CRANFIELD UNIVERSITY

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SCHOOL OF WATER, ENERGY AND ENVIRONMENT

Supervisors: Dr. Derrick Crump, Dr. Chris Walton, Prof. Naresh Magan, and Dr. Khaled Salama

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ABSTRACT

Petrol station attendants' exposure to gasoline vapours while refuelling vehicles has raised health concerns, especially in tropical countries like Saudi Arabia. This is due to the increase of gasoline vaporisation by the high temperatures and related weather conditions. This represents an increase risk of inhaling more vapours than its counterpart temperate countries. Furthermore, exposure during extended working hours (12 hrs shifts), with no vapour recovery system and the handling of gasoline containing a high percentage of volumes of toxic substances (e.g. BTEXs) have not been adequately addressed previously in Saudi Arabia. Therefore, this study was designed and carried out to investigate the validity of this concern by assessing and quantifying full shift exposures to gasoline vapours during the petrol filling process. Different exposure assessment methodologies were employed and evaluated for their suitability. The study assessed the exposures of 41 attendants via passive, active, and direct reading methods at twelve petrol stations with both high and low sales in the Eastern Province of Saudi Arabia. The study was conducted during the winter and summer months to test the seasonal variation of the pattern of exposure. The effects of the quantity of gasoline sold, the locations of the stations, weather variations (e.g. wind speed, temperature, and humidity) were tested. A purpose built mini-weather stations and modified thermometres were utilized to accurately monitor the prevailing weather conditions. Forward-looking infrared (FLIR) thermal image cameras were utilised to visualise the size and movement behaviour of the vapour plumes during petrol refuelling. Furthermore, analytical lab trials were carried out to characterise the gasoline vapour component under different temperatures. These were used to propose a new OEL. The geometric means of the personal passive results for BTEX and MTBE (0.18 ppm, 0.24 ppm, 0.09 ppm, 0.18 ppm, 1.57 ppm, respectively) were found to be relatively higher than those reported previously for Europe and North America. These results are discussed in the context of the impact that such exposure will have on people involved in this industry in petrol stations in Saudi Arabia.

Keywords: Occupational health, gasoline exposure, petrol station, BTEX, petrol station attendants, gasoline vapours OEL

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ACGIH	American Conference of Governmental Industrial Hygiene
AF	Attributable Fraction
AIHA	American Industrial Hygiene Association
API	American Petroleum Institute
BEI	Biological Exposure Indices
BM	Biological Monitoring
Bpd	barrels per day
BO	Benzene Oxide
BTEX	Benzene, Toluene, Ethylbenzene and Xylene
BTX	Benzene, Toluene, and Xylene
cm^2/s	Centimetre per second
CONCAWE	Conservation of Clean Air and Water in Europe
CNS	Central Nervous System
CYP	Cytochrome P450 enzymes
DE	Desorption Efficiency
DNA	Deoxyribonucleic Acid
EU	European Union
eV	Electron Volt
ft/min	Feet per minute

FID	Flame Ionization Detector
GGV	Group Guidance Value
g/l	Gram per litre
g/mol	Gram-mol
GSH	Glutathione
HSE	Health and Safety Executive
HSIS	Hazardous Substance Information System
HQ	Hydroquinone
IARC	International Agency for Research on Cancer
IR	Infrared Spectrum
µg/l	Micrometre
µm	Micromole
µmol	Microgram per litre
mg/m^3	milligram per cubic metre
mg/l	milligram per litre
mg/g	milligram per gram
m/s	metre per second
MN	Micronucleus Assay used for DNA damage assessment
MSDS	Material Safety Data Sheet
MTBE	Methyl-Tertiary Butyl Ether
nm	nanometre
nmol	nanomol

OEL	Occupational Exposure Limit
OSHA	Occupational Safety and Health Administration
P-450	Cytochrome P450 Enzyme
ppb	parts per billion
ppm	parts per million
PID	Photoionization Detector
RCP	Reciprocal Calculation Procedure
RF	Reference Factor
RH	Relative Humidity
RNA	Ribonucleic Acid
RON	Research Octane Number
RR	Relative Risk
S-PMA	S-PhenylmercapturicAcid
Stage I	Stage I Gasoline Recovery System at the Filling Nozzle
Stage II	Stage II Gasoline Recovery System at the Holding Tanks
STEL	Short Term Exposure Limit
t,t-MA	Trans Trans Munconic Acid
TLV	Threshold Limit Value
TVOC	Total Volatile Organic Compound
TWA	Time Weighted Average
U-B	Un-metabolized Benzene in Urine
U-MTBE	Un-metabolized Methyl Tertiary Butyl Ether

VOC Volatile Organic Compound

WEL Workplace Exposure Limit

Abbreviations are introduced within the text and re-introduced for each individual chapter

LIST OF EQUATIONS

CHAPTER 1

1. INTRODUCTION

1.1. Saudi Arabian Petrol Stations

Petrol stations in Saudi Arabia are full service stations where all fuel pumping and refuelling processes are performed by employed station attendants. The majority of the attendants work ten to twelve hour shifts remaining in the same area, and their responsibility is to pump gasoline into vehicles as the vehicles approach the station to re-fuel. A small minority of stations are individually owned, but the majority of petrol stations are owned by different companies that have a number of stations distributed throughout the country. The facility design specifications follow governmental regulations and standards that are managed by the Civil Defence Directorate of the Saudi Ministry of Interior. The operational eligibility and qualification standards are controlled by the Saudi Ministry of Municipality and Rural Affairs (MOMRA, 2014). As per literature review and interview meetings with representatives from concerned governmental sectors and the management of major service station companies, it was found that there were no occupational exposure monitoring programs carried out or available for public petrol stations or the attendants (Eastern Province Civil Defense, 2012; Eastern Province MOMRA, 2013).

The gasoline is brought to the petrol stations by tanker trucks and emptied into underground storage tanks. Consequently, this causes exposure concerns regarding the attendants. Such processes take around 30 minutes and are usually performed by one worker

assigned during the shift. No gasoline vapour recovery system is required by the Saudi government and thus none are fitted with such systems.

Studies in other countries have recorded health symptoms that were reported by petrol station attendants, such as headaches, fatigue and throat irritation which have been thought to be related to the exposure to gasoline and its components (ATSDR, 2014; Tunsaringkarn et al., 2012). These reported health symptoms could be regarded by occupational health practitioners and researchers as an indication that these workers might be overexposed to harmful gasoline constituents. Questions have been raised in Saudi Arabia among the public and in newspapers, such as the Asharq Al-Awsat, concerning the health effects that are related to the exposure of petrol station attendants to gasoline.

Public concern about the health risks related to petrol station attendants in Saudi Arabia needs to be addressed via a thorough exposure investigation by competent occupational health practitioners (Sharayah, 2007). In an attempt to meet this requirement, a current study (meta-analysis) was conducted to review literature pertaining mostly to international gasoline exposure at full service stations; the goal of this study (meta-analysis) was to design and implement an assessment of the various exposure studies. The literature review includes gasoline component exposure assessments via air sampling and biological monitoring. Based on the reviewed literature; benzene, toluene, ethylbenzene and xylene (BTEX) and the fuel additive methyl tertiary butyl ether (MTBE) were found to have been assessed in the majority of the gasoline occupational exposure assessment studies, although surprisingly none had been carried out for public petrol stations in Saudi Arabia. Therefore, these components have been included in the preliminary field exposure assessments that are ongoing in Saudi Arabia

for the present study. The participants for the current study and who accepted the invitation to participate were 50 expatriate males from different East-Asia countries with age range from 20 to 45 years. Some important information about the participated petrol station attendants was collected via questionnaire survey as summarised in Table 0-1. Most of the participants' age ranged from 30-35 years. Most of the participated subjects have more than five years of experience in the petrol stations. Furthermore, the questionnaire survey revealed that none of the petrol station attendants wear personal protective equipment such as respirators or gloves while pumping gasoline fuel. Around half (24) of the study participated attendants live in accommodation that is the vicinity of the petrol stations. Around quarter (12) of the sampled attendants smoke cigarettes.

1.2. Manufacturing of Saudi Gasoline and Local Distribution

Gasoline is the major automotive fuel consumed in Saudi Arabia. Diesel fuel, in contrast, is much less consumed as it is mostly used for trucks and buses. Diesel pumps comprise about 25%, (2/8) of the total number of gasoline pumps at any petrol station. Automobiles are the main transportation in Saudi Arabia for private and public use (Hamid, 2001). Taxies are the major form of public transportation used and are mostly gasoline operated vehicles.

Nevertheless, most people are inclined to drive their personal cars because it is more convenient and the gasoline is reasonably inexpensive. One of the motivations also is that the price of gasoline in Saudi Arabia is as low as 0.60 Saudi Riyals/litre compared to £1.34/litre in the United Kingdom (=£0.09/litre in 2015). The Saudi gasoline production rate has increased annually to meet the demand. Over the course of twenty-four years, from 1986 to 2010, the motor gasoline production has increased from 202,000 barrel per day (bpd) in

1986 to 375,550 (bpd) in 2010 (i.e. 86% increase) (Mundi, 2014). The increase in gasoline production is mostly due to the domestic consumption within Saudi Arabia. In addition, production increased due to an increase in international demand over the years.

In contrast, the consumption of motor vehicle gasoline fuel in Saudi Arabia has increased by more than 173% from 151,600 bpd in 1986 to 414,650 bpd in 2010. Both the production and consumption of gasoline in Saudi Arabia are shown in Figure 1-1 (Mundi,

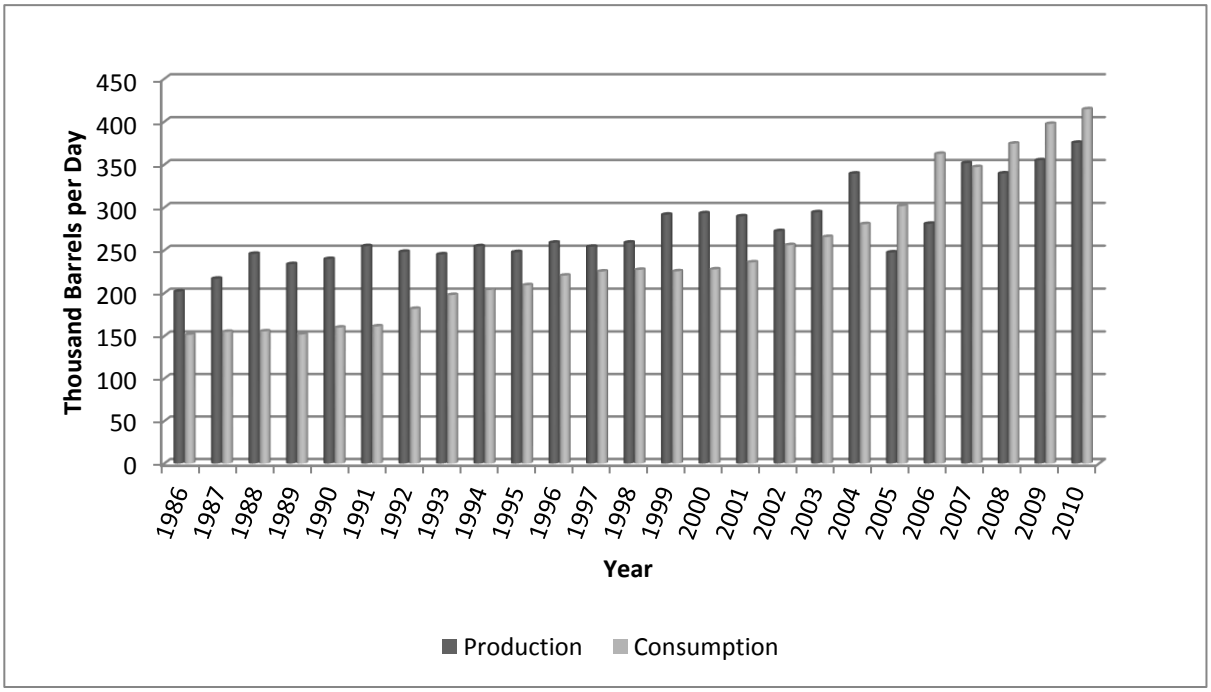


Figure 1-1: Comparison of gasoline production and combustion in Saudi Arabia (Mundi, 2014).

2014).

The consumption of gasoline fuel in Saudi Arabia has exceeded the production over the past few years. This reflects the increased demand for gasoline sold at petrol stations and thus contributes to the possible increase of exposure risks.

Furthermore, recent economic statistics show that the demand for gasoline fuel consumption increased in 2012 by 6% compared to previous years. This required the

importation of an additional 80,000 bpd of gasoline adding to the previous daily rate of 500,000 bpd in late 2011, to make a total daily consumption of 580,000 bpd (Bloomberg, 2012). This shows a vast growth in demand for gasoline, which has triggered an increase in the production rate and the building of additional petroleum refineries to meet the expected future local demand. Ground water pollution investigators have pointed out that the number of petrol stations in Saudi Arabia have reached around fourteen- thousand stations within the country (Al-Qahtani, 2012).

Saudi Arabian gasoline may be defined as a complex mixture of relatively volatile hydrocarbons, from low molecular weight compounds (naphtha), extracted from crude oil in distilled columns in refineries. This complex mixture is blended with predetermined quantities of the anti-knock fuel additive, methyl tertiary butyl ether (MTBE) to produce automotive gasoline fuel. Saudi Arabia's gasoline is produced in two octane grade ratings: Research octane numbers (RON) 91 and 95, as determined by the American Petroleum Institute (API) standard.

Gasoline is a 100% volatile mixture because it consists of components that readily vaporise into the atmosphere due to their high vapour pressure (British Petroleum, 2012; Hess, 2004). Each mixture is calibrated to meet specific market and environmental criteria of the RON, designed and recommended by the American Petroleum Institute (API). Modern gasoline contains an approximate average of 14% aromatics, 80% paraffin (aliphatic), 6% olefins, and small amounts of alcohols, ether, detergents, corrosion inhibitors, antioxidants, and oxygenates (Keenan et al., 2010). Chemically comparison of gasoline vapours and liquid is that the higher the boiling point of the gasoline component hydrocarbons, the lower the

fraction that would be in the vapour status at a specified temperature. For example, the benzene percentage as a component in gasoline liquid is greater than twice that in vapour (Berglund and Petersson, 1990).

The two gasoline types of RON 91 and 95 in Saudi Arabia contain similar volume percentages for some of these components. For example, aromatics found in Saudi Arabian gasoline, particularly BTEX and the fuel additive MTBE, differ in concentration slightly from the British gasoline in the (Anderson, 2007; Chilcott, 2007).

Overview of the Weather Conditions in Saudi Arabia

Saudi Arabia is an example of a warm climate country; hosting long summers and short winters throughout most of its regions. In the eastern region of Saudi Arabia, the monthly average temperature is around 42°C in the summer (May-October) and 26°C in the winter (November-April). The temperatures increase significantly during the months from April to

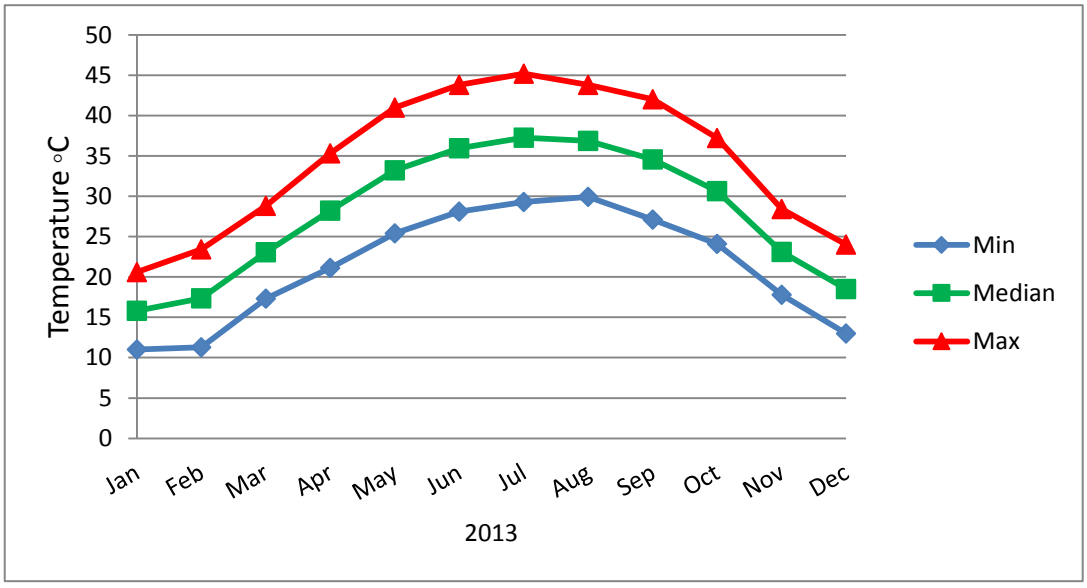


Figure 1-2: Weather monthly temperature variation during the year (Weather Online Ltd., 2014)

October. The highest temperatures occur during the months of June, July, August, and

September (Weather Online Ltd., 2014). Studies have shown that high temperatures increase the gasoline vapour exposure risks due to the increase of the liquid vaporisation as indicated in Section 1.4. Figure 1-2 illustrates the variation in the annual weather temperatures for the Eastern Province of Saudi Arabia.

In the eastern part of Saudi Arabia where this work was carried out, the vaporization effect is further influenced by Arabian Gulf weather conditions, especially the high prevailing relative humidity (Al-Garni, Sahin and Farayedhi, 1999). The wind direction predominantly blows from North-West (NW) and it is stronger during the winter than the summer (Khonkar, 2009). The monthly estimated average of wind speed throughout the year is 4.4 metre per second (m/s) with a range from 3.6 to 5.1 m/s. This is similar to many other parts of the country, except that they are less humid. In Riyadh, for example, the monthly averaged temperature is 33°C (Weather Online Ltd., 2014).

1.3. Influence of Ambient Temperature on Risk of Exposure to Gasoline Vapour

Studies have indicated that petrol station attendants' exposure risk is likely to be increased by high temperatures due to the increase volatilisation of the gasoline, especially, in tropical countries (Batterman et al., 2005; Kountouriotis, Aleiferis and Charalambides, 2014; Periago, Zambudio and Prado, 1997). In such countries, petrol station workers are likely to inhale more of these volatile compounds than in other counterpart countries where temperatures are <30°C (Pandya et al., 1975). The variance in gasoline loss from storage tanks was estimated by one of the tested petrol station in Dammam city to be around 0-20 litres during the winter and 90-100 litres during the summer (Nezar, 2014) which was also roughly agreed by other petrol stations in the same city. This clearly suggests that there is

probably an increase exposure risk to fuel vapour at petrol stations in Saudi Arabia, particularly during the hot extended summer weather conditions. Many fractions of the gasoline fuel are highly volatile and toxic, and vaporise even at relatively low temperatures (McDermott and Killiani, 1978). Aromatic hydrocarbons (e.g. BTEX) are good examples of such components.

1.4. Aims and Objectives of Current Study

Aims:

Exposure of petrol station attendants in Saudi Arabia has not been assessed previously under factors such as the high ambient temperature, 12 hour shifts and the higher content of benzene in gasoline which make the situation in Saudi different from other countries where exposure data is available. Therefore, the aim is to test the hypothesis that the attendants in Saudi Arabia are exposed to higher levels of gasoline vapours that exceed acceptable international levels.

Objectives:

To evaluate different occupational exposure assessment methodologies to determine their applicability to obtain accurate and appropriate quantitation of gasoline vapour exposure of attendants in Saudi Arabia under unique factors through the following:

1. Conducting a thorough study to assess and quantify the exposure of workers representing a range of conditions of exposure within the gasoline industry (chapter 4, 5, and 6).

2. Assessing exposure data to determine the health risks of petrol station attendants arising from harmful volatile substances in gasoline (e.g. risk of cancer, CNS impairment, and other effects) (chapter 4, 5, and 6).
3. Analytically developing occupational exposure limits (OEL) for gasoline vapour exposure, based on simulated exposure conditions (chapter 3 and 7).
4. Examining the need for further risk prevention and control strategies, to assure a safe and healthy work environment for employees, and to recommend appropriate actions, including the need for further specific research (chapter 4, 5, 6 and 9).
5. Disseminating the findings, recommendations and the assessment procedures of this research, particularly across local scientific and governmental bodies in Saudi Arabia, and as well as in the international community (appendix K).
6. Developing and testing strategies and different methodologies of air, exhaled breath, and biological benzene urine metabolites samplings for the assessment of exposure to toxic gasoline vapours, especially, for petrol stations in the eastern region of Saudi Arabia (chapter 4, 5, 6 and appendices B and C).

1.5. Outline of Thesis

Following chapter 1, which describes the extreme weather conditions where the study has been carried out, the design of a petrol station facility and operational specifications and the manufacturing and distribution of the gasoline fuel, this thesis consists of eight additional chapters as follows:

Chapter 2: Previous research and studies in the area of occupational exposures to harmful compounds in gasoline and its associated health hazards are reviewed. Various toxic effects and diseases are covered. Exposure to gasoline, as a mixture for biological additive effects are also discussed. Factors affecting personal exposures are highlighted. Different exposure assessment methods of active, passive, direct reading techniques and biological sampling are elaborated in detail. Lastly, a brief discussion about gasoline vapour recovery systems and efficiencies are pointed out.

