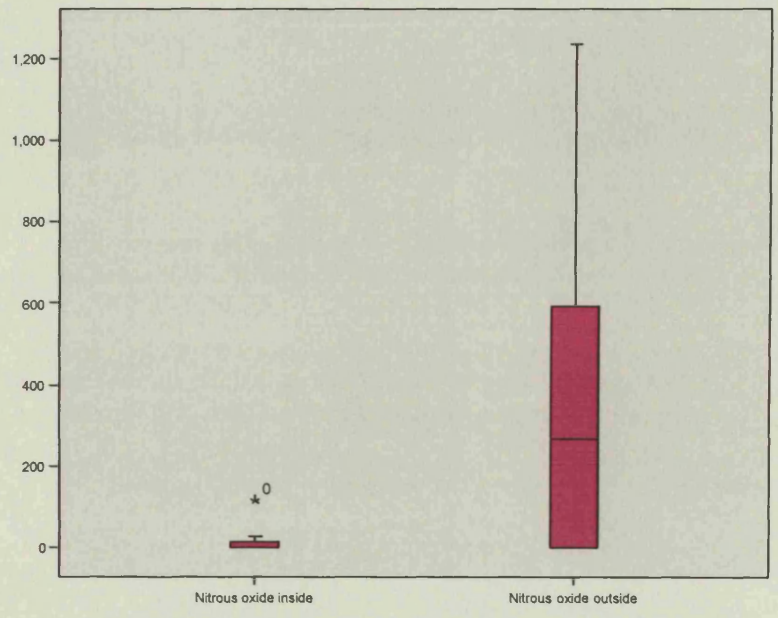


Figure 4.17 Nitrous oxide levels inside and outside households in exposed roads only

Figure 4.17 shows that there is an observed difference between the inside and outside Nitrous oxide measurements in the exposed area. To test whether there was a statistically significant difference a

Wilcoxon signed ranks test was carried out, the results are shown in table 4.21:

	Nitrous oxide outside – Nitrous oxide inside
Z	-2.366(a)
Asymp. Sig. (2-tailed)	.018

a Based on negative ranks.
b Wilcoxon Signed Ranks Test

Table 4.21 Wilcoxon signed ranks test for Nitrous oxide inside against Nitrous oxide outside in exposed areas only

The results shown in table 4.21 show that there is a statistically significant difference between inside and outside measurements of Nitrous oxide in the exposed areas (p=0.018).

To repeat this for Nitrous oxide measurements taken in the control areas, a boxplot was drawn (figure 4.18): -

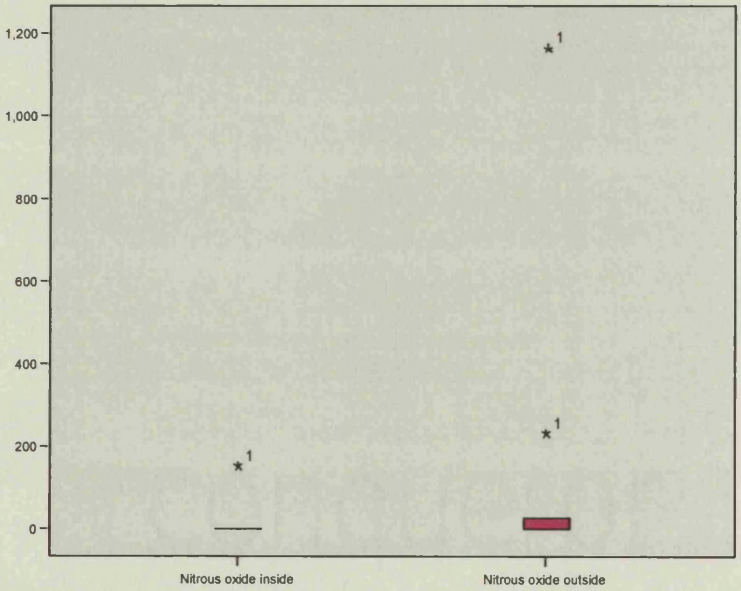


Figure 4.18 Nitrous oxide levels inside and outside households in control roads only

Figure 4.18 shows that there is a very small observed difference between inside and outside measurements of Nitrous oxide in the control areas and a Wilcoxon signed ranks test showed that there is no statistically significant difference between the inside and outside Nitrous oxide measurements in the control areas (p=0.109).

4.3 Biological monitoring

4.3.1 Biomarkers associated with indoor environment

Biological monitoring was carried out in the non-smoking group only. Each participant donated a blood sample during the week when the environmental monitoring took place. From the blood sample two biological measurements were made; the first measured carboxyhaemoglobin (COHb) levels and the second the enzyme cyclic Guanosine Monophosphate (cGMP). Blood samples were taken during the winter and also as a follow up in the summer to assess any seasonal difference.

Winter COHb results were available for 85% of subjects and cGMP measurements were available for 90% of subjects. Summer COHb results were available for 59% and cGMP data was available for 74% of the sample group. Both summer and winter COHb and cGMP measurements were available for 48% of subjects. Complete measurements of environmental concentrations as well as summer

and winter COHb and cGMP measurements were available for 31% of the sample group. Whilst there was a high rate of attendance for the biological monitoring during the initial winter months and when the monitor was in place within the home, the recall for a follow up summer measurement was not as well attended. This maybe partly due to the original hospital having closed and greater travelling being required to attend the new hospital clinic.

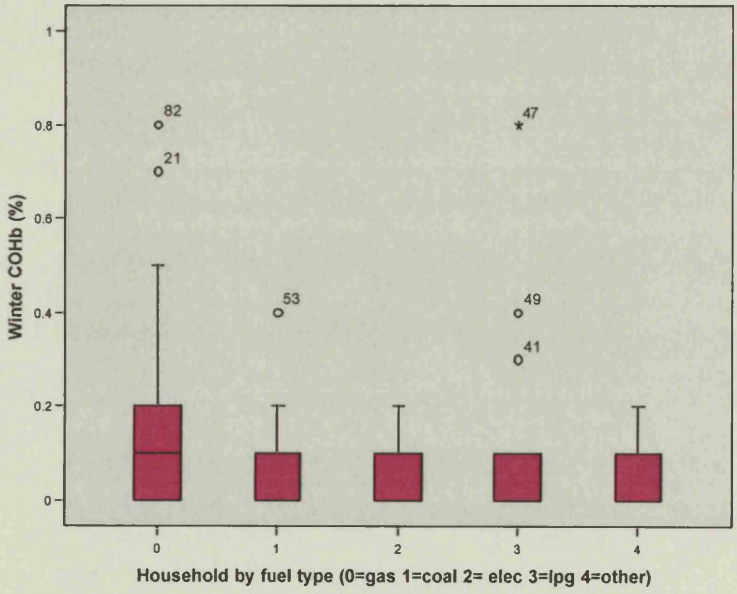
Carboxyhaemoglobin

The majority of the CoHb results received were recorded as 0%. Participants had to leave the potential CO source to travel to the hospital for the blood sample, and carboxyhaemoglobin has a half life of 2-5 hours in healthy individuals. The time between the participant leaving their home and having their blood sample taken was not recorded, the time delay and exposure to outdoor air is a possible explanation for the zero results. Further those non zero levels recorded are likely to be an underestimate of COHb levels at the point of leaving the home. For environmental CO inhaled between 1 and 3ppm, it would be expected to observe a COHb level of 0.5-0.8%, for an inhaled level of 7-50ppm, it would be expected to observe a COHb level of 1.5-8%. Reference ranges were obtained from the hospital where the analysis was undertaken for a typical week. In that week 2% of the COHb tests would be expected to be below 0.5%, 57% would be expected to be in the range 0.6-1% and

41% of tests would be expected to be above 1.1%. Table 4.22 and Figure 4.19 show the winter levels of COHb measured in each person for each of the households, grouped by fuel type: -

Fuel type (Heating)	Number of persons	persons with results	COHb Mean (%)	Range (%)	Standard deviation
Gas	58	48	0.19	0-3	0.47
Coal	11	11	0.4	0-4	1.13
Electricity	5	4	0.05	0-0.2	0.1
LPG	21	18	0.11	0-0.8	0.22
Other	4	4	0.05	0-0.2	0.12

Table 4.22 Winter Carboxyhaemoglobin levels (%) of household residents by fuel type



(Note The winter COHb scale has been reduced to show the %'s in the other fuel groups. Winter COHb levels rose to a peak of 3% in the gas group and 4% in the coal group)

Figure 4.19 Winter COHb levels by heating fuel type

An ANOVA test linked to a Tukey test confirmed that there was no statistical significance between the winter COHb results and the different heating fuel types (p=0.636).

Table 4.23 and Figure 4.20 show the summer levels of COHb measured in each of the household by fuel type.

Fuel type (Heating)	Number of persons	persons with summer results	COHb (%)	Range (%)	Standard deviation
Gas	58	29	0.22	0-4	0.75
Coal	11	4	0	0-0	0
Electricity	5	3	0	0-0	0
LPG	21	0	-	-	-
Other	4	2	0.2	0-0.4	0

Table 4.23 Summer Carboxyhaemoglobin levels (%) of household residents by fuel type

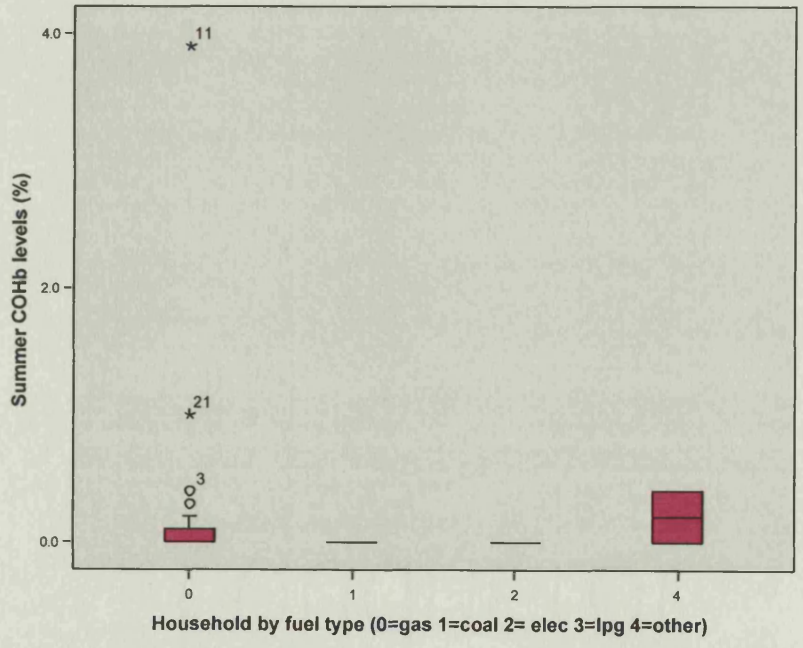


Figure 4.20 Summer COHb levels by heating fuel type

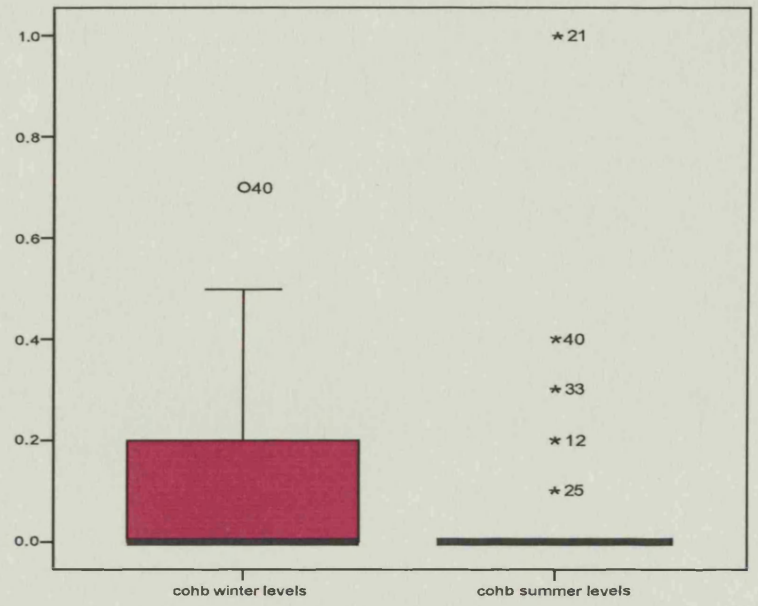


Figure 4.21 COHb winter versus COHb Summer measurements

Winter COHb concentrations ranged from 0 – 3.8% (mean = 0.18%) and Summer COHb concentrations ranged between 0 – 3.9% (mean = 0.17%) as shown in Figure 4.21. There was no statistically significant difference between summer and winter COHb measurements (Wilcoxon signed rank p=0.19). The Wilcoxon matched pairs signed-ranks test is a non-parametric test used when the same or matched subjects perform under both experimental conditions. It is applied either to paired ranked data or to paired measured non-normal data, to test the null hypothesis that the paired observations do not differ.

The median for both winter and summer carboxyhaemoglobin levels was zero, but from Figure 4.21 above it can be seen that there appears to be a greater percentage of non zero levels recorded during the winter months. The percentage of results for both seasons that were above 0.1% COHb were 10.3% for winter and 12.1% for summer, (p=0.001) and for results above 0.2% COHb were 10.3% for winter and 3% for summer, (p=0.009) The outliers present in Figure 421 were investigated further to see if there were any possible explanations but none were found.

Scatter plots of mean environmental levels versus COHb levels for all fuel groups showed no relationship but the majority of COHb results were zero.

To further investigate winter COHb levels against winter mean environmental levels, the median of means was calculated across all the fuel groups, resulting in a figure of 0.27ppm ($0.31mg/m^3$). A scatterplot was then drawn using all winter mean environmental data above this figure and plotted against all winter COHb readings. Figure 4.22 shows that there does not appear to be an observed relationship between winter COHb levels and winter mean environmental CO levels at readings above 0.27ppm ($0.31mg/m^3$).

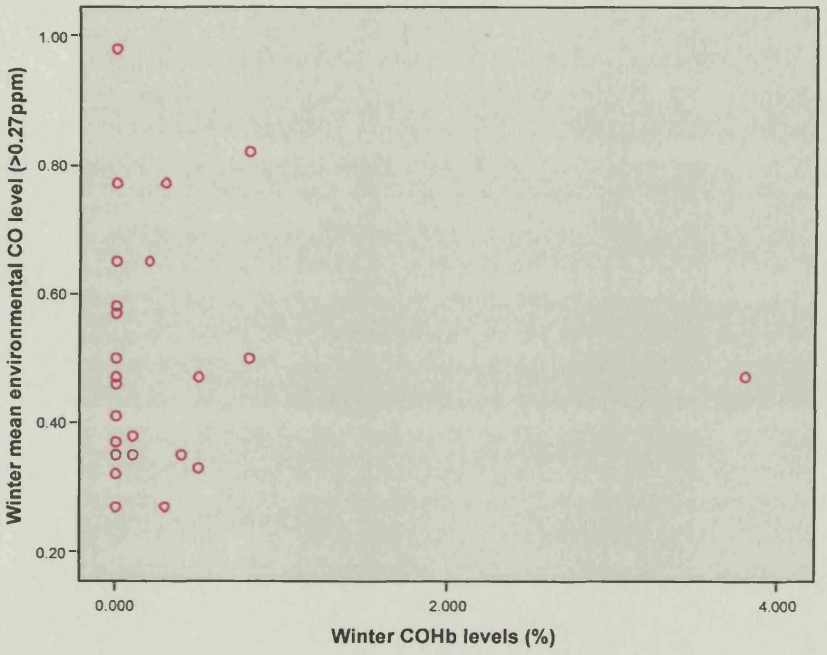


Figure 4.22 Winter mean CO levels above the median of means level for all fuel groups (0.27ppm) against the winter COHb levels

Cyclic Guanosine Monophosphate

Fuel type (Heating)	Number of persons	Persons with winter results	Mean (fmol/mg protein))	Median (fmol/mg protein)	Range (fmol/mg protein)	Standard deviation
Gas	58	48	265.9	220.0	109.2-646.3	131.9
Coal	11	11	321.9	255.3	131.0-704.2	176.5
Electricity	5	4	147.9	149.5	118.8-174.0	26.5
LPG	21	17	544.0	408.3	228-2375.6	506.3
Other	4	4	176.1	168.8	137.2-229.5	50.5

Table 4.24 Winter cGMP levels (fmol/mg protein) of household residents by fuel type

Winter cGMP concentrations ranged from 109.2 – 2375.6 fmol/mg protein (grand mean = 315.5 fmol/mg protein).

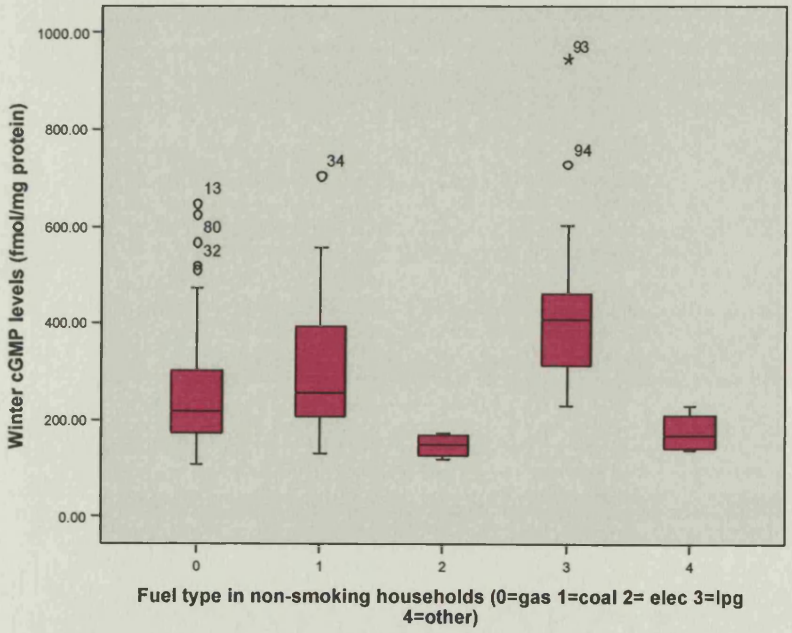


Figure 4.23 Winter cGMP levels in the different households by fuel group (non-smoking households)

The next step was to investigate which of the fuel groups were significant so an ANOVA test linked to a Tukey test was carried out.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1190686.071	4	297671.518	4.680	.002
Within Groups	5342496.408	84	63601.148		
Total	6533182.479	88			

Table 4.25 Results of the ANOVA test for the differences between winter cGMP levels in the 5 fuel groups in non-smoking households

Multiple Comparisons

Dependent Variable: cGMP Winter levels
Tukey HSD

(I) 0=gas 1=coal 2=elec 3=lpg 4=other	(J) 0=gas 1=coal 2=elec 3=lpg 4=other	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interva	
					Lower Bound	Upper Bound
0	1	-55.16699	83.42495	.964	-287.7488	177.414
	2	118.85574	130.68315	.893	-245.4780	483.189
	3	-277.28132(*)	70.13589	.001	-472.8143	-81.7483
	4	95.27241	149.59339	.969	-321.7815	512.326
1	0	55.16699	83.42495	.964	-177.4148	287.748
	2	174.02273	147.24880	.762	-236.4947	584.540
	3	-222.11433	97.58671	.163	-494.1780	49.9493
	4	150.43939	164.26291	.890	-307.5119	608.390
2	0	-118.85574	130.68315	.893	-483.1895	245.478
	1	-174.02273	147.24880	.762	-584.5401	236.494
	3	-396.13706(*)	140.14825	.045	-786.8587	-5.4154
	4	-23.58333	192.61534	1.000	-560.5789	513.412
3	0	277.28132(*)	70.13589	.001	81.7483	472.814
	1	222.11433	97.58671	.163	-49.9493	494.178
	2	396.13706(*)	140.14825	.045	5.4154	786.858
	4	372.55373	157.92918	.137	-67.7397	812.847
4	0	-95.27241	149.59339	.969	-512.3263	321.781
	1	-150.43939	164.26291	.890	-608.3907	307.511
	2	23.58333	192.61534	1.000	-513.4122	560.578
	3	-372.55373	157.92918	.137	-812.8471	67.7397

* The mean difference is significant at the .05 level.

Table 4.26 Results of the Multiple Comparisons Tukey HSD test for the differences between winter cGMP levels in the 5 fuel groups in non-smoking households

The results of the Tukey test show that median winter levels of cGMP was statistically significantly different between fuel groups' 0-Gas, 2-Electricity and 3-LPG.

Following on from these results, a scatterplot was drawn to look at the relationship between winter cGMP levels and environmental levels for the group using LPG but no correlation was observed (Spearman Ranks r=0.292).

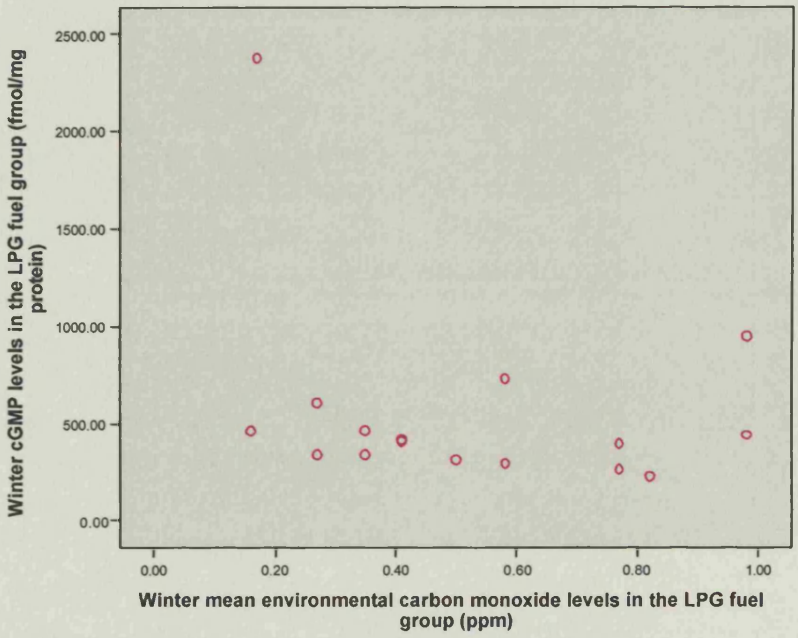


Figure 4.24 Scatterplot of winter mean environmental CO levels against winter cGMP levels in LPG households

Fuel type (Heating)	Number of persons	Persons with summer results	Mean (fmol/mg protein))	Median (fmol/mg protein))	Range (fmol/mg protein))	Standard deviation
Gas	58	47	224.2	201.5	124.28-595.52	100.5
Coal	11	10	325.4	261.5	57.83-756.7	232.7
Electricity	5	3	343.9	458.6	109.41-463.6	203
LPG	21	12	284.2	293.8	68.91-550.48	131.9
Other	4	3	194.6	209.3	150.96-223.42	9.9

Table 4.27 Summer cGMP levels (fmol/mg protein) of household residents by fuel type

Summer cGMP concentrations ranged between 57.8 – 756.7 fmol/mg protein (grand mean = 250.91 fmol/mg protein).

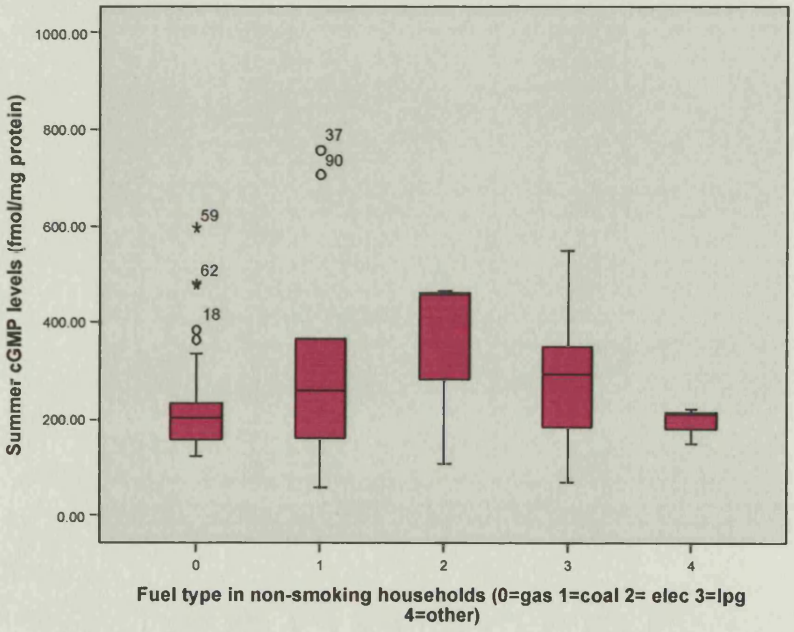


Figure 4.25 Summer cGMP levels in the different households by fuel group (non-smoking households)

An ANOVA Test showed that differences between median summer cGMP levels were not statistically significantly different between the different fuel groups.

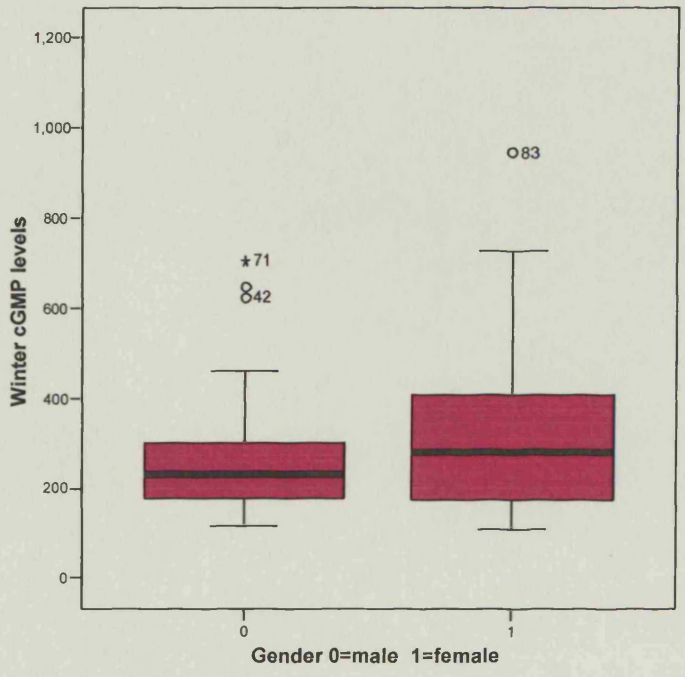


Figure 4.26 The Distribution of male and female cGMP Measurements taken in the winter

During the winter the median cGMP level for the male participants (52) was 230.8, with a median level of for the female participants (47) of 282, a difference of 22% as shown in Fig 4.26.

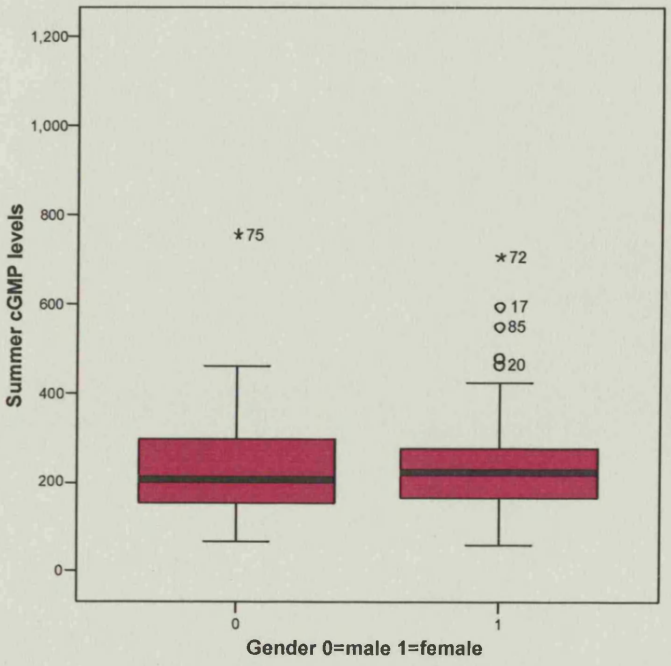


Figure 4.27 The Distribution of the male and female cGMP Measurements taken in the summer

For the summer months the cGMP levels for the male participants were 207.8, with a median level of for the female participants of 223.4, a difference of 7.5% as shown in Figure 4.27.

However, there were no statistically significant differences in cGMP levels measured in women compared to men in the summer (Mann Whitney p=0.84) or in the winter (Mann Whitney p=0.47).

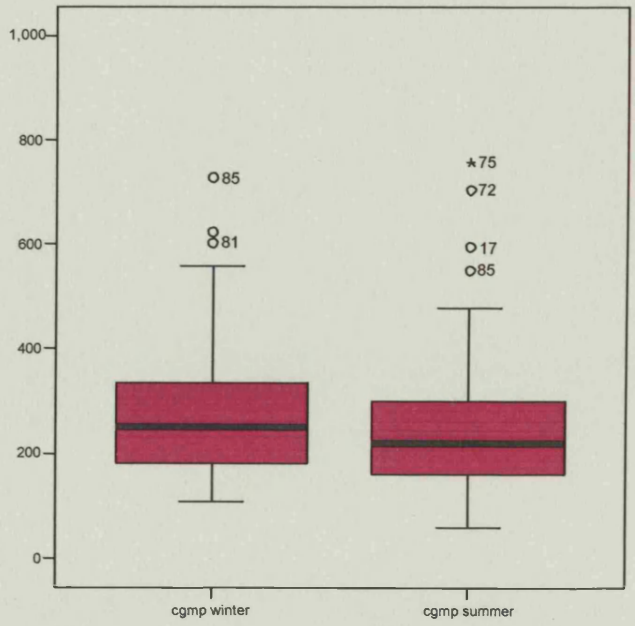


Figure 4.28 The Distribution of the cGMP measurements for the winter and the summer

The median for the winter cGMP levels was 250 and for the summer levels were 210.4, a difference of 19% as shown in Figure 4.28.