Chapter 3: This includes laboratory analysis of the gasoline compounds in liquid and vapour under simulated ambient temperatures, corresponding to the summer and winter seasons in Saudi Arabia. The properties and behaviour of the chemical constituents in the vapour were characterised and the individual volatility calculated at different trial temperatures. Regression analysis was also conducted to estimate the fuel temperatures in vehicle tanks to determine the compound concentrations with reference to particular known problem constituents.

Chapter 4: In this chapter, exposure to five selected toxic chemical gasoline vapour components was assessed using different air sampling techniques, namely active, passive and direct reading methods to evaluate their accuracy and efficiency in assessing exposure risk levels at the tested petrol stations. The significance of the relationship between the various factor, including the weather conditions, the quantity of gasoline pumped, and the location of petrol stations on the exposure levels, were evaluated.

Chapter 5: This discusses evaluation of exposure to five most toxic chemical gasoline vapour constituents via biological exhaled breath of petrol station workers. Actual pre- and post-

exposures to gasoline vapour testing was explored. Possible effects of different factors, such as weather conditions, quantity of gasoline pumped, living habits, and the location of the stations were evaluated.

Chapter 6: This investigates the effect of exposure to benzene, probably the most toxic component of gasoline, by using urine sampling as a biomarker. Three different benzene-related urinary metabolites of phenol, trans-trans-muconic acid (*t*,*t*-MA), and S-phynylmercaturic Acid (S-PMA) were tested to evaluate their accuracy and efficiency in assessing exposures to benzene vapour in air. The Chapter also describes the results of actual field exposure assessment to gasoline vapour, with comparisons of concentrations measured prior to and after working shifts.

Chapter 7: This chapter discusses exposures to gasoline as a mixture of chemicals with consideration of possible biological additive effects. All individual chemical components' concentrations in the gasoline vapour that were experimentally generated at two different ambient temperatures were recorded. Based on those recorded concentrations, a recommended occupational exposure limit (OEL) is proposed for exposures to Saudi Arabian gasoline vapours.

Chapter 8: This evaluates the three different exposure assessment methods studied in relation to their applicability, accuracy, and possibilities for the assessment of gasoline vapour exposure investigations. An overall assessment of the gasoline vapour exposures of Petrol station attendants in Saudi Arabia is discussed.

Chapter 9: This chapter presents the overall conclusions of the whole study and recommendations for further applications of the results.

CHAPTER 2

2. Literature Review

2.1. Exposures to Gasoline in the Industry

Gasoline in many parts of the world has been used for several purposes, such as a cleaner solvent, automotive fuel, ingredient in paints and various other applications. It is a mixture of petroleum hydrocarbons with a variety of volatile organic compounds (VOCs). The routes of exposure to gasoline components are inhalation (Singaraju et al., 2012) and/or ingestion and dermal absorption via contact (ATSDR, 2007). There are different gasoline vapour exposure scenarios that humans can experience. The public can be exposed to gasoline components (e.g. benzene) via inhalation of the air that is emitted from various sources such as industrial activities, traffic, and via releases during refuelling (IEH, 1999). The benzene urinary biomarker, s-phenyl mercapturic acid (S-PMA), was found to occur in higher concentrations in the urine of those people who worked partly outdoors (IEH, 2008). An investigation in 2004-2006 suggested that exposures to elevated VOCs air pollution, particularly benzene and ethylbenzene in urban villages in New Jersey, USA could represent a cancer risk. Other non-cancer neurological and respiratory effects have also been attributed to benzene, toluene, and xylene at levels that exceeded the USA EPA benchmarking (Wu et al., 2012). Traffic police officers and municipal employees are examples of people occupationally exposed to gasoline during their daily tasks, especially near heavy traffic. In contrast, petrol station attendants were found to exceed the exposure levels of these two

other professions (Bono et al., 2003; Rekhadevi et al., 2010). Furthermore, a cohort study reviewed the association between the personal exposure to VOCs in air and six cardiovascular endpoints in adult non-smokers during five seasons between 2004 and 2007 in Detroit, USA. The study findings suggest possible rapid impacts on the human cardiovascular system (Shin et al., 2015). With regard to the effects on the reproductive system, animal studies showed no effects either from exposing mice to high concentrations (≥20,000 mglm^3) of gasoline vapour on sperm count or on the offspring survival and growth (McKee et al., 2000).

Based on exposure data to gasoline, summarized and evaluated by the International Agency for Research on Cancer (IARC), the personal 8-hour time-weighted average (TWA) levels for bulk and drum gasoline loaders and tank cleaners have been reported as 40-850 mg/m^3 for total hydrocarbon and 1-27 mg/m^3 benzene. Levels at petrol stations and exposure of customers are reported to be lower (IARC, 1989).

For skin exposure and penetration, an experimental study was conducted by Adami, *et al.* (2006) to test penetration of the three gasoline components benzene, toluene, and xylene through human abdominal skin in an *in vitro* experiment. Results showed a much faster penetration of benzene through the skin than toluene and xylene. The permeability rates were 43.8x10^{-5}cm/hr for benzene, 6.48x10^{-5}cm/hr for toluene, and 0.84 x10^{-5}cm/hr for xylene. These rates represent passing through percentages of 0.43% benzene, 0.06% toluene, and 0.008% xylene of the tested dose. Benzene has the fastest rate because it has the lowest boiling point and the highest water solubility. Permeation time was 1 hour for benzene and

two hours for toluene and xylene. This study demonstrated how much faster benzene penetrates through human skin compared with toluene and xylene.

2.1.1. Exposure to Gasoline as a Hydrocarbon Solvent Mixture

The exposure assessment to chemical mixtures is complicated because of the complex compositions involving individual substances, with different occupational exposure limits (OEL). Some of such components in mixtures act on the same organs causing different biological effects that can be additive, synergist, potentiation, or antagonistic. The additive effect occurs when the impact is equal to the combined biological effects of two or more components. Synergy is when the combined biological effect is greater than the sum of each constituent. Potentiation is when a substance causes the effects only in combination with another chemical. Antagonism happens when the combined effects of the components is less than the individual ones (ACGIH, 2015; Fleeger and Lillquist, 2006; HSE, 2011).

Motor gasoline is a mixture of hydrocarbon components from petroleum crude oil that are relatively volatile (McDermott and Killiani, 1978). As per the ACGIH and HSE, solvent mixtures with toxic constituents that have an OEL significantly less than the group guidance values (GGV) (e.g. benzene, toluene, ethylbenzene, xylene, trim ethylbenzene, and cumene) shall be calculated individually using the additive formula, with reference to their designated OEL. Taxell, et al., (2014) tested three methods for the evaluation of the exposure to mixtures based on consolidating exposure scenario information of individual components. The first method is Dangerous Preparations Directive (DPD+) method of the Directive 1999/45/EC and which was amended “plus” for the consideration of the volatility of the substances (Institutions and Guidance, 2010). This method depends on the hazard classification of the

critical components that determine the health effects and risk management. The second method is called the Critical Component Approach (CCA) in which the mixture's critical components are determined based on their values of derived no-effects levels (DNEL). The third method depends on the selection of the most stringent risk management measures of the individual components. Required information concerning possible hazards are collected from the safety data sheets (SDS) that are listed by the chemical manufacturers for the individual substances in the mixture. Different routes of exposure, such as inhalation and skin absorption, were also recommended to be considered in the evaluation, especially for the DPD+ and CCA methods. The study concluded that the selection of the most stringent risk management measures is the most conservative approach. Furthermore, the study recommended checking for the adequacy of the consolidated scenarios of all components in the mixture for the CCA method. The CCA method was introduced by the European Union Regulation in the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) (Taxell et al., 2014).

Different occupational health protection organizations have proposed exposure standards for mixtures by grouping the constituents by their similar properties of physical, chemical and toxicological health effects. The standards also assign exposure group guidance values (GGVs). This method is also known as the reciprocal calculation procedure (RCP) and it applies to certain refined hydrocarbon solvent vapour mixtures. These mixtures may contain up to 200 constituents of aliphatic (alkane), cycloaliphatic (cycloalkane) and aromatic hydrocarbons with carbon numbers between 5 and 15 and boiling points in the range of 35-320°C (ACGIH, 2015; HSE, 2011). The RCP is calculated by dividing the fraction of the

component (or a group of components) with respect to their designated OEL (Equation 1). RCP is not considered for components with special effects such as carcinogenicity,

$$GGV_{mixture} = \frac{1}{\frac{Fa}{GGVa} + \cdots + \frac{Fn}{GGVn}}$$

Where:
$GGV_{mixture}$ = the calculated 8 hrs TWA-OEL for the mixture
Fa = Mass fraction of component "a" in the hydrocarbon mixture
GGVa = Group Guidance Value or OEL for component "a"

Equation 1: Group guidance value equation

sensitization, asthma, and others; instead they are calculated separately (not in groups) in the mixture depending on their specific OELs (Mckee, Medeiros and Daughtrey, 2005).

The USA Environmental Protection Agency (EPA) indicates that it is preferable to use mixture specific toxicity information to evaluate the risks of exposures to petroleum hydrocarbons when adequate information is available. When there is no strong evidence of an antagonistic or synergistic effects among the mixture components, it is assumed that the chemicals affecting the same target are additive (Choudhury et al., 2000).

The threshold limit value (TLV) for gasoline vapour mixture was published by the American Industrial Hygiene Association (ACGIH) in 1967 with value of 500 ppm using the additive Equation 2.

$$OEL_{mix} = \frac{1}{\frac{C1}{T1} + \frac{C2}{T2} + \cdots \frac{Cn}{Tn}}$$

where:
C_1 = concentration in air of component 1
T_1 = corresponding OEL for component 1

Equation 2: Chemical mixture component addition equation

The concern was the additive effect of different components in the gasoline vapour. During 1975 Shell Oil Company industrial hygienists developed the TLV with consideration of different exposure scenarios such as loading gasoline into tank trucks, dispensing it into automobile at petrol stations or when gasoline is misused for cleaning paint brushes or as degreasing solvent which generated more of the lighter hydrocarbons. In such scenarios, they included 21 hydrocarbons that formed 92 percent of the gasoline vapour. They also included all components present at 0.5 percent or greater (by volume). The TLV calculation of mixture gasoline vapour was carried out when benzene in gasoline mixture was 1 percent and OSHA benzene exposure standard was 10 ppm for an 8-hour per day. The gasoline vapour mixture exposure was then recalculated in 1978 to be 300 ppm using Equation 2 (McDermott and Killiani, 1978).

The TLV and other exposure governing standards are interpreted in ppm or mg/m^3 for all inhaled chemical compound that exit in gas, vapour, or aerosol. The aerosol, which is the suspended solid particles or liquid droplets in the air, are usually mass of chemicals in air by volume (mass/volume) and expressed as mg/m^3. On the other hand, the gases and vapours are parts of vapour or gas exist per million parts of contaminated air by volume (volume/volume) which is expressed as parts per million (ppm). The gases and vapours may also be expressed in mg/m^3, however, they are usually presented as ppm. Thus, ppm has been used for the gasoline vapour throughout this thesis. In some cases where it is more convenient to use smaller units (e.g. $\mu g/m^3$) or where measurements were taken from other references with mixtures of chemicals with unknown molecular weight (MW), the same units were used. To convert between mg/m^3 and ppm, the MW of the chemical substances are used. For the

industrial hygiene applications, 24.45 is used for the molar volume of air in litre at normal

$$ppm = (mg/m^3 \text{ X } 24.45)/MW$$

where:
ppm = parts per million
mg/m^3 = milligram per cubic metre
24.45 = molar volume of air in litres at 25°C and 14.6 psia
MW = gram molecular weight of substance

Equation 3: Conversion equation from ppm to mg/m^3

temperature and pressure (NTP) of 25ºC and 760 torr (ACGIH, 2015). The following equation (Equation 3) was utilised for converting from ppm to mg/m^3 and vice versa.

2.1.2. Petrol Station Attendant Exposures

It has been estimated, based on information obtained from various self-service petrol stations in different countries that every station attendant pumps an average of 2,000 L of gasoline, which contains around 5% benzene, during each 8 hours shift (Çelik, Çavaş and Ergene-Gözükara, 2003; Singaraju et al., 2012). Another study has shown an average of 300-4500 L/day of pumped gasoline per attendant (Pandya et al., 1975). Vehicle exhausts emitted during driving in/out and the restarting of engines at petrol stations can be other sources of gasoline components in the area (Nordlinder and Ramnäs, 1987). Furthermore, the presence of a petrol station roof, or anything similar, is an important consideration, as it increases the concentrations of gasoline vapours in both the inhalation zone of the attendants (near pumps) and in the general area, up to 3 metres downwind of pumps. The average increment percentage in roofed stations is 22% for near pumps and 25% downwind of the pumps, as

compared to non-roofed stations (Berglund and Petersson, 1990). This is justified by the restricted vertical air mixing limited by the roofs.

The location of the petrol stations is also an important factor, especially, for those close to highways or busy traffic. A gasoline vapour exposure study of petrol station attendants conducted by Bono et al., (2003) compared exposure levels to benzene at two petrol stations located near high traffic, approximately five-metre from the roads, and in a suburban area with low traffic in Torino and Biella cities in North-Western Italy. The study results showed that there was a difference of 22% (2.3 µg/m^3 and 10.3 µg/m^3) in comparing the two cities depending on how near they were to the road and the density of the traffic. The International Agency for Research on Cancer (IARC 1989, p.159) evaluated a large collection of human exposure data from different studies in order to designate a value for health hazard classification for gasoline. In this monograph, a number of cohort and case-control studies on gasoline exposures were collected from different countries, such as the UK, Sweden, and the USA. A review of the findings of such studies led to the conclusion of listing gasoline as a group 2B Suspected Human Carcinogen (IARC, 1989). The same conclusion was re-confirmed in 2012, during the IARC meeting on the Evaluation of Carcinogenic Risks to Humans, titled "*Diesel and Gasoline Engine Exhausts and Some Nitroarenes"* (IARC, 2014). IARC included a table (Table 2-1) of benzene occupational exposure levels in Europe and North America, in its 2012 benzene monograph. Petrol station attendants were one of the exposed groups who had an arithmetic mean for inhalation exposure of 0.03 ppm in 1999-2000 (Capleton and Levy, 2005).

Table 2-1: Typical benzene long term exposure levels in air in different occupational groups in Europe and North America (Capleton and Levy, 2005).

Occupation Type	Year	No. of Samples	Mean (ppm)
Petrol Station Attendants	1999-2000	78	0.03
Cashier at Petrol Stations	1993-98	268	0.01
Misc. Workers at Petrol Stations	1999-2001	6	0.06
Gasoline Pump Maintenance	1993-98	2	0.17

The effects of the weather conditions (e.g. temperature) and the quantities of gasoline pumped on the concentration of hydrocarbons in the air were tested by Periago, et al., (1997), at petrol stations in Southeast Spain. The study assessed exposures of twenty-one workers in six petrol stations to gasoline vapour, aiming at benzene, toluene, and xylene during two seasons; winter (March) with temperature ranging between 14-15°C and in the summer (July) with a temperature range of 28-30°C. The study results showed a significant relationship between the volume of gasoline sold and the hydrocarbon concentrations in the air for every worker sampled. This follows the concept that the evacuated volume of air with gasoline vapour from vehicle tanks is exactly equal to the volume of gasoline pumped. Therefore, it was concluded that the greater the quantity of gasoline pumped, the higher the possibility of gasoline vapour exposures.

2.2. Health Impacts of Gasoline Vapour in the Industry

A literature review (meta-analysis) was conducted by Keenan; et al. (2010) of epidemiologic studies published from 1970 to 2009, on the carcinogenicity of gasoline. The study criteria did not include acute or sub-chronic exposures, but focused only on chronic exposure. The study reviewed a total of 32 studies of which 19 were case-control studies and 13 cohort studies. Most of the case-control studies showed a low increase in relative risk

(RR). Seven of these studies showed significant correlation between gasoline exposure and different diseases including Non-Hodgkin Lymphoma (NHL), Acute Non-Lymphocytic Leukaemia, male breast cancer, renal cell carcinoma, stomach cancer, bladder cancer and larynx cancer. The highest RR was 2.8 (95% CI: 1.0-7.7) for larynx cancer. The only malignancy cases reported to be statistically significant were from two case-control studies; one with high RR of 2.1 and another, for renal cell carcinoma, with a RR of 1.6 Nine of the 13 cohort studies indicated no statistically significant increased risk for kidney cancer and only one reported increased risk of hematopoietic cancer. The evaluation of both the case-control and cohort studies did not identify an underlying pattern between gasoline exposures and specific cancer types. This is due to large confidence intervals for the small number of cases identified in each study. Table 2-2 summarizes reviewed studies and the correlations to gasoline exposures and cancer diseases in humans (Keenan et al., 2010).

Table 2-2: Meta-analysis of the carcinogenicity of gasoline (from Keenan et al. 2010)

No. of Studies	Study Type	Study Observation	Diseases
7	Case-Control	Significant Correlation to gasoline exposures	non-Hodgkin Lymphoma Acute non-lymphocytic leukaemia Male breast cancer Renal cell carcinoma Stomach cancer Bladder cancer Larynx cancer
9	Cohort	No statistically significant increased risk	Kidney cancer
1	Cohort	Statistically significant increased risk	Hematopoietic cancer

A recent review study (2012) sponsored by the British Health and Safety Executive (HSE), was carried out by a group of scientists from Cranfield University and Imperial College, London, to estimate the burden of different types of cancers for Great Britain from exposures to different carcinogenic scenarios in various occupations. Quantifying the attributable fraction (AF) was a main goal of the study to estimate the risks between exposures and non-exposures to carcinogens and the cases pertaining to mortality and registrations.

There was a sufficient evidence of benzene in petroleum refining as in the chemical industry and boot/shoe manufacturing having carcinogenicity effects in humans based on estimation of acute lymphocytic and acute monocytic leukaemia (Rushton and Romaniuk, 1997). Furthermore, exposure to benzene among motor mechanics and aviation workers were also elevated (Capleton and Levy, 2005). Part of the study was to review the AF of exposures to polycyclic aromatic hydrocarbons (PAH) of two or more benzene rings and levels of lung cancer in the industry. The review concluded that increased risk in different manufacturing industries, including petroleum products (Bosetti, Boffetta and La Vecchia, 2007; Slack et al., 2012). Oil refining and gasoline/diesel delivery were considered occupational agents or circumstances of exposure that are linked to the renal cell carcinoma (RCC) (Brown et al., 2012; Slack et al., 2012).

Although gasoline comprises hundreds of chemical components, benzene, toluene, xylene and MTBE have the highest toxicity which is reflected by having the lowest occupational exposure standards (ACGIH, 2015). Therefore, these components have been extensively studied, especially among the exposures to petroleum and its derived products.

Benzene is of most concern with regard to health effects because it is a confirmed human carcinogen, causes leukaemia, and is a neurotoxic agent, as classified by many International Agencies (e.g. IARC, 1982; ACGIH, 2015; UK Health Protection Agency, 2007; Safe Work Australia, 2012). MTBE is also of concern because of associated health effects (e.g. nephrotoxic and others) and is used as an anti-knock additive in gasoline (ATSDR, 1996). Toluene and xylene are neurotoxins and have been proven to affect the central nervous system (CNS) (ACGIH, 2015).

Based on similar studies, reports, and epidemiological data, the IARC lists gasoline vapour as a "possibly carcinogen to humans (Group 2B)". The IARC also agrees on the association between the exposure to benzene and the incidence of cancers, such as malignant melanoma, nose and stomach and prostate cancer, based on different studies. Furthermore, research has shown the possibility that benzene exposure causes Acute Myeloid Leukaemia (Jakobsson et al., 1993) and that there is a positive association between benzene exposure and Acute Lymphocytic Leukaemia (Crump, 1994; IARC, 1999), Chronic Lymphocytic Leukaemia (IARC, 1999; Rushton and Romaniuk, 1997), Multiple Myeloma and Non-Hodgkin Lymphoma (IARC, 2012). Therefore, based on this and other supportive data, benzene is classified as a Group 1, Carcinogen.

2.3. Governmental Regulations and Risk Management

The exposure assessment for gasoline vapours is determined by referencing to a gasoline mixture or to the individual components of concern. Many standards agree on similar occupational exposure limit (OEL) values for both long term eight-hour time weighted average (TWA), and short term exposure limit (STEL) for the duration of 15 minutes for

gasoline vapour in air. The European Union (EU) designates the OEL for gasoline vapour mixture (TVOC) exposure limit as 300 ppm for an eight hour exposure period and 500 ppm for the STEL (De Craecker et al., 2007). The other approach of assessing the exposures to gasoline vapours is via the evaluation of individual components. A number of the studies are focused on assessing exposures to the aromatic components of benzene, toluene and xylene (BTX) in gasoline, as being the most cause for health concerns. The exposure limits for BTX are individually listed in the EU's OEL. The EU designates 1 ppm for the 8-hour exposure limit to benzene vapour and considers skin as an exposure route. It also acknowledges that gasoline vapour can be absorbed with the remark "substantial contribution to the total body burden via dermal exposure is possible". Furthermore, the EU restricts the use of benzene by the public due to its high health hazard, including carcinogenicity. The OEL for toluene and xylene is 50 ppm and 100 ppm, respectively, for the eight-hour exposure limit. Similar to benzene, "skin notation" remark is also designated for toluene and xylene because they can penetrate through the skin and cause health effects (De Craecker et al., 2007).

In the United Kingdom, a benzene exposure limit was first established as 25 ppm for 8 hours TWA in 1966. This limit was reduced in 1977 to 10 ppm then further lowered to 5 ppm in 1991 (IEH, 1999). The Health and Safety Executive (HSE) of the UK, lists benzene as a skin absorbed (skin notation) carcinogen with a workplace exposure limit (WEL) of 1 ppm for the 8 hour TWA (HSE, 2011). HSE also amended the exposure limit for 8 hours for toluene to 50 ppm and similarly lists 50 ppm for 8-hour exposure to xylene. The chemicals are assigned 100 ppm and 100 ppm for 15 minute exposures, respectively. Additionally, HSE

increased the exposure limits to methyl tertiary butyl ether (MTBE) to 50 ppm for 8 hours and 100 ppm for 15 minute short exposures (HSE, 2011).

The Australian Government controls workplace health hazard chemical substances through the Hazardous Substances Information Systems (HSIS) that is managed under Safe Work Australia (Australia, 2012). The HSIS lists gasoline as Category 2, probably human carcinogen and mutagen, with Risk Phrases R45 (may cause cancer), R46 (may cause heritable genetic damage) and R65 (Harmful: may cause lung damage, if swallowed). The HSIS's assigned exposure limit for gasoline is 300 ppm.

In Canada, the Occupational Health and Safety Act & Regulations, sets an exposure limit of 300 ppm for time weighted average equivalent value (TWAEV) and a 470 ppm short term equivalent value (STEV) (McDonald, 2002).

In Germany, the Federal Institute for Occupational Safety and Health (German: *InstitutfürArbeitsschutz, IFA*) publishes the occupational exposure limits in the workplace for hazardous, chemical substances. The lists of chemicals are classified into Category I for substances with local effects or respiratory sensitizing effects and Category II for substances with systemic effects. Gasoline is listed as Category II (IFA, 2010). The Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) is responsible for determining the current state of research relating to the health risks posed by substances and materials used at the workplace. MAK proposes exposure values to volatile chemicals and biological tolerance values and also develops sampling methodologies for air and biological materials (DFG, 2014). IFA determines 470 ppm to be the long term occupational exposure limit value for gasoline, 1 ppm for benzene, 50 ppm for

toluene, 20 ppm for ethylbenzene, 100 ppm for xylene, and 50 ppm for MTBE. Furthermore, German's IFA lists biological monitoring values for occupational exposure to gasoline and some of its individual components of a concern aforementioned. In 2013, the German MAK-Collection document report stated that the Commission did not assign MAK exposure values to gasoline, due to several reasons, including the different types of gasoline, such as the motor gasoline (petrol), special boiling point gasolines, white spirit and pyrolysis gasolines. Thus, it was inferred from the MAK that each gasoline type would need a designated OEL. Furthermore, the Commission decided to reject mathematical evaluation of the gasoline mixture values until studies determine the actual concentrations of gasoline vapour at the workplace (DFG, 2014). ACGIH sets threshold limit values (TLVs), with time weighted averages (TWAs) for occupational health stressors. Many of the ACGIH standards are applied in the United States by the Occupational Safety and Health Administration (OSHA) and adopted internationally by other governments. The Saudi Arabian Standard Organization (SASO) adopts the USA OSHA exposure standard limits. However, they are not updated regularly with the changes by OSHA. The ACGIH uses TLV-TWA to set its exposure standards for chemicals "under which it is believed that nearly all workers may be repeatedly exposed day after day, over a working lifetime (40 years), without adverse health effects" (ACGIH, 2015). These TLVs are designed to protect healthy adult workers.

Similar to EU and others, ACGIH determined the level of 300 ppm exposure to gasoline vapour mixture over an averaged eight- hour working shift (TLV-TWA) and 500 ppm for the short-term exposure limit (STEL), allowed for a maximum of 15 minutes. ACGIH categorizes whole gasoline as a confirmed animal carcinogen, with unknown relevance to humans (A3) (ACGIH, 2015). Table 2-3 summarises some of the standards in developed countries for occupational exposure to whole gasoline.

Table 2-3: Governmental gasoline exposure limits and notations

Government	*OEL (**Short Term)	Notation
EU	300 (500) ppm	Not to be classified as human carcinogen if contains <1ppm benzene
Australia	300 ppm	Category 2, probably human carcinogen & mutagen
ACGIH & OSHA	300 (500) ppm	A3: confirmed animal carcinogen with unknown relevance to humans
Canada	300 ppm	
IARC		2B possible human carcinogen

*Occupational Exposure Limits
**Short Term Exposure Limit of 15 minutes

ACGIH also assigns exposure limits for individual gasoline components, based on their data collected on experimental and epidemiologic studies concerning health effects. It categorizes benzene as a confirmed human carcinogen (A1), based on the weight of evidence from epidemiologic studies. The ethylbenzene, MTBE, and gasoline mixtures are categorized as a confirmed animal carcinogen, with unknown relevance to humans (A3) and a cause of reproductive and kidney damage. Toluene and xylene are also listed as agents that could be carcinogenic to humans, but cannot be assessed conclusively because of a lack of data. Both toluene and xylene are listed as "not classified as human carcinogen" (A4) by the ACGIH. Both substances are neurotoxins in addition to causing reproductive impairments and eye

irritation, respectively (ACGIH, 2015). Table 2-4 summarises ACGIH's exposure limits and toxic effects of gasoline and its frequently evaluated components.

Table 2-4: Exposure limits and adverse health effects (ACGIH, 2015)

Chemical	*ACGIH TLV-TWA ppm	ACGIH Classification	Carcinogenicity	Adverse Health Effects
Benzene	0.5	A1	Confirmed human	Cancer & Leukaemia
Toluene	20	A4	Suspect human	Visual impairment & female reproductive loss
Xylene	100	A4	Suspect human	Eye Irritation & CNS impairment
MTBE	50	A3	Suspect human	Reproductive, Liver & Kidney damage

* American Conference of Governmental Industrial Hygiene (ACGIH) Threshold Limit Value (TLV-TWA)

These TLV-TWA and other similar exposure limits are predominantly designed for eight-hour working shifts. Therefore, exposure to more than eight-hour shifts (i.e. unusual working shifts) requires exposure limit adjustments. This requirement is based on the time needed for the body to physiologically recover, and to eliminate toxins after exposure every twenty-four hour period which also depends on the biological half-life of the substance (ACGIH, 2015; HSE, 2011; Verma, 2000). The biological half-life is the duration for substance and/or its metabolites to decrease to half in the target organ, tissue, or body fluid (Verma, 2000). Therefore, for the increased time of exposure, the exposure limits usually needs to be lowered. The Brief and Scala Model Equation 4 is one of the methods used to calculate reduction factors (RF) to adjust exposure limits for extended working hour shifts (Brief and

Scala, 1986). This equation is still recommended by the ACGIH for the reduction factor calculation of the adjustment for unusual working shifts.

Such adjustment calculations should be applied when determining exposure limits for ten and twelve working hour shifts at petrol stations. For example, by applying Equation 4 to the exposure of benzene with the 1 ppm occupational exposure limit value for an eight-hour period, the limit would be adjusted to (0.7 ppm for a ten -hour working shift. Similarly, this eight- hour exposure value would be modified to 0.5 ppm for twelve- hour shifts.

OSHA published guidelines in 1979 that recommends permissible exposure limits (PEL) adjustment for extended work shifts of more than eight hours per day. In this approach both pharmacokinetics and toxicological effects are considered. The rationale behind this

$$RF = \frac{8}{h} \times \left(\frac{24-h}{16}\right)$$

Equation 4: Unusual working shift exposure limits adjustment

Where:

RF: reduction factor used to estimate adjusted exposure limits

h: working time in hours

approach is that the severity of the toxic effects on the target organ is subject to the concentrations that reaches that organ. There are two proposed equations (equations 5 and 6) to calculate the PELs for daily and weekly exposures.

$$\textbf{Weekly Equivalent PEL} = \textbf{8 hr PEL x } \frac{\textbf{40 hours}}{\textbf{hours of exposure in a week}}$$

Equation 5: OSHA permissible exposure limits for weekly extended work shifts

$$\textbf{Daily Equivalent PEL} = \textbf{8 hr PEL x } \frac{\textbf{8 hours}}{\textbf{hours of exposure in a day}}$$

Equation 6: OSHA permissible exposure limits for daily extended work shifts

By applying OSHA's equation, the equivalent PEL becomes less affected than that by the Brief Scala's formula. For example, the benzene PEL for ten hours is 0.8 ppm and 0.67 ppm for twelve hours shifts.

The TLVs of the ACGIH, particularly for the three BTX components in gasoline, have been reduced over the years since first established in 1946, as shown in Figure 2-1. In contrast, the MTBE exposure limit was increased to 50 ppm from 40 ppm in 2005 (ACGIH, 2015). These are examples of the on-going research regarding assessments of health hazards due to exposure and making changes to the standards based on new evidence. This is called Notice of Intended Changes (NIC) that is applied on the TLVs of the ACGIH exposure standards (ACGIH, 2015).

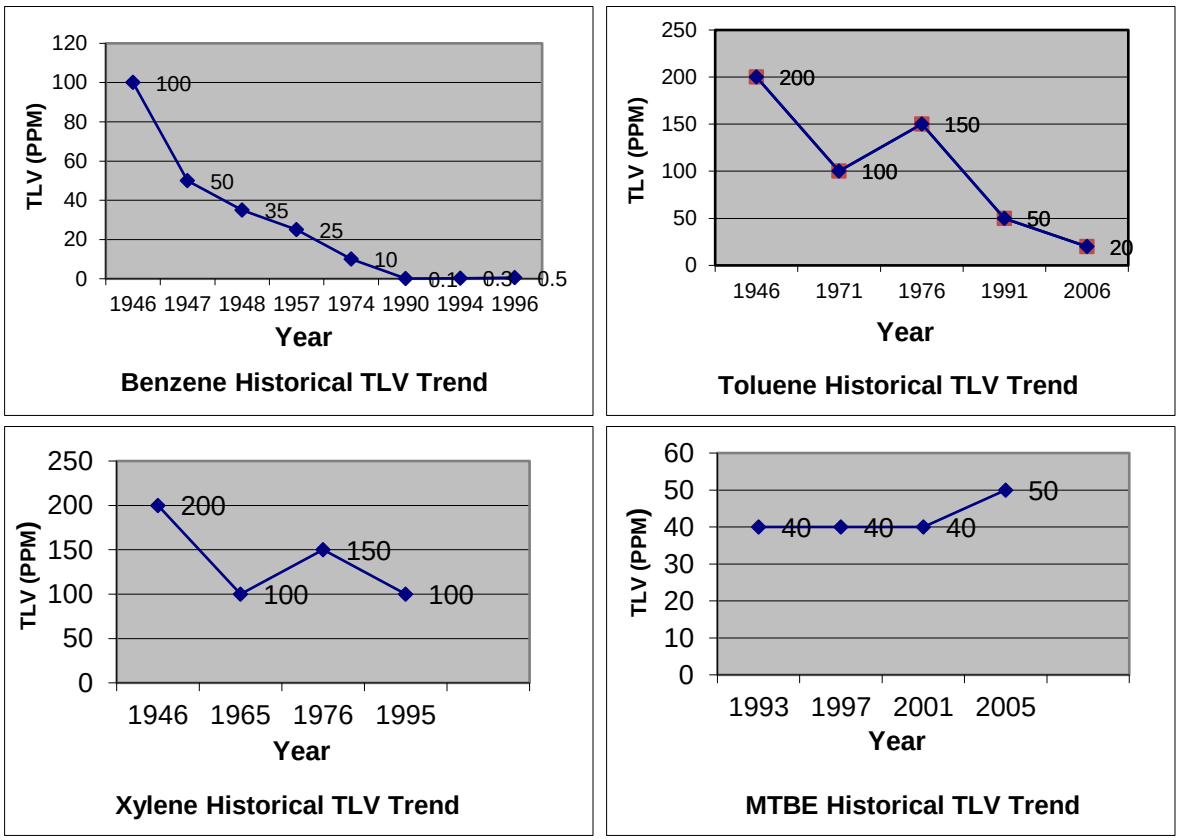


Figure 2-1: ACGIH historical changes of occupational exposure standards (TLV-TWA) (from ACGIH, 2015; ACGIH, 2005; AIHA, 1998).

2.4. Methods of Exposure Assessment to Gasoline Vapours

Statistics play major roles in assessing occupational exposures in the workplace. It is a mechanism of collection, summarising and analysing data on quantitative characteristics of population. The descriptive statistics (obervational studies) are means applied to study and analyse measurable facts and results. Descriptive statistics examples are means, medians, modes, standard deviations, ranges, and others. Biostatitstics is branch that studies matters of health concerns and diseases among occupational population via recorded oveservations. The occpational health practitiners and industrial hygienists use biostatitstics as tools to test the presence of workers' health concerns and the relationships between environmental conditions and the development of injuries and sickness to set control measures (Janicak, 2007). The recorded obesrvations are most useful when summarised in analytical data format (e.g. tables, graphes, charts, etc.) to help exploring causal relationaships among multiple variables and the concerned matters (International Labour Office, 1983).

Normal distribution is the shape we would want the data to be to give the accurate estimation of the real mean. In such distribution, the arithmetic mean and median are similar. In the log-normally distributed data, the geometric mean (GM) and median are similar. The log-normal distribution is used for data that are not normally distributed (skewed). Usually, the environmental and biostatistics data are not normally distributed. This is because a few of the results can be high while the majority of the results are grouped closely. If such data are treated as normally distributed a large weight would be applied to the outlying values and wrong deductions may be made. Therefore, to solve such issue, some methods of

transforming (e.g. logarithms) data are performed (Harrison, R. M., 1999) and GMs are considered. Note that the GM of zero is zero, therefore, it is applied for values greater than one (Vijay P. Singh , Sharad K. Jain, 2007). Most of the occupational exposure data distribution tend to be lognormal (ECHA, 2012). The GM and geometric standard deviation (GSD) are the best that characterise lognormally distributed data. This is because GM depends on values rather than the total of data as in the arithmetic mean. This means GM is less affected by very high or low values than the arithmetic mean (Kumagai and Matsunaga, 1994).

Paired *t* test is a statistical tool that is applied to compare two measurements as of before and after (pre- and post) treatment carried out on the same subject. It is used to test the hypothesis between two variables. It is also called dependents observations. The vaiables are assumed to be continuous data (not desticnt) and every subject has two measures. Simply, if the treatement made no effects, then the mean difference between the measurements would be zero (0) and the null hpothesis is kept. However, if the treatment has made changes, then the mean difference would not equal zero and the null hypothesis is rejected (Janicak, 2007; Kuzma and Bohnenblust, S., 2001).

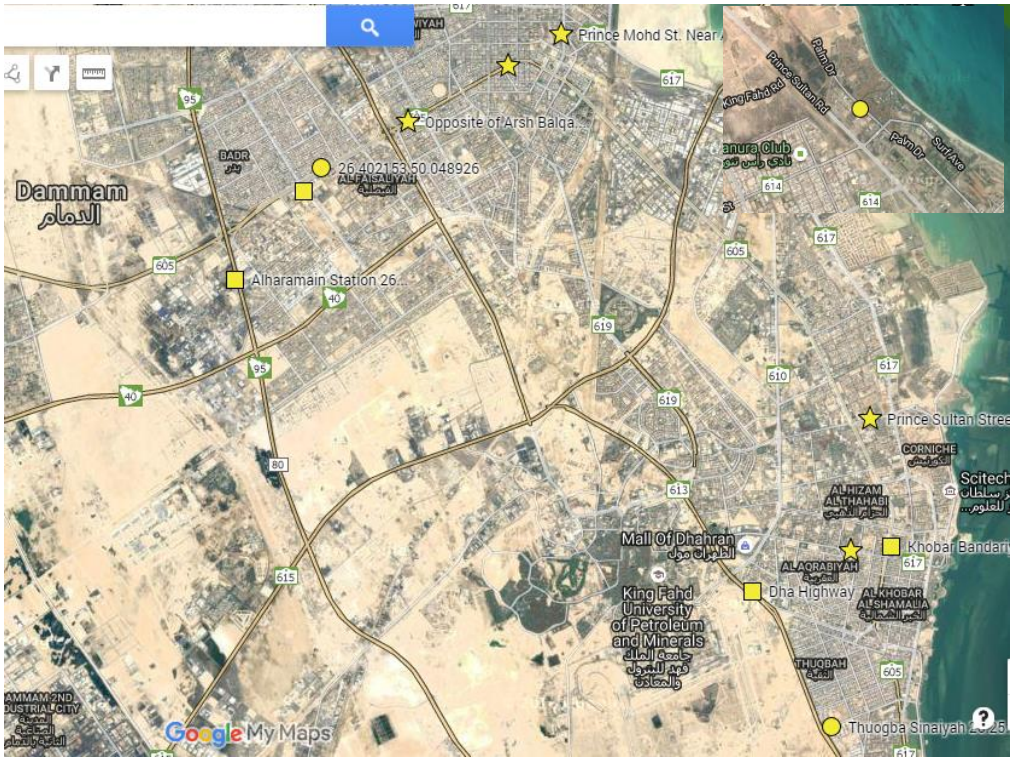
Statisticians (Janicak, 2007; Kuzma and Bohnenblust, S., 2001) suggest that prior to conducting exposure monitoring studies, it is essential to have a solid sampling plans and experimental exposure monitoring design. Furthermore, selecting a sample from a population is very important to draw inferences about that population. In the case of sampling for exposures to gasoline vapours of workers at petrol stations, the method of selecting a sample of petrol station attendants from the overall attendant population is reliable because of their

similar and specified characteristics including the number of working hours, the nature of the work activities, the exposure scenarios and to the same gasoline fuel, especially in Saudi Arabia, and others. This is called convenience sampling method due to particular similar aforementioned characteristics and profession (Kuzma and Bohnenblust, S., 2001).

The selection of different types of petrol stations as of their locations (near highways, surrounded by tall buildings, or in open areas) is considered a stratified sampling. This is because they are subgroups of the general petrol stations. The determination of the desired sample size is important to obtain statistically meaningful results. Therefore, it is statistically suggested that when the sample size is greater than around 30 it approximates the normal distribution of the population. Thus the larger the sample size that more reliable it is (International Labour Office, 1983; Janicak, 2007; Kuzma and Bohnenblust, S., 2001).

In light of all aforementioned statistical information, this thesis sampling strategy and exposure monitoring design for petrol station attendants was conducted. Moreover, in reference to other studies that tested the effects of the quantities of gasoline pumped and quantities of gasoline pumped (Periago, Zambudio and Prado, 1997), and the locations of the petrol stations (Bono et al., 2003) on the attendants' exposure levels to gasoline vapour, the sampling plans were structured. The tested petrol station locations were selected based on their proximity to highways/busy roads (speed limit of 60 km/hr or more, number of cars passing-by is more than 20 cars/hour during 7:00 to 17:00 on weekdays), limited air movement (near-by buildings of less than 15 metres distance from the fuel pumps with height of more than 7 metres on at least two sides), and the volumes of gasoline sold of high (> 15,000 L/day) and low (< 15,000 L/day) from the pre-assessment survey and interviews with

the stations managements. Figure 2-2 illustrates the three petrol station categories and their locations on an overview map. Furthermore, Table 2-5 summarises the designed and monitored variables set for the study.



Open Area Location Petrol Station
Surrounded by Buildings or Near Highway
Surrounded by Buildings and Near Highway

Figure 2-2: Top view map illustrating the tested gasoline stations according to their location category (Courtesy: Google Maps 2016)

The personal exposure monitoring methods of active and passive are the most commonly applied methods to assess exposure by inhalation (Weisel, 2010). Other techniques such as biological samples of urine and blood are used for the measurement of total personal exposure, including inhalation. The following two sections describe each method in detail.

Table 2-5: Monitored variables and types of petrol stations

Designed variables	Monitored variables
Volume of gasoline pumped: • High sale >15,000 L/day • Low sale <15,000 L/day	Environmental Conditions: • Wind movements around the monitored subjects
Location Classification: • Near highways/busy roads (speed limit of 60 km/hr or more, number of cars passing-by is more than 20 cars/hour during 7:00 to 17:00 on weekdays) • Surrounded by buildings (near-by buildings of less than 15 metres distance from the fuel pumps with height of more than 7 metres on at least two sides) • Open area	• Relative humidity during the sampling • Ambient air temperatures

2.4.1. Active Air Samplers

Personal air sampling is the technique most commonly used to assess occupational inhalation exposure to airborne contaminants in the workplace. The purposes of such assessment can be used to compare measured concentrations of airborne chemicals to applicable exposure standards for compliance and protection, or to evaluate the performance and effectiveness of exposure control measures. Area air sampling is the assessment of air concentrations in the workplace. This complements personal air exposure evaluation and they should have correlated levels of concentrations (Fleeger and Lillquist, 2006). Active air sampling requires employing mechanical air movers (pumps), to pull airborne contaminants into a sampling device such as an adsorbent tube or treated filter in which the contaminants are trapped or collected. The air sample is taken from the workers' breathing zone, which is in the radius of 6-9 inches near the nose and mouth (Huey, 1996). The air sampling pumps are calibrated to draw constant and accurate air flow in pre-determined amounts per measured

time. The air flow rate depends on the sampling methodology recommended for the desired contaminants.