The median value for winter cGMP levels in the Gas fuel group was 220, with a median value for the summer cGMP levels of 202; the difference translates to an effect size of 9%

The median value for winter cGMP for the LPG fuel group was 408, with a median value for the summer cGMP levels of 294; the difference translates to an effect size of 39%.

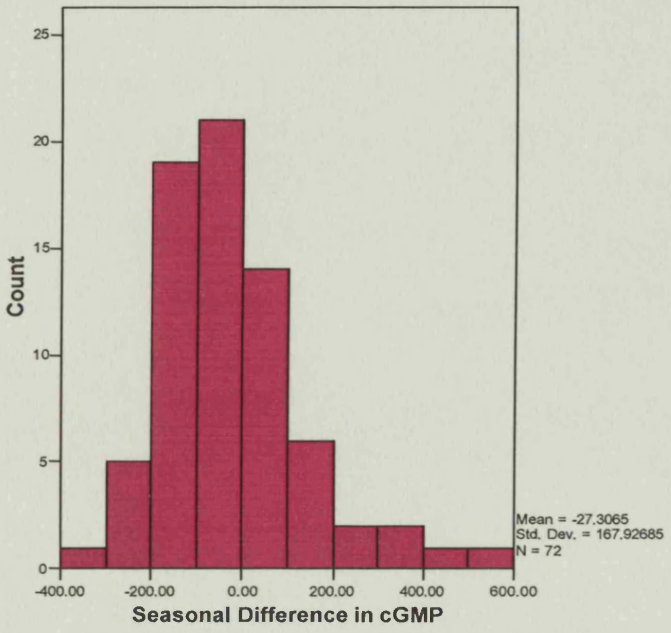


Figure 4.29 The Histogram for the difference in cGMP Measurements between the winter and the summer

The Seasonal Difference in cGMP was analysed using the Wilcoxon Signed Ranks Test. The results of this analysis are as follows: -

cGMP summer - cGMP winter			
	N	Mean Rank	Sum of Ranks
Negative Ranks	46	36.34782609	1672
Positive Ranks	26	36.76923077	956
Ties	0		
Total	72		

Test Statistics	
	cGMP summer - cGMP winter
Z	-2.008986267
Asymp. Sig. (2-tailed)	0.04453859

Table 4.28 The Seasonal Difference in cGMP Summer and Winter cGMP levels

For the 72 subjects who submitted both summer and winter blood samples, the winter cGMP levels were significantly higher (Wilcoxon signed rank p=0.04).

This was further investigated by looking only at the gas and LPG fuel groups using the Wilcoxon Signed Ranks Test. The results are as follows: -

For the 45 subjects who submitted both summer and winter blood samples from the Gas fuel group, there was no significant difference in the summer and winter cGMP levels (Wilcoxon signed rank p=0.103).

		N	Mean Rank	Sum of Ranks
cGMP Summer – cGMP Winter	Negative Ranks	28(a)	23.64	662.00
	Positive Ranks	17(b)	21.94	373.00
	Ties	0(c)		
	Total	45		

	cGMP summer – cGMP winter
Z	-1.631(a)
Asymp. Sig. (2-tailed)	.103

Table 4.29 The seasonal difference in cGMP Summer and Winter cGMP levels in the Gas fuel group

For the 12 subjects who submitted both winter and summer blood samples in the LPG fuel group, the winter levels were significantly

higher (Wilcoxon signed rank p=0.003). Whilst there was no correlation between the winter cGMP levels and the number of hours the heating was on, there was a weak correlation between winter cGMP levels and the time the participant spent in the living room for subjects in all the fuel groups. [r=0.261, p=0.013] The correlation for the LPG group appeared to be stronger but did not achieve statistical significance [r=0.386, p=0.126]

A scatterplot was drawn to see if a relationship could be observed between the COHb and cGMP levels taken during the winter months, the correlation for all fuel groups did not show statistical significance [r=0.187], the test was repeated for the gas group only and the LPG group only, again no correlation was found [r=0.535], [r=0.340].

4.3.2 Biological Monitoring associated with the outdoor environment

	Number of Samples	Mean level umol/mol	Range umol/mol	Standard deviation
s-PMA	52	1.75	0.2-7.7	1.19
s-PMA (control group)	12	1.32	0.3-3.4	0.76
s-PMA (exposed group)	40	1.89	0.2-7.7	1.28

Table 4.30 s-PMA results-All areas

A total of 52 samples were collected for s-PMA, with a mean pma/creatinine ratio of 1.75 umol/mol (range 0.2-7.7 umol/mol).

In the exposed group there were 40 samples with a mean of 1.89 umol/mol (0.2-7.7 umol/mol), and in the control group there were 12 samples, with a mean of 1.32pma/creatinine ratio umol/mol (0.3-3.4 s-PMA/creatinine ratio umol/mol).

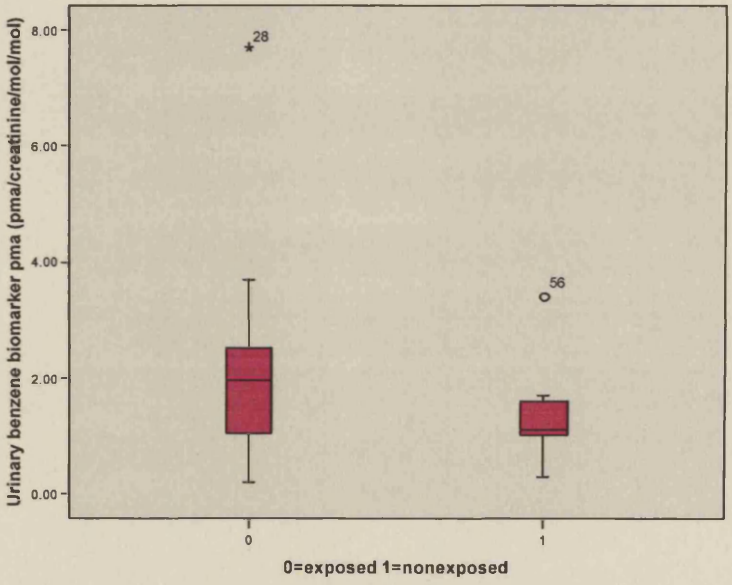


Figure 4.30 Boxplot illustrating the urinary s-PMA in the exposed and control groups

Figure 4.30 shows s-PMA levels in the exposed and control groups. The median level of s-PMA in the exposed group was 1.95, with a median level of 1.1 in the control group; the difference translates to an effect size of 77%.

The results of a Mann Whitney test were that that there is not a statistical difference between s-PMA levels in the exposed and control groups (p=0.91).

To investigate whether there was a relationship between the environmental Benzene levels sampled and the urinary s-PMA levels obtained from the participants of the study, a scatterplot was drawn including all the results from both microenvironments, the results are shown in figure 4.31: -

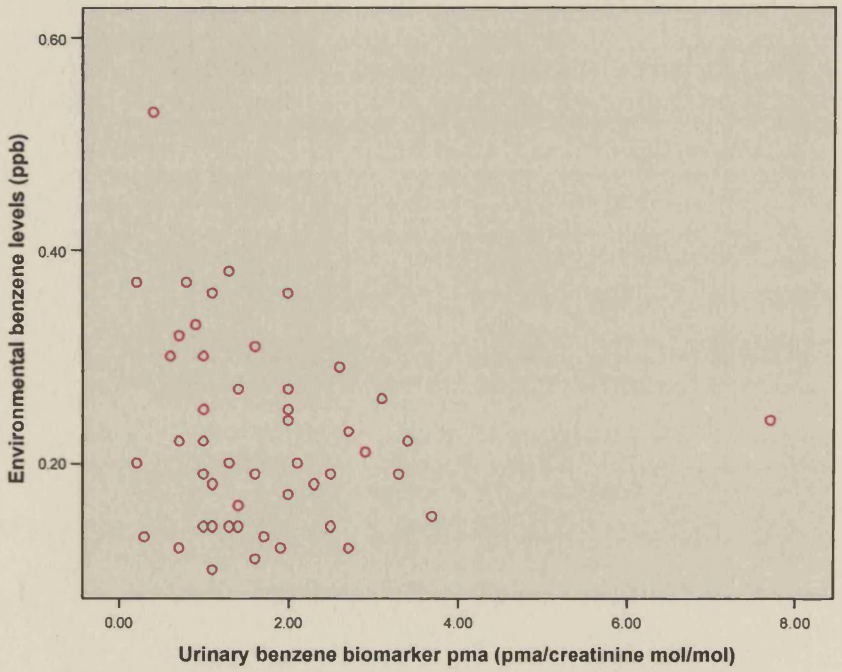


Figure 4.31 Scatter plot of environmental Benzene against urinary sPMA (all samples)

The results of the scatter plot shown in figure 4.31 show that there is no correlation between the atmospheric Benzene levels and the s-PMA levels, a Pearson correlation confirmed this (p=0.270, r=-0.161).

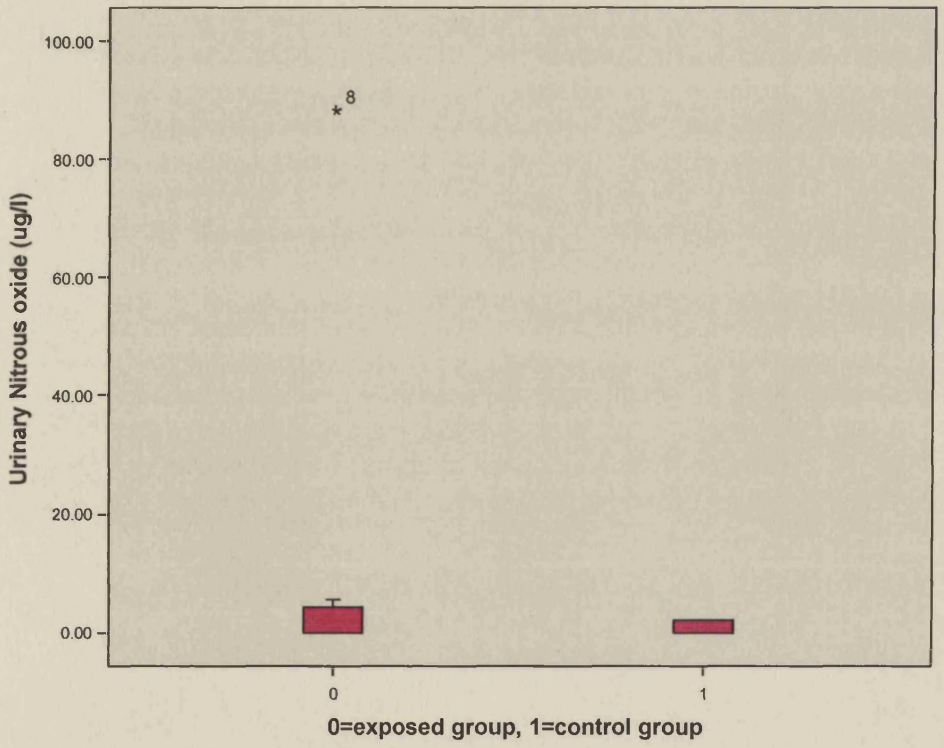
		Benzene	s-PMA
Benzene	Pearson Correlation	1	-.161
	Sig. (2-tailed)		.270
	N	66	49
s-PMA	Pearson Correlation	-.161	1
	Sig. (2-tailed)	.270	
	N	49	52

Table 4.31 Results of the Pearson correlation for Benzene and s-PMA – all areas

	Number of Samples	Mean level ug/l	Range ug/l	Standard deviation
Urinary Nitrous oxide	17	183.8	0-1863.4	515.1
Urinary Nitrous oxide (control group)	5	232.5	0-1160	518.7
Urinary Nitrous oxide (exposed group)	12	163.5	0-1863.4	535.9

Table 4.32 Urinary Nitrous oxide results-All areas

A total of 17 urinary samples were collected for Nitrous oxide, with a mean of 183.8ug/l (0-1863.4ug/l). In the exposed group there were 12 samples with a mean of 163.49 (0-1863.4ug/l) and in the control group there were 5 samples, with a mean of 232.54 (0-1160ug/l).



(Note The Urinary Nitrous oxide scale has been reduced to illustrate the differences between the exposed and non-exposed groups at the lower concentrations)

Figure 4.32 Urinary Nitrous oxide levels in the exposed and control groups

Figure 4.32 shows that there is very little difference between the two microenvironments for the urinary Nitrous oxide measurements. Nitrous oxide urine outliers are 8 which is in Ty Glas Road and, 25 which is in Llandaff road both of which are in the exposed areas, and then 29 which is in Deepfield close, which is in a control area.

4.4 Comparison between Pollutants

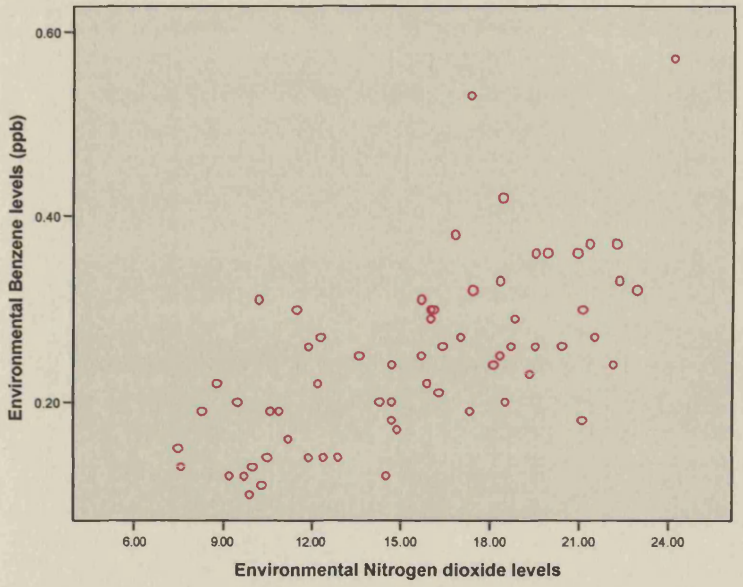


Figure 4.33 Scatterplot of Environmental Benzene against Environmental Nitrogen dioxide for all samples

The scatterplot (figure 4.33) illustrates that there appears to be a correlation between the environmental Benzene and Nitrogen dioxide levels. A Pearson test confirmed that the correlation ($r=0.65$) was statistically significant. ($p<0.001$)

A further scatterplot was then drawn reducing the data to only the exposed areas, figure 4.34: -

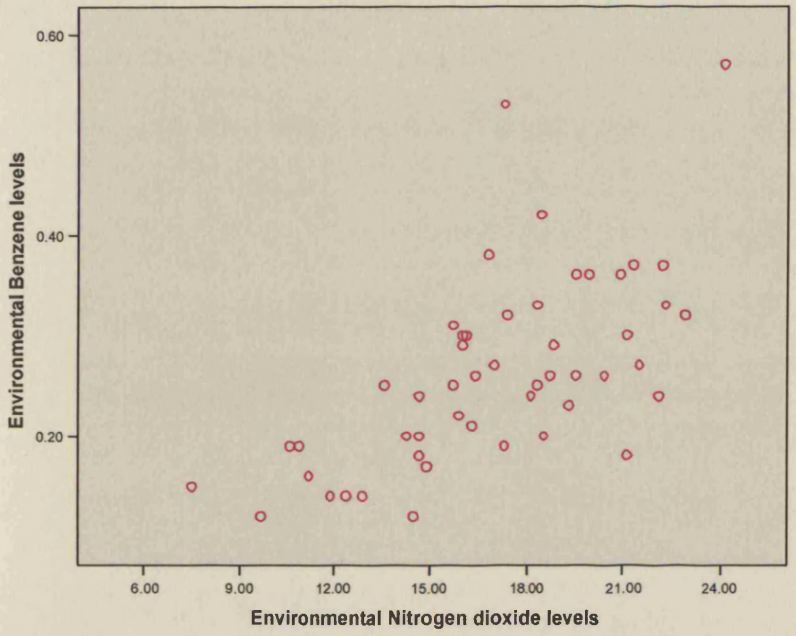


Figure 4.34 Environmental Benzene levels against Environmental Nitrogen dioxide levels for exposed areas

There was a statistically significant correlation ($p<0.001$) between environmental Benzene and environmental Nitrogen dioxide levels in the exposed group ($r=0.65$).

A further scatterplot was then drawn reducing the data to only the control areas, figure 4.35: -

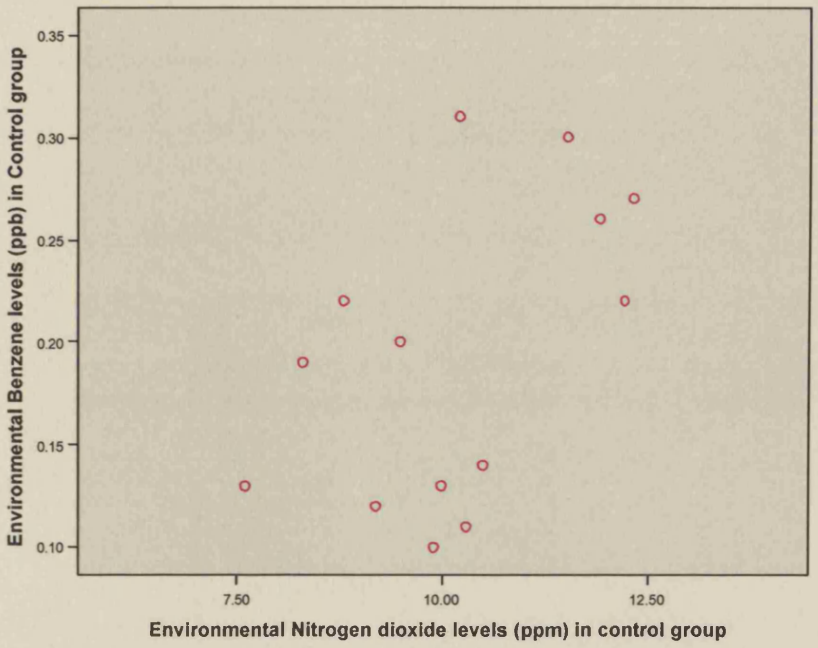


Figure 4.35 Environmental Benzene levels against Environmental Nitrogen dioxide levels for control areas

There was not a statistically significant correlation between environmental Benzene and environmental Nitrogen dioxide levels in the control group (p=0.067, r=0.503).

		Benzene	Nitrogen dioxide
Benzene	Pearson Correlation	1	.503
	Sig. (2-tailed)		.067
	N	14	14
Nitrogen dioxide	Pearson Correlation	.503	1
	Sig. (2-tailed)	.067	
	N	14	14

Table 4.33 Results of the Pearson correlation for Benzene and Nitrogen dioxide – all areas

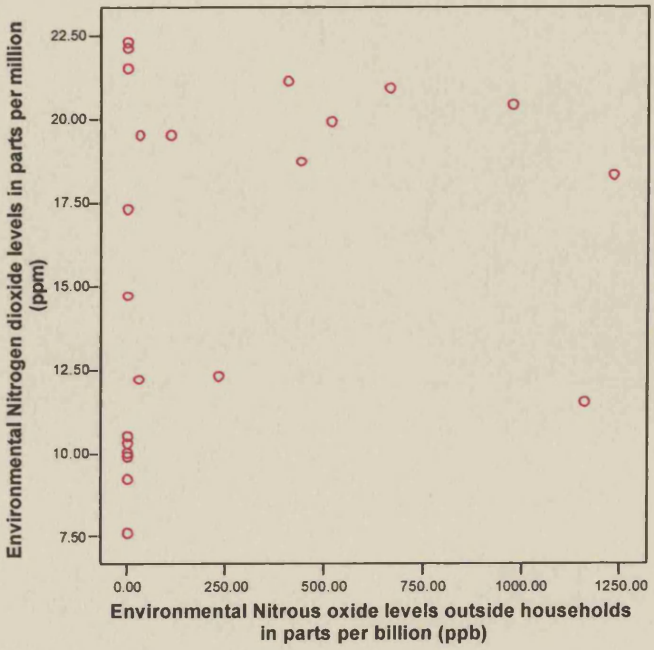


Figure 4.36 Scatter plot illustrating the distribution of environmental Nitrous oxide levels against environmental Nitrogen dioxide outside households (all results)

The scatterplot shown in figure 4.36 shows that there doesn't appear to be a correlation between the environmental Nitrogen dioxide and environmental Nitrous oxide levels measured. A Pearson correlation confirmed that there was no correlation (p=0.267, r=0.247).

Environmental		Nitrogen dioxide	Nitrous oxide (outside)
Nitrogen dioxide	Pearson Correlation	1	.247
	Sig. (2-tailed)		.267
	N	68	22
Nitrous oxide (outside)	Pearson Correlation	.247	1
	Sig. (2-tailed)	.267	
	N	22	22

Table 4.34 Results of the Pearson correlation for Nitrogen dioxide and Nitrous oxide (outside) – all areas

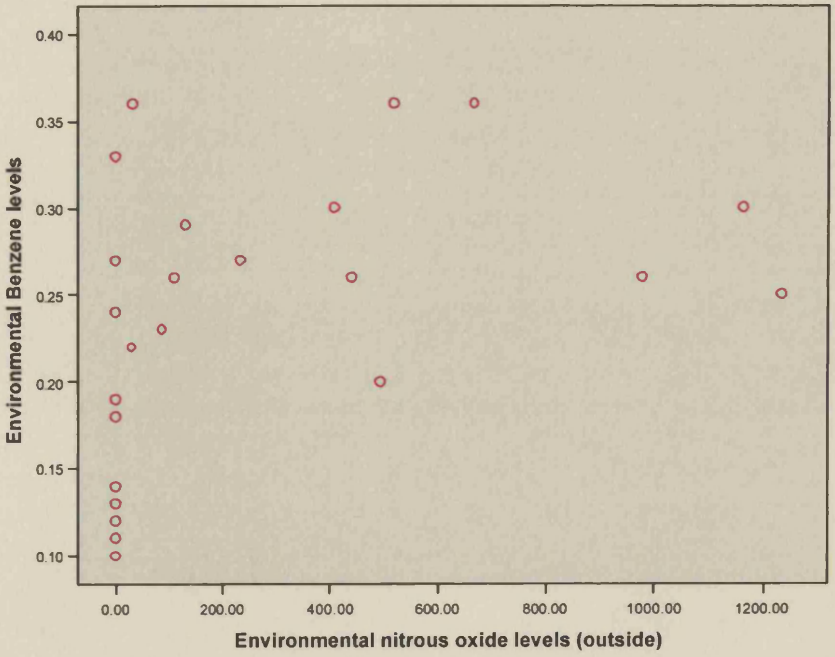


Figure 4.37 Scatterplot illustrating Environmental Benzene levels against Environmental Nitrous oxide levels in all samples

The scatterplot (figure 4.37) illustrates that there does not appear to be a correlation between Environmental Benzene and Environmental Nitrous oxide although a Pearson correlation test showed that there was a weak correlation (p=0.037, r=0.418).

		Benzene	Nitrous oxide outside
Benzene	Pearson Correlation	1	.418(*)
	Sig. (2-tailed)		.037
	N	66	25
Nitrous oxide outside	Pearson Correlation	.418(*)	1
	Sig. (2-tailed)	.037	
	N	25	25

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

Table 4.35 Results of the Pearson correlation for Benzene and Nitrous oxide (outside) – all areas

The results demonstrate that there is a correlation between Benzene and Nitrogen dioxide levels for all areas. There appears to be no identified correlation between Nitrous oxide and the other two environmental variables. The results also indicated that there appeared to be no identified correlation between the environmental concentration and their associated biomarkers of exposure.

CHAPTER 5 DISCUSSION AND LIMITATIONS OF THE RESEARCH

An objective of the thesis was to investigate the indoor concentration of carbon monoxide in homes using different types of fuel for heating and to determine if the differential in exposure led to differences in the concentrations of cGMP in blood platelets. The households were situated in both rural and urban areas and within urban areas at locations with differing traffic flows. However the ambient indoor levels were low regardless of geographical area. The mean environmental concentrations over a seven day period in non-smoking homes were less than 1 ppm (1.15mg/m^3).

There was a wide variation in the individual measurements of cGMP. In the winter the lowest median value of cGMP was observed in the group using Electricity as fuel and the highest median was observed for the group using LPG. The results show that during the winter the group using LPG have much higher levels of cGMP than other groups and the difference between medians (86%) was statistically significant for the Gas and LPG groups. For summer measurements no statistically significant differences in median cGMP were observed between any of the groups and the difference between medians for the Gas and LPG groups was 46%.

Further, for the group using LPG, cGMP levels were statistically significantly higher in the winter than in the summer with a difference between means in paired measurements of 91%. These findings are consistent with an effect of heating type on cGMP in this group. The environmental measurements were made over a period of 1 week and should be reasonably representative of the chronic exposures which actually determine cGMP measurements.

The significance of smoking in the home was also investigated and in homes containing a smoker the mean concentrations measured over a period of two days ranged from 0.2 – 21 ppm (0.23-24mg/m^3). The risk posed by environmental tobacco smoke is accepted and reflected in legislation on smoking in Public places. However, this may result in more smoking in the home and importantly the potential exposure of children. We recorded concentrations in one smoking household which exceeded the WHO one-hour short-term guidelines value. This highlights the potential for children to be exposed to undesirable environmental concentrations in households with a smoker.

Population exposure

This data and previous studies indicate that population exposure to CO is relatively low. However, even a large study (Raw GJ et al., 2004) surveyed only 830 homes and therefore the possibility remains

that undesirable indoor concentrations could occur at a prevalence of as high as approximately 1 in 1000 and previous studies have not been large enough to detect this. Analogously a proportion of the 250,000 domestic Gas appliances condemned annually may have had high levels of CO and the size of this proportion is unknown. Croxford et al (2005) as part of the UK Government Fuel Poverty programme, monitored 56 homes for Carbon monoxide, the occupants of the homes were either on income support, over 60 years old or a single parent family. Out of the 56 homes monitored, 13 (23%) had Carbon monoxide levels above the WHO 8 hour guidelines for outdoor ambient air 8.6ppm (9.85mg/m^3), of these 6 exceeded the WHO 1 hour level of 25ppm (29mg/m^3) and 3 exceeded 30 minute guidelines of 50ppm (57mg/m^3). As a result of the findings a CO gas safety expert was employed to investigate 10 of the 13 homes where levels had exceeded guidelines. The report showed high levels were due to old, poorly installed and poorly maintained gas fires and gas cookers.

Limitations of the research

Sample selection

There was little variation in the observed environmental concentrations of carbon monoxide in the homes heated by different fuel types. Very few homes using only electricity were recruited and therefore very useful information on homes with no carbon monoxide

generated indoors was lacking. Future studies should ensure that enough people are recruited from this group. Subsequent to the sample selection a publication (Croxford, 2005) highlighted that homes and individuals suffering from fuel poverty had a higher prevalence of higher indoor carbon monoxide concentrations than other homes. Therefore such homes should be included in future studies to extend the range of indoor carbon monoxide concentrations to be studied.

Confounding

The median concentrations of carbon monoxide in the homes heated by LPG were almost twice those in homes heated by gas. However the concentrations in all homes were very low and therefore the absolute difference in environmental carbon monoxide between homes heated by gas and LPG would appear to be insufficient to explain the marked observed associated difference for cGMP in blood platelets. Therefore a confounding variable would seem to be responsible. Acute cold can effect measured concentrations of cGMP but the magnitude of the difference observed in cGMP between seasons for the LPG group would not seem to be explicable by the seasonal difference in temperature. Further to this, although indoor temperature was not measured, it is very unlikely that chronic indoor temperature difference is the explanation for the observed difference in cGMP in the winter between groups heating their homes

by either gas or LPG. The most likely confounder (because of its known effect on cGMP) is Nitric oxide emitted by gas appliances. Environmental monitoring of Nitric oxide is expensive but this data indicates that it is necessary to incorporate environmental monitoring of nitric oxide into future research. The role of Nitric oxide indoors has been overlooked in previous research on the assumption that it is immediately converted to nitrogen dioxide. This is only partly true for the outdoor environment and possibly not true for exposure of individuals indoors in proximity to a constantly emitting source i.e. the heating source.

Timing of blood collection

Ideally one would wish to collect blood samples from an individual within the home to reflect the blood concentration of cGMP in equilibrium with the indoor environmental concentration of cGMP. However there are practical difficulties involved which relate to the researcher's being allowed and able to take blood samples. There is little data on the temporal relationship between Carbon monoxide exposure and the concentration of cGMP in blood platelets. However it is unlikely that the delay (typically 0.5-1 hour) between leaving the home and donating a blood sample at a local hospital would have had an appreciable effect on the concentration of cGMP in blood platelets before leaving the home. A similar argument applies for COHb. The relationship of COHb decrease with time on

removal from a source of exposure is known but in practice the subject were exposed to concentrations in ambient air when travelling between home and the hospital and ambient levels are approximately the same as measured levels.

Representativeness of sample measurements

It was not the intention of this study to obtain a representative measurement of environment carbon monoxide concentration for different fuel types and no claim is made that the reported measurements are representative. The intention was to sample residences using particular categories of heating which it was believed a Priori Might have different levels of indoor carbon monoxide and then to investigate if such differences gave rise to differences in cGMP.

Carboxyhaemoglobin

The majority of the COHb results obtained for participants were zero. This may have been a consequence of low exposure or the clearance of CO from their blood in the time between leaving their residence and donating a blood sample at the hospital. Whilst the majority of results for both winter and summer were zero nevertheless more than 10% of measurements for winter and summer were in excess of 0.1% COHb and 10% of winter measurements and 3% of summer measurements were in excess of

0.2% COHb. Although this (may) indicate that there are a greater proportion of individuals with higher levels of COHb in the winter than the summer the absolute values of COHb are not significant for health. COHb from endogenous CO formation is reported to amount to 0.7% on average. Individual winter and summer measurements of COHb ranged between 0 and 3.9% but no relationship was observed between environmental levels and levels of COHb. There was no statistically significant difference between levels of COHb in the different fuel groups.

Another objective of the thesis was to investigate outdoor concentrations of benzene in urban and more rural environments and to determine if the differential in exposure led to differences in sPMA in the urine of exposed subjects. Nitrogen dioxide was also determined in the two different microenvironments.

The level of air pollution within the city of Cardiff is low in comparison to major urban areas within the UK (e.g. Birmingham or London) and traffic flow even in highly trafficked areas are similarly less. Despite this, differences in mean levels of Benzene of 50% ($p<0.001$) and in mean levels of NO_2 of 64% ($p <0.001$) were found between the heavily trafficked and miniminally trafficked microenvironments. There was a relatively strong correlation ($r=0.65$) between Benzene

and NO_2 in the trafficked microenvironment but the pollutants were not correlated (r=0.067) in the non trafficked areas.

The lower range of values recorded for Nitrogen dioxide in both the exposed and the control areas was the same, suggesting that there is a background level of approximately 7.5ppb in the Cardiff area. Similarly this was also the case with Benzene, again suggesting that 0.1ppb is the background ambient level for the city of Cardiff.

Sampling was carried in two main batches, one during the late winter, early spring and one during the late summer, early autumn. It was interesting therefore to observe a statistically significant difference between the two sampling periods with significantly higher levels for Benzene, Nitrogen dioxide and Nitrous oxide being recorded during the late winter, early spring. This may be explained by the fact that during winter, extended periods of cold, calm weather conditions can predominate allowing what are known as 'temperature inversions' to form. Temperature inversions consist of a layer of cold air at ground level under a layer of warm air. They occur mainly at night because of a rapid cooling of the earth. Air pollutants get trapped in the lower cold air layer, and the pollution concentrations build up.

Background s-PMA/creatinine ratios for non-occupationally exposed individuals who are non-smokers have been reported to vary from 0-1.8umol/mol. The levels measured in the less urban group were consistent with there reported values but the levels in the urban group were higher. The median level of s-PMA in the urban group (1.95 µmol/mol) was 77% higher than in the less urban group (1.1 µmol/mol) but the difference was not statistically significant due to the small sample size of control group (12) and exposed group (40). s-PMA may not be a sufficiently sensitive biomarker to delineate groups whose mean environmental exposure levels differ by 50% and where mean exposure is relatively low i.e. 3ppb (9.5mg/m^3). The Benzene results from the study show higher mean environmental and urinary levels than a previous pilot study carried out in the city of Swansea (Matthews, 2001)

The results show that there was twice as much Nitrous oxide in urban areas than the less urban areas. Outdoor levels of Nitrous oxide were 20 times higher than indoor levels of Nitrous oxide and there was a significantly statistical difference between indoor and outdoor Nitrous oxide measurements in the urban group ($p=0.008$) but this relationship was not observed in the less urban group ($p=0.109$). A difference between indoor measurements for the two groups may have been expected as there is a hypothesis that outdoor levels influence indoor levels, therefore it may have been

expected that in exposed areas, Nitrous oxide may have diffused into the indoor environment, increasing the levels present. This relationship may not have been detected due to the low numbers of houses monitored. Because of the small number of results collected for Nitrous oxide it is not possible to draw anything other than preliminary observations.

The results of the environmental sampling carried out in the urban and less urban areas within the city of Cardiff, show that there is compliance with the UK air quality strategy, and WHO guidelines that are in place for Benzene and Nitrogen dioxide.

The 2007 Air Quality Strategy reported that objectives for Benzene are being met, but highlight that with current projections, the UK will fail to meet its objectives for Nitrogen dioxide, whilst these are expected to be small exceedences and in highly populated areas therefore significant numbers of people are likely to be affected. The urban concentrations of NO_2 have not been declining as fast as those of NO. The most likely explanation of this is a change in the percentage of road traffic emissions directly emitted as NO_2, this can be linked to the increasing numbers of light duty diesel vehicles especially cars having been fitted with oxidation catalysts and heavy duty vehicles being fitted with catalytically regenerative particle traps.

Since 1997 every local authority in the UK has been required to carry out a review and assessment of air quality in their area. Where the national air quality objectives are unlikely to be achieved the local authority must declare an air quality management area, and put in place a local air quality action plan. Two areas within the study area have been declared an air quality management area; The Philog in northwest Cardiff and Cardiff west. The pollutant declared for both areas is Nitrogen dioxide.

Environmental awareness amongst the general population is currently at an increased level due to increased media coverage and the governments of the developed world striving to limit the effects of climate change. As a result of this, in the UK there is an increasing awareness within the general population of the desirability of reducing their 'Carbon footprint'. One of the key objectives is to reduce the number of car journeys, particularly single occupancy journeys and encourage walking and cycling and the greater use of public transport. Many UK cities are currently considering the possible implementation of congestion charges to limit the volume of traffic entering the city centre and therefore reducing the amount of pollution generated. Whilst this may have a minimal effect in the short term, the changing attitudes towards transport ideally will have a positive effect on reducing pollution levels within city centres.

The UK Government published 'The Future of Transport White Paper' in 2004 stating that vehicles must continue to get cleaner, quieter and less harmful to the environment. In the last decade, emissions from all road transport have fallen by approximately 50% despite an increase in traffic, this is due to tighter vehicle emission and fuel standards agreed at European level and reflected in UK legislation, levels are expected to fall a further 25% over the next decade.

Limitations of the study

Sample selection

The urban and less urban streets were selected on the basis of more or less trafficked microenvironments and in order to explore how different environmental concentrations of benzene and nitrogen dioxide and nitrous oxide might be between the two environments. An inescapable disadvantage of sitting the study in Cardiff is that even the more heavily trafficked residential streets are less trafficked than the same category of street in, for example, London or Birmingham.

Traffic data was obtained for 8 of the 17 streets classified as heavily trafficked. However no traffic data was available for the other streets classified as heavily trafficked or for any of the less heavily trafficked less urban streets. Therefore there may have been some

misclassification of exposure category for certain streets. However we consider this not to have had a major effect, in particular for the less trafficked streets. Further, any misclassification of streets is likely to have led to a diluting effect and to a somewhat underestimate of the true differences between the two different microenvironments.

Representativeness of sample measurements

Subject to the proviso outlined above, the measurements are representative for Cardiff but no claim is made for their general representativeness in terms of other cities.

Biological sampling

Only one measurement of sPMA was made in urine for each individual. Thus a measurement on a single day may not truly represent the chronic levels of the biomarker over more prolonged periods of time.

CHAPTER 6 CONCLUSIONS AND FURTHER WORK

Environmental concentrations of carbon monoxide indoors were measured in real-time over a period of one week and integrated measurements of benzene and nitrogen dioxide concentrations outdoors were obtained over a period of one month. cGMP was measured in the blood platelets of subjects and sPMA in their urine. The first objective of the thesis aimed to measure chronic residential exposure of the elderly to Carbon monoxide in homes using different fuels and to examine if chronic exposure affects the enzyme cyclic GMP in blood platelets. This research investigated whether the elderly may be at particular risk from CO exposure by virtue of the protracted time they spend indoors and the amount of heating used.

The mean environmental concentrations over a seven day period in non-smoking homes were less than 1 ppm (1.15mg/m^3). There was evidence that indoor ambient levels varied by type of fuel used for heating. The households were situated in both rural and urban areas and within urban areas at locations with differing traffic flows. However the ambient indoor levels were low regardless of geographical area. This data indicates that the chronic exposure of the elderly to CO in the home is not excessive provided heating devices are not incorrectly installed or malfunctioning. The majority of the COHb results obtained for participants were zero. The results show raised Carbon monoxide levels in homes utilising Liquid

Petroleum Gas as their fuel type. This may have been a consequence of low exposure or the clearance of CO from their blood in the time between leaving their residence and donating a blood sample at the hospital. There was no statistically significant difference between levels of COHb in the different fuel groups.

Environmental concentrations of carbon monoxide indoors were low but despite this in homes heated by liquid petroleum gas (LPG) the concentration of cGMP in subjects' blood platelets were twice those in subjects using other types of heating. Further, for the LPG group the difference between paired measurements for the winter and summer seasons were 91%. Exposure to emissions from LPG heating substantially affected cGMP concentrations in blood platelets but this is very unlikely to be caused by the low levels of carbon monoxide measured. We hypothesize that Nitric oxide may be responsible for the differences observed in cGMP.

The lack of correlation between cGMP and environmental measurements may relate to the kinetics of cGMP production and persistence or indeed further highlight that the effect on cGMP was in fact as a result of exposure to Nitric oxide rather than carbon monoxide. The environmental measurements were made over a period of 1 week and may not be representative of the chronic exposures which actually determine cGMP measurements.

A differential in ambient concentrations of benzene and nitrogen dioxide was hypothesized for residential areas of differing urbanicity in particular with respect to traffic flows. The second objective of the thesis was to measure outdoor ambient benzene concentrations in residential areas of differing urbanicity, in particular in respect of traffic flow, and to investigate if differential exposure is reflected in a urinary biomarker of exposure. Ambient Nitrogen dioxide which is known to reflect traffic flow was also investigated.

The results of the ambient environmental sampling in the differing residential areas, show that there is compliance with the UK air quality strategy, and WHO guidelines that are in place for Benzene and Nitrogen dioxide for the city of Cardiff. Substantial differences between mean concentrations of benzene (37%) and nitrogen dioxide (65%) were observed between urban and less urban areas but this differential was not reflected by any difference in toxic uptake as measured by sPMA in urine.

The differential in urinary biomarker of benzene exposure, s phenylmercaptuirc acid (sPMA) was investigated in people residing in urban and less urban microenvironments. The levels of s-PMA recorded exceed what would normally be expected from non-occupationally exposed, non-smokers. The median level of s-PMA in the exposed group (1.95 μmol/mol) was 77% higher than in the

control group (1.1 µmol/mol) but the difference was not statistically significant due to the small sample size of control group (12) and exposed group (40). s-PMA may not be a sufficiently sensitive biomarker to delineate groups whose mean environmental exposure levels differ by 50% and where mean exposure is relatively low i.e. 3ppb (9.5mg/m^3).

Further work

Since cGMP is recognised to be an important enzyme in the regulation of vascular tone and it also acts as a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis and it also relaxes smooth muscle tissues. The research findings which appear to show increased cGMP levels by heating type for very small increases in environmental carbon monoxide warrant further investigation. Further investigation should be made into the possible confounding variable, Nitric oxide, which is also emitted by gas appliances (because of its known effect on cGMP). Environmental monitoring of Nitric oxide is expensive but this data indicates that it is necessary to incorporate environmental monitoring of nitric oxide into future research. The role of Nitric oxide indoors has been overlooked in previous research on the assumption that it is immediately converted to nitrogen dioxide. This is only partly true for the outdoor

environment and possibly not true for exposure of individuals indoors in proximity to a constantly emitting source i.e. the heating source.

It maybe inferred from the not inconsiderable acute domestic poisoning incidents that a greater number of sublimal and unrecognised poisoning occurs within the population. Surveys have shown that particular susceptible groups (Croxford) are more at risk. Future work should consider cGMP levels in conjunction with environmental monitoring of Nitric oxide and investigate the effects of interventions at reducing exposure in such groups.

Differences in mean levels of Benzene of 50% ($p<0.001$) and in mean level of NO_2 of 64% ($p<0.001$) were found between trafficked and non-trafficked environments. This highlights that epidemiological studies investigating links between those exposures and health outcomes must take microenvironment exposure into account if exposure misclassification is to be avoided e.g. one or two central urban monitoring sites are not sufficient. Measurements shall also be undertaken throughout the year to identify any seasonal changes. S-PMA and N_2O in urine do not appear sufficiently sensitive to act as biomarkers of environmental exposure. However since determination of personal exposure and uptake is an ultimate goal, there is a continuing need to test novel biomarkers of exposure.

REFERENCES

AEA Technology plc.UK Nitrogen Dioxide Diffusion Tube Network Instruction Manual AEAT – 3675: version 1.5. 2003.

Adams KF, et al. Acute elevation of blood carboxyhaemoglobin to 6% impairs exercise performance and aggravates symptoms in patients with ischemic heart disease. Journal of American College of Cardiology. 1988;12: 900-909.

Allred EN, et al. Short term effects of Carbon monoxide exposure on the exercise performance of subjects with coronary heart disease. New England Journal of Medicine. 1989;321:1426-1432.

Alm S, Mukala K et al. Personal Carbon monoxide exposures of preschool children in Helsinki, inland: levels and determinants. Atmospheric Environment; 2000;34: 277-285.

Amodio-Cocchieri R, Del Prete U et al. Evaluation of Benzene exposure in children living in Campania (Italy) by urinary trans, trans-muconic acid acid assay. Journal of Toxicology and Environmental Health Part A 2001;63(2):79-87.

Antonoine SR, DeLeon IR et al. Environmentally significant volatile organic pollutants in human blood. Bull Environmental Contamination Toxicology.1986;36:364-371.

Astrup P. Some physiological and pathological effects of moderate Carbon monoxide exposure. British Medical Journal. 1972. 4:447-452.

ATSDR Toxicological profile for Benzene. September 1997. US Department of health and Human Services.

Aubard Y. Carbon monoxide poisoning in pregnancy. British Journal of Obstetrics and Gynaecology 2000, 107:833-838.

Bailie RS, Pilotto LS et al. Poor urban environments: Use of paraffin and other fuels as sources of indoor air pollution. Journal of Epidemiology and Community health. 1999;53;9;585-586.

Ballester F, Tenias J.M et al. Air pollution and emergency hospital admissions for cardiovascular diseases in Valencia, Spain. Journal Epidemiology and Community Health. 2001;55:57-65

Barbieri A, Accorsi A et al. Lack of sensitivity of urinary Trans, Trans-Muconic acid in determining low level (ppb) Benzene exposure in children. Archives of Environmental Health 2002;57(3):224-228.

Bechtold WE, Sabourin PJ et al. A reverse isotope dilution method for determining Benzene and metabolites in tissues. Journal of Analytical Toxicology. 1988, 12:176-179.

Beevers S, Carslaw D. Evaluation of Local Transport Measures in Tackling National Air Quality Strategy (NAQS) Objectives. SEIPH Report to the Department of the Environment, Transport and the Regions. 1998.

Benowitz NL. The use of biologic fluid samples in assessing tobacco smokeconsumption. In: Grabowski J, editor. Measurement in the analysis and treatment of smoking behaviour. Rockville:National Institute on Drug Abuse;1983, p6-26.

Berry RW, Brown VM et al. Building Research Establishment report. Indoor Air Quality in Homes: Part 2. The Building Research Establishment Indoor Environment Study. Garston, Watford: Building Research Establishement. 1996.

Bertoni G, Ciuchini C et al. Monitoring of ambient BTX at Monterotondo (Rome) and indoor-outdoor evaluation in school and domestic sites. Journal of Environmental monitoring, 2002;4(6):903-909.

Biersteker KH, De Graaf et al. Indoor air pollution in Rotterdam homes. International Journal of Air and Water Pollution. 1965 9:343-350

Bois FY, Jackson ET et al. Population Toxicokinetics of Benzene. Environmental Health Perspectives. 1996; 104 (supplement 6):1405-1411.

Boogaard PJ, Sittert NJ. Suitability of S-Phenyl mercapturic Acid and trans-trans-Muconic Acid as biomarkers for exposure to Low Concentrations of Benzene. Environmental Health Perspectives. 1996;104 supplement 4; 1151-1157.

Brown RH. Environmental use of diffusive samplers:evaluation of reliable diffusive uptake rates for Benzene, toluene and xylene. Journal of Environmental Monitoring, 1999,1,115-116.

Bruce EN, Bruce MC. A Multicompartment Model of Carboxyhemoglobin and Carboxymyoglobin Responses to Inhalation of Carbon Monoxide. Journal of Applied Physiology, 2003, doi:10.1152/japplphysiol.00217.

Brugnone F, Perbellini L et al. Breath and blood levels of Benzene, toluene, cumene and styrene in non-occupational exposure. International Archives of Occupational Environmental Health, 1989;61:303-311.

Brugnone F, Perbellini L et al. Reference values for blood Benzene in the occupationally unexposed general population. International Archives of Occupational Environmental Health 1992;64:179-184

Brugnone F, Perbellini L et al. Benzene in environmental air and human blood. International Archives of Occupational Environmental Health 1998;71(8):554-559

Buckingham C, Clewley C et al. Atmospheric Emissions Inventory for four Urban Areas: Merseyside, Bristol, Southampton, Portsmouth and Swansea and Port Talbot. London Research Centre/RSK Environment, ISBN 1-85261-269-2. 1997.

Buckingham C, Sadler L et al. Glasgow, Middesborough and West Yorkshire Atmospheric Emissions Inventory. London Research Centre, ISBN 1-85261-285-1. 1998.

C J Dore, J D Watterson et al. UK Emissions of Air Pollutants 1970 to 2004. UK Emissions Inventory Team, AEA Energy &Environment: December 2006.

Campbell G, Stedman JR et al. A survey of Nitrogen dioxide concentrations in the United Kingdom using diffusion tubes. Atmospheric Environment.1994,28,477-486.

Carbon Monoxide. Expert Panel on Air Quality Standards. Department of the Environment. HMSO. ISBN 0 11 753035 2. 1994

Cardiff and Vale NHS Trust. Medical Biochemistry and Immunology Servies. 2005.

Chaitman BR, Dahms TE et al. Carbon Monoxide and cardiac Arrhythmias. Research Report 52. The Health Effects Institute. 2000

Chan A.T., 2002. Indoor–outdoor relationships of particulate matter and Nitrogen oxides under different outdoor meteorological conditions. Atmospheric Environment, 2002;36 (9):1543–1551.

Chief Medical Officer Welsh Assembly Government. 2007. Carbon Monoxide. Crown copyright.

Coburn R.F. Mechanisms of Carbon monoxide toxicity. Prev. Med. 1979; 8:310–322,

Cocheo V, Sacco P et al. Urban Benzene and population exposure. Nature, 2000;404(6774):141-142.

Collins JJ, Ireland BK et al. Evaluation of lymphopenia among workers with low-level Benzene exposure and the utility of routine data collection. Journal of Occupational and Environmental medicine 1997;39(3):232-237.

Corgi. Carbon Monoxide Report. 2007.

Coward SK. Brown VM et al Exposure to air pollutants in English homes. Journal of Exposure Analysis and Environmental Epidemiology. 2004;14 Suppl 1:S85-94.

Crebelli R, Tomei F et al. Exposure to Benzene in urban workers: environmental and biological monitoring of traffic police in Rome. Occupational and Environmental medicine 2001;58(3):165-171.

Croxford B, Fairbrother L. Low cost, accurate monitoring of indoor Carbon monoxide concentrations. Indoor and built environment; 2005;14;3-4:235-240.

Crump DR. Volatile organic compounds in indoor air. Issues Environmental Science Technology.1995;4:109-124.

Dawson SV. Schenker MB. Health effects of inhalation of ambient concentrations of Nitrogen dioxide. American Review of Respiratory Disease.1979;120(2):281-92.

Department for Transport. The Future of Transport - White paper CM 6234. Crown Copyright 2004.

Department of Health. Letter from the Chief medical Officer, London: DOH 1998 (PL/CMO/9815).

Department of the Environment. Benzene. Expert Panel on Air Quality Standards. 1994. ISBN 0 11 752859 5.

Ding X, Liu G et al. Change of Carbon monoxide in plasma and tissue during acute hypoxia. Chinese medical Sciences Journal. 2003;18 (1):50-53.

EPAQS (1994b) Expert Panel on Air Quality Standards, Carbon Monoxide, December 1996, ISBN 0117530352

EPAQS (1996) Expert Panel on Air Quality Standards, Nitrogen dioxide, December 1996, ISBN 017531359

Farrow A. Greenwood R et al. Nitrogen dioxide, the oxides of Nitrogen, and infants' health symptoms. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Archives of Environmental Health.1997; 52(3):189-94.

Ferriman A. BMA demands ban on smoking in enclosed workplaces in the United Kingdom. British Medical Journal 2004;329:70

Flo K, Hansen M et al. Effect of probenecid, verapamil and progesterone on the concentration dependent and temperature sensitive human throcyte uptake and export of guanosine 3', 5' cyclic monophosphate (cGMP). Scandinavian Journal of Clinical and laboratory Investigation 1995;55(8):715-721

Fruin SA, Denis MJS et al. Reductions in human Benzene exposure in the California South Coast Air basin. Atmospheric Environment, 35(6):1069-1077

Fustinoni S, Buratti M et al. Biological and environmental monitoring of exposure to airborne Benzene and other aromatic hydroCarbons in Milan traffic wardens. Toxicology Letters, 1995;77(1-3):387-392.

Gajdos P.H, Conso F et al. Incidence and causes of Carbon Monoxide Intoxication: Results of an Epidemiologic Survey in a French Department. Archives of Environmental Health. 1991;46 (6): 373-376.

Gilli G, Scursatone E et al. Geographical distribution of Benzene in air in north western Italy and personal exposure. Environmental health Perpectives. 1996; 104(6) Supp 6 1137-1140.

Gobba F, Rovesti S et al. Inter-individual variability of Benzene metabolism to trans, trans-muconic acid and its applications in the biological monitoring of occupational exposure. Scienece of the Total Environment 1997;199(1-2):41-48.

Goldstein BD. Melia RJ et al. The relation between respiratory illness in primary schoolchildren and the use of gas for cooking--II. Factors affecting Nitrogen dioxide levels in the home. International Journal of Epidemiology.1979; 8(4):339-45.

Gonalez-Flesca N, Vardoulakis S et al. BTX Concentrations Near a Stage !! Implemented petrol station. Environmental Science and Pollution Research, 2002;9(3):169-174.

Gouveia N, Fletcher T. Time series analysis of air pollution and mortality: effects by cause, age and socio-economic status. Journal of Epidemiology and Community Health; 2000;54: 750-755.

Gruenke LD, Craig JC et al. Quantitative analysis of Benzene by selected ion monitoring/gas chromatography/mass spectrometry. Journal of Analytical Toxicology.1986;10:225-232.

Guerin MR, Jenkins RA et al. The Chemistry of Environmental Tobacco Smoke: Composition and Measurement. Chelsea, MI: Lewis Publishers. 1992.

Hardy KR, Thom SR. Pathophysiology and treatment of Carbon monoxide poisoning. Journal of Toxicology and Clinical Toxicology. 1994;32:613-629.

Hargreaves KJ. The development and application of diffusion tubes for Air Pollution measurement. PhD thesis, University of Nottingham.1989.

Harrison R.M, Thornton C.A et al. Personal exposure monitoring of particulate matter, Nitrogen dioxide, and Carbon monoxide, including susceptible groups. Occupational and Environmental Medicine. 2002;59(10): 671-679.

Harrison RM, Colbeck I et al. Comparative evaluation of indoor and outdoor air quality – chemical considerations. Environmental Technology letters. 1988;9:521-530

Harrison RM, Leung PL et al. Analysis of incidence of childhood cancer in the West Midlands of the United Kingdom in relation to proximity to main roads and petrol stations. Occupational and Environmental medicine, 1999;56(11):774-780.

Harrison RM, Shi JP. Sources of Nitrogen dioxide in winter smog episodes. Science of the Total Environment. 1996; 189/90:391-399.

Harwell Scientifics standard operating procedure HS/GWI/1015 issue 8.

Harwell Scientifics standard operating procedure HS/GWI/3015.

Hasselblad V. Eddy DM et al. Synthesis of environmental evidence: Nitrogen dioxide epidemiology studies. Journal of the Air & Waste Management Association. 1992;42(5):662-71.

Hayes RB, Yin SN et al. Benzene and the dose related incidence of hematologic neoplasms in China. Chinese Academy of preventive Medicine-National Cancer Institute Benzene study group. Journal of the National

Health Protection Agency (HPA). National Poisons Information Service Annual Report 2006/2007. ISBN 978-0-85951-602-0. 2007.

Hernandez-Viadel M, Castoldi AF et al. In vivo exposure to Carbon monoxide causes delayed impairment of activation of soluble guanylate cyclase by nitric oxide in rat brain cortex and cerebellum. Journal of Neurochemistry, 2004;89:1157-1165.

Hotz P, Carbonnelle P et al. Biological monitoring of vehicle mechanics and other workers exposed to low concentrations of Benzene. International Archives of Occupational and Environmental Health, 1997;70(1):29-40.

Hutchinson D, Clewley L. West Midlands Atmospheric Emissions Inventory. London Research Centre, ISBN 1-85261-243-6.1996.

IARC (1987): Monographs Supplement 7, 120-121. International Agency for Research on Cancer (IARC), Lyon, France.

IARC (1990): Monographs on the Evaluation of Carcinogenic risks to Humans. Volume 49. Chromium, Nickel and Welding. International Agency for Research on Cancer (IARC), Lyon, France.

Inoue Y, Nakao M et al. Thermoregulatory responses of young and older men to cold exposure. European journal of Applied Physiology Occupational Physiology. 1992;65, 492-498.

Institute for Environment and Health. IEH assessment on Indoor Air Quality in the Home (2): Carbon Monoxide. 1998. ISBN 1 899110 16X

Institute for Environment and Health. IEH assessment on Indoor Air Quality in the Home (Chapter 2 Nitrogen dioxide) 1996. ISBN 1 899110 05 4

Institute for Environment and Health: Indoor air quality in the home:Final report on DETR contract EPG 1/5/12. 2001

Jarvis D, Chinn S et al. Association of respiratory symptoms and lung function in young adults with use of domestic gas appliances. Lancet. 1996;347:426-431.

Jo W-K, Moon K-C. Housewives' exposure to volatile organic compounds relative to proximity to roadside service stations. Atmospheric Environment 1999;33(18):2921-2928

Jo W-K, Oh J-W. Exposure to methyl tertiary butyl ether and Benzene in close proximity to service stations. Journal of the Air and waste management Association, 2001;51(8):1122-1128.

Kapturczak-Hill N, Agarwal A. Carbon monoxide: from silent killer to potential remedy. American Journal Physiology Renal Physiology. 2006;290:F787-788.

Kim YM, Harrad S et al. Concentrations and sources of VOC's in urban domestic and public microenvironments. Environmental Science and amp;Technology. 2001;25(6):997-1004.

Kirk PWW, Hunter M et al. British indoor air quality:Regional and local variations in the concentrations of three environmental tobacco smoke components. Environmental Technology Letters. 1988;9:437-448

Kuller L.H, Radford E.P. Epidemiological bases for the current ambient Carbon monoxide standards. Environmental health Perspectives; 1983;52: 131-139.

Lagorio S. Crebelli R et al. Methodological issues in biomonitoring of low level exposure to Benzene. Occupational Medicine. 1998;48(8) 497-504.

Lai HK, Kendall M et al. Personal exposures and microenvironment concentrations of $PM_{2.5}$, VOC, NO_2 and CO in Oxford, UK. Atmospheric Environment. 2004;38(37);6399-6410.

Lambert W.E, Colome S.D et al. Application of end-expired breath sampling to estimate carboxyhaemoglobin levels in community air pollution exposure assessments. Atmospheric Environment. 1988; 22(10): 2171-2181.

Laquatra J, Maxwell L.E et al. Indoor Air Pollutants:Limited-Resource Households and Child Care faclilities. Journal of Environmental Health 2005;67(7):39-43

Lee CR, Yoo CI et al. Hematological changes of children exposed to volatile organic compounds containing low levels of Benzene. The Science of the Total Environment, 2002;299:219-228.

Lee HS, Kang BW et al. Relationships between indoor and outdoor air quality during the summer season in Korea. Atmospheric Environment.1997;31:1689-1693.

Leopardi P, Zijno A et al. Analysis of micronuclei in peripheral blood lymphocyes of traffic wardens: effects of exposure, metabolic genotypes, and inhibition of excision repair in vitro by ARA-C. Environmental and Molecular Mutagenesis, 2003;41(2):126-130.

Leppert J, Ringqvist A et al. Cold exposure increases cyclic guanosine monophosphate in healthy women but not in women with Raynaud's phenomenon. Journal of Internal Medicine; 1995;237: 493-498.

Linn W.S, Hackney J.D. Short term human respiratory effects of Nitrogen dioxide: determination of quantitative dose-response profiles, phase II. Exposure of asthmatic volunteers to 4ppm NO_2. Atlanta GA Coordinating Research Council Inc.
1984

Lynge E, Anderson A et al. Risk of cancer and exposure to gasoline vapours. American Journal of Epidemiology, 1997;145(5):449-458.

Maines MD. The heme oxygenase system: a regulator of second messenger gases. Annuals Rev Pharmacology Toxicology. 1997;37:517-554.

Mann J.K, Tager I.B et al. Air pollution and hospital admissions for ischemic heart disease in persons with congestive heart failure or arrhythemia. Environmental Health Perspectives. 2002;110(12) 1247-1252.

Mannino DM, Siegel M et al. Environmental tobacco smoke exposure in the home and worksite and health effects in adults: results from the 1991 National Health Interview Survey. Tobacco Control 1997;6(4):296-305

Marks G, Brien J et al. Does Carbon monoxide have a physiological function? Trend Pharmacological Science. 1991; 12:185-188.

Matthews IP, Fielder H et al. A Pilot study of the feasibility of using urinary phenyl mercapturic acid (PMA) as a biomarker for environmental Benzene exposure. 2001

Melia RJ. Florey Cdu V et al. Childhood respiratory illness and the home environment. II. Association between respiratory illness and Nitrogen dioxide, temperature and relative humidity. International Journal of Epidemiology. 1982;11(2):164-9.

Melia RJW, Florey C et al. Association between gas cooking and respiratory disease in children. British Medical Journal. 1977;2:149-152.

Meredith T, Vane A. Carbon monoxide poisoning. British Medical Journal. 1988;296:77-78.