In the USA, regulatory authorities such as OSHA and research institutes, such as the National Institute for Occupational Safety and Health (NIOSH), recommend analytical methods for occupational exposure air sampling and analysis. In the United Kingdom, the methods for the determination of hazardous substances (MDHS) are recommended by the Health and Safety Executive (HSE). MDHS 70 is an example method for sampling collection and analysis of airborne gases and vapours (HSE, 1993). Furthermore, the International Organization for Standardization (ISO) publishes the standard of indoor, ambient and workplace air-sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography. This standard is divided into two parts: ISO16017-1 for guidance on pumped sampling and ISO 16017-2 for guidance on passive sampling. Such standards detail procedures for air sampling, with all required equipment, calibration, calculations and other supporting information. Such standards are commonly applied in the EU states, including the UK. For volatile contaminants, such as benzene, toluene, ethylbenzene, xylene (BTEX), a Tenax sorbent tube is recommended by ISO 16017 for both pumped and passive sampling procedures (ISO, 2001; ISO, 2003). Other sources are published literature referenced, such as Annals of Occupational Hygiene, the American Industrial Hygiene Journal, Applied Industrial Hygiene, or Analytical Chemistry.

The air sampling pumps are lightweight (~340 grams) and intrinsically safe battery-operated ones which can be used in flammable or explosive environments without causing igniting sparks (SKC, 2012). They can be pocket- sized and can be attached to the workers'

belts while allowing normal activity during the personal air sampling period. The battery packs in the personal air sampling pumps are capable of operation for an extended time that exceeds the eight-hour working shift (Monteith, L. and Rubow, 2001). It is very important that the air pumps are capable of maintaining a constant airflow recommended in sampling methodologies. This is to accurately calculate the amount of contaminants collected from a known volume of air pulled through the sampling device (e.g. mg of contaminants mass over m^3 or ft^3 of air). Constant airflow is controlled by pressure-compensating devices. Pressure-compensation is achieved with built-in devices inside the air pumps ,which are designed to detect airflow changes and cause the pump's performance to increase or decrease to maintain the constant flow (Huey, 1996). Thus, pumps can maintain the desired constant air flow rate for the entire sampling period.

2.4.1.1. Air Pumps Calibration

For best results, it is recommended to calibrate air flow before and after the sampling event under similar ambient conditions, such as temperature, humidity and pressure, with the entire sampling train (e.g. pump, filter, tube, and hose) assembly. Calibration is usually conducted against a primary standard flow metre, which is based on measurable linear dimensions, such as the length and diametre of a cylinder. Examples of such devices are spirometres, bubble metres, and Mariotte bottles. In the bubble metre, the bubble indicator is pulled upward by the air pump and the speed of the bubble travelling in the numbered glass tube is measured by calculating the distance it passes in a certain recorded time (ml/seconds), as shown. Using basic lab glass burette connected to air pumps to measure bubble movement is the basic concept behind the contemporary calibrators with built-in sensors that

automatically detect the movement of the bubbles and calculate the flow rates (Dietrich, 2003). Such modern calibrator with built-in bubbler indicator and air pump with floating ball in a rotametre is shown in Figure 2-3.

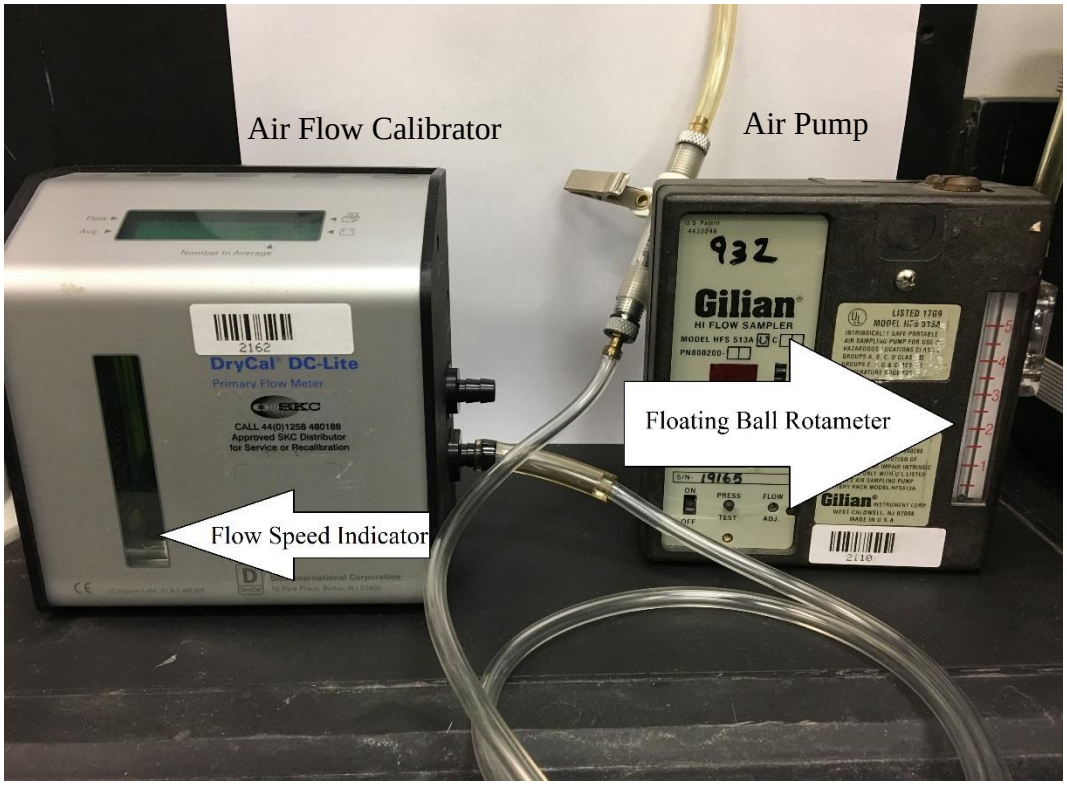


Figure 2-3: Personal air pump connected to air flow calibrator showing the floating ball rotametre and flow speed indicator

2.4.1.2. Sampling Media

Sampling media is a part of a device that collects the airborne contaminants when the contaminated air is pulled through them. The most important points to consider for collection efficiency are the interfering compounds, humidity and temperature effects, the required measuring range, and the physical state of the contaminants (McCammon, C. and Woebkenberg, 1998; Saalwaechter et al., 1977). Temperature and humidity play important roles in the collection efficiency of the sampling media. Furthermore, air sampling flow rate

is another important factor that can affect the collection efficiency. Sampling methodologies (e.g. NIOSH) recommend certain ranges of flow rates appropriate for the contaminants to be captured and retained. The standard 100 mg/50 mg charcoal absorbent tube is designed with a backup section that is about 50% of the front section size of the tube (McCammon, C. and Woebkenberg, 1998). The purpose of the backup section is to verify if the contaminants have migrated through the front section due to saturation, very high flow rate, and/or extreme ambient air conditions. OSHA indicates in Section 3.9 (reporting results) of its organic vapour analytical procedure that if the backup section contains 25% or more of the analyte of the front section, the sample should be considered saturated and therefore, be disregarded (OSHA, 2000). It is also recommended in the OSHA technical manual (OTM) that flow rates should be lowered, or the air volume reduced to half of the maximum of the recommended range in the sampling methodology when sampling in more than 90% humidity, or if the concentration of the organic vapours is high for efficient sampling.

Activated charcoal material is the most used sorbent for collecting non-soluble or reactive gas and vapour (First, 2001) of volatile organic compounds recommended in OSHA and NIOSH sampling methodologies (Pendergrass, 2003; Potter, 1987). Coconut shell and petroleum-based charcoal are commonly used in the industry, due to their high ability and large surface area (>1000 m^2/g) to adsorb organic contaminants (Dietrich, 2003). The purpose of activating the charcoal is to improve the ability of adsorption and increase porosity and surface area. The activation of the charcoal sorbent is conducted in two processes: chemical activation and physical reactivation. In the chemical activation, the material is treated chemically, with acid or a strong base to remove unwanted components and to

catalyse the material. Then the material is physically treated in a process called carbonization where the material is heated at (600-900°C) in the absence of oxygen. Lastly, the activation/oxidation process is done by exposing the carbonized material to an oxidizing atmosphere at high temperature (600-1200°C), which in turn increases the porosity of the material (Díaz-Terán et al., 2001). The activated charcoal is able to trap and retain large amounts of the desirable contaminants (e.g. hydrocarbons), and efficiently respond in the desorption process of the trapped contaminants at the laboratory.

Tenax TA (Poly (2,6-diphenyl phenylene oxide) is another sampling media to collect/trap airborne contaminants. The contaminants are basically trapped on the sorbent materials and then transferred to the gas chromatography (GC) via the thermal desorption process (heating of the sorbent material). Thermal desorption (TD) is the technique used to extract analytes. The thermal extraction method provides a very high extraction efficiency that can reach 100% ,which makes it 1000-fold more efficient than the solvent extraction method (Woolfenden, 2010). Tenax TA is used for certain chemicals with a boiling point of 80-200°C. Each analyte has a specific retention volume estimated per gram of the Tenax TA sorbent material. For instance, benzene maximum retention volume is 19 litres per gram (l/g) of the Tenax TA estimated at 38°C (US EPA, 1984). Caro J. et al. (2009) determined LODs and LOQs for a sample volume of 1 litre for the BTEX and the MTBE. These results are shown in Table 2-6.and LOQs for a sample volume of 1 litre for the BTEX and the MTBE. These results are shown in Table 2-6.

Table 2-6: LOD and LOQ of Tenax TA for BTEX and MTBE (Caro and Gallego, 2009)

Compound	LOD (ng)	LOQ (ng)
Benzene	0.03	0.1
Ethylbenzene	0.07	0.2
Toluene	0.03	0.1
Xylene	0.05	0.2
MTBE	0.03	0.1

Limit of Detection (LOD) and Limit of Quantification (LOQ)

2.4.1.3. Active Air Sampler Selection Considerations

Active sampling is considered reliable due to extensive testing by organizations, such as OSHA, HSE, NIOSH, and others for regulatory compliance purposes. Moreover, the calibration and accuracy of airflow and collected volumes increase the validation precision. The backup section of the charcoal tube adds high assurance reliability (OSHA, 2008). The various new air pumps are designed with different air flow ranges from a few millilitres per minute to 10-20 litres per minute. This is for the consideration of collecting the same volumes of air in a shorter time possibility.

Air pump servicing is another consideration, as there are technical difficulties associated with the time it takes to calibrate the lack of required skills for calibration, achieving required airflow, interference with workers' job and pump aging and maintenance, including repair and recharging. Furthermore, the air pump instrument performance can be affected by electromagnetic radiation (e.g. near walkie-talkies or high-voltage equipment). Battery charging and maintenance of the pumps can also be other disadvantages, as well (Dietrich, 2003; Monteith, L. and Rubow, 2001).

2.4.2. Passive Air Sampler

Passive air sampling is the technique of collecting airborne gases and vapours via a diffusion mechanism, with no requirement for a sampling pump. This process is driven by the physical static force of the air, which facilitates the permeation of the contaminants into the device and traps them in a sorbent material. The contaminants' mass transfer rate follows Fick's first law of diffusion, which is basically the movement of molecules from a high concentration area (ambient air) to a low concentration area (inside the passive sampler at the sorbent surface) (Huey, 1996).

Equation 7 is Fick's first law of diffusion of the material:

Example: For the 3M organic vapour passive monitor the validated sampling rate for gasoline is 30.5 cc/min. This is the laboratory verified sampling rate; (3M, 2012). I it was used to sample an eight-hour shift, the equivalent air volume sampled would be:

Vol.: 30.5 cc/min X 480 minute (8-hrs) = 0.0146 m^3

If the lab reported a mass of 7.6 mg then the concentration would be:

Conc.: 15.3 mg ÷ 0.0146 m^3 = 1,050 mg/m^3 (exceeds the gasoline standard of 900 mg/m^3 or 300 ppm).

$$J = D\,(A/L)\,(C_1\text{-}C_0)$$

Where:

J = diffusion transfer (mass/time)
D = diffusion coefficient (cm^2/s)
A = Cross-sectional area of diffusion path
L = Diffusion path length
C_1= Ambient concentration of contaminant (mass/vol.)
C_0= Concentration of contaminant at collection surface (mass/vol.)

Equation 7: Fick's first law of molecules diffusion from high to low concentrations.

Every analyte has a specific diffusion in air coefficient value (D) and an uptake rate that depends on temperature, pressure, and the physical properties of the analyte and the geometry of the sampler. Such properties are determined via experiments and made available by testing agencies. OSHA publishes validated sampling and analytical methods for diffusive sampling of different chemicals as shown in Table 2-7.

SKC manufactures the organic vapour monitor (OVM) diffusive sampler 575-001 badges and they have validated uptake rates for BTEX and MTBE to the US OSHA, NIOSH and the American National Standard Institute (ANSI) protocols (OSHA, 2008; SKC, 2014). The diffusive sampler validation included full and bi-level protocols. The full protocol means that the badges passed NIOSH requirements for sampling rate, desorption efficiency, humidity effects, reverse diffusion, storage stability and interfering compounds. In the bi-level category, members of a homologous series (compounds with the same general formula such as methane, ethane, propane, etc.) passed full validation and the rest passed partial (SKC, 2014).

Table 2-7: OSHA validated sampling and analytical methods that permit diffusive sampling

Analyte	Method	Sampler
Benzene	Full Validation 1312 OSHA 1005 (OSHA Sampling and Analytical Method; Benzene)	SKC 575-001 SKC 575-002 3M 3520
Ethyl Benzene	Bi-level OSHA 1002 (OSHA Sampling and Analytical Method: Xylenes (o-, m-, p-isomers) Ethylbenzene)	SKC 575-001 SKC 575-002
Toluene	Bi-level OSHA 111 (OSHA Sampling and Analytical Method; Toluene)	SKC 575-001 SKC 575-002 3M 3520
Xylene (o, m, p isomers)	Bi-level OSHA 1002 (OSHA Sampling and Analytical Method: Xylenes (o-, m-, p-isomers) Ethylbenzene)	SKC 575-001 SKC 575-002 3M 3520
MTBE	1352 Full Validation	SKC 575-001 3M 3520

The sampler sorbent material types are manufactured in different materials depending on the desired contaminants to be collected. For hydrocarbons or VOCs, activated charcoal, also used in the active samplers, is also used for the passive ones. The passive samplers (Figure 2-4) are small and can be clipped onto the worker's collar to be near the nose and mouth of the breathing areas. The sampling process starts once the cap cover is removed and the air contaminants start diffusing through the membrane.

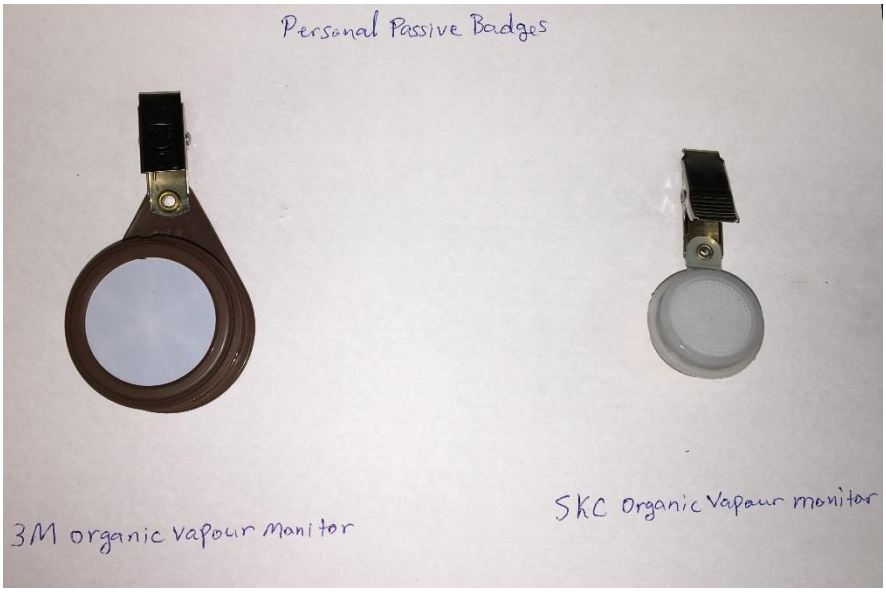


Figure 2-4: Badge type passive sampler with clip for personal air sampling for volatile organic vapours (from SKC 2014; 3M 2012).

There are other inorganic passive samplers, such as badges that are used for mercury vapour exposure assessments. Anasorb C300 sorbent capsules are made by SKC and validated by both OSHA ID-140 and MDHS 16/2 (SKC, 2011). Other types such as GABIE and RADIELLO badges are used for VOC exposure assessments (Carrieri et al., 2006).

2.4.2.1. Passive Air Sampler Selection Considerations

The passive badges are considered easy to use and require little technical training to operate. Passive samplers can be used to asses both long or short term exposures (ISO, 2003). However, the accuracy in estimating the short time (e.g. 15 minute) exposures is something to be considered for such a sampling method (Dietrich, 2003). Bartley (1986) suggested errors, which can reach 10% for estimating short term personal exposure when using passive samplers. This is due to the fluctuation of the contaminants', concentration in air and the ability of the sampler to respond to the concentration changes, which would be negligible

when sampling for longer periods such as for 8 hours (Bartley, 1986; Feigley and Chastain, 1982). Furthermore, Bartley (1986) suggested other possible losses from the sorbent, such as transient release to the air space of the sampler and transfer of analyte material to/from the sampler structure (e.g. walls).

High wind speed can affect the collection efficiency by disturbing the contaminant diffusion mechanism and conversely in a stagnant air space (<25 ft/min), "starvation" can occur (Dietrich, 2003; ISO, 2003). This means that there would be less air movement to replace analyte at the sampler surface at the same or higher rate than it is depleted by the sampler itself by its collection. Therefore, windscreens are used to avoid or minimize the high wind or turbulence effects.

2.4.3. Gas Chromatography

Gas chromatography (GC) is a widely used analytical versatile technique in the industry to analyse both chemical gas and liquid into their components by volume or mass (weight) (ASTM International, 1996). Its initial applications were analysis of gas and vapour from very volatile components followed by gas-liquid chromatography. It has been utilised to solve many problems in variety of fields such as drugs and pharmaceuticals, environmental studies, clinical chemistry, food and pesticides, and petroleum industry (Groby, R. L. and Barry, E. F., 2004). In the oil and gas industry, GC technology is used to analyse the gas and liquid hydrocarbons into their constituents with a very high accuracy. One of the recent challenging advantages is analysing heavy chemical components of as high as C_{80} within a reasonable time (hours) in little samples (Dandekar, 2013).

Generally, the essential parts of a GC units include injecting port, a porous packed column, a carrier gas (e.g. helium), temperature adjustable oven, and a detector (Figure 2-5). The separation process starts by injecting the sample via the injection port into the heated zone after which the sample vapour is carried by a gas carrier through the separation column and the detected components are recorded as a chromatogram (ASTM International, 1996; Groby, R. L. and Barry, E. F., 2004). The sample's components are separated according to their boiling points (BP) (isotherms) as they are eluted from the sample mixture starting with light components or low BP (high volatile) and ending with heavy molecule components or high BP.

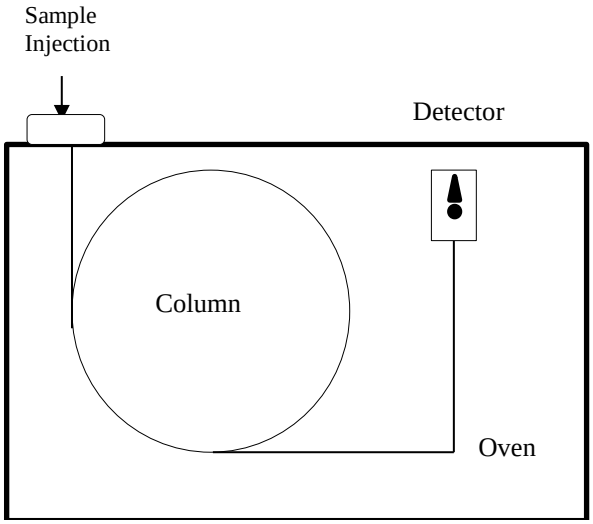


Figure 2-5: Simplified diagram example of main parts of gas chromatography machine [adopted from (Dandekar, 2013)]

The separated components are carried out to the detectors at the end of the column that determine their concentrations based on their areas under the detector response-retention time curve as shown in Figure 2-6. Analytical results are obtained by infrared spectroscopy (IR) and mass spectrometry (MS). Furthermore, flame ionization detector (FID) and photoionization detector (PID) are commonly used in combination with GC for hydrocarbon and other organic compounds detection (Danesh, 1998). The FID and PID's mechanisms are detailed in the ionization detection section of this thesis. The GC can also be used for other non-hydrocarbon components but with different detectors known as thermal conductivity

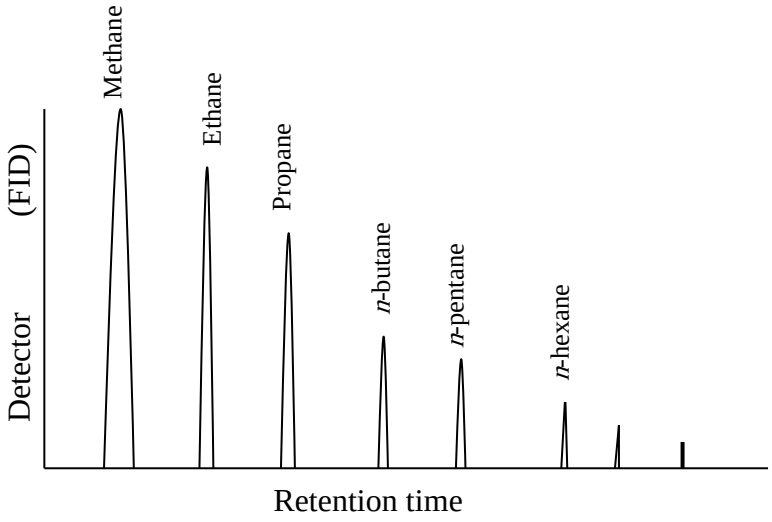


Figure 2-6: A gas chromatogram schematic illustrating peaks of eluted constituents [adopted from (Dandekar, 2013)]

detectors (TCD).