Minnegasco. In Carbon Monoxide Toxicity. P526. 1997.

Miro O, Casademont J et al. Mitochondrial cytochrome c oxidase inhibition during acute Carbon monoxide poisoning. Pharmacology Toxicology 1998; 82;4;199-202.

Morris RD, Naumova EN et al. Ambient Air Pollution and Hospitalization for Congestive Heart Failure among Elderly People in Severn Large US Cities

Mosqueron L, Momas I et al. Personal exposure of Paris office workers to Nitrogen dioxide and fine particles. Occupational Environmental Medicine. 2002;59: 550-556.

Mott JA, Wolfe MI et al. National Vehicle Emissions Policies and Practices and Declining US Carbon Monoxide–Related Mortality. Journal of the American Medical Association 2002;288:988-995.

Muir B, Hursthouse A et al. Application of diffusion based surveys in the district wide assessment of Benzene and select volatile organic compounds in urban environments-a case study from Renfrewshire, Scotland. Journal of Environmental Monitoring, 2001, 3, 646-653.

Mukherjee P, Viswanathan S. Carbon Monoxide modelling from transportation sources. Chemosphere; 2001;45(6-7) 1071-1083.

Pantazopoulou A, Katsouyanni K et al. Short term effects of air pollution on hospital emergency outpatients visits and admissions in the greater Athens, Greece area. Environmental research 1995;69(1):31-36

Parmet S. Journal of the American Medical Association. 2002;8:1036

Perbellini L. Environmental and occupational exposure to Benzene by analysis of breath and blood. British Journal of Industrial Medicine 1988;45:345-352

Plaisance H, Sagnier I et al. Performances and application of a passive sampling method for the simultaneous determination of Nitrogen dioxide and sulphur dioxide in ambient air. Environmental Monitoring and Assessment. 2002;79: 301-315.

Poloniecki J.D, Atkinson R.W et al. Daily times series for cardiovascular hospital admissions and previous day's air pollution in

London, UK. Occupational and Environmental medicine. 1997;54:535-540.

Prikryl P, Rysanek K et al. Effect of cold stress on catecholamines, cyclic AMP and cyclic GMP in hardened and unhardened men. Activitas Nervosa Superior. 1982;24 (1) 32-33.

Prockop DJ and Kivirikko KI. Relationship of hydroxyproline excretion in urine to collagen metabolism. Annals of International medicine, 1967;66:1243-1266.

Quantification of the effects of air pollution on health in the United Kingdom. Chapter 5 Nitrogen Dioxide. Committee on the Medical Effects of Air Pollution. Department of Health. ISBN 0 11 322102 9 1998

QUARG: 1993, 'Urban Air Quality in the United Kingdom', First report of the Quality of Urban Air Review Group, Department of the Environment, London, UK.

Raw G.J, Coward S.K.D. Exposure to Nitrogen dioxide in homes in the UK: a pilot study. Building Research Establishment Occasional paper. 1992.

Raw GJ, Coward SKD et al. Exposure to air pollutants in English homes. Journal of Exposure Analysis and Environmental Epidemiology 2004;14:585-594

Reynolds P, Von Behren J et al. Traffic patterns and childhood cancer incidence rates in California, United States. Cancer causes and Control, 2002;13(7):665-673.

Rinsky RA, Hornung RW et al. Benzene exposure and hematopoietic mortality: A long-term epidemiological risk assessment. American Journal of Industrial medicine, 42(6):474-480.

Roger L.J. Pulmonary function, airway responsiveness, and respiratory symptoms in asthmatics following exercise in NO_2. Toxicology and Industrial Health, 1990;6: 155-171.

Ross D, Coward SKD et al. An investigation into levels of Carbon monoxide in homes with unflued non-electric portable heaters. Proceedings of healthy Buildings 1997;3;117-122.

Ross D. Continuous monitoring of NO_2, CO, temperature and humidity in UK homes. Indoor Air 1996, Japan; 1;513-518.

Ross D. Continuous monitoring of NO_2, CO, temperature and humidity in UK homes;in Yoshizawa S, Kimura K-I, Ikeda I, Tanabe S-I, Iwata T (eds):Proceedings of the 7th International Conference on Indoor Air Quality and Climate. Tokyo, Institute for Public health 1996:513-518

Samet JM. Lambert WE et al. Nitrogen dioxide and respiratory illness in children. Part I: Health outcomes. Research Report - Health Effects Institute. 1993;(58):1-32; discussion 51-80.

Samet JM. Utell MJ. The risk of Nitrogen dioxide: what have we learned from epidemiological and clinical studies?. Toxicology & Industrial Health. 1990;6(2):247-62.

Schmidt HHHW, Lohmann SM et al. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biophys Acta. 1993;1178:153-175.

Sheps DS, et al. Lack of effect of low levels of carboxyhaemoglobin on cardiovascular function in patients with ischemic heart disease. Archives of Environmental health. 1987;42: 108-116.

Smith MT, Rothman N et al. Molecular cytogentics of humans exposed to Benzene in dermally exposed male rats. American Industrial Hygiene Association Journal.1996;49:506-511.

Snyder SH, Jaffrey SR et al. Nitric oxide and Carbon monoxide: parallel roles as neural messengers. Brain Research Reviews 1998;26:167-175

Sonander H, Stenqvist O et al. Urinary Nitrous oxide as a measure of biologic exposure to Nitrous oxide anaesthetic contamination. Annals of Occupational Hygiene; 1983;vol 27 1: 73 – 79.

Speizer FE. Ferris B Jr et al. Respiratory disease rates and pulmonary function in children associated with NO2 exposure. American Review of Respiratory Disease. 1980;121(1):3-10.

Spengler JD, Samet JM et al. Indoor Air Quality Handbook. Mc Graw-Hill. 2000. ISBN 0-07-445549-4.

Spix C. Anderson HR et al. Short-term effects of air pollution on hospital admissions of respiratory diseases in Europe: a quantitative summary of APEA study results. Archives of Environmental Health. 1998;53:1; 54-65.

Stedman JR. Revised High Resolution Maps of Background Concentrations in the UK:1996. NETCEN Report to the Department of the Environment, Transport and the Regions. 1998.

Stewart RD. The effect of Carbon Monoxide on humans. Journal of Occupational Medicine. 1976, 18, 304-309.

Suematsu M, Wakabayashi Y et al. Gaseous monoxides: a new class of microvascular regulator in the liver. Cardiovascular Research. 1996;32:679-686.

Suk WA. Collman G et al. Human biomonitoring: research goals and needs. Environmental Health Perspectives. 1996;104 Suppl 3:479-83.

Synder R, Hedi CC. An Overview of Benzene Metabolism. Environmental Health Perspectives. 1996;104 (supplement 6) :1165-1171.

The Air Quality (Wales) Regulations 2000. ISBN 0-11-090111-8
The Air Quality Strategy for England, Scotland, Wales and Northern Ireland. Defra 1997.

The Air Quality Strategy for England, Scotland, Wales and Northern Ireland (Volume 1). Defra 2007

The Air Quality Strategy for England, Scotland, Wales and Northern Ireland Working together for Clean Air. Department of the Environment, Transport and the Regions in partnership with the Scottish Executive, The national Assembly of Wales and the Department of the Environment in Northern Ireland. Section 6 Nitrogen Dioxide 2000

The Carbon Monoxide and gas safety Society. Press pack Claygate Surrey; C)-GAS, 1997.

Thom SR, Xu YA et al. Vascular endothelial cells generate peroxynitrite in response to Carbon monoxide exposure. Chemical research Toxicology. 1997;10:1023-1031.

Travis CC. Tissue dosimetry for reactive metabolites. Risk Analysis, 1990;10(2):317-321.

Turner M, Hamilton-Farrell MR et al. Carbon monoxide poisoning: An update. Journal of Accident and Emergency medicine. 1999; 16;2;92-96.

US Department of Health and Human Services. Agency for Toxic Substances and Disease Registry. 1997. Toxicological Profile for Benzene.

Van Bel F, Latour V et al. Is Carbon monoxide-mediated cyclic guanosine monophosphate production responsible for low blood pressure in neonatal respiratory distress syndrome?. Journal of Applied Physiology. 2005;98:1044-1049.

Van Uffelen B.E, de Koster B.M et al. Carbon Monoxide enhances human neutrophil migration in a cyclic GMP-dependent way. Biochemical and Biophysical research Communications. 1996;226, 21-26.

Verma A, Hirsch DJ. Carbon monoxide: a putative neural messenger. Science. 1993;259:381-384.

Vogelaere P, Dekunder G et al. factors enhancing cardiac output in resting subjects during cold exposure in air environment. Journal Sports medical Physiology. Fitness. 1992;32, 378-386.

Wald WS, Howard S et al. Association between atherosclerotic diseases and carboxyhaemoglobin levels in tobacco smokers. British Medical Journal. 1973;1:761-765.

Walker E. Hay A. Carbon Monoxide poisoning. British medical Journal. 1999; 319:1082-1083.

Wallace L. Environmental Exposure to Benzene: An Update. Environmental health Perspectives. 1996;104: 1129-1136.
Wallace LA. Human exposure to environmental pollutants: a decade of experience. Clinical and Experimental Allergy 25:4-9.

Walsh JT, Andrews R et al. Haemodynamic and hormonal response to a stream of cooled air. European Journal of Applied Physiology. Occupational Physiology. 1995; 72, 76-80.

Weaver LK. Carbon Monoxide poisoning. Critical care Clinics 1999; 15;2;297-317.

Welsh Air Quality Forum. The Air Quality Monitoring database for Wales 4th Annual Report April 1998-March 1999 ISBN 1 90 15 17 00 9
WHO (1987) Air Quality Guidelines for Europe (European series No. 23), Copenhagen, Denmark, World health Organisation Regional Office for Europe.

WHO (1994) Update and revision of the Air Quality Guidelines for Europe, Copenhagen, Denmark, World Health Organisation Regional Office for Europe.

WHO (2000) World Health Organisation. Air Quality Guidelines for Europe. WHO Regional publications, European series No 23, Copenhagen, WHO Regional Office for Europe; 1987 ISBN 9289013583

Wiech C, Raw GJ. Asthma, dust mites, ventilation and air quality:study design and initial Carbon monoxide results; in Maroni M (ed):Healthy Buildings '95. Milan, University of Milano and International Centre for pesticide Research 1995:425-430

Wilson RC, Saunders PJ et al. An epidemiological study of acute Carbon monoxide poisoning in the west Midlands. Occupational Environmental Medicine. 1998;55;723-728.

Wong O, Raabe GK. Non-Hodgkin's lymphoma and exposure to Benzene in a multinational cohort of more than 308,000 petroleum workers, 1937 to 1996. Journal of Occupational and Environmental medicine 2000;42(5):554-568.

World Health Organisation (WHO) (2000a) Fifty third World Health Assembly: Verbatim records of Plenary meetings and list of participants, 15-20 May 2000, WHO, Geneva.

World Health Organisation (WHO) 1996. Updating and revision of the Air Quality Guidelines for Europe. Report on a WHO working group on Volatile Organic Compounds. Brussels, Belgium, 2-6 October 1995.

World Health Organisation. Air quality guidelines for Europe. Second Edition WHO publication 2002. ISBN 92 890 1358 3.

World Health Organisation. Environmental Health Criteria: Carbon Monoxide (2nd edition). 1999 ISBN 92 4 157213 2

World Health Organisation. 1987b. Nitrogen dioxide. In Air Quality Guidelines for Europe, pp297-314. European series 23. World Health Organisation Regional Office for Europe. Copenhagen: WHO Regional Publications.

Yang W, Lee K et al. Characterisation of indoor air quality using multiple measurements of Nitrogen dioxide. Indoor Air. 2004;14: 105-111.

Yu R, Weisel CP. Measurement of the urinary Benzene metabolite trans, trans-muconic acid from Benzene exposure in humans. Journal of Toxicology and Environmental health 1996;48:453-477.

APPENDIX 1

Recruitment information

Letter to resident / Appointment letter

«URN»
«House_number» «subnumber» «Name» «Road»
«Deptroad»
«posttown»
«locality»
«locality2»
«postcode»

Please help us by filling in this short questionnaire.

Please tick ONE box on each line

I am willing to take part in the study and for someone from the University of Wales College of Medicine to visit my home to obtain more details	Yes ☐ No ☐
Does anyone in your house regularly smoke cigarettes, cigars or a pipe	Yes ☐ No ☐
Which fuel is used for heating your house? **Do you have central heating?**	electricity ☐ gas ☐ oil ☐ other ☐ Yes ☐ No ☐
Please tell us your telephone number	☎----------

My name is .....

My date of birth is .....

Please tick a box to show when it would be most convenient for a researcher to contact you

	Weekday Morning	Weekday Afternoon	Weekday Evening	Weekends	Anytime
The best time to contact me is:					

Thank you for your time.

Dr. Ian Matthews

Information Sheet / Telephone recruitment information – Carbon monoxide

- Every form of heating (except electric heating) gives off very small amounts of 2 gases called Nitrogen dioxide and Carbon monoxide.

- many people spend a great deal of time indoors particularly in the winter and medical researchers now think it is possible that even these very low levels of gases might effect health by: -

    - Making respiratory (chest) breathing problems worse
    - Decreasing the ability of blood to carry oxygen from the lungs to the organs of the body (e.g. heart)

- We are carrying out a survey which measures the levels of these gases in the home and in the blood.

- We will visit and:
    - Leave a small gas monitor (size of a packet of butter) in your living room for 7 days
    - Ask you to complete a 4 page questionnaire

- A small sample of blood will be taken by a qualified technician in the normal way. We will make an outpatient appointment for you at Neath general hospital at a convenient time. We will pay 25p per mile for own car travel or arrange transport

- We would then like to take another blood sample sometime in the summer months, and then we will compare both of these samples.

- Smoking is known to affect the health effects we want to measure so we will not be investigating smokers

- Certain medicines can effect the measurements on blood that we will make. Please tell me the names of any medicines which you are taking (including sprays)

List of medications resulting in exclusion from the Carbon monoxide study

Angitak
Buccal tablets
Cedocard retard
Chemydur
Coro-Nitro pump spray
Deponit
Elantan
Elantan LA
Glyceryl Nitrate
Glyceryl Trinitrate
Glytrin spray
GTN 300 mcg
60 Imazin XL
Imdur
spray
Isib 60XL
Ismo
Ismo retard
Isocard
Isodur
Isoket
Isoket retard
Isordil
Isosorbide mononitrate
Isorbine dinitrate
Isotard
Isotrate
MCR 50
Minitran
Modisal XL
Monit
Monit SR
Monit XL
Monomax
Monosorb XL
Nitrocine
Nitro-dur
Nitrolingual pump

Nitromin
Nitronal
Percutol
Sorbid SA
Suscard
Sustac
Transiderm-Nitro

Consent form

ID .....

Name .....

Address .....

.....

.....

I have read the information sheet about the Housing and Health Study.

I understand that my participation is entirely voluntary and that I may decline to take part in any aspect of the study or withdraw from it at any time.

I understand that all information will be treated in strict confidence.

I agree to the following procedures (delete any that you do not agree to):

- *Answering a questionnaire on respiratory and cardiovascular symptoms*
- *The siting of a small air sampler in my home for 7 days to measure Carbon monoxide*
- *To blow into a Carbon monoxide measuring meter*
- *To donate a venous blood sample on one occasion in the winter and on one occasion in the summer by prior arrangement and at a time which is convenient for me.*

Signed: .....

Date: .....

APPENDIX 2

Analysis methods – Biological samples

1. Carboxyhaemoglobin

Analysis of COHb using the ABL625 series analyser.

The method used was visible absorption spectroscopy

1. The blood sample was transported to the cuvette positioned in the hemolyzer unit. The temperature of the cuvette was regulated to 37°C.

2. 1µL of the sample was ultrasonically hemolyzed in the cuvette at a frequency of about 30kHz in order to rupture the walls of the red blood cells so that their content was mixed with the blood plasma, giving an optically clear solution. To eliminate air bubbles in the sample and to enhance hemolyzation, an over-pressure of one atmosphere was maintained throughout hemolyzation and measurement.

3. Light from a 4 Watt halogen lamp was sent to the cuvette via an infra-red filter and a bioconvex lens.
   The voltage across the halogen lamp was regulated by a thermostatted photodiode so that the amount of light sent to the cuvette had a constant intensity.

4. The light transmitted through the cuvette was guided to the spectrometer via an optical fiber.

5. The light passed through a slit that directed it towards a combined mirror and concave grating.

6. The grating separated the light into 128 single wavelengths and the mirror focused the 128 light signals on a photodiode array.

7. The photodiode array had 128 diodes or pixels, one for each wavelength, which converted the monochromatic light signals to currents.

8. The currents and therefore the intensity of the light signals were measured at each of the 128 diodes, which form the basis for the absorption spectrum for a particular sample.

9. The spectrum was sent to the analyser's computer, where the calculations of the oximetry parameter values were made.

2. Cyclic Guanosine Monophosphate (cGMP)

1. Without delay collect venous blood into 6x4ml pre-prepared vacutainers. When all containers are full gently invert them to thoroughly mix the contents. **They are on no occasion to be shaken.**

2. Centrifuge the blood samples at 1000rpm for 10 minutes.

3. Following centrifugation uncap the vacutainers and using a plastic (**never glass**) disposable pipette carefully collect the upper layer of platelet-rich plasma into clean 2x4.5ml plastic "Rohren" tubes. Equal volumes should be added to each tube. Plasma from individual vacutainers may be mixed to complete this step. Care should be taken to remove only the upper layer. Leave behind the layer formed between the plasma and the red blood cells. Recap the vacutainers and discard the red blood cells.

4. Cap the 2 plastic tubes containing the platelet-rich plasma and centrifuge at 3000rpm for 10 minutes.

5. Following centrifugation uncap the tubes and using a plastic disposable pipette carefully collect the upper layer of cell-free plasma into clean 2x4.5ml plastic "Rohren" tubes, leaving behind the cell pellet. Cap all tubes. There should now be 4 tubes (2 with cell-free plasma and two with a cell pellet). Either place all tubes immediately on dry ice or freeze at –20°C. All tubes should then be stored at -70°C for long term storage.

Where practicable cGMP samples were collected on a weekly basis. Aliquots of blood samples were centrifuged before being frozen for subsequent analysis of cyclic GMP by radioimmunoassay.

The vaccutainers were supplemented with Zaprinast (10microM) that is a cGMP phospodiesterase inhibitor. Zaprinast prevents the breakdown of cGMP.

Platelet pellets were resuspended in 1ml of ice cold 65% (v/v) ethanol to extract the cGMP from the cells. Following centrifugation at 3000rpm for 10 minutes at 4°C the resulting supernatant was removed and evaporated to dryness. The pellet of cell debris was dissolved in 1ml of 1M sodium hydroxide solution and assayed for protein content using a commercially available kit (Biorad). The dried sample was resuspended in the appropriate assay buffer and the cGMP content measured by a commercially available

radioimmunoassay kit (Amersham Biosciences). The cGMP content of the sample was then normalised to the total pellet protein concentration.

3. S-Phenylmercapturic Acid (s-pMA)

The samples were allowed to reach room temperature before proceeding with the assay.

1. Open the foil bag containing the coated plate. Check the desiccant tablet remains blue, indicating that the plate has remained dry. Each plate has sufficiently coated wells to assay one set of standards and quality control samples and 22 specimen samples.

2. Determine how many wells are required to perform the assay in duplicate i.e. 2 wells each for the 6 calibrators, 2 quality control samples and each of the specimen urine samples.

3. Dispense 80ul reagent 1 (25ml phospate buffered saline containing 0.004% gentomycin) to each well required for the assay.

4. Dispense 10ul of each of the calibrators, QC's and specimen urines into the appropriate wells.

5. Dispense 80ul 'working primary antibody' (50ul of primary antibody with 12mls diluent) into each well.

6. Mix by holding the plate on the bench with one hand whilst tapping the side of the plate gently with the other hand.

7. Incubate at room temperature for 2 hours.

8. Decant the contents of the wells into a sink.

9. Wash the plate 3 times with the working wash buffer and blot on tissue paper to drain.

10. Dispense 100ul reagent 3 (15mls of secondary antibody at working strength) into each well.

11. Incubate for one hour at room temperature.

12. Decant the contents of the wells into the sink.

13. Wash the plate 3 times with working wash buffer and blot on tissue paper to drain.

14. Dispense 100ul reagent 4 (15mls 3,3',5,5'-tetra-methyl-benzidine (TMB) liquid substrate system).

15. Read the optical densities of the wells at 650nm using a plate reader.

The plate reader has the facility to enter a template and calibrator values to calculate the unknowns. The calibrators, QC's and specimen urines were positioned on the plate to correspond with the template.

4. Urinary Nitrous oxide

1. A 10ml urine sample was taken from the sample bottle directly into a 20 ml gas-tight vial equipped with a silicone septum.

2. The gas-tight vial was incubated in a water bath at 37°C for twenty minutes and then a 50 µl sample of the headspace gas above the urine was taken using a gas tight syringe and injected directly into the gas chromatograph. All such Gas Chromatograph injections were performed in triplicate.

3. The gas chromatograph was a Hewlett Packard 5890, Series 2, fitted with Electron Capture Detection. A HP Plot Q, 30m x 0.53 mm I.D. column was used and the carrier gas was 5% methane in argon with molecular sieve and oxygen trap.

4. Conditions were; column flow rate $6ml min^{-1}$, split vent 30ml $minute^{-1}$, septum purge $3ml min^{-1}$, auxiliary gas $50ml min^{-1}$, anode purge $3ml min^{-1}$, oven temperature 30^0C, injector temperature 80^0C and detector temperature 250^0C.

5. The retention time for Nitrous oxide was 3.8 minutes. External calibration was performed by injecting 50 µl Nitrous oxide calibration gas (24ppm in air) supplied by Alltech.

APPENDIX 3

Standard Operating Procedure: Carbon monoxide monitors

(1) Each time a piece of sampling /monitoring equipment is to be used the
User should check the general condition for signs of damage and moisture. Any appearance of these would be a contra-indication for use.

(2) All electrical cables, plugs and sockets which are intended to be used
Should likewise be checked for signs of wear or damage, the appearance of any such signs would prohibit use.

(3) The users of any electrical sampling / monitoring equipment should be
Satisfied that the correctly rated fuses are fitted before connecting to any
supply.

(4) Following checks (1), (2) and (3) but before connecting to the domestic
Mains electricity supply the item of sampling / monitoring equipment should
be subjected to a portable appliance test. Only on completion of satisfactory tests for Earth Continuity, Insulation and Current Leakage should any item of
equipment is considered safe for connection to the domestic electricity supply. These inspections and tests should be performed before every use of any item of sampling / monitoring equipment in a private domestic dwelling.

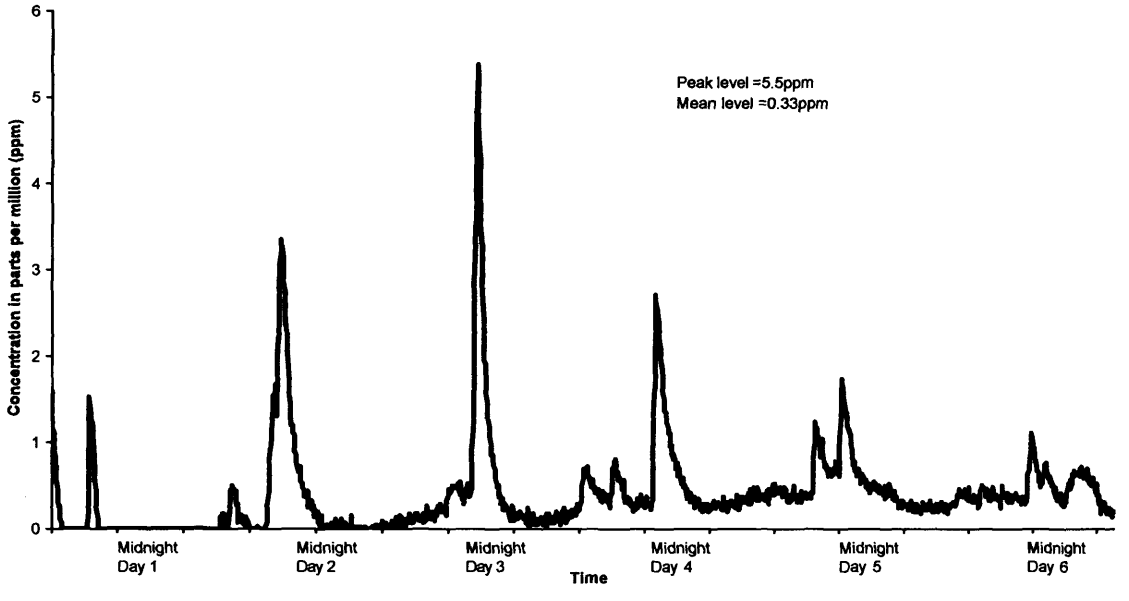
(5) All equipment should be used in accordance with agreed protocols and in
the form and manner intended by design and function. The use of other
accessories, extension cables, procedures etc. which might compromise operating safety should be strictly avoided.

(6) Details of all safety inspections, electrical testing and operating procedure should be recorded in a log-book for periodic scrutiny by project supervisors.

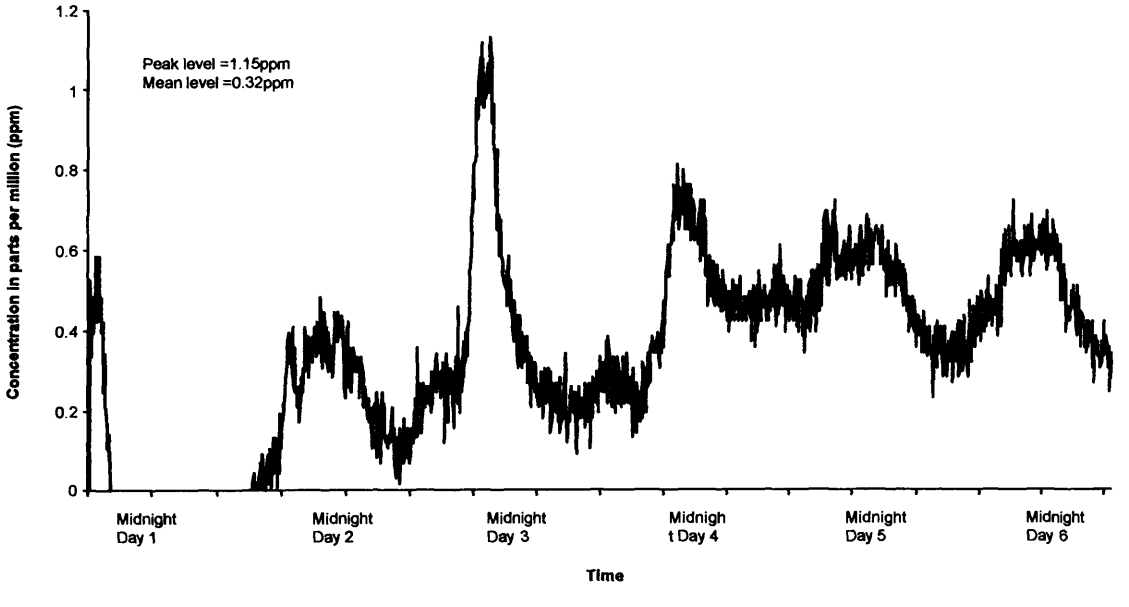
APPENDIX 4 – Environmental monitoring graphs of Carbon monoxide in non-smoking households grouped by heating fuel type

Gas

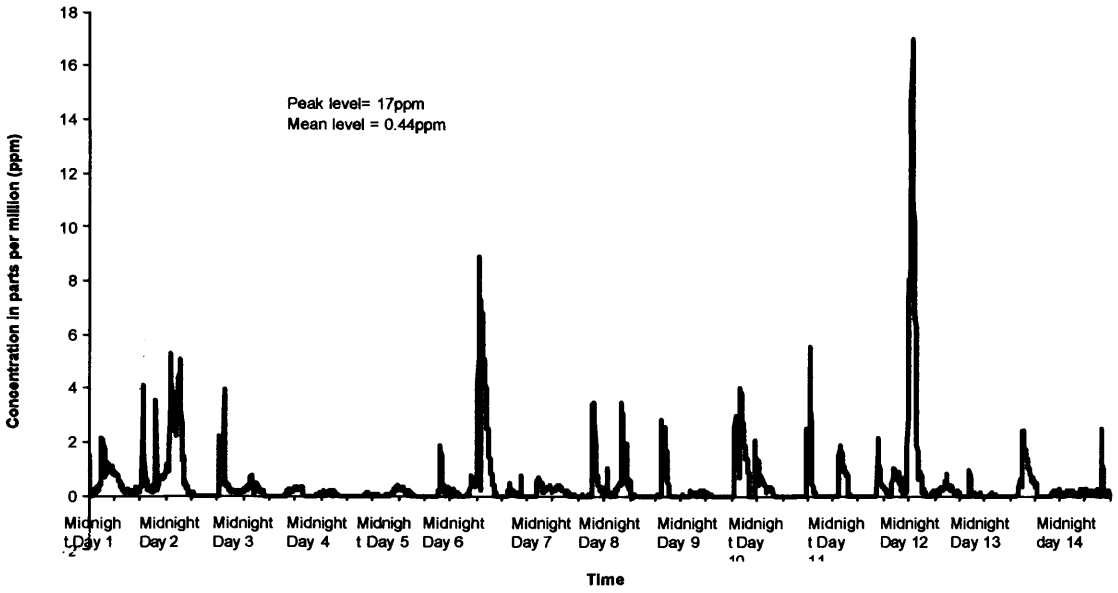
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 2)