As shown in Figure 2-6 chromatogram is a graphical display from which chemical components are identified and their concentrations are determined based on retention times and peaks generated during the component separation when passing through the detector. The ordinate axis gives the concentration based on detector response and the abscissa axis indicates the time. Each peak refers to a component in a mixture. The retention time, which

is the time from injecting the sample till the appearance of a peak (tip of the peak), is a characteristic of the identity of the component.

The component is determined by comparing and matching its retention time and peak with a known reference's characteristics under the same conditions. However, because of some affecting analysis conditions, peak shape might differ from the ideal. Thus peak height may no longer represent the true concentrations. Therefore, the area under the peak is considered for determining the concentration. The concentration of a component in a mixture is calculated as a percent of the total as shown in Figure 2-7.

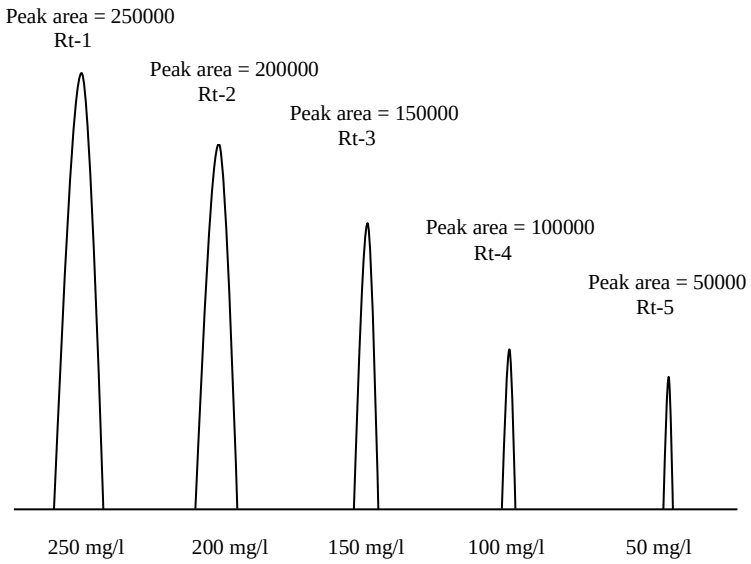


Figure 2-7: Peaks areas of different components with interpreting concentrations

In Figure 2-7 the peak areas are measures that represent concentrations of each component. Each of such areas is determined in accordance to the total area. Furthermore, the retention time (Rt) helps in identifying the entity of each component.

Many studies are being generated around the GC technology and its applications of eluting chemical components via different methods with different efficiency. The American Society for Testing and Materials (ASTM) is an international organization that develops and publishes technical standards including GC pertaining methodologies. Table 2-8 includes some of such studies related to petrol component analysis.

2.4.4. Air Grab Sampling

Table 2-8: Compositional analysis studies for petroleum products

Study Title	Designated No.	Compounds Examples	Purpose
Detailed Analysis of Petroleum Naphthas through n-Nonane by Capillary Gas Chromatography	D5134	- *n*-Butane - Benzene - Toluene - Xylene (*m & p)* - Ethyl Benzene	To determine hydrocarbon components of petroleum naphthas
Determination of Individual Components in Spark Ignition Engine Fuels by 100–Metre Capillary (with Precolumn) High-Resolution Gas Chromatography	D6730	- Benzene - Toluene - Methyl-t-butyl ether (MTBE) - Ethylbenzene - *p*-xylene	To determine individual hydrocarbon components of spark-ignition engine fuels and their mixtures containing oxygenate blends
Determination of C2 through C5 Hydrocarbons in Gasolines by Gas Chromatography	D2427	Olefins: - Propylene - Pentene Paraffins: - Propane - Isobutane	To determine the two (C2) through five (C5-) carbon paraffins and mono-olefins in gasolines.
Boiling Range Distribution of Gasoline and Gasoline Fractions by Gas Chromatography	D 3710	- Toluene - *p*-Xylene - *n*-Propylbenzene - *n*-Butylbenzene	To determine the boiling range distribution of gasoline and gasoline components

Grab sampling of air is a basic air sampling method, which is conducted by obtaining a certain volume of air collected into a bag, flask, bottles, canister or other means of containers in a very short time, of less than 5 minutes (Huey, 1996). The USA EPA has developed

method TO-14 and TO-15, which is designed for collecting air contaminants into specially-prepared stainless steel canister and Tedlar bags. The collected air is then analysed by different techniques (e.g. GC) at the laboratory or utilized as input to a direct reading instrument. Another grab technique is the colorimetric methods of which the tested gas or vapour is pulled through media that changes colour depending on reactions with the substances of interest and their concentrations. Grab samples are rarely used to estimate long term exposure (e.g. 8 hours) and are mostly used for peak exposure assessment or intermittent events to compare to ceiling limit standards (Rae, 2014).

2.4.4.1. Grab Sample Selection Considerations

Grab sampling is a quick process and usually takes all existing chemicals in the air at the sampling area. Depending on the compatibility between the chemicals and the material of the collection means, most of the samples can be retained for a long time before analysis. The vacuum canister is made of stainless steel and contains chromium to increase the stability of the collected compounds. Therefore, the type of canister, the reactivity of the material, and the condition of storage are important factors in selecting the collection containers (Król, Zabiegała and Namieśnik, 2010). Colorimetric samples give instant results by recording the change of colour. These kinds of devices are inexpensive, lightweight and easy to carry around to different locations, such as confined spaces (Sensidyne, 2015).

One of the clear disadvantages of the grab samples is that they, except canisters, cannot measure the entire working shift exposure (TWA). The closest estimation to the entire exposure working time is via integrated measurements, which are not a complete measurement of the exposure period. Furthermore, due to chemical reaction of the sampled

contaminants and the collection container materials, this method is not compatible for many chemicals such as hydrogen sulphide, nitrogen dioxide, and sulphur dioxide (Huey, 1996).

2.4.5. Direct Reading for Gas and Vapour Instruments

Direct reading instruments have been increasingly used and quickly developed in the industrial hygiene field for gas and vapour exposure assessment in the workplace. In recent instrumentation development, direct reading technology can be used for area monitoring and surveying (Smith et al., 2007). This is because they are small, lightweight and portable due to the possibility of their battery powered feature. Such instruments have been widely used in industry to instantly measure hazards associated with exposure to harmful chemicals, including volatile organic compounds in confined spaces. They are used to check the effectiveness of engineering control (e.g. fume extractors), personal exposures to contaminants at different locations during shifts, etc. (Todd, 2003; Woebkenberg and McCammon, 2001).

2.4.5.1. Ionization Technology

Ionization is one of the technologies used in direct reading of instruments to detect contaminants and record their concentrations. In this concept, the electrons of the compounds are energized (ionized) to change to a higher orbital and then the emitted energy of the compounds are measured as the electrons return to their original orbital. The commonly used ionization sources are photoionization and flame ionization. Such sources are built within detectors that measure the emitted energy and interpret it into concentrations of parts per million (ppm). Photoionization detectors (PID) and flame ionization detectors (FID) can both be used to detect organic compounds (hydrocarbons/aromatics) in the air (Thermo, 2015).

Photoionization Detectors (PID)

Photoionization uses ultraviolet lamps to ionize chemical molecules. Such lamps are available in different voltages to cause ionization of any species of contaminants, with an ionization potential less than the energy emitted by the lamp. When the molecule absorbs a photon through adequate energy provided, it loses an electron and becomes positively charged. Such positively charged molecules are received on a negatively charged collector electrode. This creates signals at the collector that is proportional to the amount of the ionized species (Rae, 2015). The mostly used lamp is the range of 10-11 electron volts (eV). Ambient humidity should be considered with the PID due to water interference effects (Barsky, Hee and Clark, 1985).

Flame Ionization Detector (FID)

Flame ionization uses a hydrogen/air flame to pyrolyse gas contaminants and produces ions and electrons inside an electrode gap. This process enables a flow in the gap and causes a current to flow in an external circuit. The current is measured by an electrometre, which in turn, converts it into concentrations. FID is a sensitive detector for organic compounds and not responsive (resistant) to water vapour. Compounds such as chlorine and sulphur in the air can affect its accuracy by reducing its response (Thermo, 2015; Woebkenberg and McCammon, 2001).

2.4.5.2. Infrared (IR) Detectors

Infrared (IR) spectrometry is another direct reading instrument that can be used for detecting organic compounds (e.g. hydrocarbons). This technology uses electromagnetic

spectrum wavelengths from 770 nanometre (nm) to 1000 µm. The wavelength 3.25-3.35 µm is the commonly used range for aromatic hydrocarbons (Baskins and Durham, 1982). Molecules of the tested compounds (contaminants) absorb IR and due to its low energy, it can merely cause vibration or rotation of the molecules. IR spectrometry follows the Beer-Lambert Law (Equation 8) due to the dependency of absorption of the wavelength:

$$A = \varepsilon bc$$

Where:

A = absorbance
ε = molar absorptivity
b = path length
c = concentration

Equation 8: Beer-Lambert law applied for the absorption of the infrared spectrometry by species molecules

The absorbance "A" depends on the energy absorbed and the remaining, as the incident energy is I_o and I is the energy remaining or not absorbed ($A = \log I_o/I$). Through the application of Beer's law, Equation 8, the concentration of the contaminants of interest is calculated depending on the absorption of the energy and the length of the path through the sample; The longer the path length in the instrument the more sensitive the detection (Todd, 2003).

2.5. Biological Exhaled Breath Sampling

Breath sampling has developed increasingly over the years for the assessment of VOC exposures at workplaces (Dyne and Wilson, 1997). Generally, VOC species are originated from cells (endogenous) and conversely externally (exogenous). Experimentally, in assessment of VOC exposure, if the concentrations in blood and breath are high and low in ambient air, it may indicate that the blood/cells are the sources of the detected VOCs

(endogenous). In contrast, if there is high concentrations in the ambient air and low in blood and breath, it may indicate that the VOC origin is exogenous (Mochalski et al., 2014).

The exhaled breath exposure assessment is more sensitive than the blood for VOC detection (Cao and Duan, 2006). The breath occupational exposure assessment is an exogenous method that captures the compounds in exhaled breath vapour (EBV) in the nanomolar range (Alonso and Sanchez, 2013; Amorim and de L. Cardeal, 2007). In this method, the exhaled breath is collected from the deep parts of the lungs (alveolar) or what is also called end-tidal, into an appropriate container. In this area, gases are continuously and rapidly exchanged between the pulmonary blood and the lungs, thus, the exhaled breath represents an indication of contaminant concentrations in the blood. These may be present as a consequence of exposure to chemicals through different routes of entry (e.g. inhalation and skin) into the human body. Gage et al., (1977) tested the collection of exhaled breath from volunteers in impermeable Plastigas bags (aluminum foil-plastics). The study was carried out on occupational exposures to fuel handlers including petrol station attendants, road tanker drivers, and seamen on coastal tankers. The collected breath was transferred into silica gel absorption tubes before it was desorbed and analysed by gas-liquid chromatography. This study concluded the possibility of detecting very low concentrations in the range of µg/l of VOCs including benzene using breath samples (Gage, Lagesson and Tunek, 1977). Wilson (1986) reviewed a number of breath sampling studies conducted to assess volatile compound exposures and concluded that breath samples can be a useful non-invasive alternative method to blood analysis.

Another study was conducted on five different occupations including traffic police officers, parking garage attendants, petrol station attendants, roadside storekeeper, and underground storekeepers. The study was conducted in Taegu, the third largest city in South Korea, to measure VOCs (i.e. benzene, toluene, ethylbenzene, and xylene) exposure via pre- and post-work breath concentrations that were matched and compared to controls. For the petrol stations, ten attendants (six males and four females), ranging in age from 21-33 years, were included, from five stations. The attendants spent an average of 6-8 hours daily, working at the petrol stations. The results showed that petrol station attendants had the highest post-work breath concentrations among the five occupations (Jo and Song, 2001).

Testing of petrol station attendants for BTEX has always showed a significant increase between the pre- and post-work breath concentrations and between exposed and non-exposed subjects (Alonso and Sanchez, 2013; Jo and Song, 2001). The gasoline component benzene is mainly taken up by the inhalation route of exposure. The blood-air equilibrium occurs after about 30 minutes of the inhalation exposure and about fifty percent (50%) in this equilibrium is retained in the body. The elimination, in contrast, follows in two phases: initially a short half-life of 2.4-2.8 hours followed by a slow half-life of 11-29 hours, with half-life depending on the dose (Ernstgård et al., 2014). A review summary for VOC lung retention from human volunteer studies, with exposure of at least 2 hours showed a large range of retention times of between 20-90%. The retention rates decrease for a continuous exposure to VOCs when the concentrations of the compounds increases towards equilibrium between absorption and metabolism and/or elimination (Jakubowski and Czerczak, 2009). The retention time for the BTEX and MTBE in particular is listed in Table 2-9.

Table 2-9: Lung Retention of VOC based on Human Volunteer Studies (Jakubowski and Czerczak, 2009)

Compound	No. of Subjects	Lung Retention %
Benzene	28	50-62
Ethylbenzene	6	49
Toluene	27	50-57
Xylene	32	60-75
MTBE	6	43-45

A novel device for collecting breath samples was developed by Dyne et al., (1997). The device is designed to capture the end-tidal air portion, which is then transferred into an automated thermal desorption system (ATD) to be analysed by gas chromatography and mass spectroscopy (GC-MS). This breath capture sampler was evaluated based on volunteer exposures to VOCs in industries such as shoe manufacturing, ink and coatings, and dry cleaning facilities. The experiment was conducted using 500 breath samples from 24 volunteers who were exposed to ten different solvents, including benzene, toluene, xylene, n-hexane. The method demonstrated high sensitivity, as low as 1 nmol/l (i.e. ng/kg) (Dyne and Wilson, 1997).

Another device known as “Bio-VOC” breath air sampler was (Figure 2-8) developed by Markes International in collaboration with the Department of Epidemiology, University of Wales College of Medicine, Cardiff, and Public Health of the Toxicology department of the UK Health and Safety Laboratory (HSL).

Figure 2-8: Bio-VOC Breath Sampler kit (Markes, 2014)

The device captures the last 100 ml from the end-tidal air of the exhaled deep breath of the alveolar portion in the lungs. The tested subjects are instructed to exhale through a cardboard mouthpiece into the plastic Bio-VOC sampler. It is necessary to explain to the workers to keep exhaling until the lungs were emptied and, therefore, capturing the end-tidal air. The collected breath in the Bio-VOC sampler is then transferred into a stainless-steel absorbent median (e.g. Tenax tubes) by steadily plunging it out. The captured chemical contents are then extracted by thermal desorption method (Markes, 2014).

Furthermore, the same researchers tested two methods of environmental and alveolar air assessments for six petrol station attendants for exposure to BTEX and MTBE. The study found that the most abundant compounds in ambient air were significantly correlated (r = 0.9) with the highest concentrations in the alveolar air. It was also found that the concentrations of VOCs in alveolar air were always lower than the ambient air. Another study by Chen et al. (2002) found significant correlations between exhaled breath and ambient personal air monitoring for toluene, xylene and ethylbenzene for petrol station attendants in Taipei City, Taiwan. A multiple regression analysis showed that the toluene concentrations

in exhaled breath were highly correlated with the personal air monitoring and the amount of gasoline sold ($r^2 = 0.762$); the concentrations of xylene in the exhaled breath were correlated with concentrations of the personal air monitoring and the wind speed ($r^2 = 0.665$). Ethylbenzene levels were too low to determine any relationships.

2.6. Biological Analysis of Gasoline Vapour Exposure Metabolites in Urine

Biological monitoring (BM) is conducted to assess workers' exposures to chemicals via different body entry routes at their workplaces. Biological monitoring can be a useful complementary approach to inhalation studies, especially if the chemical can be absorbed through the skin (HSE, 2011). Similar to the inhalation exposure limits mentioned earlier in the previous section, organizations, namely ACGIH, recommends BM values to assess exposure to different chemicals. These values represent concentrations of chemical indicators (biomarkers) in the biological media of exposed individuals as evidence of the substance uptake. ACGIH defines the Biological Exposure Indices (BEI) as "generally indicate a concentration below which nearly all workers should not experience adverse health effects (ACGIH, 2015). Table 2-10 lists the biological sampling data recommended by the ACGIH and used for monitoring exposures to benzene. It shows three metabolite biomarkers for benzene exposure, chemical identification method in urine, and the recommended sample collection time for post-exposures. The table also includes the concentrations of the determinants in the urinary creatinine of which they should not be exceeded and within which exposed persons should not experience adverse health effects. Urine sampling is easier to collect because it is not as invasive as blood withdrawal.

Table 2-10: The benzene biomarkers in urine for biological monitoring (from ACGIH, 2015)

Metabolite Biomarker	Exposure Chemical	BM Method	Sample Collecting Time	Exposure Limit	Reference
Phenol	Benzene exposure detection	Phenol concentration in urine	End of shift	250 mg/g creatinine	*ACGIH-BEI
***t,t,*-MA**	Benzene exposure detection	***t,t,*-MA** Concentration in urine	End of shift (9hrs)	500 µg/g creatinine	ACGIH-BEI
***S*-PMA**	Benzene exposure detection	***S*-PMA** Concentration in urine	End of shift (9hrs)	25 µg/g creatinine	ACGIH-BEI

* American Conference of Governmental Industrial Hygienist-Biological Exposure Indices.

The biological monitoring guidance values (BMGV) are set, based on the relationship between the biological concentrations and health effects or between biological concentrations and exposure at the levels of the workplace exposure limits (WEL), and/or based on industrial best practice recommendations. Exceedances of BMGV deem the necessity for investigation into workplace control measures and evaluation of their efficiency (HSE, 2011).

Gasoline components can be detected in the body using biological methods via urine and blood samples. For gasoline exposure, there are several biomarkers that are used to estimate body exposure/burden to components. There are different determinants listed and used in several occupational BM studies such as the concentrations of phenol in urine, *trans*, *trans*-muconic acid (*t,t,*-MA), *S*-phenylmercapturic acid (*S*-PMA), mandelic acid, phenylglyoxylic acid, hippuric acid, and methylhippuric acids as well as comet assay and micronucleus tests (Campo et al., 2011; Manini et al., 2010; De Palma et al., 2012; Rekhadevi et al., 2010; Singaraju et al., 2012). For benzene, which is known as the most toxic component in the gasoline, phenol concentration in urine is one of the metabolites commonly used to assess the benzene exposure levels via different exposure routes (Pandya et al., 1975; Singaraju et

al., 2012). Phenol in urine has been used to detect exposure to benzene in high levels that exceeds the OEL of 1-5 ppm (Weisel 2010; Boogaard 1995) . However, phenol in urine is not specific for exposure to benzene as it can be influenced by phenyl group components in food and medicine (European Commission, 1991). The *t,t,*-MA and *S*-PMA are other benzene biomarkers which were predominantly measured in a number of other studies. However, *t,t*-MA has been discussed as possibly influenced by the sorbic acid in food (Carbonari et al., 2016; Carrieri et al., 2006; European Commission, 1991).

It should also be considered that genetic variation (polymorphism) within the human population may affect the function and efficacy of any enzyme activity causing effects on the metabolism and the excretion of biomarkers. Some studies concentrated on three categories of polymorphism influencing benzene metabolism for polymorphic genes association with benzene biotransformation, oxidative stress, and DNA repair mechanism (Carbonari et al., 2016). A study conducted on biomonitoring of petrol station attendants evaluated effects of gender from exposure to benzene in the gasoline using *t,t*-MA urinary biomarker. The study was carried out in Rio Grande do Sul, Brazil on 20 men and 20 women of petrol station attendants and matched with same number of control subjects with no history of occupational benzene exposure. The study suggested potential effect of gender on the metabolite levels with increased median *t,t*-MA in women petrol station attendants (Moro et al., 2017). Furthermore, another study was conducted on 70 traffic policemen and 40 employees of the city Bologna, Italy. The study showed that median micronucleus (MN) frequency was significantly (P=0.007) higher in female indicating genotype dependant effects. Moreover, men with glutathione-S-transferase M1 (*GSTM1*)-null genotype had significantly higher MN

frequency than men with active *GSTM1* genotype (Angelini et al., 2011; Hoyos-Giraldo et al., 2009). It was indicated no association of *S*-PMA excretion with the studied polymorphism (Angelini et al., 2011). Another study also found that the enzyme activity of the polymorphic *GSTT1* and *GSTMA* can affect the concentration of *S*-PMA in urine (Carbonari et al., 2016). Manini *et al*. (2006) also found higher levels of urinary *S*-PMA in individuals carrying wild-type genotype than heterozygous and the mutant genotypes.

A study measured concentrations of five polycyclic aromatic hydrocarbons (PAHs) metabolites for exposed Middle Eastern adults. The urinary concentration of one of the metabolites (hydroxyphenanthrenes) was significantly higher among Arabs and Druze study participants (N = 56) compared to Jewish participants (N = 183) and not for the metabolite 1-hydroxypyrene. The higher hydroxyphenanthrenes concentrations were found for the participants consumed grilled food once a month or more (Levine et al., 2015).

The absorbed organic solvents (e.g. BTX) in the gasoline vapour via inhalation exposure get into the arterial circulation through the alveoli. This means that the solvents are distributed widely into the body before they are metabolized by the liver for degradation and excretion via urine and potentially bile. Such solvents are converted to water-soluble substances through oxidative and conjugative reactions through the involvement of the enzyme P450 and the glutathione pathways. As results from the biotransformation, the metabolites are formed and excreted. Such metabolites are used for the biological monitoring (Teaf, 2000). Benzene undergoes biotransformation mainly in the liver and can also be metabolised in the lungs and bone morrow (Carbonari et al., 2016). It is metabolised in the liver by CYP2E1 enzyme to benzene oxide (BO) which is the source of different metabolites

(e.g. phenol, *S*-PMA, and *t,t*-MA), as shown in Figure 2-9, due to its electrophile characterisation that makes it bind to macromolecules (Sungkyoon K. et al. et al., 2006). Phenol is then formed through spontaneous rearrangement of the BO which undergoes further CYP oxidation to form hydroquinone (HQ). Another different path of CYP oxidation of BO associated with ring opening forms the muconaldehydes which also are capable of binding to macromolecules to ultimately form *t,t*-MA. Furthermore, the reaction of small amount (1% of total benzene update) of the BO for detoxification via conjugation with glutathione (GSH), by glutathione-S-transferases (GSTs), cause excretion in urine as *S*-PMA (Carbonari et al., 2016; Melikian et al., 1999; Schettgen et al., 2008; Sungkyoon K. et al. et al., 2006). Unlike *t,t*-MA there is unknown dietary sources that would possibly cause production of *S*-PMA, therefore, excretion of *S*-PMA is only referred to exposure to benzene (Schettgen et al., 2008).

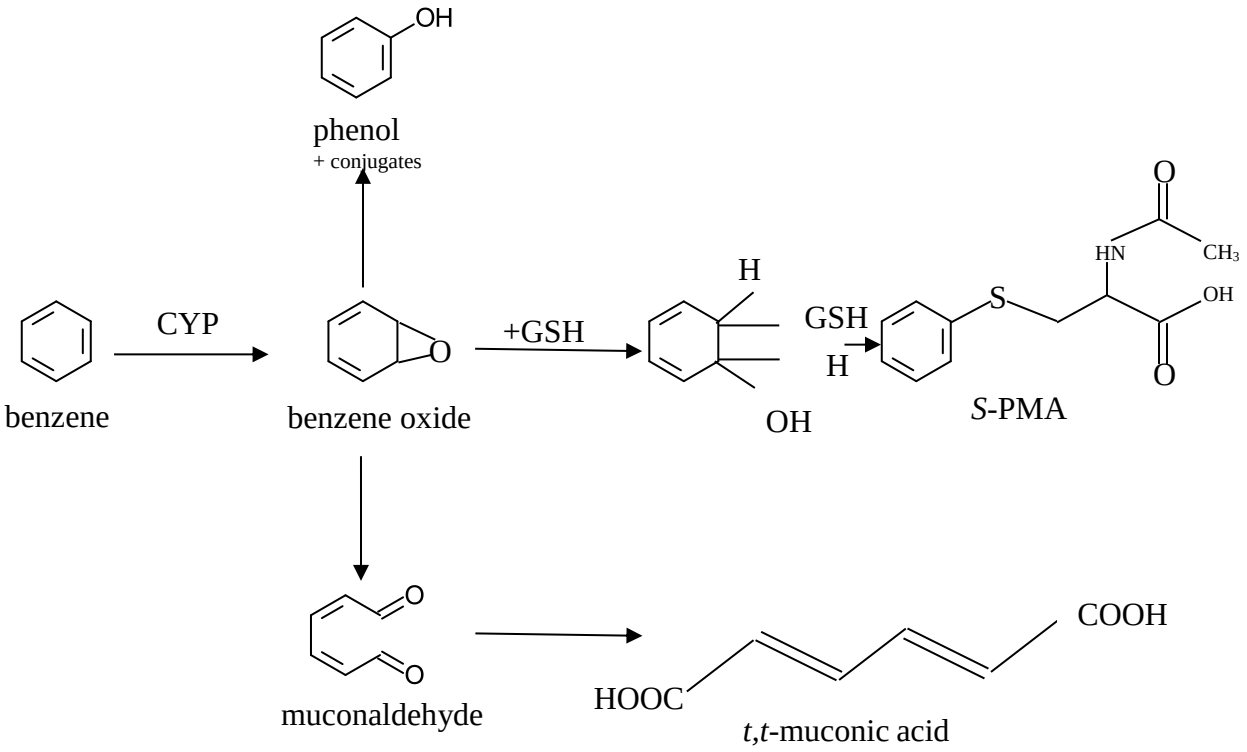


Figure 2-9: Metabolic pathways of benzene and the formation of three urinary biomarkers

Toluene's biomarker is hippuric acid metabolite in urine which is mainly generated in the liver as primary site. This metabolism transformation of the toluene is initiated by the cytochrome P450 enzymes. The main formation pathway of the hippuric acid is through the metabolism of toluene via hydroxylation of the methyl group to form benzyl alcohol which is then oxidized to form benzaldehyde and benzoic acid. The conjugation of glycine and benzoic acid produces the hippuric acid (

Figure **2-10**). This is considered the main route of detoxification and elimination of toluene (Aspey et al., 2008; US EPA, 2010).

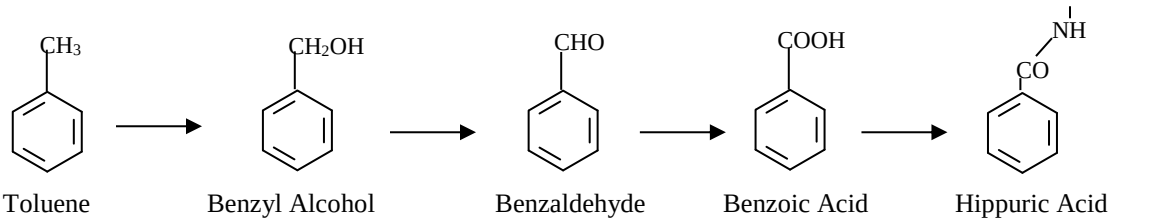


Figure 2-10: Metabolic transformation pathway of toluene and its hippuric acid metabolite (Aspey et al., 2008)

The xylene's principal biomarker in urine is methylhippuric acid. The metabolic pathway of xylene is through oxidation biotransformation that converts xylene to methyl benzylalcohol then to toluic acid (methylbenzoic acid). The last formation of *o-, m-, and p-* methylhippuric acid (toluric acid) metabolites is through the conjugation of toluic acid with glycine as shown in Figure 2-11 (Aspey et al., 2008).

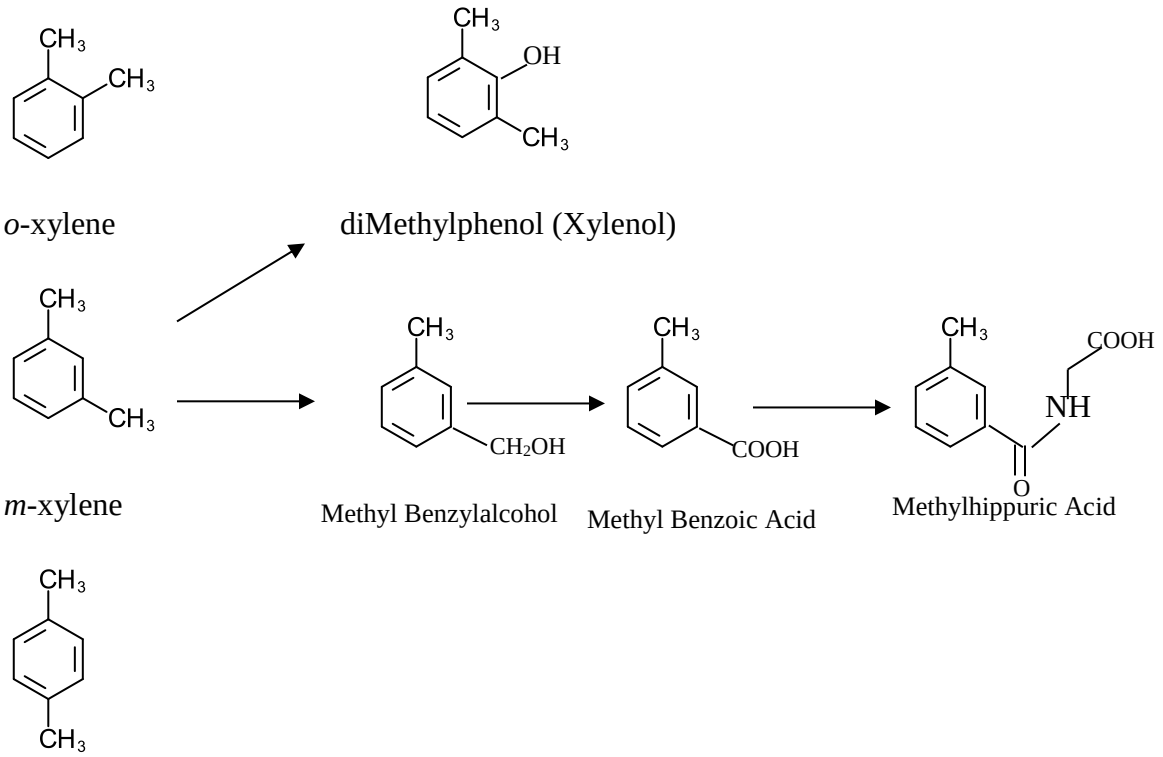


Figure 2-11: Metabolic transformation pathway of xylene and its methylhippuric acid metabolite (Aspey et al., 2008)

In the metabolism process of MTBE, the cytochrome P450 enzyme forms tertiary butyl alcohol (TBA) and formaldehyde via oxidation as shown in 2Figure 2-12. The formaldehyde is very reactive and mostly metabolised in the liver. The TBA is further oxidized into 2-methyl-1,2,propanediol and α-hydroxyisobutyric acid (Ahmed, 2001; Brady, J.F., Xiao, F., Ning, 1990). In humans' liver, CYP2A6 is the main P450 isoform responsible to metabolise MTBE (Ahmed, 2001; Hong et al., 1997). Variable portion of the MTBE (20-70%) is eliminated via exhalation and the remaining is eliminated via urine (Ahmed, 2001; Miller, M.J., Ferdinandi, F.S. Klan, M. Andrews, L.S., Fielding Douglas, J. and Kneiss, 1997). The pharmacokinetics of MTBE in humans showed very quick elimination from blood but

significant amount of TBA remains for long after exposure was stopped. The complete metabolism of MTBE in humans was estimated with half-life of 7.8-17 hours with 35-69% was recovered as metabolites in urine (Ahmed, 2001; Amberg, Rosner and Dekant, 1999).

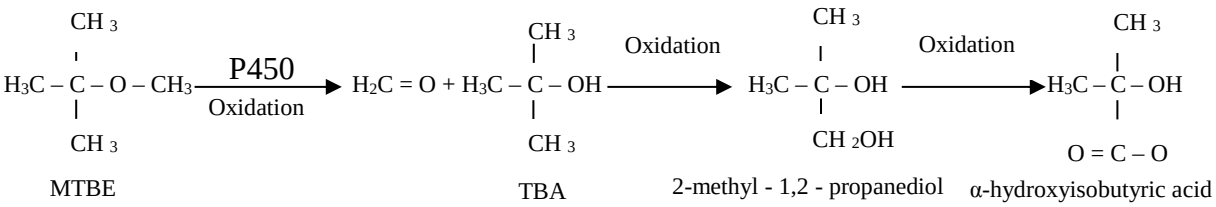


Figure 2-12: Structural metabolic pathway of MTBE adopted from (Ahmed, 2001).

A comparison study was conducted by Carrieri *et al*, (2006) on 33 petrol station attendants in northern Italy using both environmental (personal air samples) and biological assessments to find out which of the two biomarkers, *t,t*-MA and *S*-PMA is most correlated with the environmental exposure. Air samples were collected during entire working shifts using diffusive (passive) samplers. Urine samples for the biological exposure assessment were collected before and after work shifts. All of the petrol stations used in the study were designed with gasoline exposure reduction techniques; vapour extraction systems on pumps, self-service stations (i.e. vehicles filled by owners) and the fuel contained less than 1% benzene. Results showed lower concentration values of benzene than the recommended exposure standard TLV-TWA by ACGIH (air sample: 0.044 mg/m^3; *t,t*-MA: 171 µg/g

creatinine; and *S*-PMA: 2.7 µg/g creatinine). Significant increases of S-PMA (p<0.0001) and *t,t*-MA (p<0.005) were found in the end shift samples compared to the beginning of the shift samples. No significant relationship was observed for airborne benzene concentration and benzene end shift metabolisms (*S*-PMA and *t,t*-MA). Furthermore, the study suggests that the environmental exposure assessment is better for individual exposure assessment than bio-monitoring although the biological exposure assessment is useful for group exposure assessments. Comparison of *S*-PMA and *t,t*-MA studies showed that excretion of *S*-PMA was higher in smokers because of the benzene in cigarettes. The benzene level can fluctuate from 20 to 90 µg per cigarette (Carrieri et al., 2006; European Commission, 1991). This study concluded an important fact to be considered when sampling for benzene exposure using the biological marker *S*-PMA for smokers and second-hand smokers, as it can be a confounding bias. This drawback can be eliminated or minimized by determining the cotinine marker in urine among the subjects. Cotinine is considered a reliable indicator metabolite of nicotine for tobacco in smokers. It is more dependable than the nicotine in urine itself due to its higher half-life (15-19 hr) in the body (Hagan, Ramos Jr and Jacob III, 1997; Lee So Ryong, 2014). The determinant cut-off value for smokers/second-hand smoker and non-smokers is 100 $\mu g.L^{-1}$ (Lee So Ryong, 2014). This value was also determined from laboratory studies and statistically analysed results (Tranfo, 2014). Storage temperature of the collected urine samples to be analysed can affect the level of cotinine, as it can increase it to almost double over 30 days storage at elevated temperatures (Hagan, Ramos Jr and Jacob III, 1997).

A study by De Palma *et al*., (2012) found a significant correlation between gasoline airborne contaminant exposure and the corresponding urinary biomarkers, unmetabolised

urinary benzene (U-B), *t,t*-MA, *S*-PMA, and MTBE (U-MTBE) when conducted across sectional gasoline exposure studies for 102 petrol station attendants in the city of Parma, Italy. Biological monitoring was conducted for BTEX and additionally MTBE. The study suggests the applicability and validity of utilizing bio-monitoring for BTEX and MTBE at low concentration exposures. The study further compared gasoline vapour exposure of attendants in full service and self-service stations and found higher exposure levels at the full service station (De Palma et al., 2012).

Other biomarker studies have been conducted on petrol station attendants to evaluate damage and repair of DNA, using comet assay (sensitive technique for the detection of DNA damage) after exposures to gasoline vapour during their regular daily working shifts. These are considered to thus have carcinogenic effects. A Comet assay that damaged DNA in the exposed group when compared to controls even after exposure to levels lower than the occupationally acceptable standards (Keretetse et al., 2008; Rekhadevi et al., 2010). Such damage was attributed to exposure to BTX causing genetic changes. Exposure time was also correlated with DNA damage and repair levels (Rekhadevi et al., 2010).

Another biomarker study by Manini et al., (2010) was conducted on 239 workers (policemen, taxi drivers, and petrol station attendants) in Parma City, Italy, to investigate the association of occupational exposures to low levels of benzene in gasoline and nucleic acid oxidation (damage of DNA and/or RNA). The median airborne level of benzene exposure for petrol station attendants, in particular, was 0.011 ppm. The study used the biomarkers *t,t*-MA, *S*-PMA in urine and the nucleic acid oxidation. Generally, from the three occupations, significant correlations were found among biomarkers and nucleic acid oxidation.

Furthermore, the study found that the urine metabolites were produced more in smokers than non-smokers. The nucleic acid oxidation (8-oxodGuo) was also detected at higher levels in smoker petrol station attendants compared to the corresponding non-smoking group ($p<0.05$). The study indicated that this was an important confounding factor, especially when considering low levels of benzene exposures (Kirkeleit et al., 2006; Manini et al., 2010). This study concluded that there is a significant association between exposure to benzene and the oxidation damage to nucleic acids. RNA seems to be more susceptible than DNA to nucleic acid oxidation due to exposure to benzene (Manini et al., 2010).

Singaraju et al., (2012) applied a micronucleus (MN) assay to assess DNA damage (cytogenetic damage) among petrol station attendants and non-exposed control groups. They used exfoliated buccal cells (cells from the cheek) obtained from petrol station attendants. The study suggested that MN frequency is an effective measurement tool (Pitarque et al., 1996; Rekhadevi et al., 2011) to assess DNA damage from exposure to toxic chemicals. This had the advantage of being a non-invasive method (rather than using blood). Furthermore, it was suggested that several sampling times are required during a 7 to 21 day period from the exposure to determine the optimal peak expression of the MN for DNA damage (Singaraju et al., 2012).

A similar study of utilizing the MN frequency test was conducted by Celik *et al.,* (2003) in Mersin city in south Turkey to assess cytogenetic damage from occupational exposure of petrol station attendants to gasoline vapours that included benzene. The study included 50 attendants with 50 matched age and sex controls. The analysis of buccal cells showed that the MN and nuclear abnormalities were significantly higher in the petrol station attendants

than the control subjects with the conclusion that station attendants are at risk of cytogenetic damage, especially, smokers.

Sister chromatid exchange (SCE) is a test of mutagenicity that is conducted to examine the exchange of genetic materials between two identical sister chromatids. The number of exchanges per chromosome pair during mitosis is an important matter in determining the mutagenicity. Therefore, the frequency of sister chromatid exchange is a tool used to indicate possible formation of tumours (Lacquaniti et al., 2012). Çelık and Akbaş (2005) investigated SCE in peripheral blood lymphocytes from 30 petrol station attendants (15 smoker + 15 non-smokers) matched with 30 controls (15 smoker + 15 non-smokers) from 12 stations in Mersin, Turkey. The results showed that there are significant differences in SCE values ($P < 0.01$) in the petrol station attendants indicating occurrence of genotoxic effects on the lymphocytes. Furthermore, the tested phenol levels in urine showed a significant difference in the mean values of 7.24 to 19.85 mg/g/L for the control and exposed workers, respectively.

2.7. Laboratory Analysis and Desorption Methodology

2.7.1. Desorption of Collected Contaminants

Suitably equipped analytical laboratories are required to analyse airborne hydrocarbon contaminants collected via active and passive sampling methods using absorbent materials (e.g. charcoal). The airborne contaminants are desorbed (recovered) via solvent and thermal extraction processes. Carbon disulphide (CS_2) is the most commonly used solvent for desorbing contaminants, due to its high desorption efficiency and less interference with GC analysis (Pozzoli, Cottica and Ghittori, 1982). The solvent solution that contains the extracted contaminants is then injected into the GC for separating the individual components of

interest. The thermal process is the technique for desorbing analytes from Tenax samplers of the Polydiphenyl oxide and Poly 2,6-diphenyl-*p*-phenylene oxide material for active and passive sampling mechanisms (ISO, 2003).

Thermal desorption is conducted by exposing the sorbent containing the contaminants to elevated temperatures. The desorbed contaminants are driven by an inert gas (e.g. nitrogen, helium, argon) through the GC separation column. This method is capable of measuring very low concentrations of parts per billion (ppb) of VOCs (Jian et al., 2012). The GCs can be attached to photoionization or flame ionization detectors to measure the concentrations of the individual contaminants coming through the GC column.

2.7.2. Desorption Efficiency

The desorption efficiency (DE) or recovery coefficient is a value used to determine the actual amount of the contaminants that can be recovered from the sorbent material expressed as a percentage. The DE is determined via experiments of injecting known volumes, typically 0.1, 0.5, 1.0, or 2.0 times the target concentration (e.g. OEL) of the analyte, under certain conditions and flow rate, then the recovered amount is measured as a percentage. The DE depends on the solvent used for the desorption process. CS_2, as mentioned earlier, is one of the highly efficient solvents for desorbing analytes from charcoal sorbent. Lepera and Colacioppo (2002) determined 75% DE for most of the 22 compound mixtures tested, including benzene, toluene, xylene and ethylbenzene desorbed. A mixture of 99:1 carbon disulfide-dimethylformamide was used to desorb the solvents of interest from the charcoal adsorbent. This experiment was conducted to evaluate occupational exposures to multi solvent mixture.

Pozzoli et al, (1982) proposed a simple method for estimating desorption efficiency from air samples collected in the field, without referring to a prepared standard. The proposal is basically using double elution of the absorbent (i.e. charcoal) by the same amount (e.g. 5 ml) of the CS_2 and calculating the DE, by dividing the second time collected desorbed organic substance by the first time collected amount. A strong correlation was illustrated when the results from this proposed method was compared to the traditional method of using or referring to standards.