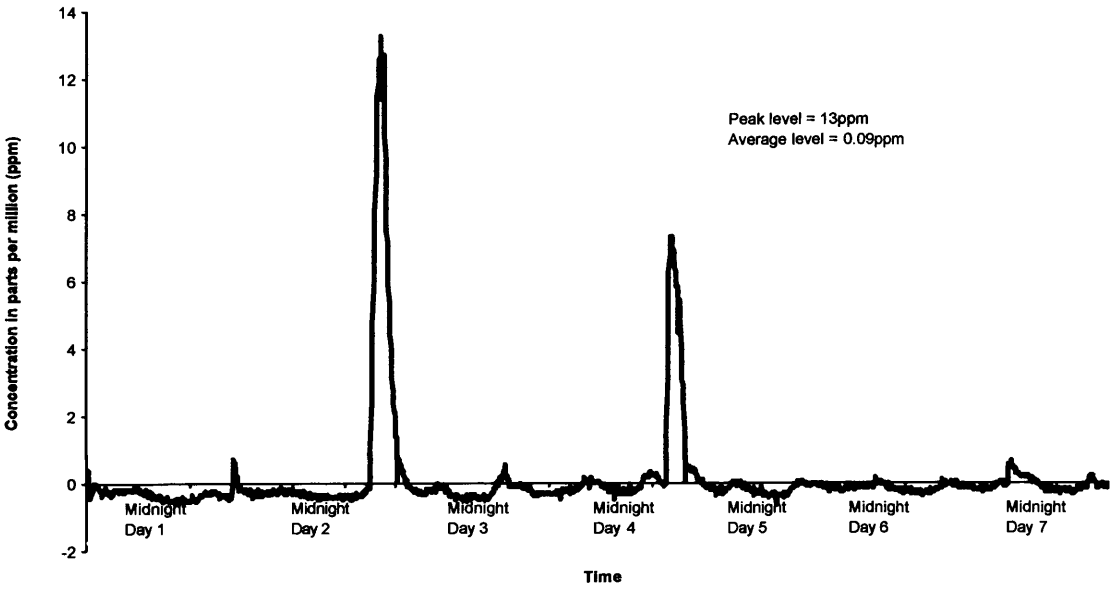
Continuous Carbon Monoxide measurements in a Home, fuel type Gas (Household 4)



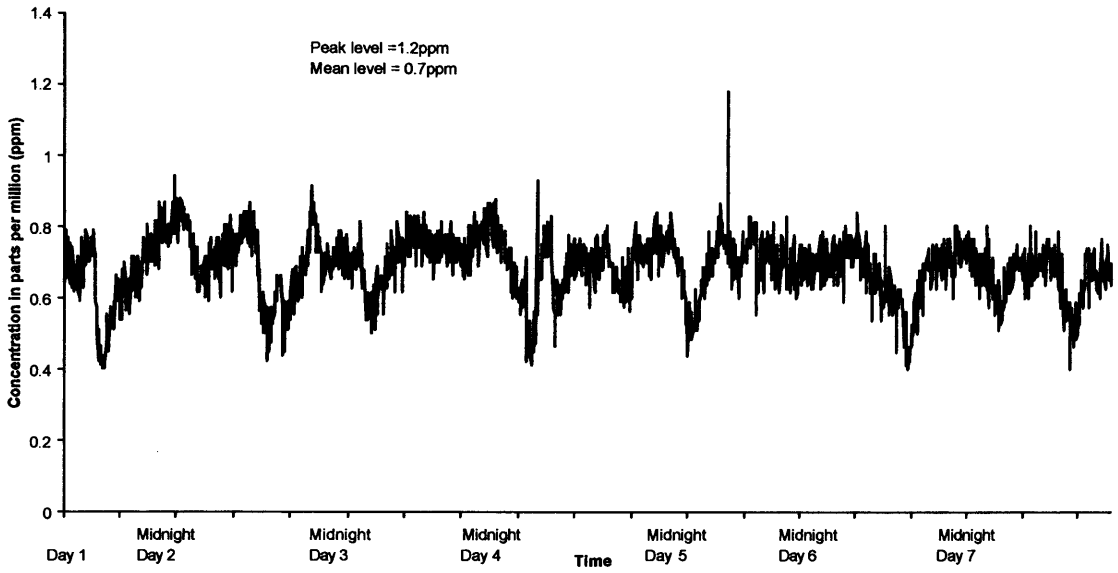
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 9)



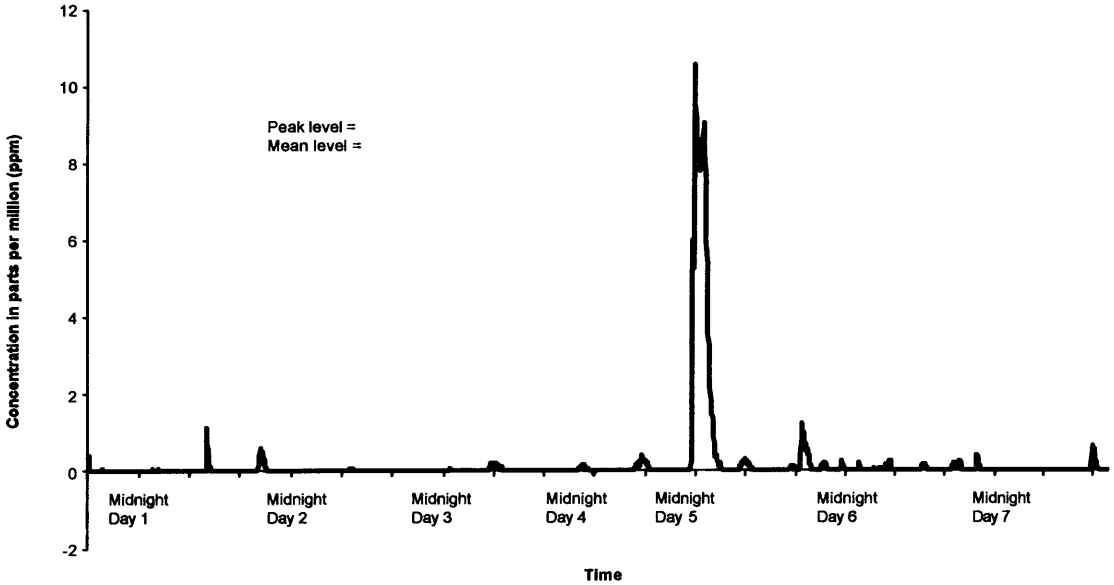
Continuous carbon monoxide measurements in Home, Fuel type Gas (household 11)



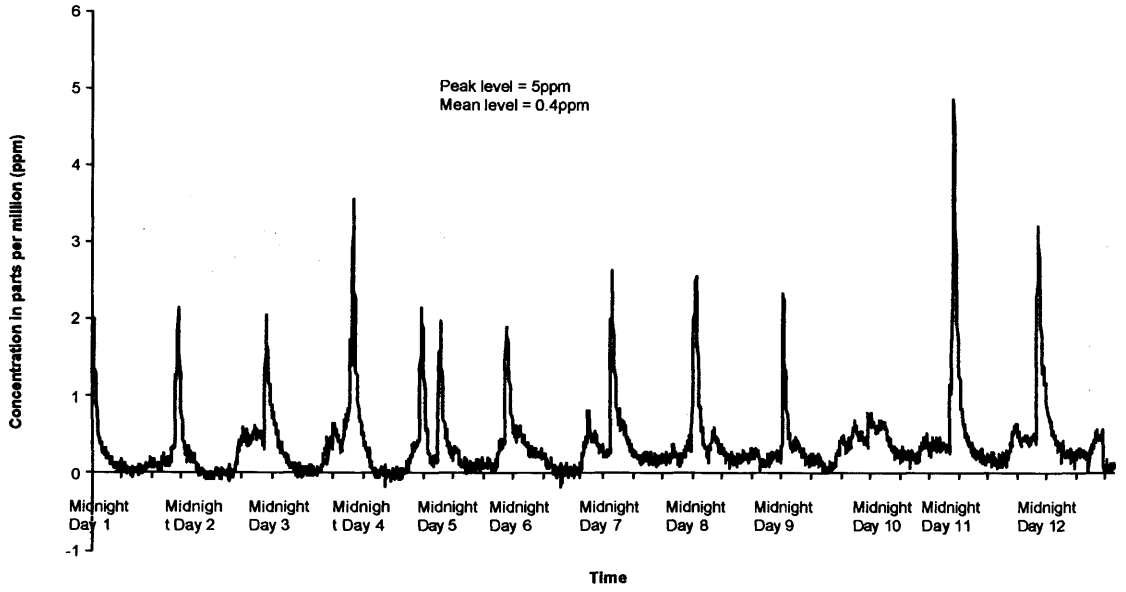
Continuous Carbon Monoxide levels in a Semi detached house - Fuel type Gas (Household 14)



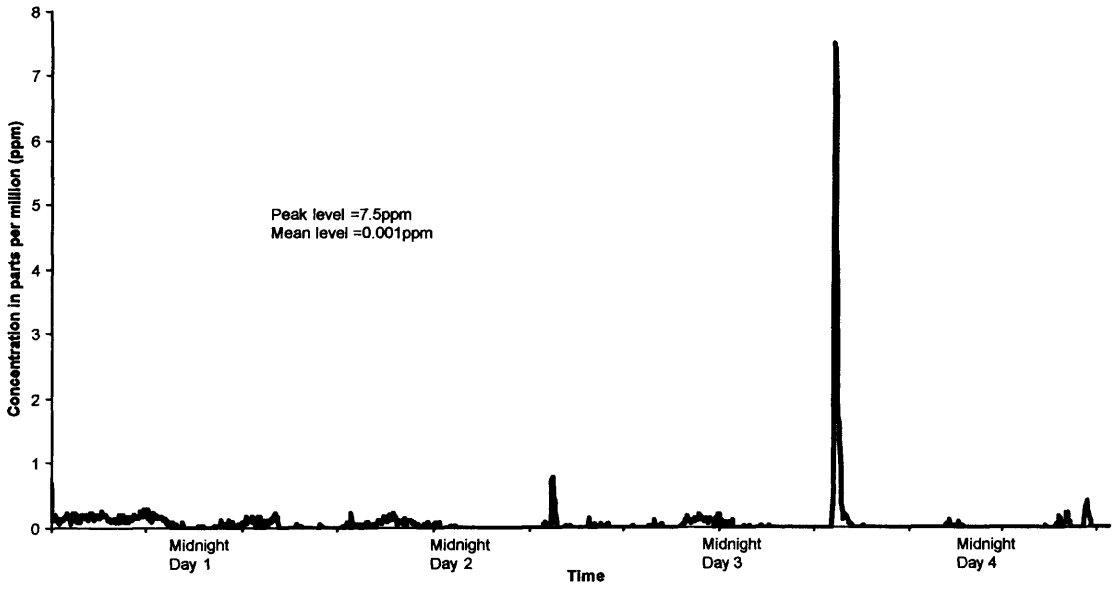
Continuous Carbon Monoxide measurements in a Home, fuel type Gas (Household 15)



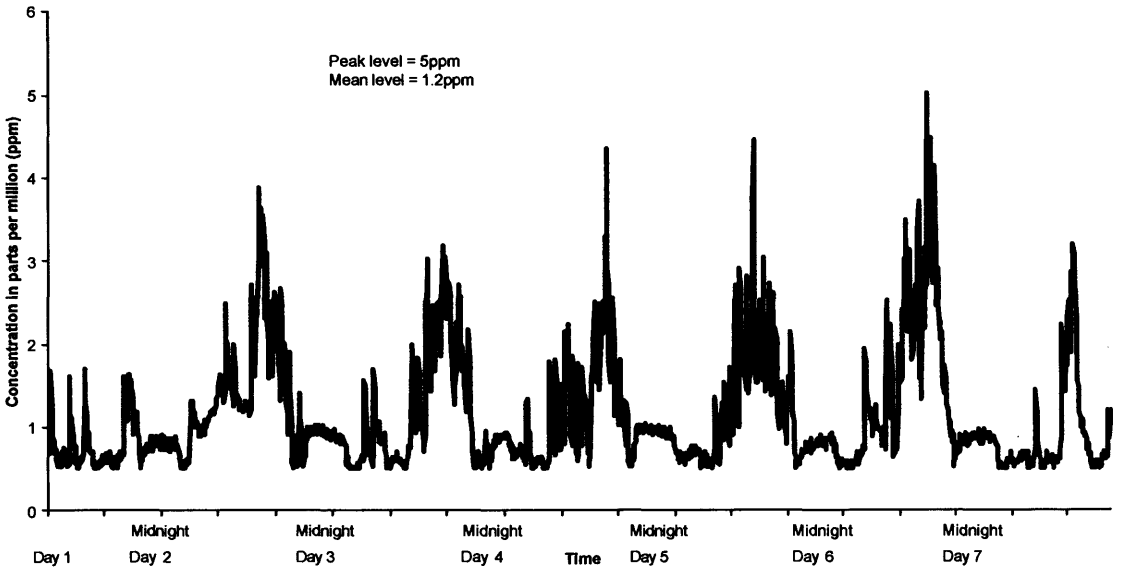
Continuous Carbon monoxide measurements in Home, fuel type Gas (Household 21)



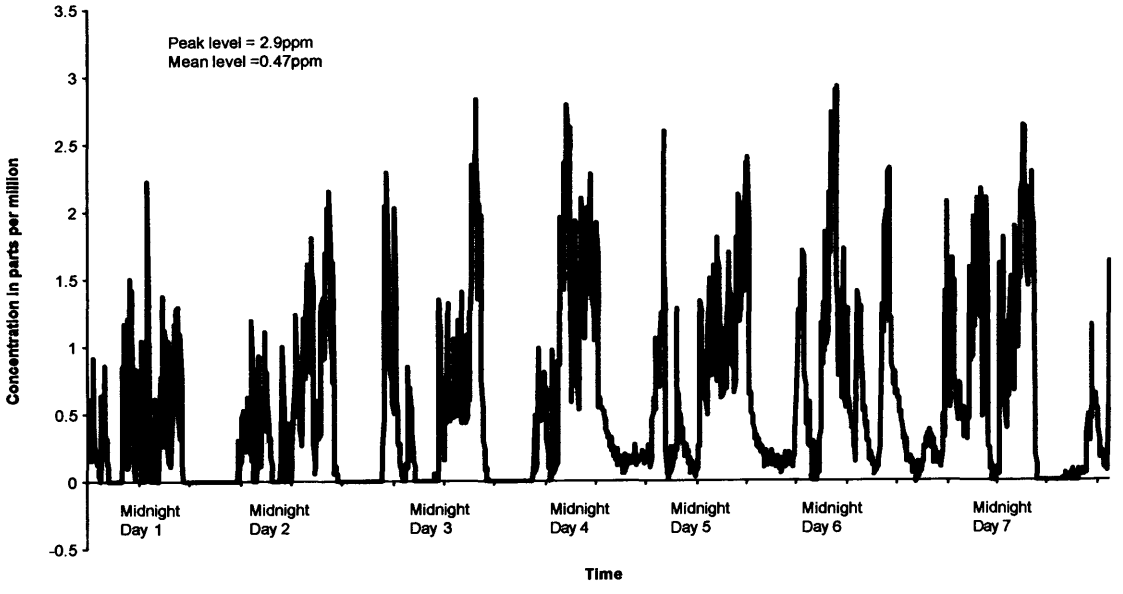
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 24)



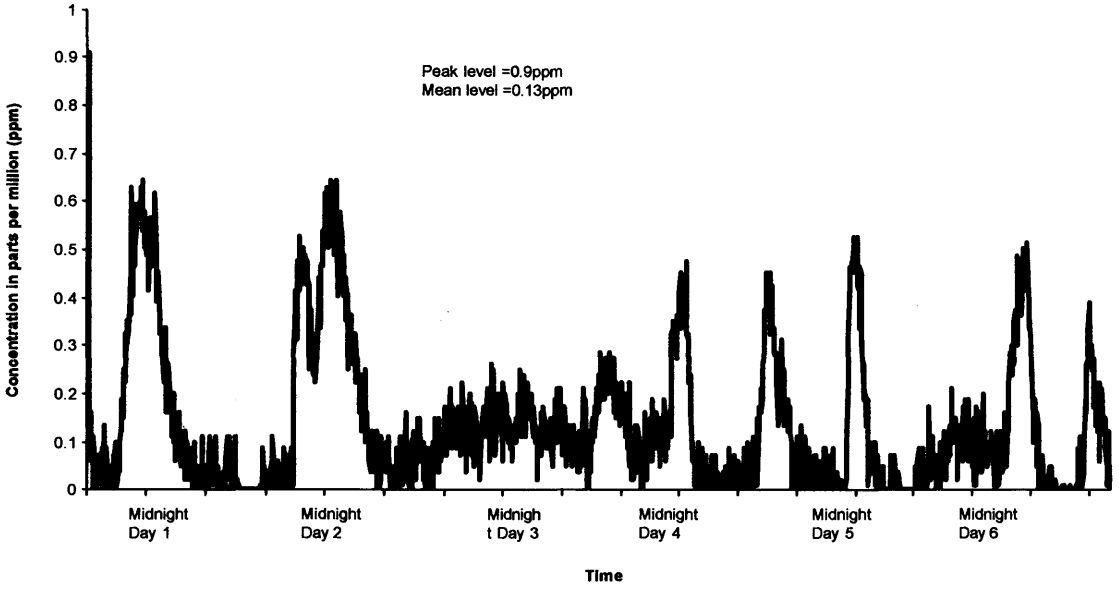
Continuous carbon monoxide measurements in a terraced house, fuel type gas (Household 28)



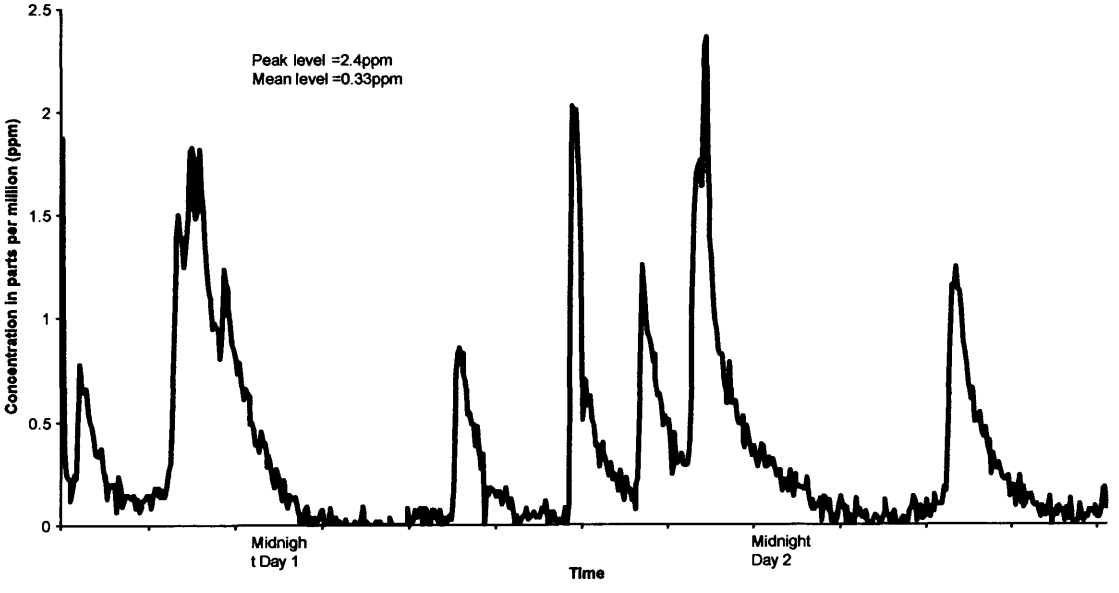
Continuous Carbon monoxide measurements in Home, fuel type Gas (Household 30)



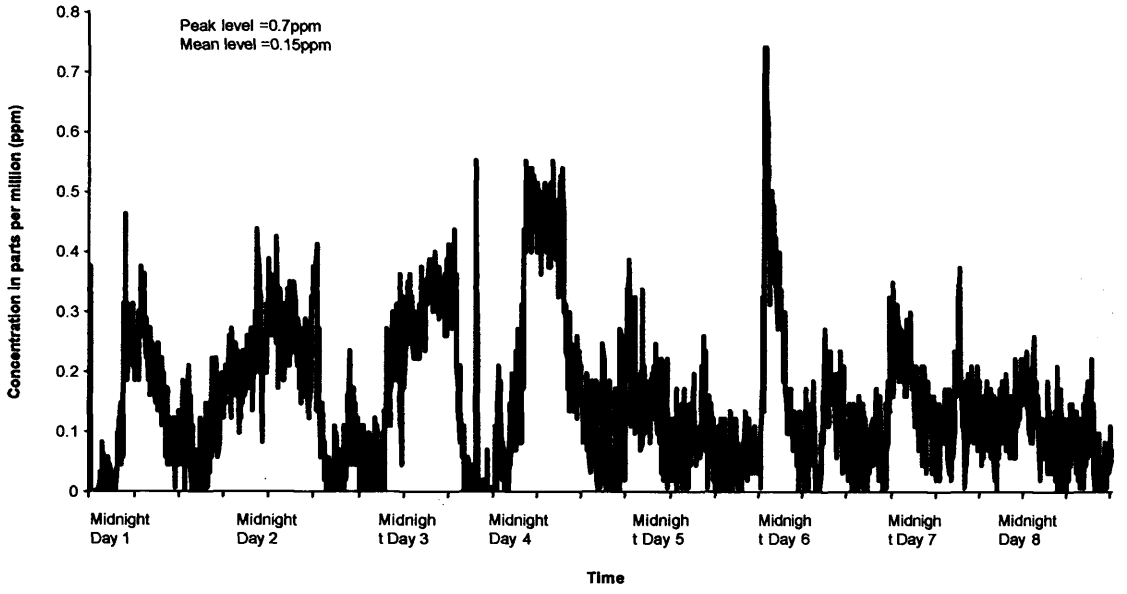
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 32)



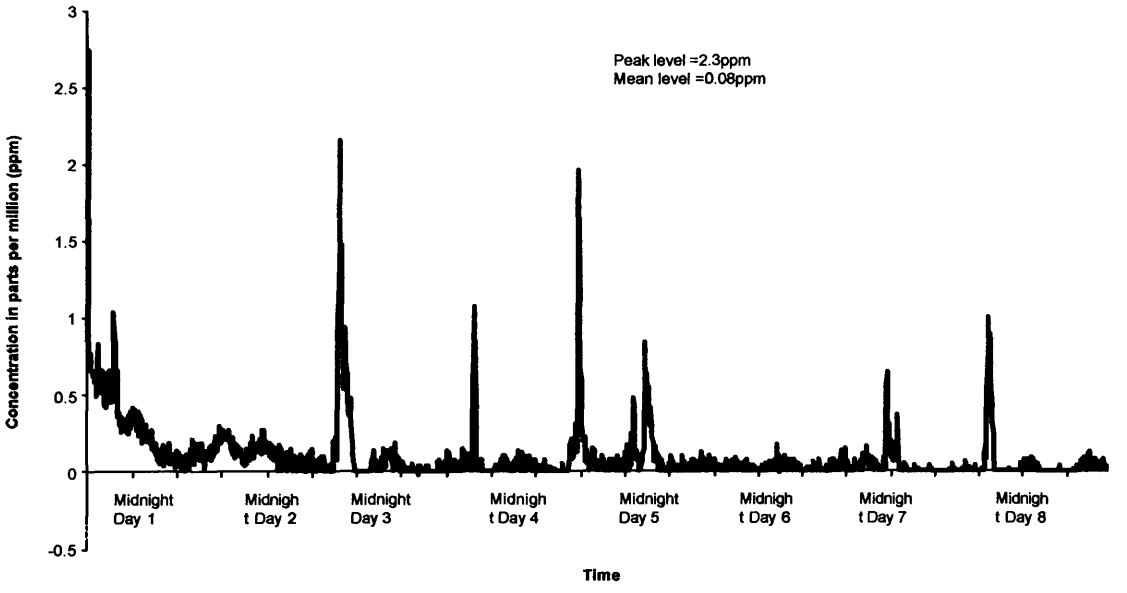
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 33)



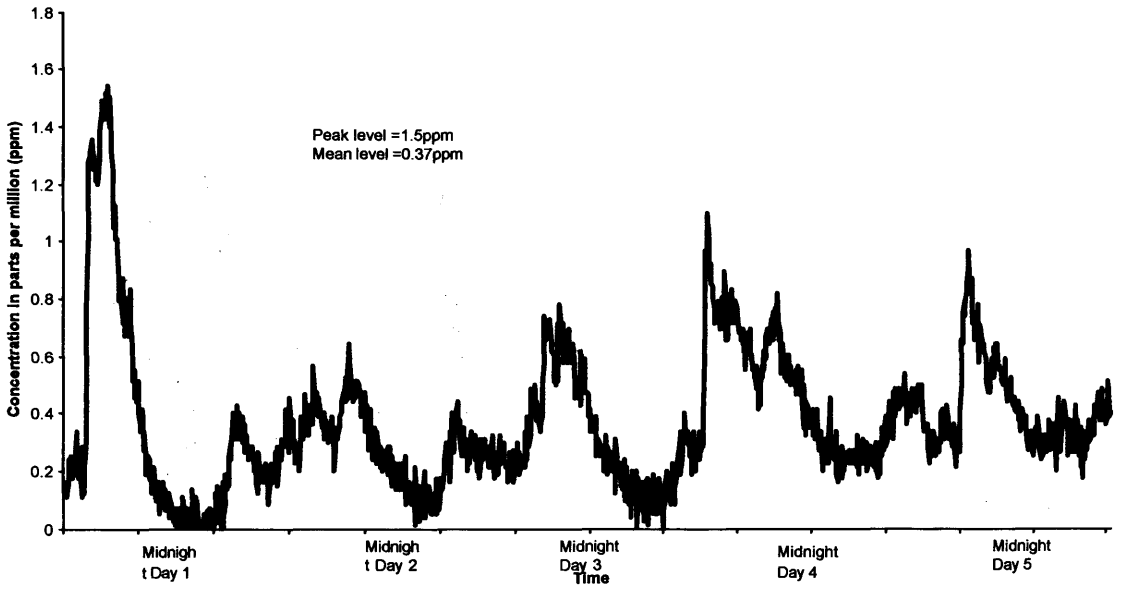
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 34)



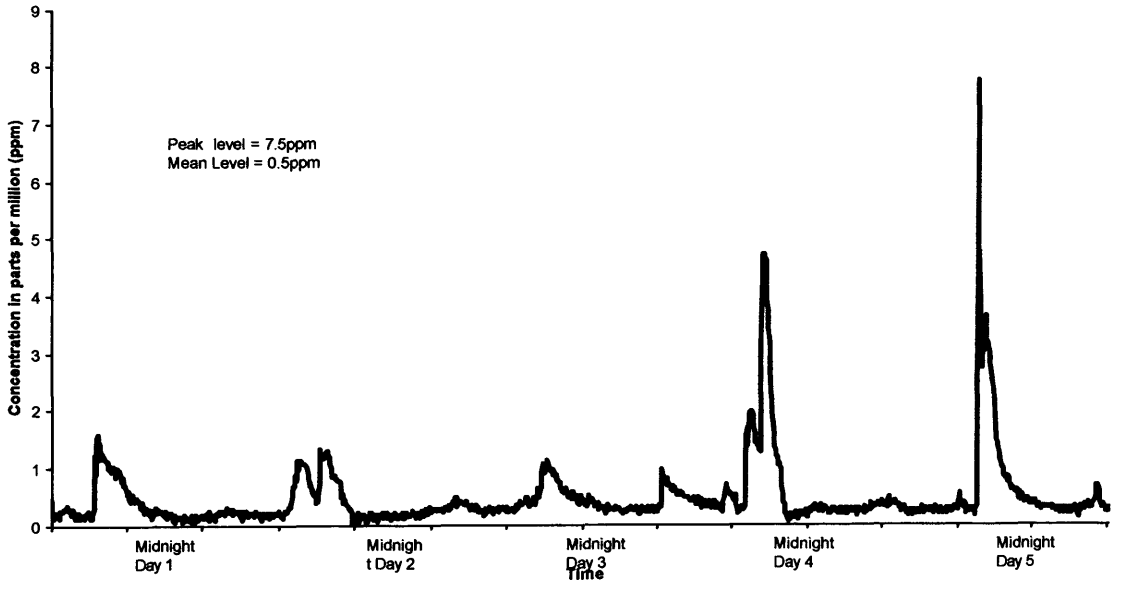
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 35)



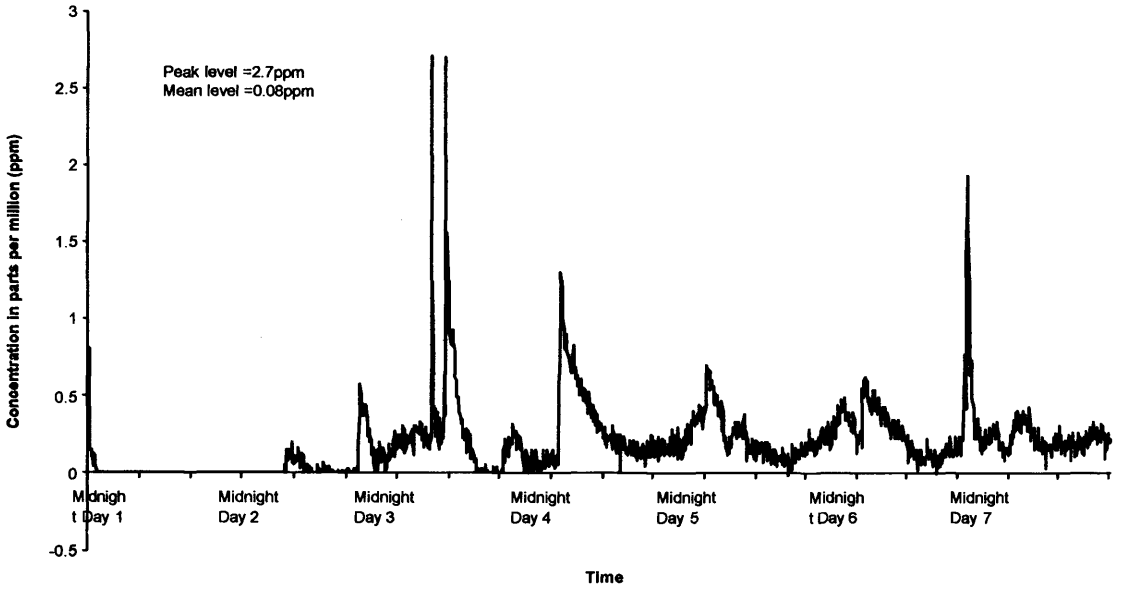
Continuous Carbon Monoxide measurments in Home, fuel type Gas (Household 37)