2.8. Gasoline Vapour Exposure Control Measures

Governmental efforts have been exerted for the protection of the ozone layer under the ozone abatement program through the reduction in VOC emissions. An example of an ozone and health protection measure is the use of safer VOC containing gasoline fuel that contains less benzene and other harmful aromatics. The European Directive 98/70/EC limited the benzene level, in particular, for health protection, to a maximum of 1% in gasoline (European Commission, 1998). In the United States, the current benzene content of gasoline fuel is 1% by volume. Further reductions have been requested by the Environmental Protection Agency (EPA) to all petroleum refineries within the USA to produce gasoline with 0.62 vol. % benzene (US EPA, 2007).

Engineering control of vapour recovery/extraction systems has also been applied as a control measure to minimize gasoline vapour releases. The European Directive 94/63/EC recommended the application of vapour recovery systems for facilities and mobile transportation of gasoline with classified toxic, carcinogenic, or teratogenic components. The vapour control requirements apply to classified facilities, including petrol stations with

certain amounts of emission/losses percentages of the throughputs and are implemented within a determined time. For petrol stations, the target is to reduce annual loss of gasoline during loading/unloading to below 0.01 w/w % of the throughput (European Commission, 1994). Vapour recovery has been applied in the USA since 1980s and was initially required by the Clean Air Act (CAA) in 1990 as part of ozone protection policies (CT, 2011). This in turn has helped reduce personal exposures at petrol stations and in the surrounding vicinity (Gonzalez-Flesca, Vardoulakis and Cicolella, 2002).

A typical gasoline vapour recovery system consists of a Stage I system, which works to return gasoline vapour from the main holding tanks (usually underground tanks) into the tanker trucks during refilling. The Stage II system works to recover vapour released from refuelling vehicle tanks (Four Corners Air Quality Task Force, 2007). Stage II requires special design dispensing nozzles to eliminate released amounts of gasoline vapour going into the atmosphere by returning the vapour back to the main holding tanks. A simplified diagram of stages I and II are displayed in Figure 2-13.

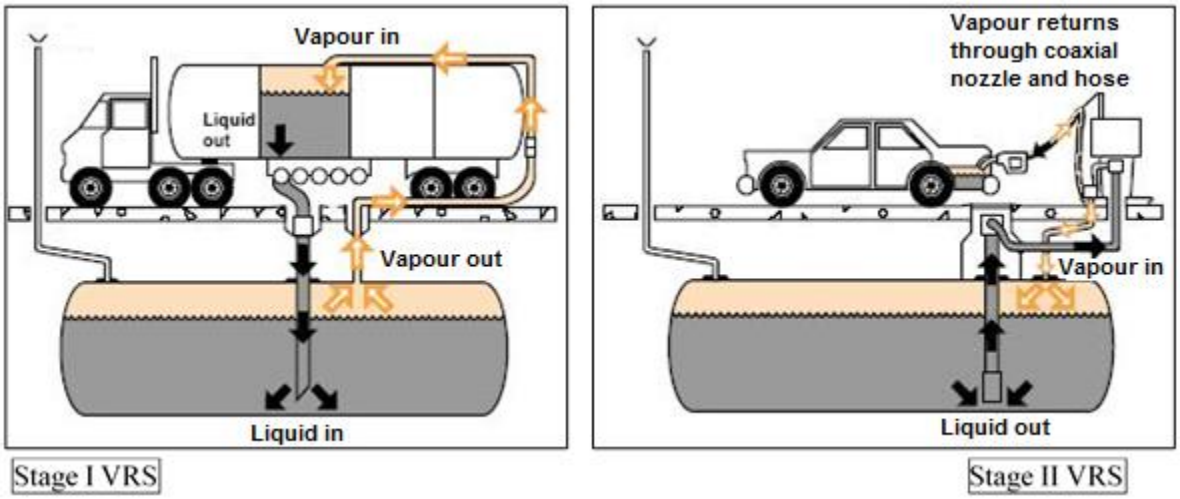


Figure 2-13: Stage I and II of Gasoline Vapour Recovery Systems (VRS) (CT, 2011)

Stage II systems are available in either a Balance-Type or a Vacuum Assist-Type stage. The Balance-Type transfers vapour from vehicle tanks into underground storage tanks based on pressure differentials. The nozzle is equipped with an accordion-like rubber piece (Figure 2-14) that tightly seals around the filling pipe onto the tank opening lip to allow the transfer of the gasoline vapour back into the holding tanks.

Figure 2-14: Balance-Type Stage II Nozzle of Gasoline Vapour Recovery Systems (Photo by Ahmed Alyami, 2008).

The vacuum assist stage II type system requires a vacuum source (e.g. pump) to move vapour from the vehicle tank to the holding tanks. This design requires a different type of nozzle, which is especially designed with holes (perforated) around the tip of the nozzle (OEC, 2011) to facilitate extracting gasoline vapour into the line to the holding tanks Figure 2-15. Studies by the Minnesota Pollution Control Agency estimated that the efficiency of Stage I and II vapour recovery systems can reach up to 95% (Four Corners Air Quality Task Force, 2007), which may be economically recycled in addition to achieving the minimization of health hazard risks. Another study (Berglund and Petersson, 1990) showed that vapour recovery can reduce both personal exposure by up to 99% and the release of

Figure 2-15: Vacuum-Type Stage II Nozzle of Gasoline Vapour Recovery System (OEC, 2011)

vapour into the atmosphere by 95%. In Mexico City, a vapour recovery system applied at 10 petrol stations showed a reduction of more than 80% (Cruz-Nunez, Hernandez-Solıs and Ruiz-Suarez, 2003).

The third type of gasoline vapour recovery system is known as on board refuelling vapour recovery (ORVR). This system is designed to adsorb gasoline vapour in the vehicle tank air as it is replaced with liquid gasoline during refuelling. The system blocks the vapour inside the tank from escaping to the atmosphere and diverts it to an adsorbent canister. The recovered vapour can be returned to the vehicle engine to be used. This system is built in specifically designed gasoline operated vehicles that were first introduced in 1998 (US EPA, 2012).

Gonzalez-Flesca *et al.,* (2002) verified the efficiency of the Vapour Recovery System stage II, by investigating the recovery at the vehicles' tanks of an example system installed at a station in Yvelines, France. The study considered the influence of weather factors (wind speed, wind direction and temperature), amount of gasoline sold and contributions of nearby road traffic in the assessment of the recovery of BTX vapours. Concentrations of BTX, especially, benzene, were higher at the 0.2 metre height than at 2 metres. This was attributed to product spillage and shorter distances to cars' exhausts. Furthermore, the concentration

level of BTX near the pumps was four times higher than outside the station (background). The study also found that the wind speed had a significant effect on the vapour recovery efficiency. There was a critical wind speed value above which vapour recovery is no longer effective. The higher the wind speed (3.5 m/s), the lower the recovery efficiency.

A study on customer exposure to gasoline vapour components, including benzene and MTBE, during refuelling was conducted at two petrol stations in Finland during the summer of 1996. The stations are equipped with a stage I vapour recovery system, and the gasoline contained 2.7% MTBE and 0.75% benzene. The customer refuelling time average was estimated to be 69 seconds. The study results showed that MTBE had a mean value of 0.91 ppm in the customers' breathing zone compared to an average of 0.030 ppm in the ambient air. The benzene exposure level was 0.28 ppm and 0.003 ppm in the area. The study was conducted in an average wind speed of 1.4 $m.s^{-1}$ and normal temperature of 21˚C (Vainiotalo et al., 1999).

Overall, the available literature suggests that monitoring is required to evaluate and determine the relative risks to petrol pump attendants in Saudi Arabia where no previous studies of this type have been previously conducted.

CHAPTER 3

3. Gasoline Content Characterization

3.1. Introduction

The objective of this chapter was to analytically study the chemical composition in the vapour from Saudi Arabian produced gasoline mixtures at different ambient simulated temperatures in the laboratory. This was to produce chemically speciated data of the gasoline vapour and to determine the behavioural characterisation of the chemical constituents under different environmental conditions. This would be beneficial to evaluate the health risks to petrol station attendants from exposure during their 8-12 hr shifts. The gasoline mixture has a flashpoint of – 43ºC and a wide boiling point range (27-221ºC) (ATSDR, 2014; British Petroleum, 2012). Thus, many of the hydrocarbon components in the mixture vaporise at room temperature (25ºC). It should be also borne in mind that the concentrations of each chemical component varies with temperatures.

Currently there are a lack of studies to clarify such notion, therefore, information provided here will help in evaluating the risks in the extremely hot weather conditions in many parts of Saudi Arabia. Thus it was important to obtain information on the vaporisation rates at high temperatures (45°C). Analytical testing was carried out for standard gasoline mixtures that have predetermined composition specifications. The liquid and vapour of the gasoline mixtures was analysed using gas chromatography (GC) for both 91 and 95 Research Octane

Number (RON) gasolines used in the country. The vapour mixtures were further characterised via a headspace technique and analysed by GC.

The vapour samples were analysed at two temperatures, 25°C and 45°C, to simulate the temperatures inside vehicle fuel tanks and the underground storage tanks during the summer and winter seasons. The analysis of the summer and the winter gasoline formulas was carried out to examine any differences in the gasoline mixtures and the chemical components. The gasoline vapour profiling at the two temperatures would also help to better understand the vaporisation behaviour of the individual compounds and their abundance in the vapour mixtures.

3.2. Material and Methods

For the purpose of determining the vehicles' and underground tank temperatures, a digital KTJ TA318 ExoTerra, 1.5 volt battery operated indoor and outdoor dual sensor thermometre (Figure 3-1), with measurement range of -50ºC to 70ºC and ±1ºC accuracy, 25% to 98% RH (±5% RH) was used. The metre was modified by adding a 1.5 m Tygon flexible tube along the external sensors' wire to enable reaching the gasoline liquid inside the tanks. The same instrument was also utilised in the verification of the water bath temperature prepared for heating samples in the laboratory. The temperature measurements were taken ten minutes after the insertion of the sensor into the tanks to allow for measurement stabilisation. The instrument displayed the inside and outside measured temperatures on the digital screen. Measurements were taken from fuel tanks of different vehicles on randomly selected days with various ambient temperatures during the summer and winter of 2013-2014. The

measurements were taken just after the vehicles had been parked, similar to a refuelling scenario at petrol stations.

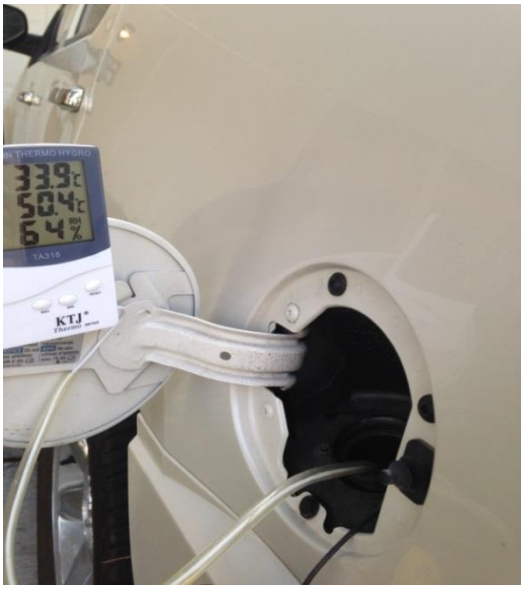


Figure 3-1: A modified dual sensor digital thermometre used to measure the gasoline temperatures inside and outside vehicle tanks and underground storage tanks

The gasoline fuel samples were brought to the laboratory in one-litre gas sealed cap glass bottles by The Cary Company 1195 W. Fullerton Ave, Addison IL 60101 and stored at 0°C in an explosion proof cooler (GFL), especially designed for the laboratory storage of hydrocarbon products. A GC analyser (Model 6890N Agilent Technologies) connected to a flame ionization detector (FID) was used for all tests. The gasoline sample (0.5 ml) was transferred to a 1ml vial by gas-tight syringe injection and then placed in a temperature adjusting water bath (Figure 3-2) for 60 minutes. The samples were removed from the water bath and then put into the auto sampler tray for the vapour to be extracted and injected into the GC immediately (Figure 3-3). This process was conducted in a very quick time (few seconds) to avoid temperature change. Furthermore, a GC temperature controlled circulating water bath (Figure 3-4) was available but was not used as the aforementioned method was

found to be satisfactory. This device's function basically is to heat the water and circulate it through the GC vial trays hollows to provide the vials with the required temperature.

Figure 3-2 Heated water bath with temperature digital indicator for heating gasoline in vials.

The different temperatures tested were 25°C and 45°C for both gasoline grades of 91 and 95 to represent the summer and winter temperature simulation. Each temperature trial was tested with a new gasoline sampling vial.

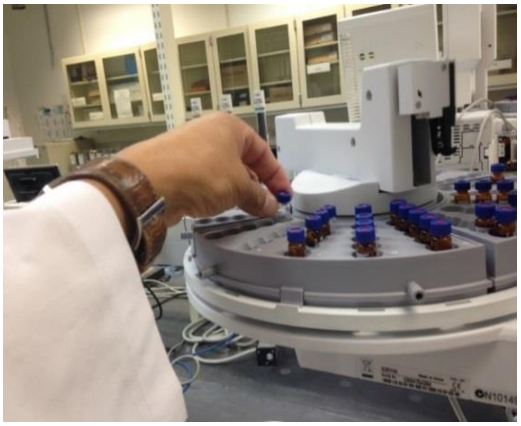


Figure 3-3: A gas chromatography vial tray with the GC auto sampler

Figure 3-4: Example of GC temperature controlled circulating water bath

Two separate gasoline liquid samples of both gasoline grades (91 and 95) were analysed at each designated temperature (25 ºC and 45 ºC). A similar process was followed for the gasoline vapour analysis at each temperature (Table 3-1).

Table 3-1: The number of samples analysed per gasoline type and temperature

RON	Liq./Vapour	25ºC	45ºC
91	Liquid	2	2
	Vapour	6*	6*
95	Liquid	2	2
	Vapour	3	3

* Three samples from the winter formulae

The GC syringe collects a vapour volume of 1 µl (ASTM International, 2008) at the depth of 0.2 mm into the vial headspace and this was injected into the column with a temperature of 275°C. Helium was the carrier gas through a 100 m x 0.25 mm ID capillary column (RESTEK Rtx-100-DHA by Restek Corporation, U.S., 110 Benner Circle, Bellefonte, PA 16823) coated with 0.5µm of methyl silicon stationary phase.

The liquid gasoline sample was kept at 25°C and injected directly into the GC. The results of the separated compounds were prepared for comparison with others tested in the vapour state. Results of components were reported via GC associated software "Hydrocarbon Experts" sorted by chemical groups (e.g. paraffin, aromatics, olefins, etc.), carbon numbers, separation time, volume percentages, and mol-fraction. The procedure (ASTM International, 1996) was applied to calculate percentage and mol-fraction of the individual components. This procedure reports to the nearest 0.1 % using the following equation:

$$Component\ percent\ = \frac{S \times A \times 100}{B \times (100 - S)}$$

Where:
S = percentage of standard
A = peak area of the component
B = peak area of the internal standard

Equation 9: Calculation of percentages of components of gasoline mixture separated via GC

Sample components is determined from the chromatogram by comparing peak areas with known amounts of calibration standards (std.). The following is an example for benzene component calculation:

Benzene: Sample wt. = 21.441; Sample vol.% = 149.32 (100/sp.gr.); area of std. = 15.97172; Std. wt. = 0.6266; Std. % (std. wt./(sample wt.+std. wt.)*100) = 2.839457; A = 0.43761; benz sp.gr. = 0.8844; benz wt. % = ((std % / area of std.)*benz A) = 0.08; benz vol fraction = (benz wt.% / benz sp.gr.) = 0.09; benz vol.% = (100/vol%* benz vol fr) = 0.07.

Headspace analysis was used to examine the volatility of gasoline compounds with the use of the GC. The concentration of each compound in the vapour phase (V) was determined and compared to its original concentration in the liquid phase (L). The partition coefficient

(K) is the equilibrium distribution of an analyte between the liquid phase and vapour phase (Restek, 2000).

3.3. Results

The results of the measured vehicle tank temperatures are listed in Table 3-2. Eleven separate measurements were taken from various vehicles during different ambient

Table 3-2: Temperature measurements inside and outside vehicle fuel tanks

Car Type	Temp. In (C°)	Temp. Out (C°)
Ford Windstar 2001	22	17
Ford Expedition 2010	24	19
Ford Mercury 2007	22	17
GMC Yukon 2012	32	28
Honda Accord 2004	20	19
Mercedes Benz 1998	32	21
Ford Windstar 2001	35	32
GMC Yukon 2012	50	34
Ford Windstar 2001	43	35
Ford Crown Victoria 2010	47	35
Ford Crown Victoria 2010	51	37

temperatures. Some of the measurements were repeated for the same vehicles for different temperatures.

The chemical compounds in the gasoline liquid were separated using the GC at 25°C. Appendix D lists all detected liquid components of the gasoline. The average numbers of 66 and 73 components were detected in the 91 and 95 RON, respectively. The components' volumes ranged from 0.09 to 13.7% in 91 and 0.077 to 14% in 95. The compounds' ranges are listed by chemical groups in Table 3-3. The paraffin, iso-paraffin and the aromatics were the largest volume groups. These were the compounds in the range of C_5-C_7. The oxygenates had small volumes because it is a singular compound (MTBE) compared to other groups of components. The olefins and n-olefins were the smallest groups.

Table 3-3: Component grouping of the analysed grades of gasoline liquids at 25°C

Group	RON 91 (n=66) % Vol Range	RON 95 (n=73) % Vol Range
Paraffin	21-22	17-18
iso-Paraffins	29-30	27.7-29
Aromatics	30-32	34-35
Naphthalenes	0.1-0.21	0.1-0.2
Olefins	0.09-0.1	0.10-0.15
Naphthenes	3.5-3.6	2.3-3.2
n-Olefins	0-0.4	0.7-0.8
iso-Olefins	0.12-0.26	0.4-1.1
Oxygenates	13.3-13.7	13-14

n: average number of compounds detected in the mixture liquid.

The results of the gasoline vapours analysed at 25°C and 45°C for grade 91 gasoline is shown in Table 3-4. The list of all gasoline vapour components detected by the GC is

Table 3-4: Component grouping of gasoline 91 vapour at different temperatures

Group	25°C (n = 18) (%Vol.)	45°C (n = 19) (%Vol.)
Paraffin	24-30	20-30
iso-Paraffins	23-36	29-40
Aromatics	19-47	26-38
Naphthenes	6-7	5.2-5.5
iso-Olefins	0.00-0.36	0.00-1.80
Oxygenates	16-22	17-19

n: average number of compounds detected in the mixture vapour.

presented in Appendix D. The average number of components at 25°C and 45°C were 18 and 19, respectively. The volume ranges of the paraffins, iso-paraffins and the aromatic groups were also the largest at both temperatures followed by the oxygenate and naphthene.

The iso-olefins groups were either not detected or detected in very minimal volumes. The naphthalene, olefins, and n-olefin groups were not detected at both tested temperatures. The number of components in the vapour phase ranged between 22-23% of the liquid gasoline phase at temperatures 25°C and 45°C, respectively. The same two temperatures were

examined for the gasoline 95, which resulted in fewer numbers of components n = 12 at 25°C and n = 15 at 45°C as shown in Table 3-5. Similar to the gasoline liquids and the 91 grade vapour results the paraffin, iso-paraffins, and aromatics were present in the largest volumes. This was followed by the oxygenate and then naphthenes. The iso-olefins were present in small amounts at 45°C but was not found at the 25°C. The olefin group was not detected in

Table 3-5: Component grouping of gasoline 95 vapour at different temperature

Group	**25°C (n = 12) (%Vol.)**	**45°C (n = 15) (%Vol.)**
Paraffin	18-37	18-22
iso-Paraffins	26-37	26-33
Aromatics	31-39	38-52
Naphthenes	0.00-3.34	0.00-1.39
Olefins	Nil	Nil
iso-Olefins	Nil	0.00-0.62
Oxygenates	20-21	18-20

n: average number of compounds detected in the mixture vapour.

either grade at both temperatures. The number of the components in both gasoline grades increased at higher temperatures as shown in Figure 3-5.

The volumes of the most toxic components of concern (BTEX), detected in the vapour of the two gasolines, are shown in Table 3-6. The compound group volumes in the gasolines 91 and

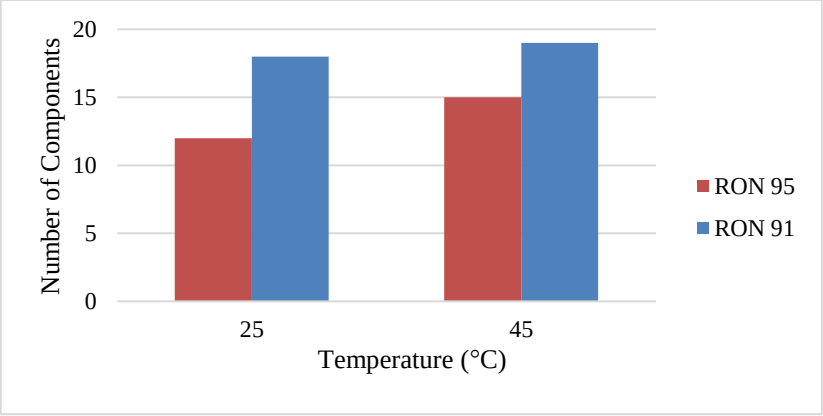


Figure 3-5: Average number of gasoline compounds tested under two temperatures

95 were recorded individually per component. The increasing trend in the concentrations at the higher temperature varies amongst these components. The clearest trend in increasing

Table 3-6: BTEX volume percentages in the gasoline vapour

	25°C		45°C	
Gasoline Grade (RON)	91	95	91	95
	(%Vol)		(%Vol)	
Benzene	3.15	3.9	3.35	4
Ethylbenzene	1.56	1.8	1.34	1.9
Toluene	14	17	13.2	19
Xylene	2.6	3.4	2.89	4

concentration were for benzene and xylene.