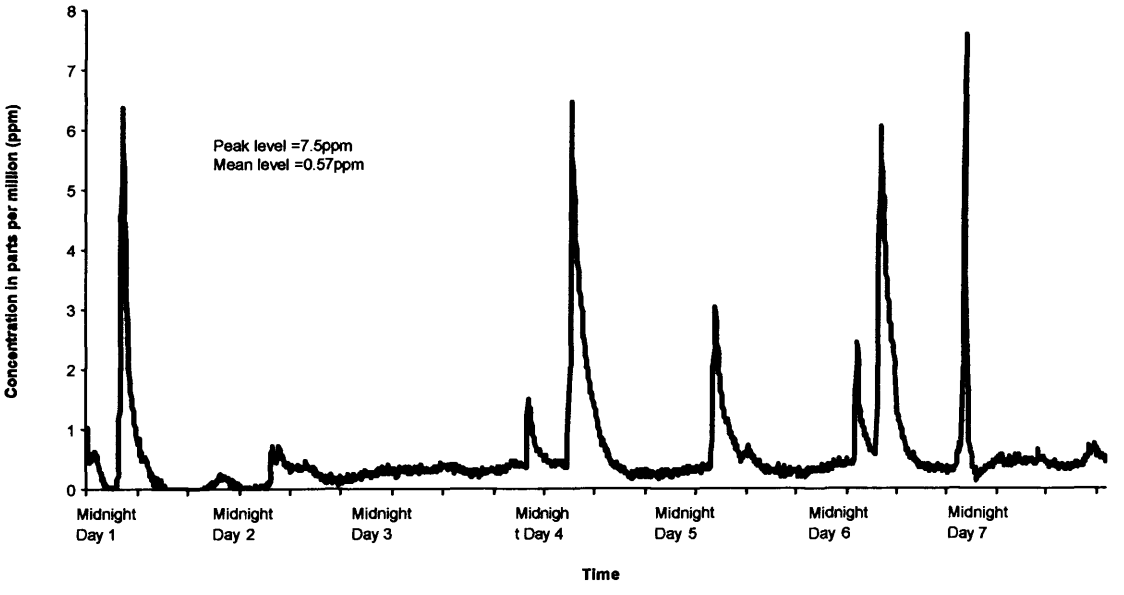
Continuous Carbon Monoxide measurement in Home, fuel type Gas (Household 38)



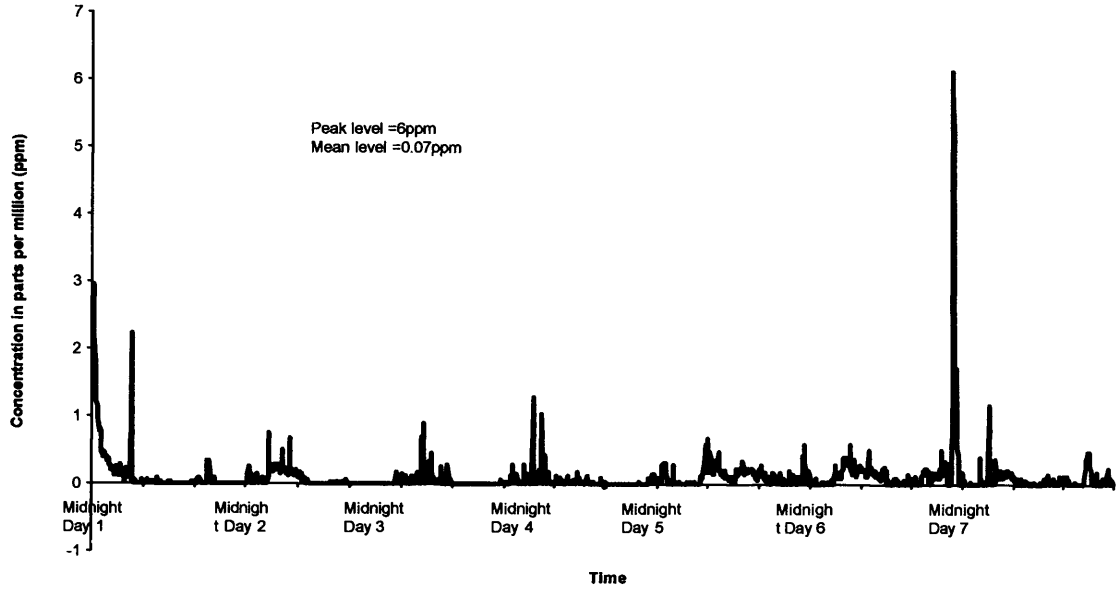
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 40)



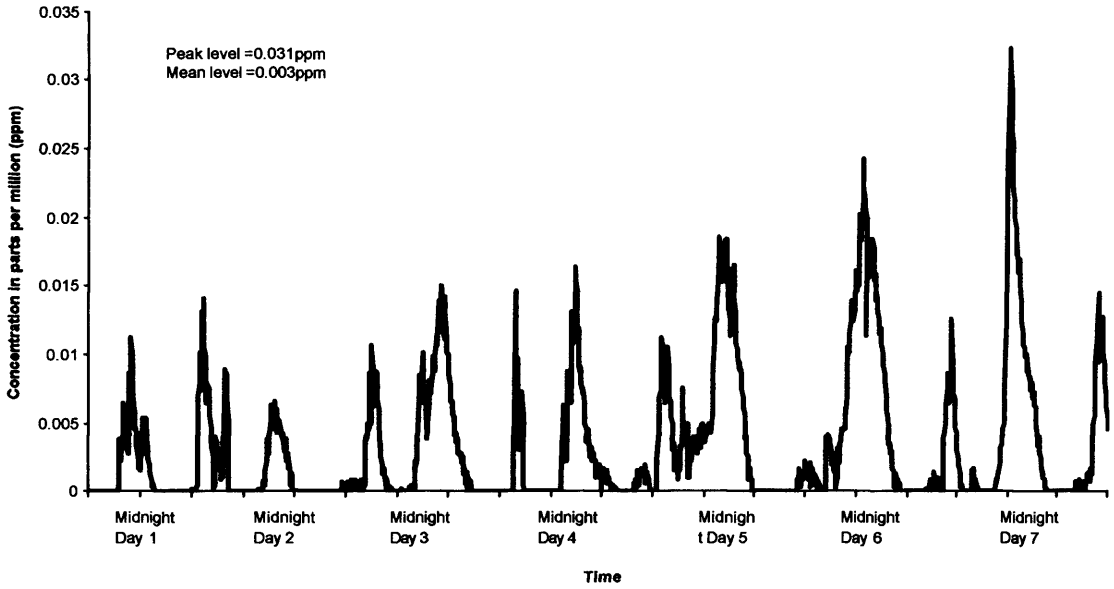
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 42)



Continuous Carbon Monoxide measurement in Home, fuel type Gas (Household 44)



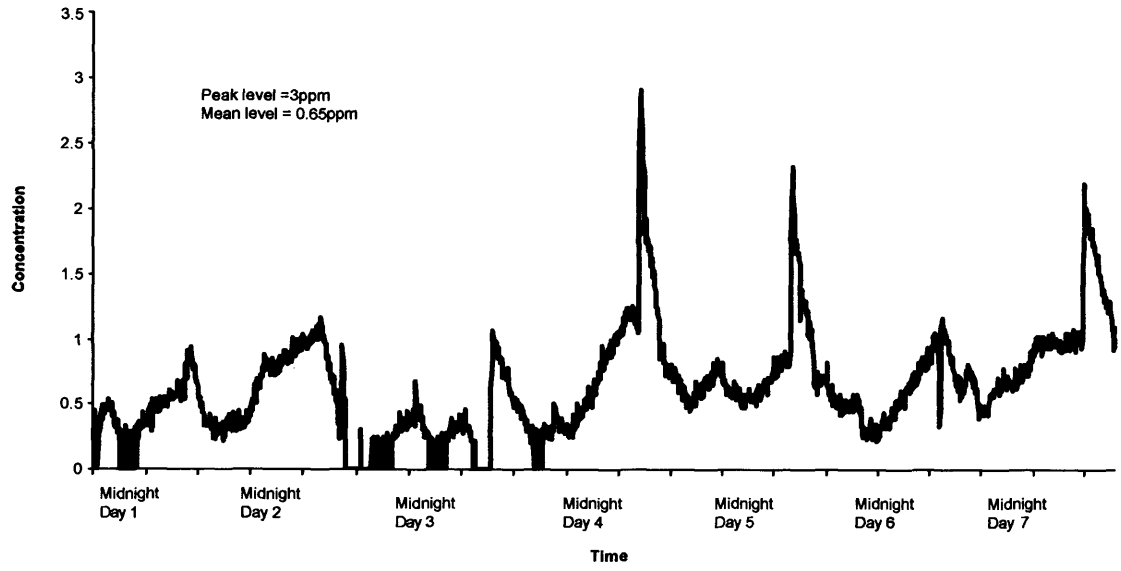
Continuous Carbon Monoxide measurements in Home, fuel type Gas (Household 48)



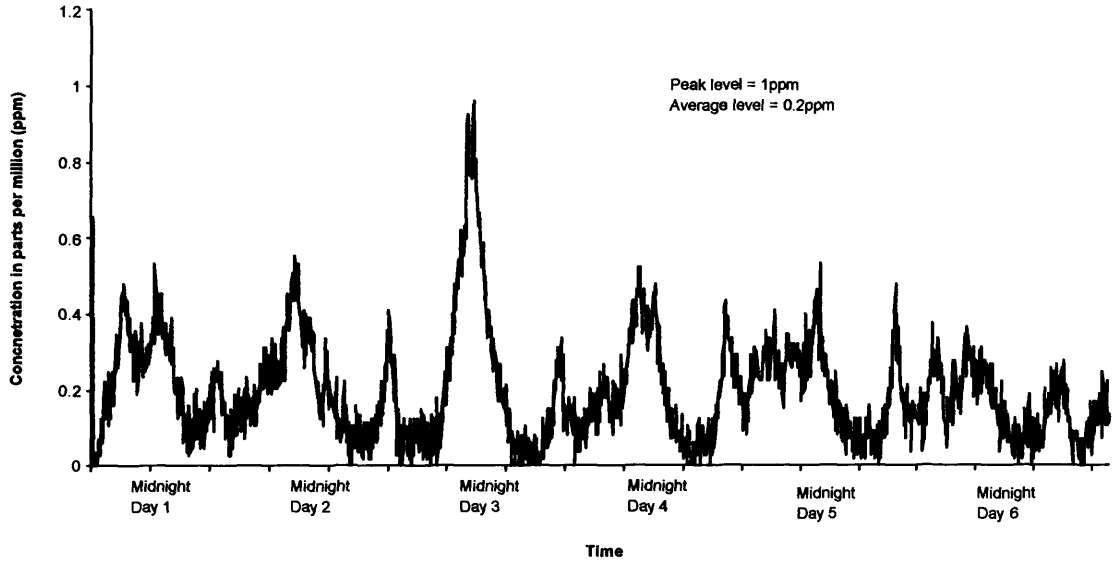
No graphs were available for Households 5, 7, 10, 13, 16, 17, 19, 20, 23, 25, 26, 31, 35, 39 and 41.

Coal

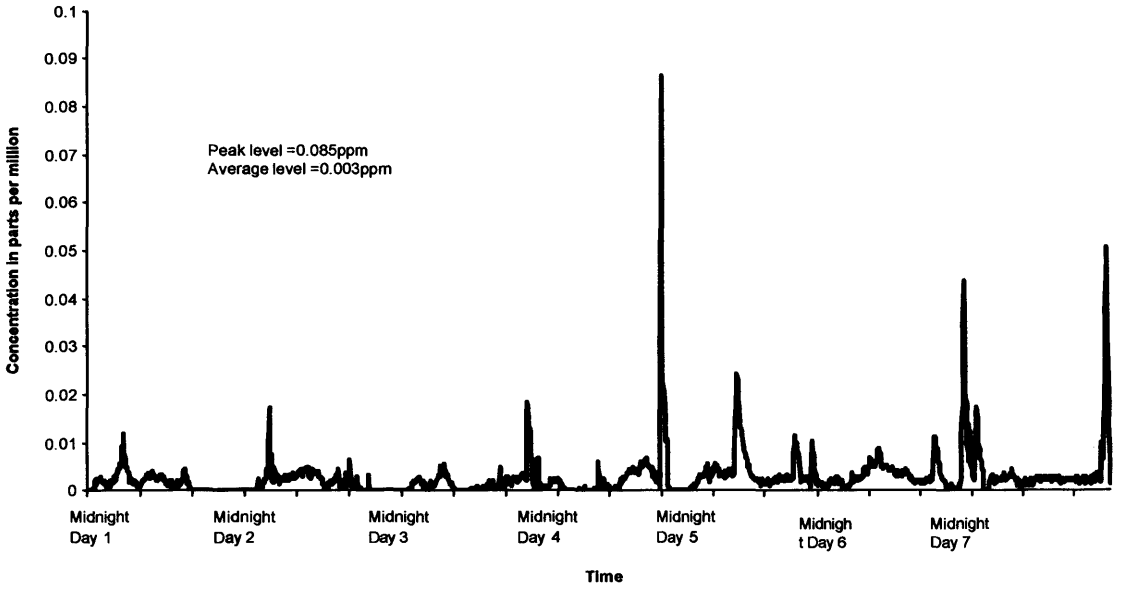
Continuous carbon monoxide levels in a semi detached House, fuel type coal (Household 6)



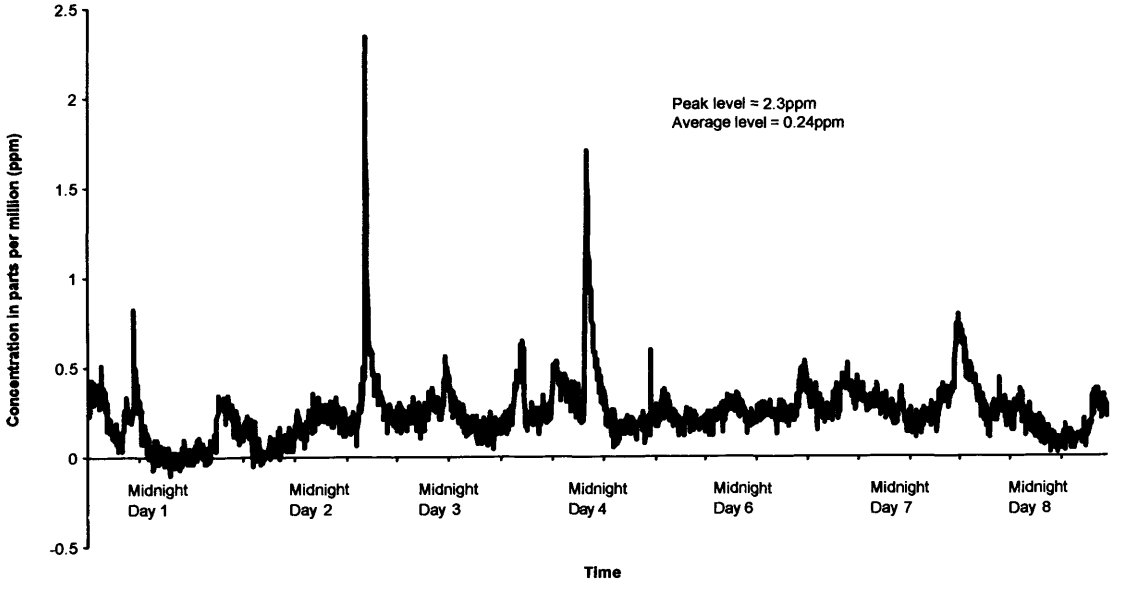
Continuous carbon monoxide levels in Home, fuel type Coal (Household 18)

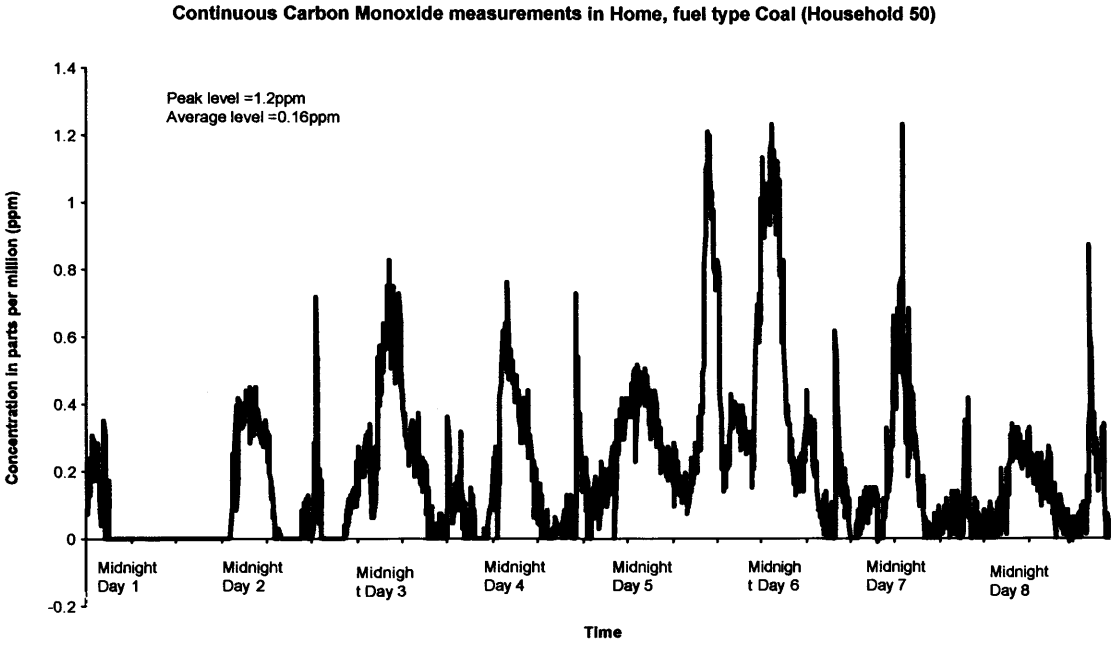


Continuous carbon Monoxide measurements in Home, fuel type Coal (Household 47)



Continuous Carbon monoxide measurements in Home, fuel type Coal (Household 49)

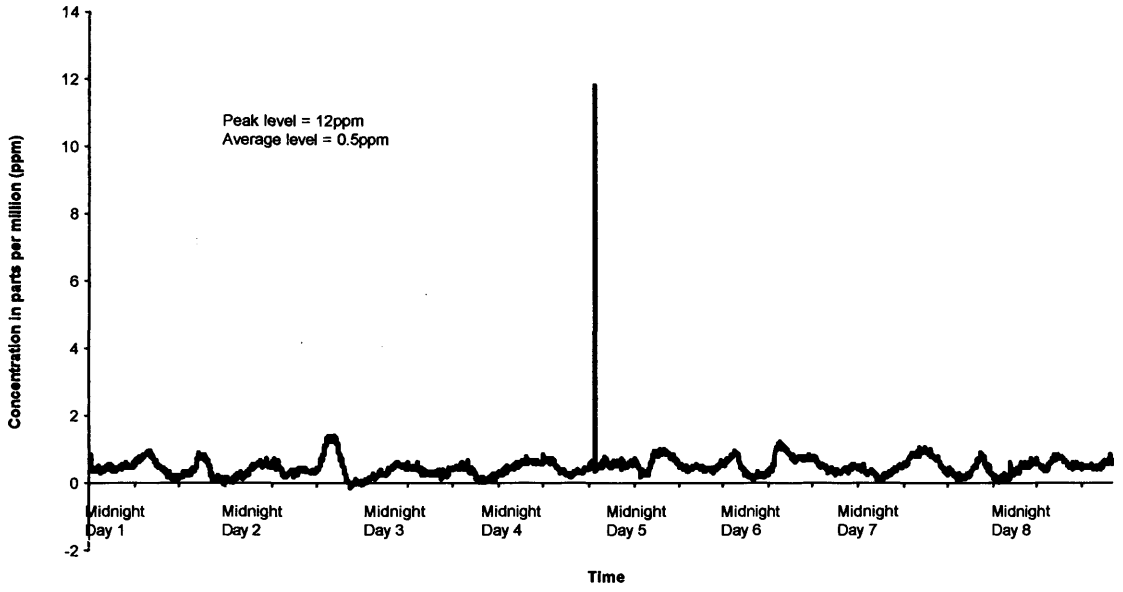




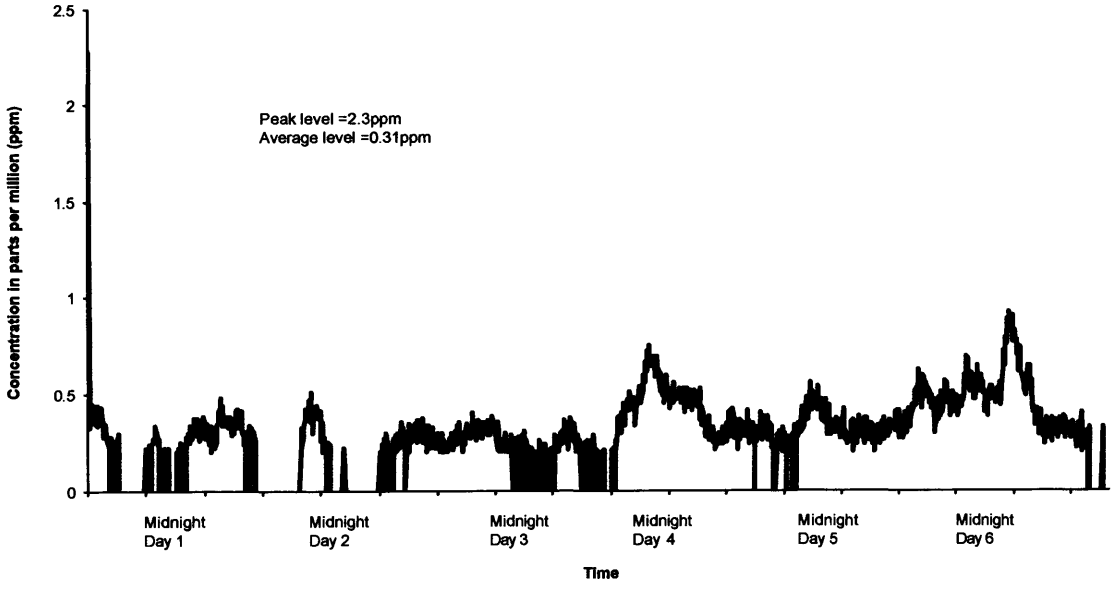
No graphs were available for Households 1 and 46.

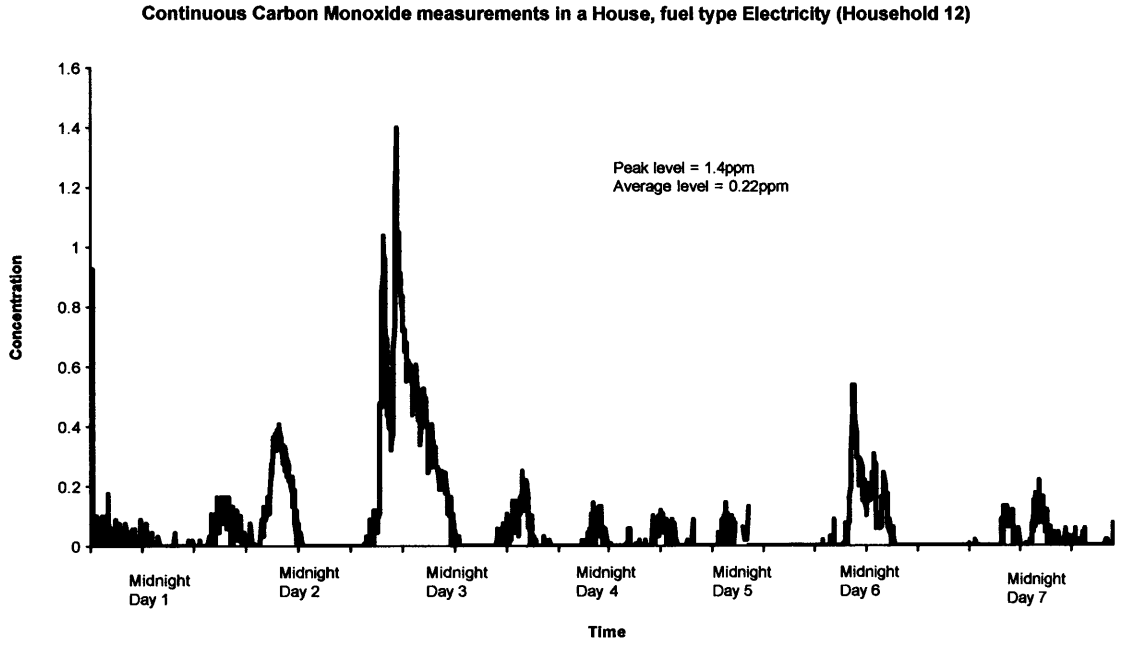
Electricity

Continuous Carbon Monoxide measurements in a House, fuel type Electricity (Household 45)



Continuous Carbon Monoxide measurements in a House, fuel type Electricity (Household 27)

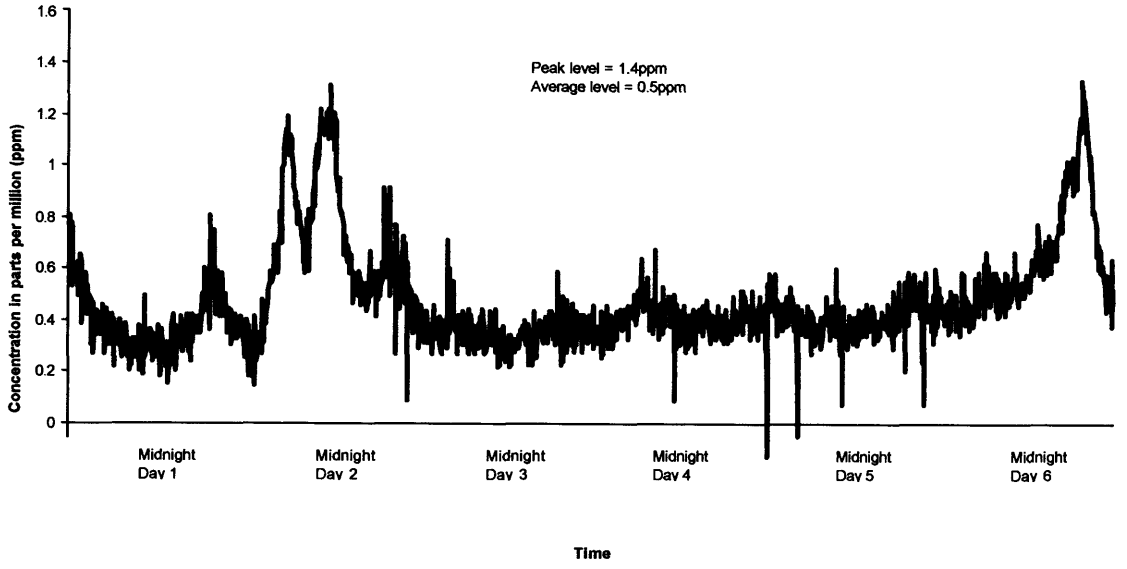




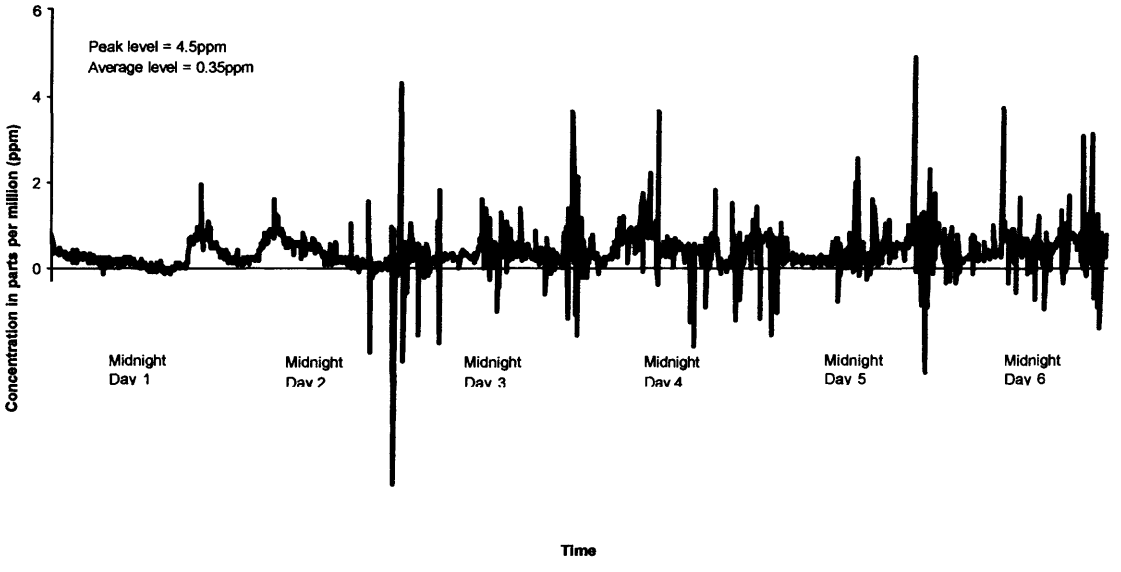
No graph available for Household 8

Liquid Petroleum Gas (LPG)

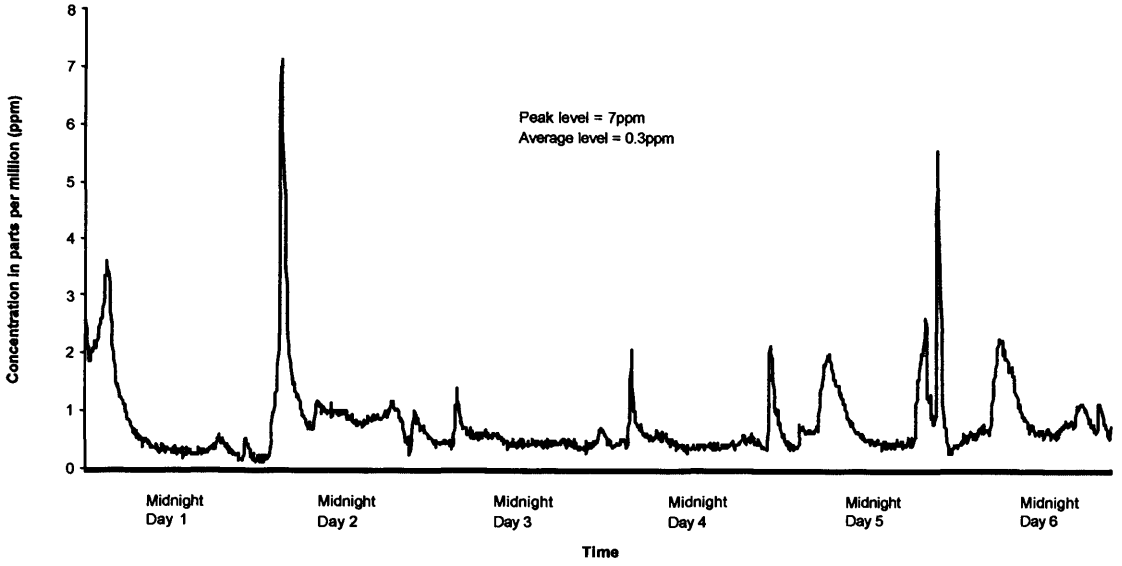
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 1)



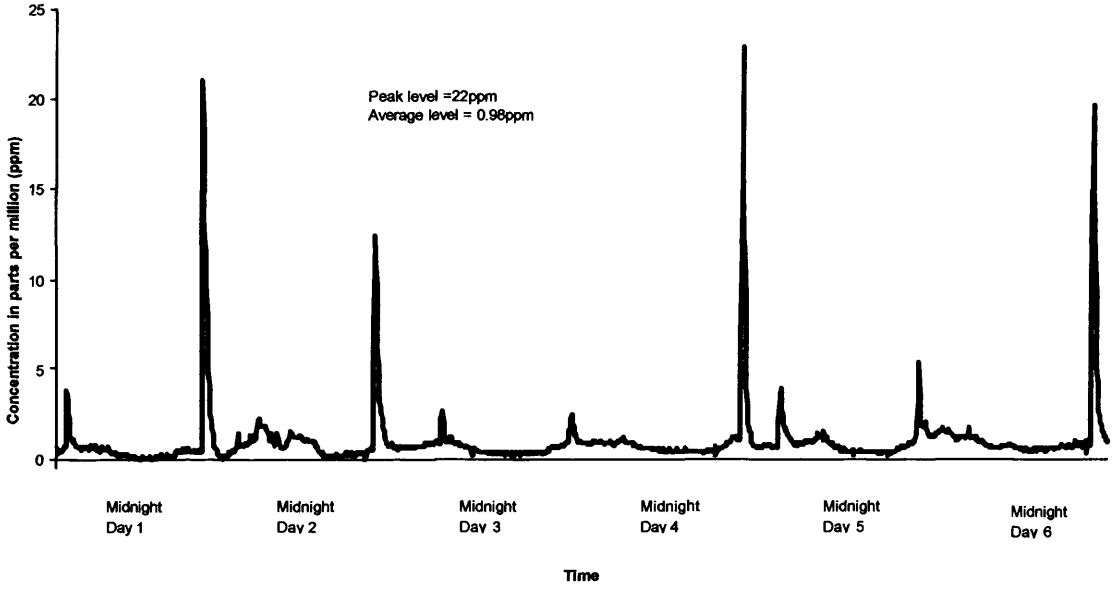
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 2)



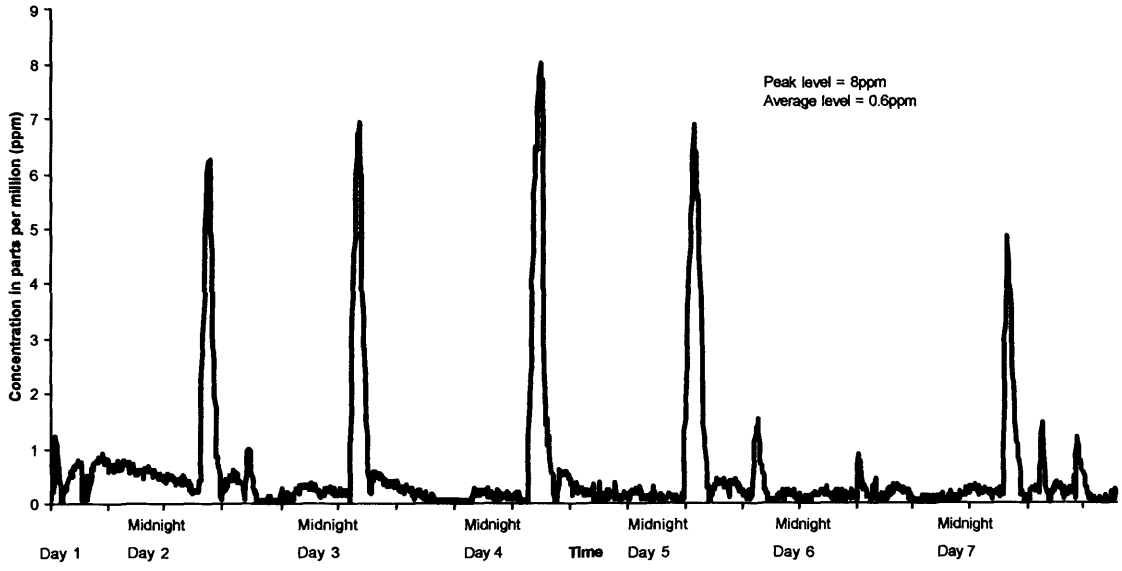
Continuous Carbon Monoxide measurements in a Static Mobile Home, feul type LPG (Household 3)



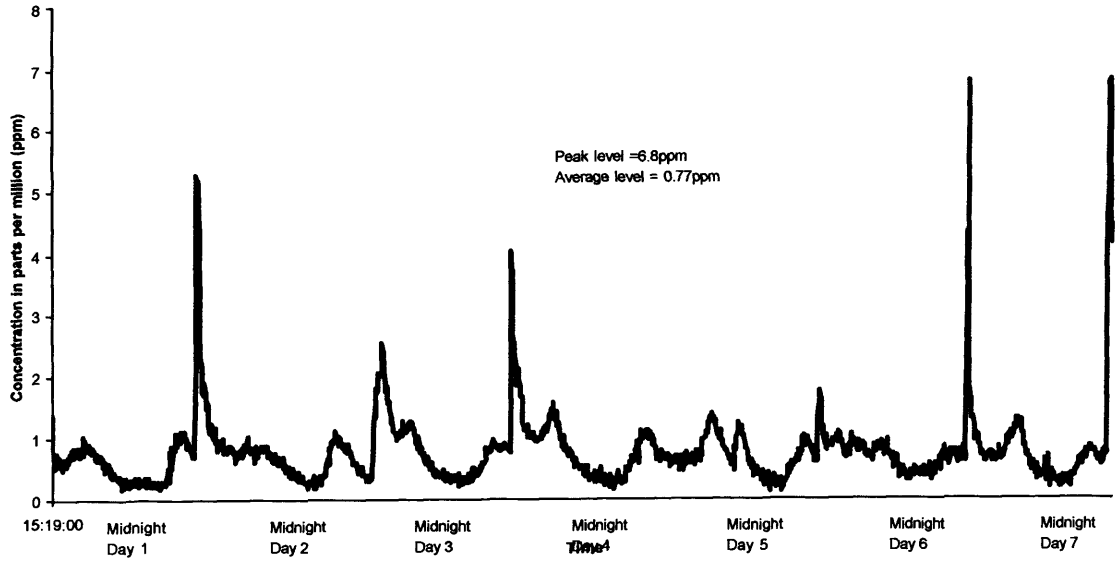
Continuous Carbon Monoxide measurements in a Static Mobile Home, fuel type LPG (Household 4)



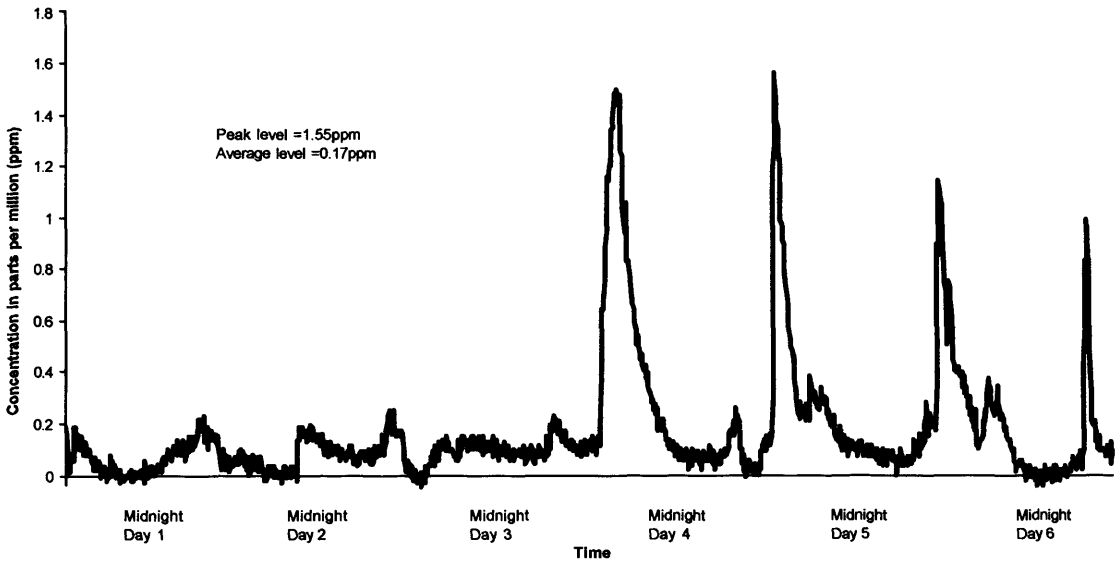
Continuous carbon monoxide measurements in a Static Mobile Home, fuel type LPG (Household 5)



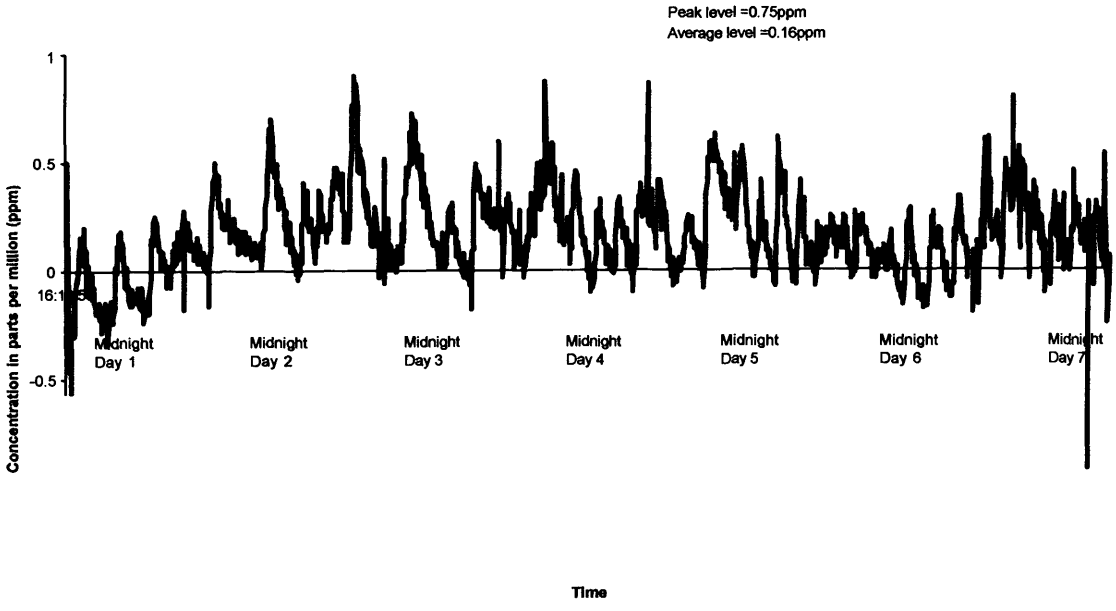
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 6)



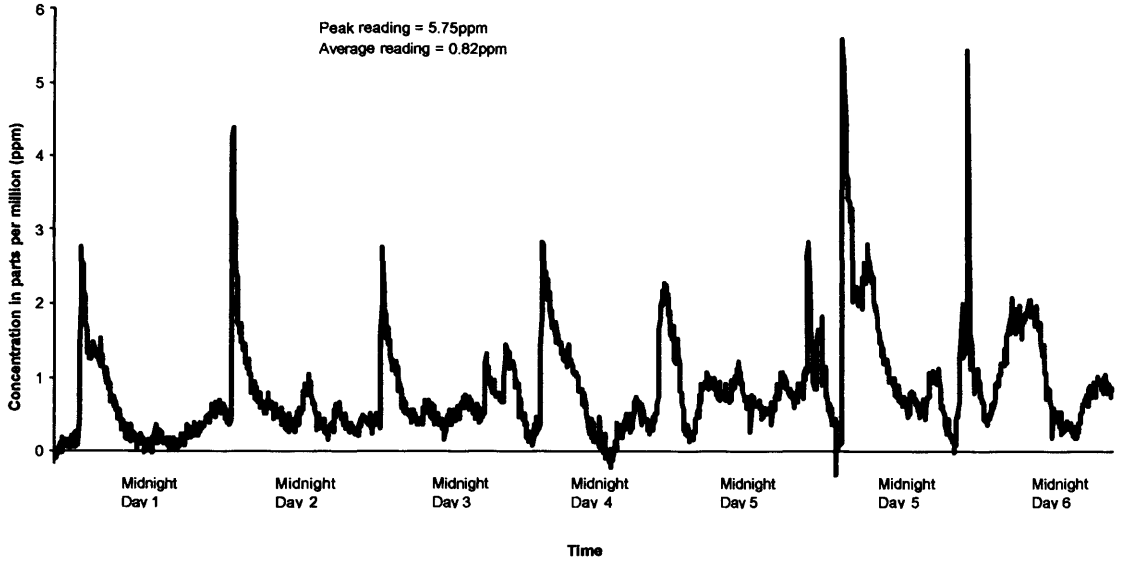
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 7)



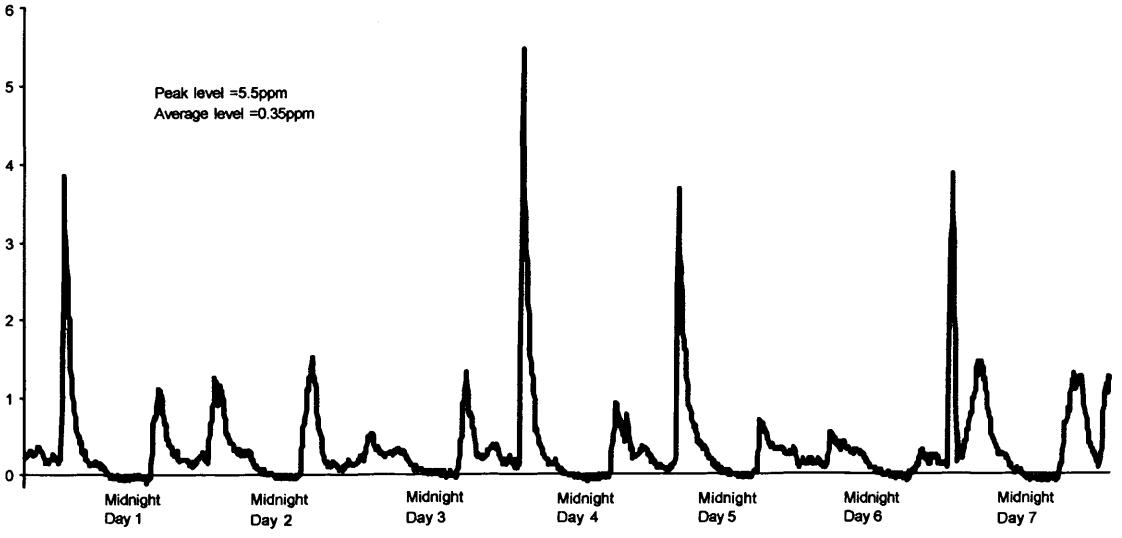
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 8)



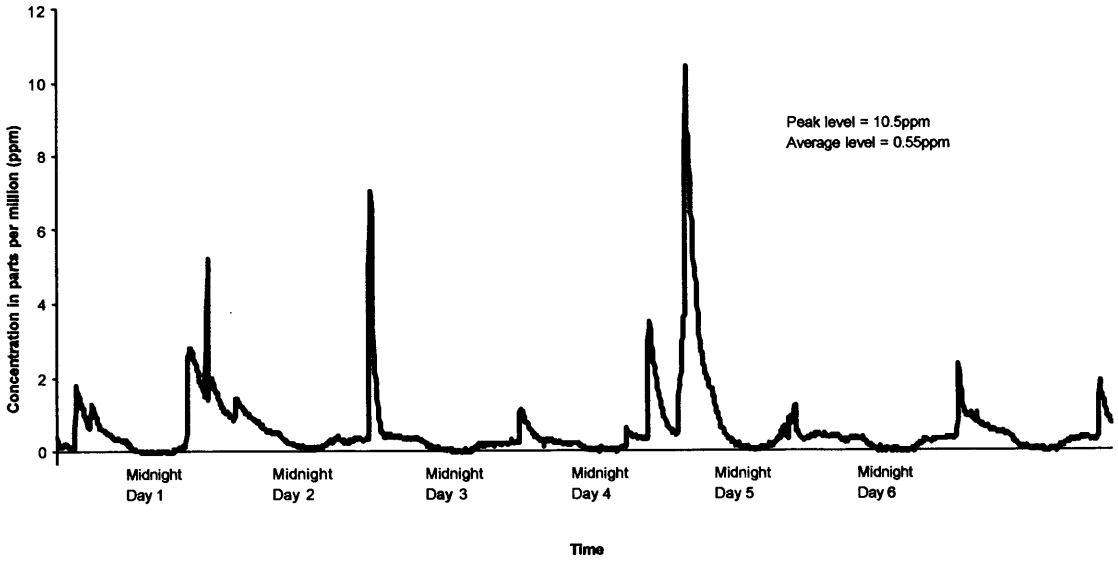
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 9)



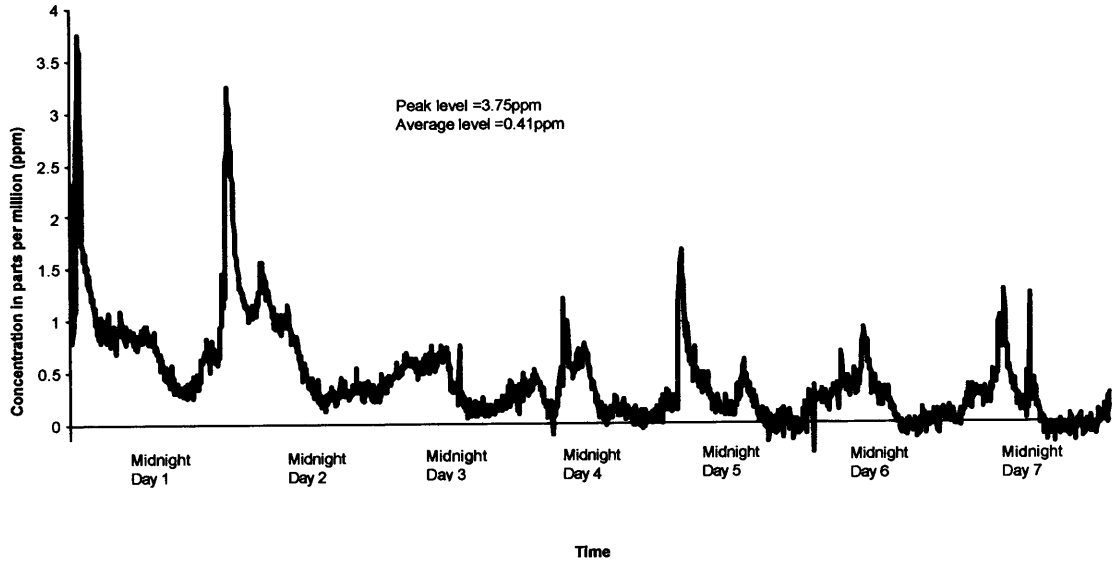
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 11)



Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 13)



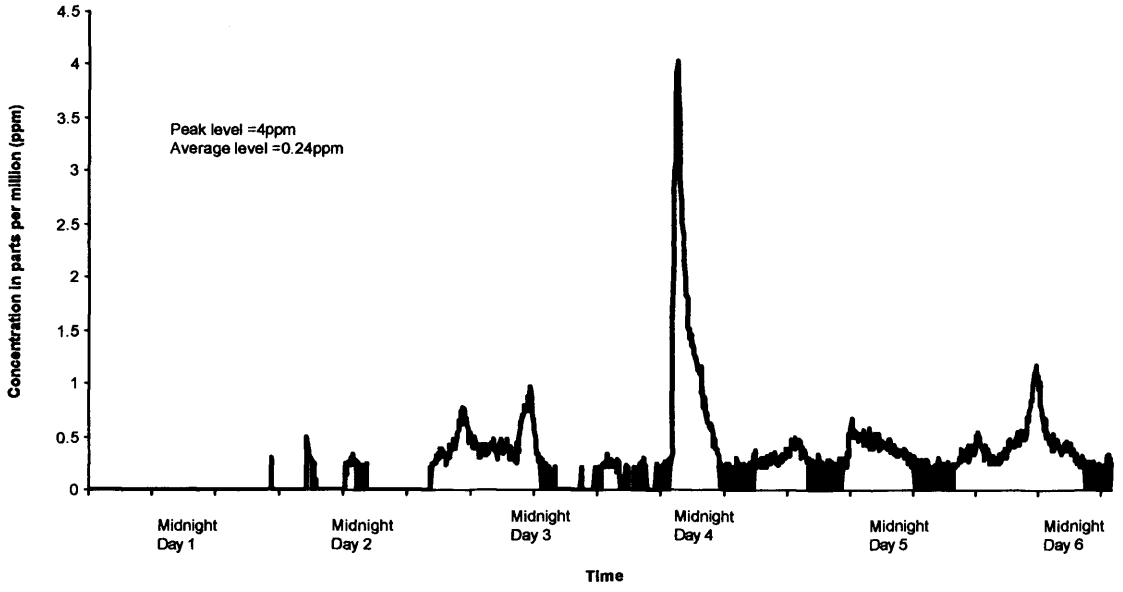
Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 14)



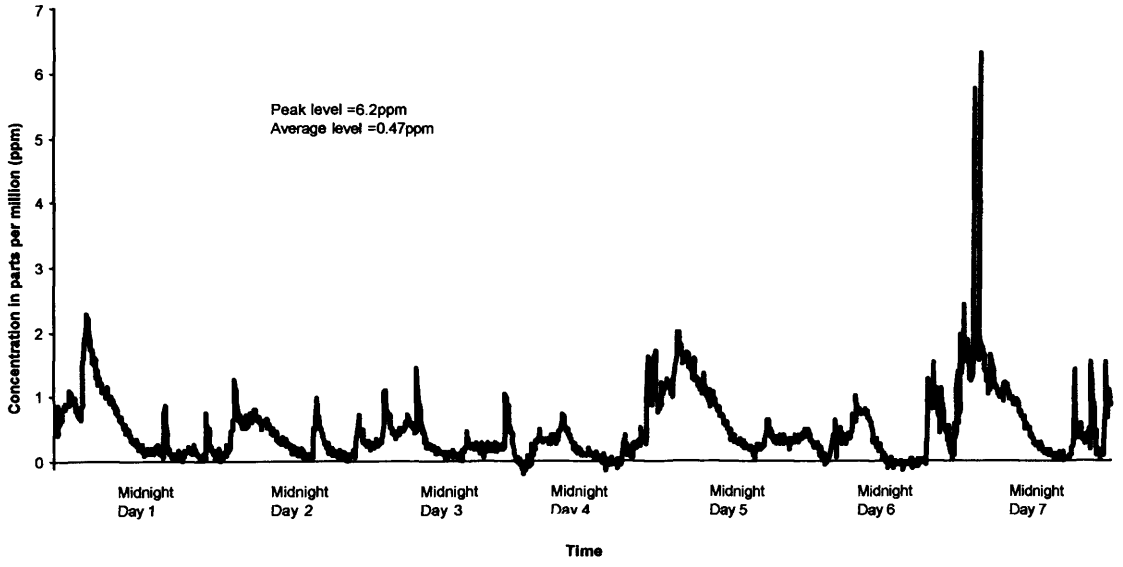
No graph available for Static Mobile Home (Household 12)

Oil

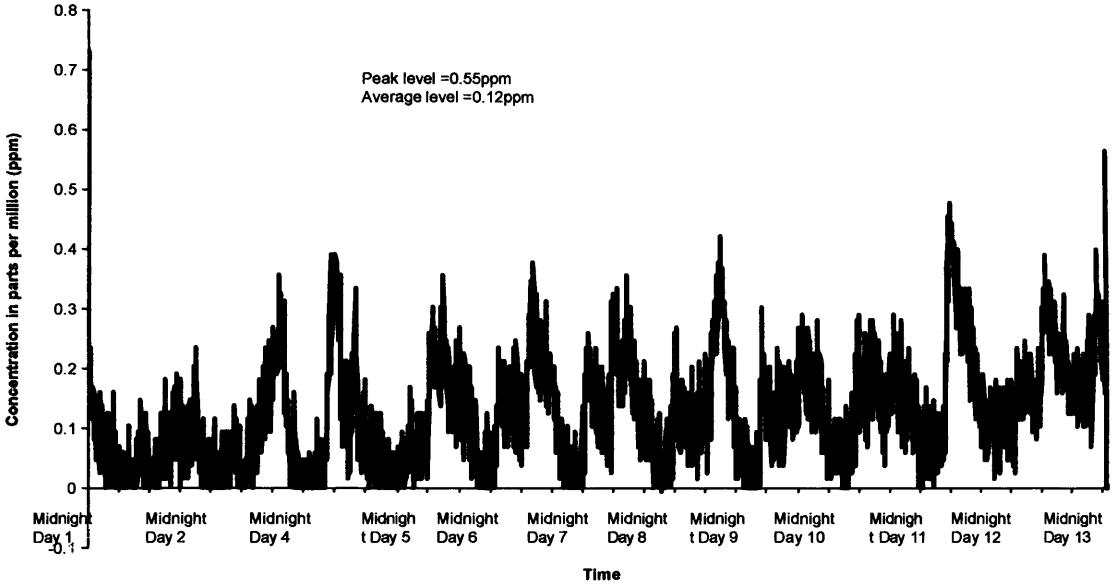
Continuous Carbon Monoxide measurements in a House, fuel type Oil (Household 3)



Continuous Carbon Monoxide measurements in a Static Mobile Home (Household 10)



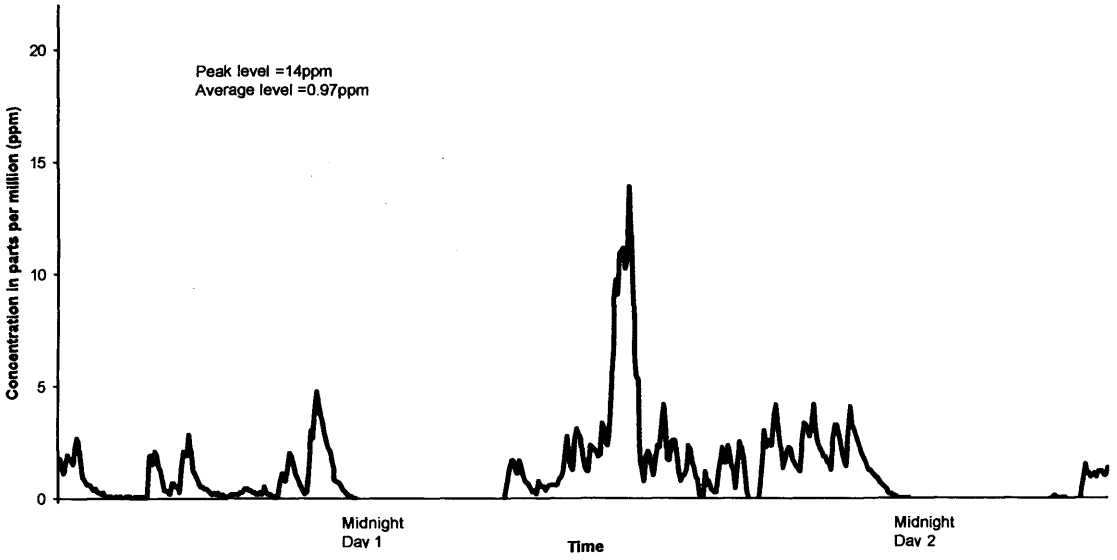
Continuous Carbon Monoxide measurements in a House, fuel type oil (Household 22)



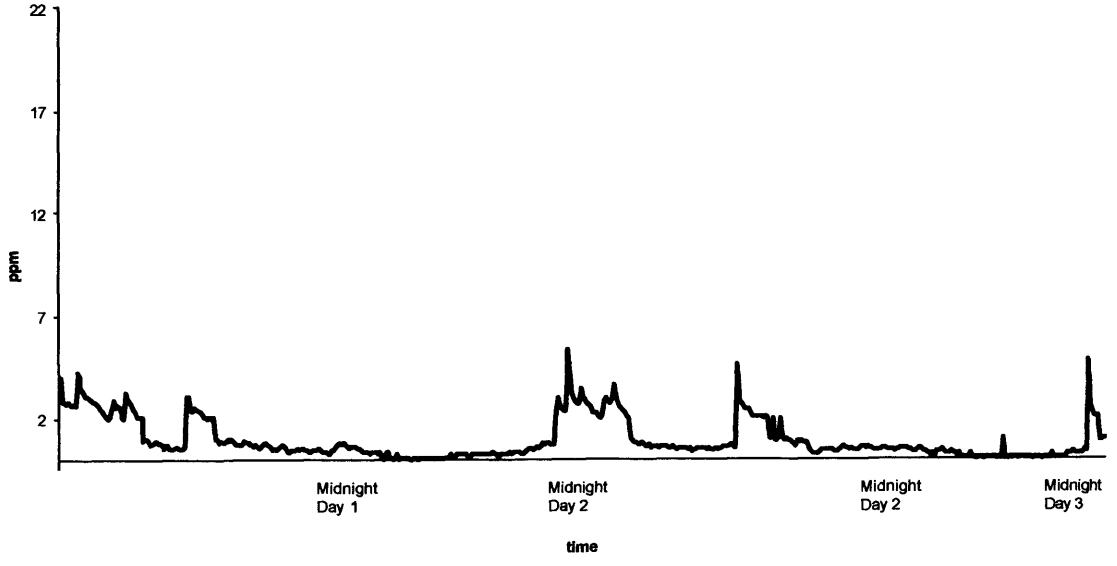
APPENDIX 5 – Environmental monitoring graphs of Carbon monoxide in smoking households grouped by heating fuel type

Gas

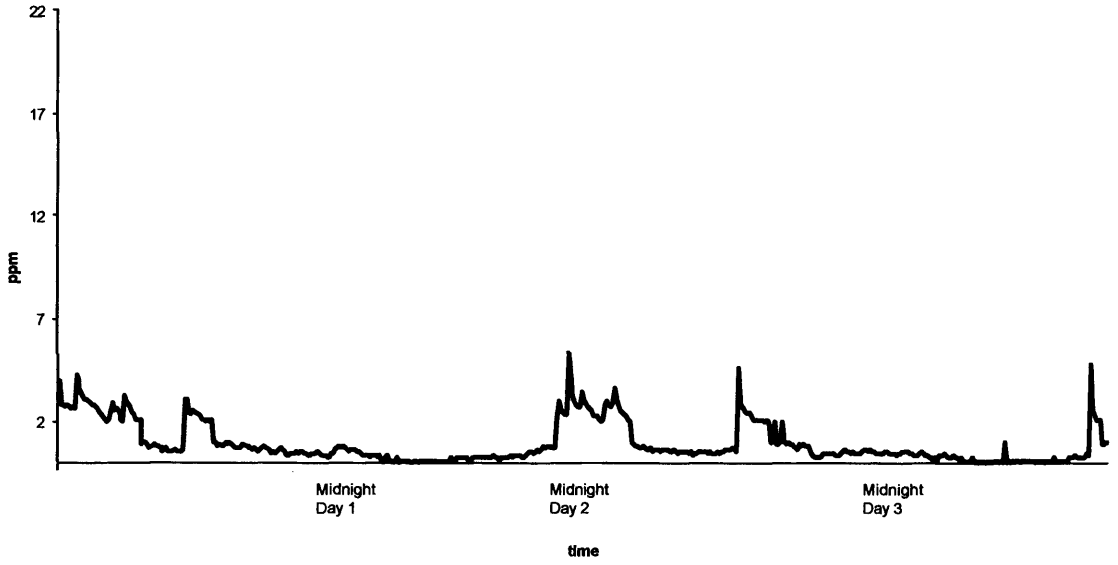
Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker Household 8)



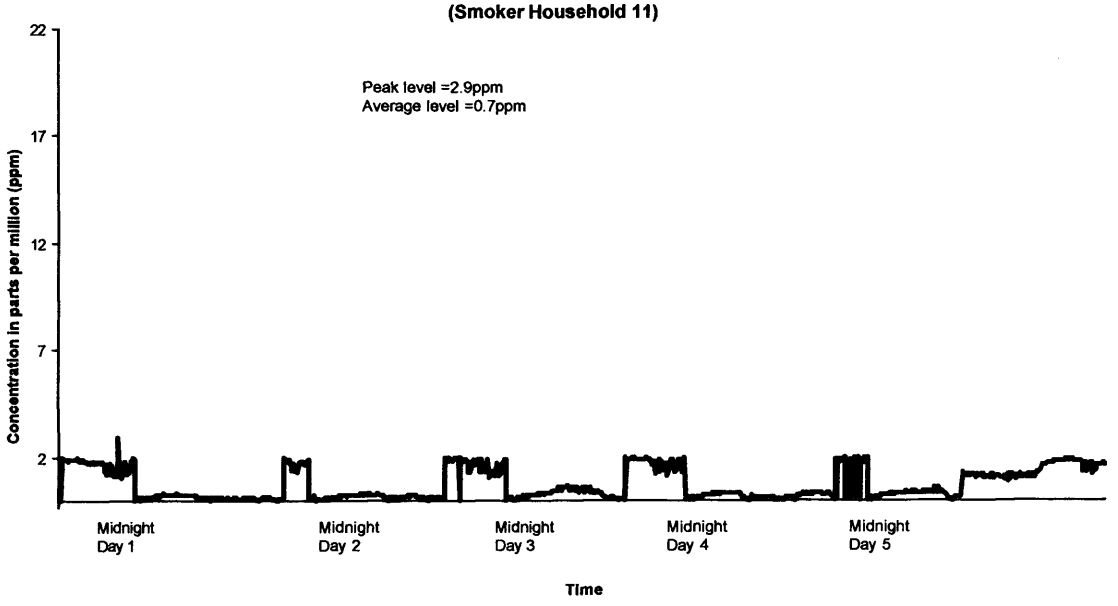
Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker Household 9)



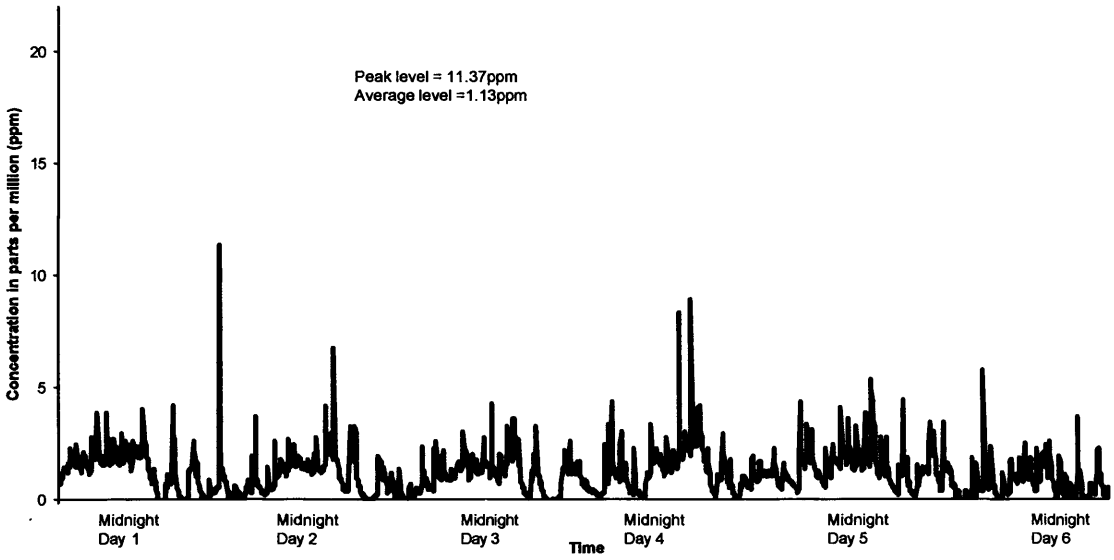
Continuous Carbon Monoxide levels in a static mobile home, fuel type gas (Smoker Household 10)



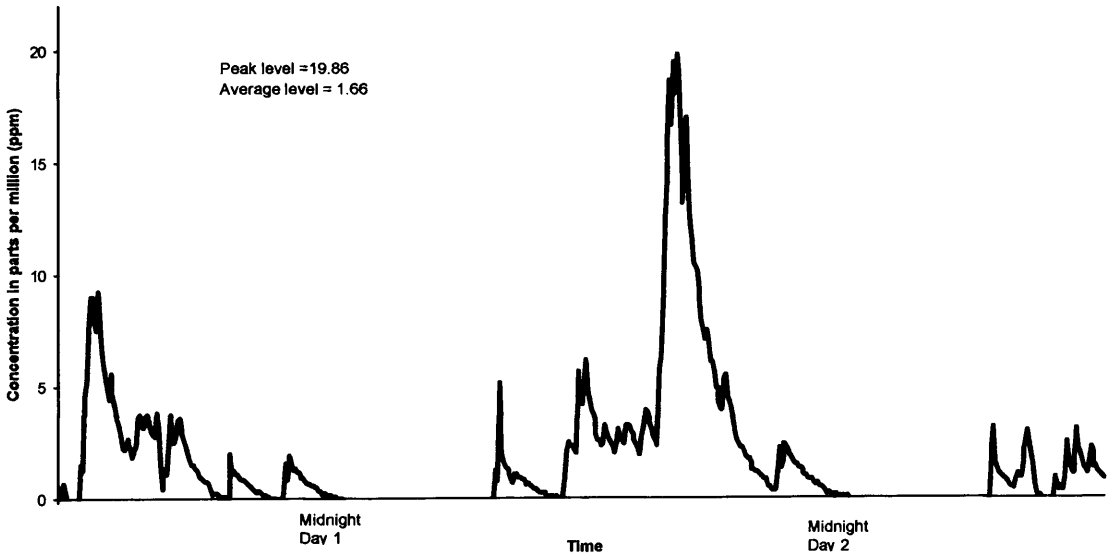
Continuous Carbon Monoxide levels in a static mobile home, fuel type gas (Smoker Household 11)



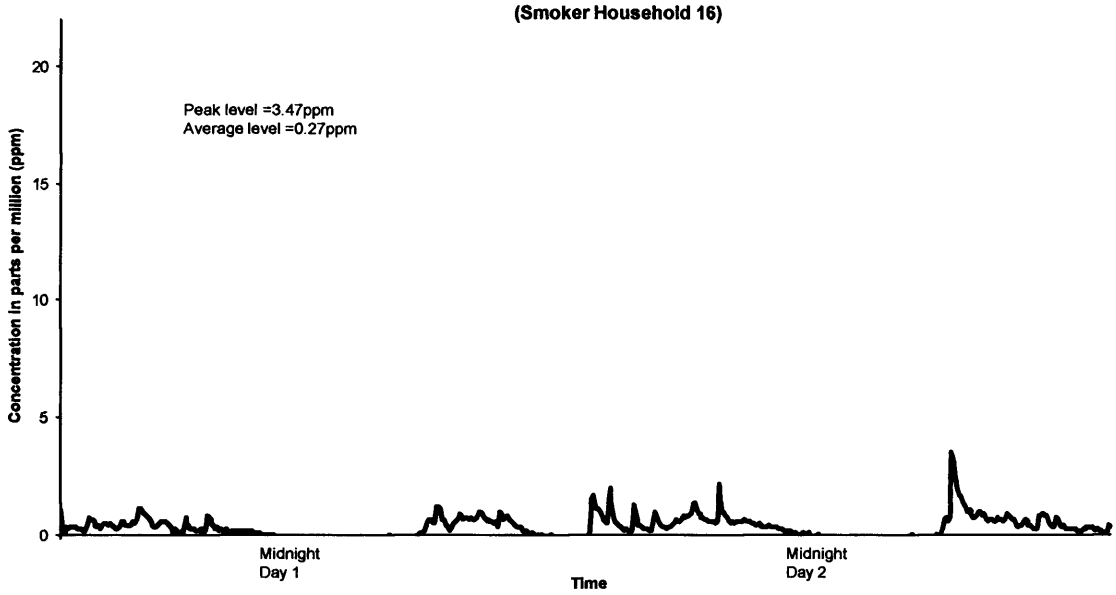
Continuous Carbon Monoxide levels in a static mobile home, fuel type gas (Smoker Household 12)



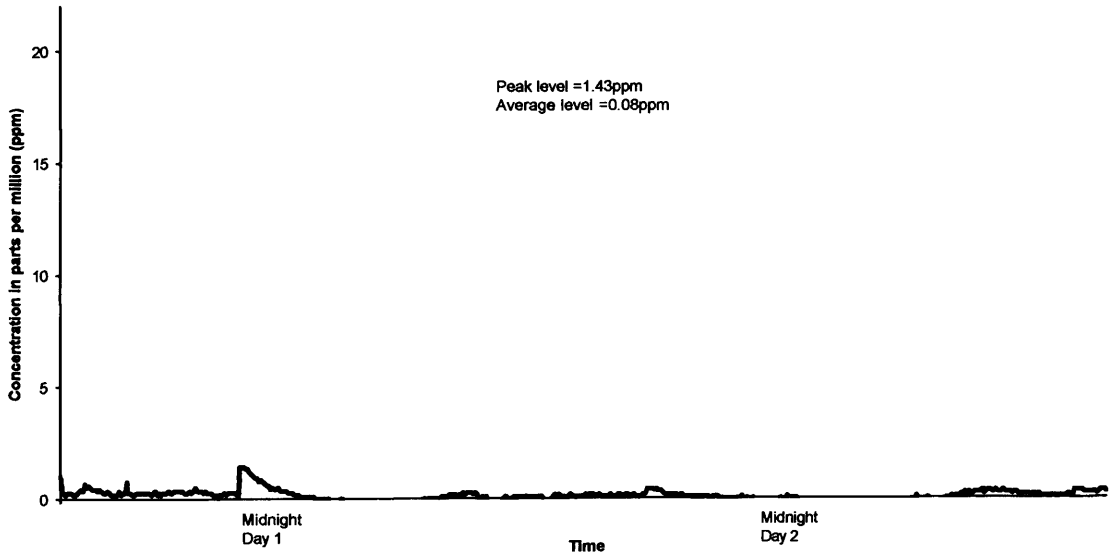
Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker household 13)



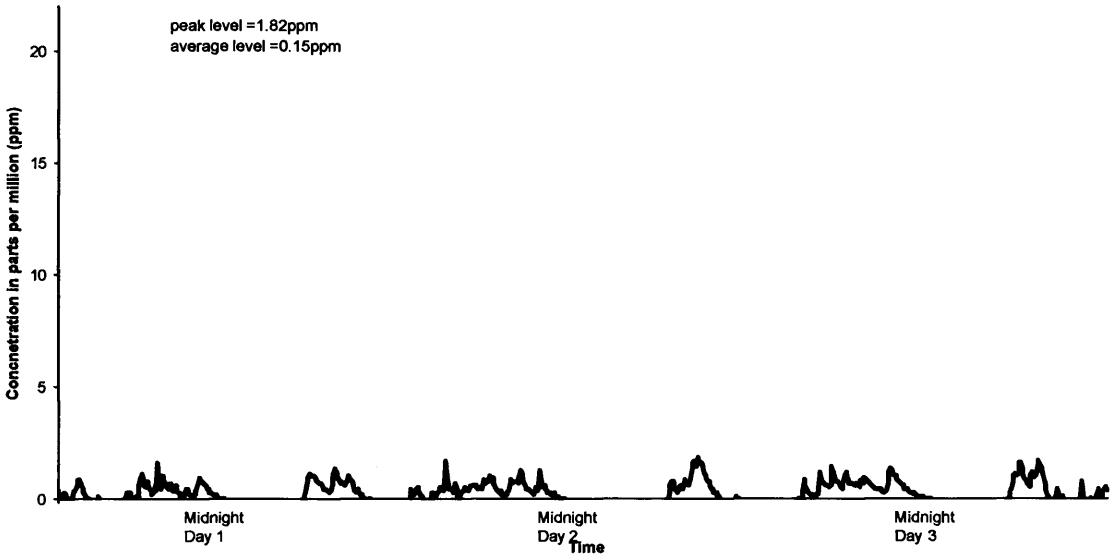
Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker Household 16)



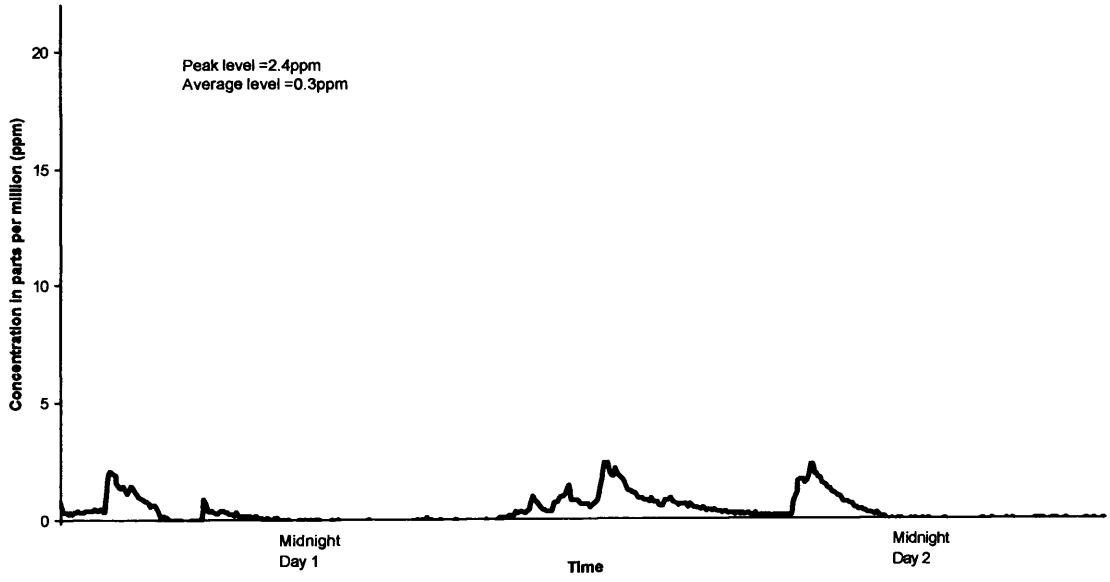
Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker household 17)



Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker household 18)



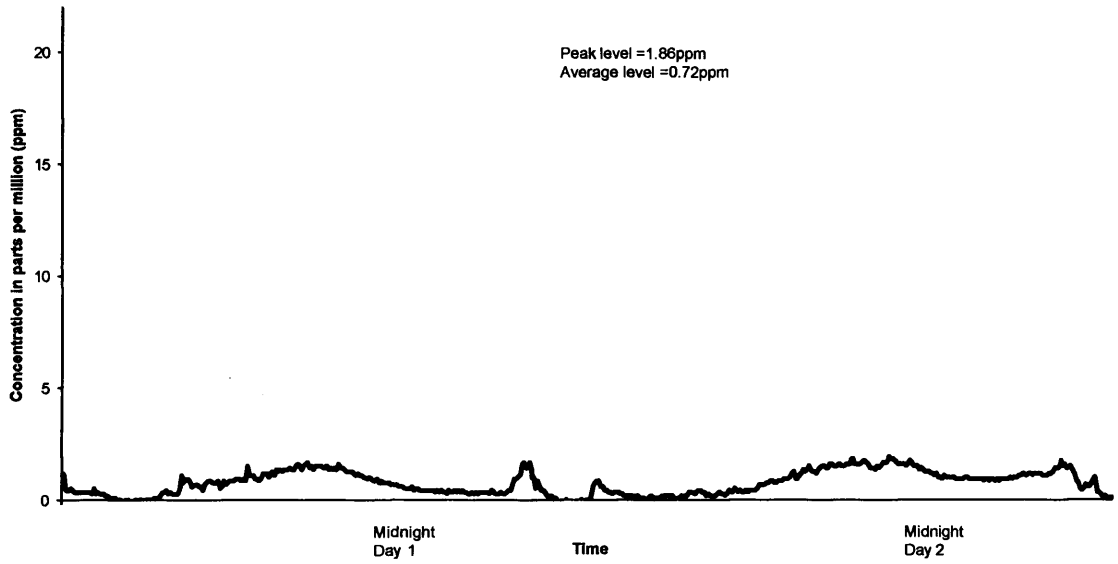
Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker household 22)



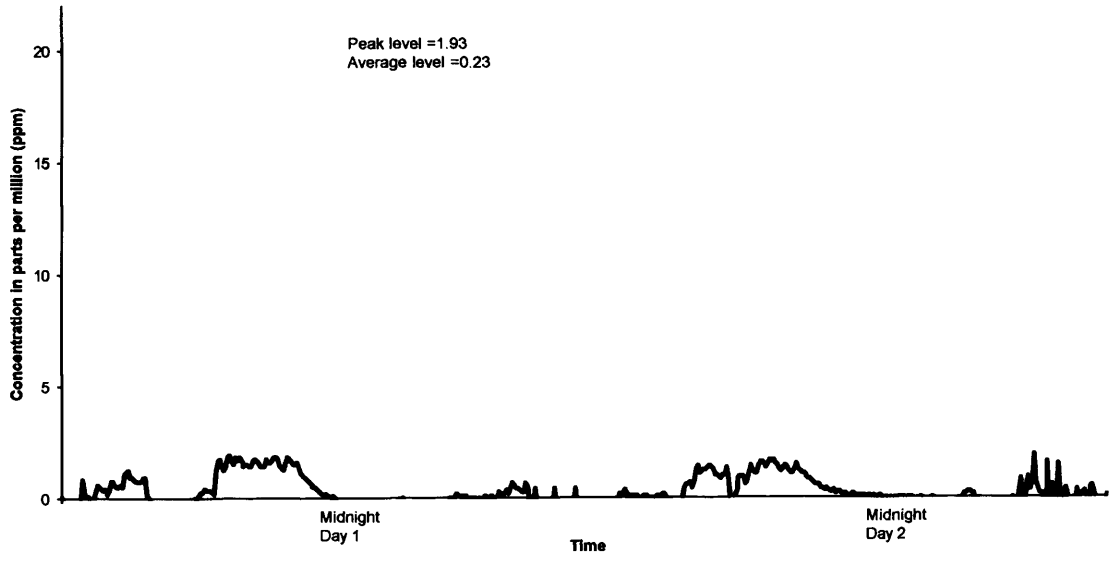
No graphs are available for Smoking Households 14, 20 and 21

Coal

ContinuousCarbon monoxide levels in a static mobile home, fuel type coal (Smoker Household 7)



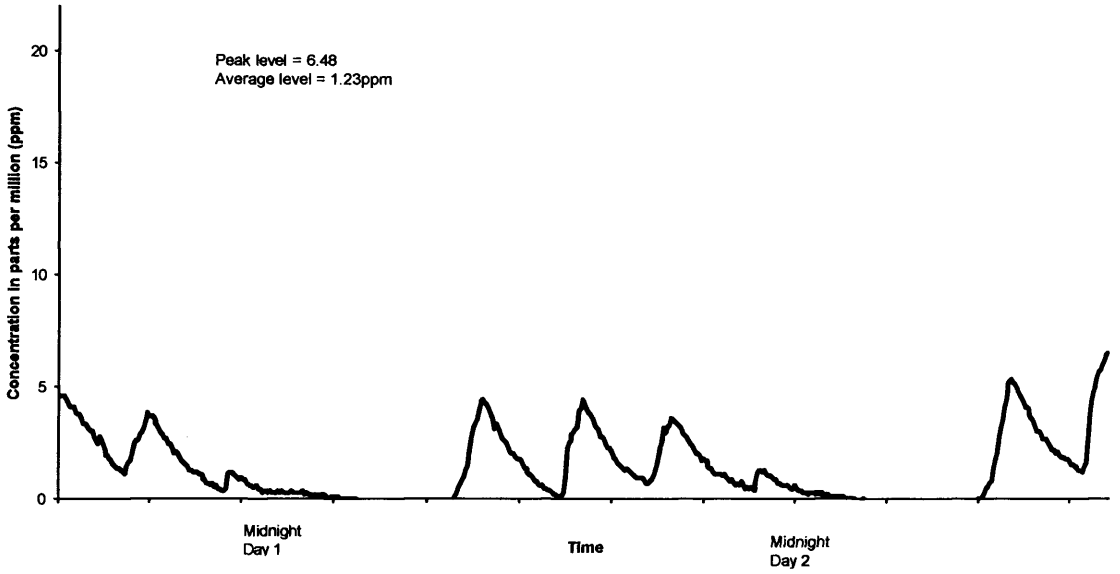
Continuous Carbon Monoxide levels in a statsic mobile home, fuel type coal (Smoker household 15)



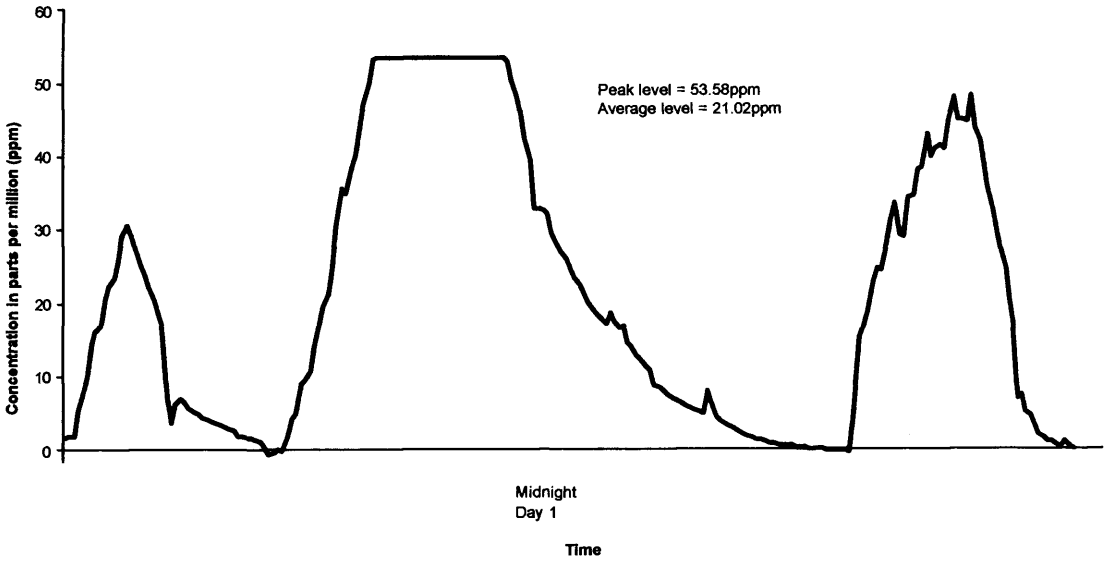
No graph available for Smoking Household 19 and 23

Liquid Petroleum Gas (LPG)

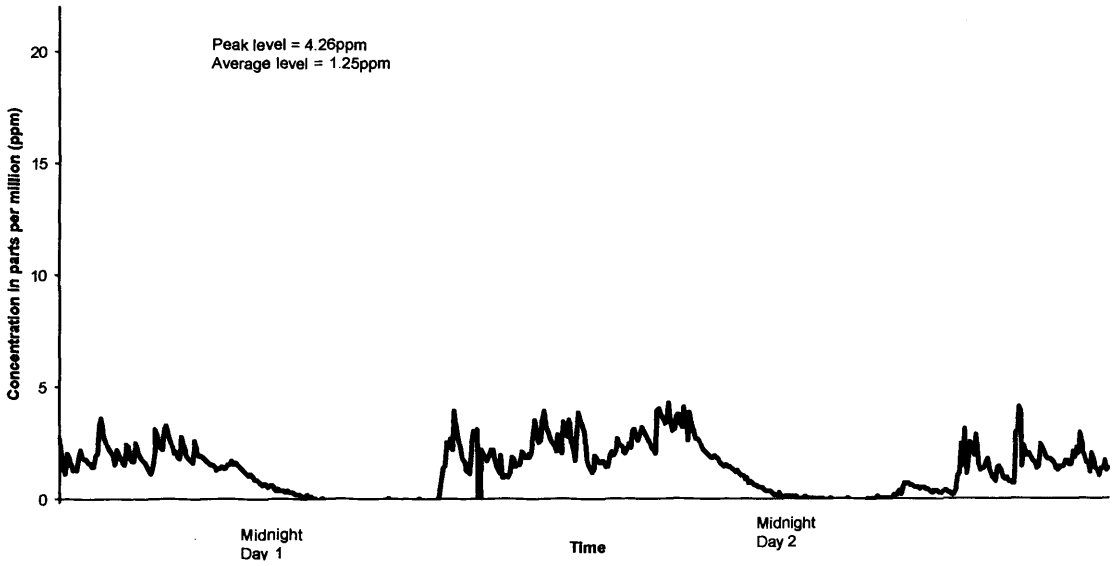
Continuous Carbon Monoxide levels in a static mobile home, fuel type LPG (Smoker Household 1)



Continuous Carbon Monoxide levels in a static mobile home, fuel type LPG (Smoker Household 2)



Continuous Carbon monoxide levels in a static mobile home, fuel type gas (Smoker Household 4)



Continuous Carbon Monoxide levels in a static mobile home, fuel type LPG (Smoker Household 5)