Due to the similarity of the characteristics of the two tested gasolines, they were considered as one gasoline mixture of both 91 and 95. The volumes of each chemical group from the two gasolines for the same temperature were summed. The volumes of the light compound groups like paraffins and iso-paraffins decreased at the higher temperature when the aromatics and naphthenes increased. The oxygenate (MTBE) was either the same or slightly decreased at higher temperature. These results are shown in Figure 3-6.

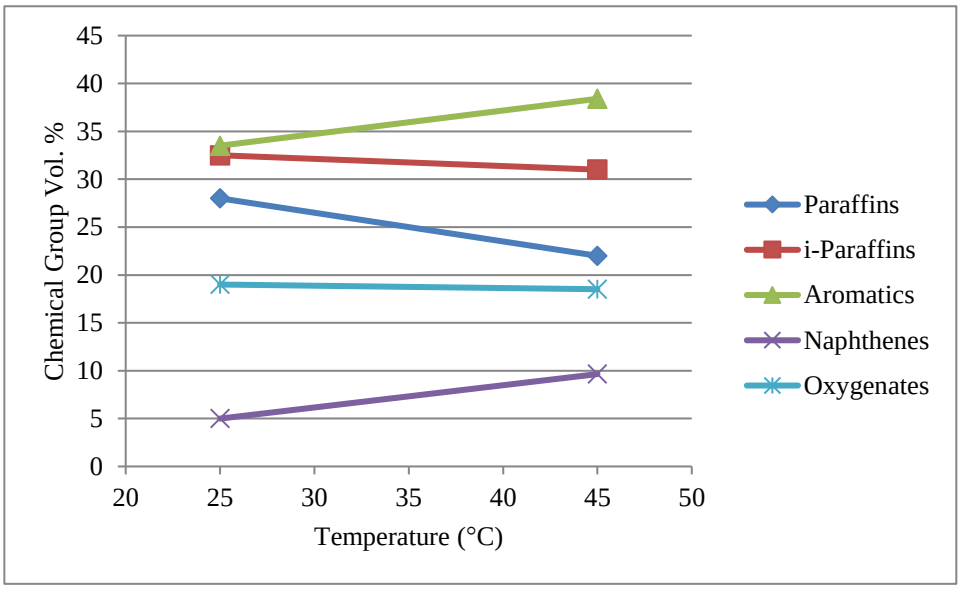


Figure 3-6: Behaviour of gasoline component groups at the two temperatures tested

The volatility of each chemical group was calculated with respect to the tested temperatures using Equation 10:

$$K = L / V$$

Where:
K: the partition coefficient
L: the concentration of compound in the liquid phase
V: the concentration of compound in the vapour phase

Equation 10: Calculating partition coefficient values to determine compound volatility

The mean compound values in liquid were divided by their pertinent mean compound values in vapour of both 91 and 95 combined to calculate the volatility coefficient "K". The results are shown in Table 3-7.

Table 3-7: Volatility coefficient (K) of the averaged 91 and 95 gasoline component groups concentrations in two tested temperatures

Group	**K* for 25°C**	**K for 45°C**
Paraffin	0.71	0.91
i-Paraffins	0.98	1.03
Aromatics	0.99	0.86
Naphthenes	0.67	0.35
Oxygenates	0.72	0.75

**Note: Lower K value means more volatile compound*

Furthermore, the volatility of the aromatics BTEX was calculated separately and is shown in Table 3-8. The naphthene compounds were the highest volatile gasoline vapour at both temperatures. In contrast, the iso-paraffins showed the lowest volatility response among the tested compounds. On the other hand, the benzene was the highest volatile compound among the tested aromatic chemical groups. Xylene was the lowest volatile among the same group.

Table 3-8: Volatility of BTEX in 91 and 95 combined gasoline at two tested temperatures

	K for 25°C	K for 45°C
Benzene	0.60	0.57
Ethylbenzene	0.94	0.98
Toluene	0.63	0.60
Xylene	1.04	0.91

3.4. Discussion

The fuel temperature inside vehicle tanks was found to be consistently higher than the ambient temperature for the cars that stopped at the petrol stations for refuelling during the summer and winter. The variation ranged from 1 to 16°C with larger ranges during the summer. A regression analysis for both temperatures inside and outside the tanks was conducted to test the estimation of the internal tank temperature with reference to the ambient temperature. Figure 3-7 shows a regression coefficient (R^2= 0.88) for this relationship. This

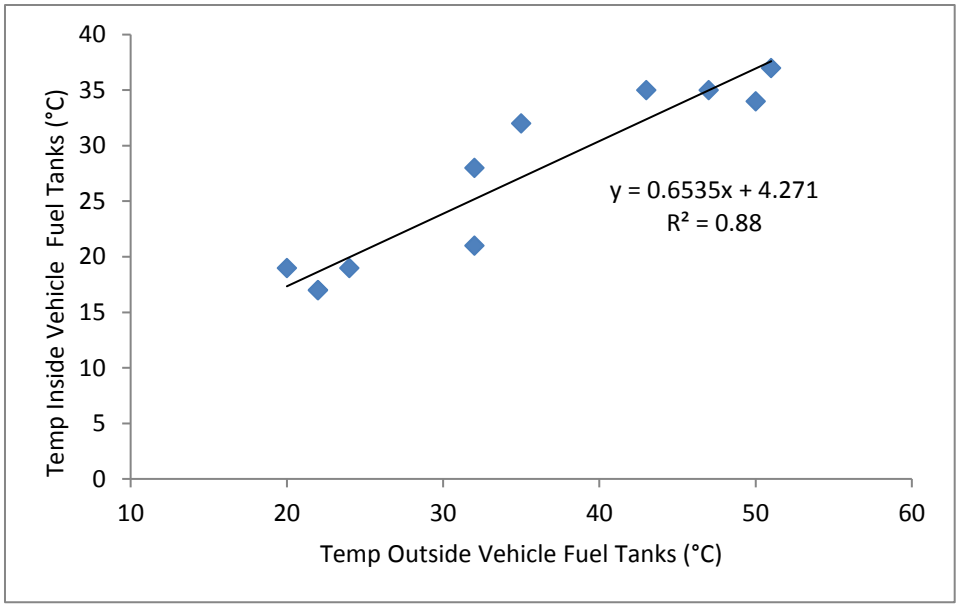


Figure 3-7: Regression analysis of internal and external temperatures of fuel tanks

helps in approximating the internal vehicle fuel tank temperature with reference to the outside ambient temperature.

The analytical results of the two gasoline grades of 91 and 95 revealed their similarity in the properties and characteristics for both liquid and vapour phases during the hot and cold conditions. When the gasoline compound concentrations are considered, the higher temperature would be more of concern, especially, with regard to the C_5 components which are 52% and 44% in the parraffins and iso-paraffins group, respectively. This is in addition to the aromatics and oxygenates. Such concepts should be considered for sampling for the worst case scenarios which play important roles for taking the personal assessment in tropical countries like Saudi Arabia where temperatures reach >45°C (Weather Online Ltd., 2014).

The results of the gasoline content breakdown analysis for different temperatures showed consistent relationships between the concentration values for every two compounds in a mixture. Therefore, the concentration range of one constituent can be estimated by referring to another. For example, if the average result for benzene concentration in the vapour mixture was 2.25 ppm the MTBE concentration would be approx. 11.70 ppm. Therefore, such relationships can be used as reference factors. Such concepts can be applied when using benzene specific instrumentation (e.g. Ultra-Rae 2000) to detect benzene in vapour mixtures and estimate other existing compounds of interest. Table 3-9 shows the theoretical multiplier factors between every two tested aromatic compounds for alternatively estimating the other

Table 3-9: Estimation factors of gasoline vapour compound concentrations

	Benzene	**Ethylbenzene**	**Toluene**	**Xylene**	**MTBE**
Benzene	1:1	1:2.18	4.38:1	1:1.11	5.2:1
Ethylbenzene	2.18:1	1:1	9.57:1	1.93:1	11.35:1
Toluene	1:4.38	1:9.57	1:1	1:4.92	1.18:1
Xylene	1.11:1	1:1.93	4.92:1	1:1	5.85:1
MTBE	1:5.2	1:11.35	1:1.18	1:5.85	1:1

approximate concentration in gasoline mixture vapour. Such analytical and interpretation methodology can be applied to other mixture products of interest to publicise content concentrations.

3.4.1. Measuring Gasoline Compound Concentrations in Liquid vs. Vapour at Different Ambient Simulated Temperatures

The gasoline is 100% volatile at its boiling point (>200ºC) (British Petroleum, 2012; Hess, 2004), which means that all components completely evaporate into the atmosphere at the individual components' boiling points of 26-221ºC. Because the higher temperature causes faster evaporation of the entire compound, some light compounds (e.g. isobutene and normal butane) exist in higher concentrations in vapour mixture when compared to the liquid. Hence, the chemicals in the mixture evaporate at different temperatures and percentages (McDermott and Killiani, 1978). The evaporation of the gasoline mixture constituents occurs gradually, starting at their flash points when the chemicals start vaporising, and increase with the temperature until reaching their individual optimal boiling points at which the chemicals reach their maximum evaporation stage. Therefore, some components exist predominantly in the vapour mixture at different temperatures. This study analytically determined the concentration of each compounds in the vapour mixture with reference to the liquid concentration values at certain temperatures. With consideration of the RVP = 8.6, the maximum possible concentration of the gasoline vapour in air can be calculated by dividing the concentration in

liquid at atmospheric pressure (14.6 psia) and multiplying by 10^6 to convert this to ppm via Equation 11below:

$$\frac{RVP(mix)}{AP} \text{ X } 10^6 = \text{ppm}$$

Where:
RVP (mix) = Reid Vapour Pressure (8.6 psia)
AP = Atmospheric Pressure (14.6 psia)
ppm = Maximum part per million value (589,041ppm)
Equation 11: Calculating maximum possible concentration of components in air

This means theoretically that the maximum gasoline vapour concentrations in air can reach up to 589,000 ppm (assuming ideal gas behaviour).

3.4.2. Determining Gasoline Compound Individual Volatilities at Different Ambient Simulated Temperatures

Every individual chemical in the gasoline mixture has a different volatility. The tendency for the liquid fuel to evaporate, depends on various factors, including the relative temperature. The volatility of the gasoline mixture is adjusted by adding or reducing light (low boiling point) compounds to meet the required fuel specifications. Exceedance of the required volatility causes vapour lock, which in turn blocks the vehicle fuel line and the flow of the gasoline liquid to the engine (McDermott and Killiani, 1978). Therefore, the gasoline volatility or vapour pressure is adjusted depending on the season. Furthermore, an oxygenating agent, MTBE, is added into the mixture to adjust knocking or pre-detonation inside the engine (US EPA, 2013).

The compounds with a low volatility coefficient K value and high vapour pressure are more volatile and tend to partition readily into the vapour phase. Temperature increases vapour pressure for which the value of K decreases and more of the compound passes into the headspace phase (Tipler, 2013). In this study, K was calculated for the same compounds under

the effects of two different temperatures to determine and compare their volatilities at ambient simulated conditions during the hot and cold seasons. Benzene had the lowest K value among the aromatic BTEX which means that it is the highest volatile component. This is due to its high vapour pressure. The increase of the tested temperatures showed small effects to the increase of the general concentrations of the compounds as illustrated by other studies e.g. (Periago, Zambudio and Prado, 1997).

3.4.3. Published Estimates of Gasoline Vapour Compound Concentrations

The Saudi gasoline is standardized for the chemical constituents and volatility specifications that are determined for the two grades of 91 and 95. The mixture components' percentages, such as that for the benzene and MTBE are controlled within specified ranges, which are determined by the government. This is applied for both gasoline grades which also have similar chemical contents. This was analytically verified in the laboratory through GC analyses. As mentioned in the previous section, some gasoline components predominate in the vapour mixture at different temperatures, due to their volatilities and the same concept can be used for correlating contents. For example, when using a gasoline compound specific vapour analyser other component can be estimated in the same vapour mixture. Reference factors have been determined using such concepts.

3.5. Summary and Conclusion

The temperature of the gasoline inside vehicle tanks that are being refuelled at the petrol stations is always higher than the ambient temperature, even during the winter time. The Saudi Arabian produced liquid gasoline was analytically tested at 25°C for both grades of 91 and 95 and detected 66 and 73 compounds respectively. Based on the laboratory analytical testing,

the behaviours and characterisations of the two gasoline grades are similar. Therefore, either of the gasoline types can be used to evaluate gasoline vapour exposures. The gasoline component vaporisation is slightly affected by the temperature for most of the gasoline components. The concentrations of the tested analytes were higher at 45°C verses the 25°C.

The gasoline vapour compounds can be estimated using a known compound concentration in the same mixture and under the same conditions via the developed multiplier matrix. Furthermore, the theoretically measured compound concentrations in a GC vial headspace can be extrapolated to estimate exposure environment concentrations with considerations to different ambient temperatures and atmospheric pressure. The concentrations of the compounds in vapours are more than in liquid phases. The volatility coefficient K was calculated for different gasoline compounds under the winter and summer simulated temperatures. Benzene had the lowest K value among the aromatic BTEX, thus, the highest volatile. Concentration ranges of some compounds in the gasoline mixture can be estimated by referencing to others' known concentrations.

CHAPTER 4

4. Factorial Effects on Personal Exposures Assessment to Gasoline Vapour Contaminants

4.1. Introduction

The occupational exposure scenario of petrol station attendants to gasoline vapours in Saudi Arabia differ to some extent from that in Europe and the United States due to a number of differences, especially the high ambient temperatures (45ºC) during the summer, full service type stations, lack of vapour recovery systems, and differences in the concentrations of gasoline mixture components. Work was carried out in Dammam and Al-Khobar cities, in the Eastern Province in Saudi Arabia, which are examples of large and busy metropolitan parts of the country and thus good areas for this type of study. The objective of this Chapter was to examine the level of exposure of petrol station attendants to the most toxic components in the gasoline vapour namely; benzene, toluene, ethylbenzene, xylene (BTEX) and the oxygenated additive (anti-knocking), methyl tert-butyl ether (MTBE). Such components have the lowest exposure limits set by different organizations such as US OSHA, ACGIH, HSE, and MAK (ACGIH, 2015; DFG, 2014; HSE, 2011; OSHA, 2006) among others in the gasoline mixture. Benzene was the component of greatest concern because of its confirmed carcinogenicity.

Active and passive sampling methods were applied, as well as direct reading instrumentation. The different sampling methods were employed to test the differences

among them in detecting contaminants and the levels of exposures. The direct reading method was used for longer sampling period (15 minute) that usual application of instant reading measurements. Furthermore, area sampling for measuring the contaminants levels in the working areas were applied using different sampling methods for the same comparisons purposes. The variations between the levels of contaminants detected by personal exposure assessment and area measuring levels were tested. The exposure assessments were collected during the summer and winter. In the winter months, the ambient temperature ranged from 18-37˚C, mean relative humidity 33%, and wind speed averaged 1.90 (m/s). In contrast, the summer sampling conditions were 26-41˚C for temperature range, 13% for relative humidity, and 1.6 (m/s) for wind speed average. To support the study of the impact of weather and climate on worker exposure, a mini-portable weather measurement station was custom-made to closely and accurately monitor such variables. Results were described statistically and correlation analysis was conducted for a range of factors (i.e. weather factors, quantity of gasoline sold, location of the petrol stations) to assess their impact on the level of occupational exposure to benzene.

4.2. Materials and Methodologies

The study was carried out at twelve pre-selected petrol stations none of which were fitted with gasoline vapour recovery systems. Category considerations of high (>15,000 L/day) and low fuel sale (<15,000 L/day), if near a heavy traffic highway or not, and if surrounded (enclosed) by buildings or not. Selected station categories are detailed in the Chapter 2 Literature Review. Depending on the location of the petrol station, the exposure concentrations were compared. This was to test the influence of the petrol station locations

with different set-ups on the worker exposure levels. Two of the petrol stations were very large, operated by 6 and 9 attendants, respectively, with daily sales of >50,000 L/day. The other stations were operated by between 2-4 attendants.

The quantity of gasoline pumped per worker was calculated by averaging the amount of gasoline sold during the shift divided by number of workers in the same shift. The personal passive exposure assessments were sampled for full shifts of 10 to 12 working hours or at least for 80% of the shift in some cases. All tested petrol stations operated for 24-hours. Samples were taken during the day shifts (worst case scenario) because they are busier than night shifts.

A total of 41 male petrol station attendants and 4 non-exposed (control) subjects were sampled. The petrol stations and participants were assigned random numbers according to which samples were taken to avoid any bias. All workers were expatriates, predominantly from different parts of Asia including India, Pakistan, Bangladesh, Nepal, and the Philippines with an age range of 20-45 years. The control participants were supervisors who spent their shifts inside offices and away from the gasoline pumps.

The OSHA 7 sampling method for organic vapours extracted via carbon disulphide (CS_2) and analysed by GC-FID. Table 4-1 illustrates statistical precision information for the diffusive samplers, reported by the analysing laboratory.

Table 4-1: Laboratory reported diffusive sampler analytical information

Analyte	Flow Rate (cc/min)	Reporting Limit (µg)	Recovery %
Benzene	16	1	95.80
Toluene	14.5	3	95.55
Ethylbenzene	12.9	3	96.35
Xylene	12.8	6	94.20
MTBE	13.6	3	NE*

*Not enough data points

Two diffusive badge types were used (SKC 575-001 and SKC 575-002) with the former one used in the majority (94%) of cases. Fifty and twenty-five passive samples were used for personal and area monitoring, respectively. Four non-exposed (control) subjects personal and area monitoring were also used.

Ten personal active air samples and one area were used for the sampling which were collected on charcoal tubes, 226-1 coconut shell (100 mg/50 mg) connected to SKC low flow (50-200 ml/min) pumps model 222 (Table 4-2). The active air sampling pumps were calibrated before and after sampling and the average flow rate was used for the analysis. All three types of collecting media used were validated by the US OSHA (OSHA, 2008). The air samplers were attached to the workers' collars near the breathing zones (6-9 inches around nose) as recommended by Huey (1996).

Table 4-2: Total number of samplers used for area and personal at petrol stations

	Personal			Area		
	Active	Passive	Control	Active	Passive	Control
Benzene	12	50	4	1	25	1
Toluene	12	50	4	1	25	1
Ethylbenzene	12	50	4	1	25	1
Xylene	12	50	4	1	25	1
MTBE	12	50	4	1	25	1

The sampling media were collected, cap-sealed and kept refrigerated at <5ºC (Pendergrass, 2003) after each sampled shift. Samples were analysed by the industrial

hygiene laboratories of Bureau Veritas located in the USA and accredited by the American Industrial Hygiene Association (AIHA) following NIOSH method No. 1550 for petroleum analysis detailed in Appendix J.

The weather factors, including ambient air temperature, relative humidity, and wind speed were measured per minute and hour intervals using a specially designed portable mini-weather station. The unit was always placed near attendants' working locations at 1.5 metre height. The unit consist of an ambient weather sensor (Weather Hawk 5.10), a 12 volt battery pack, and a Campbell Scientific CR8500 data logger all fitted into a 2.5 kg fiberglass box (Figure 4-1).

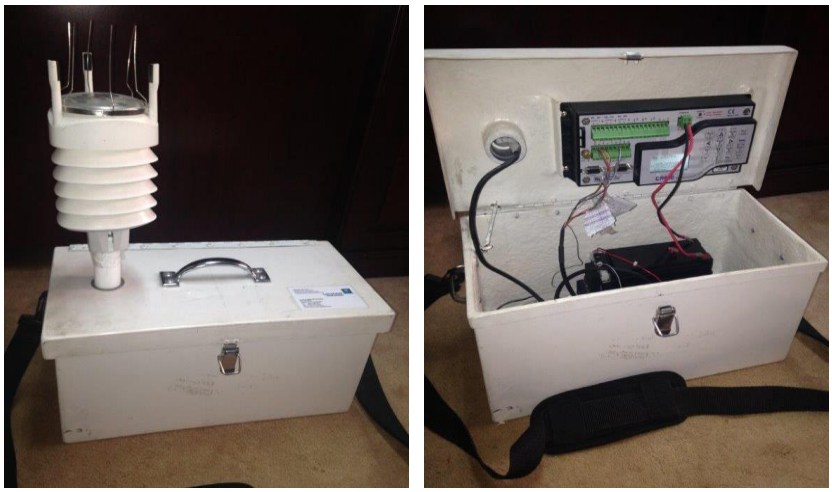


Figure 4-1: Portable mini-weather station designed and used in these studies

Handheld direct reading photo-ionising detectors (PID) were used for instant (direct) measurements. MiniRae 2000 and 3000 models were used to measure the gasoline vapour as total volatile organic compounds (TVOC). The UltraRae 2000, which is benzene specific, included a filtering tube for direct measurement of this component in the gasoline vapour. The instruments were calibrated against 100 ppm isobutylene span for VOC measuring and 5 ppm benzene for benzene measurement. The calibration gas cylinders were purchased from

Rae Industrial Scientific Corporations, Dammam, Saudi Arabia. Fresh air calibration was also applied prior to every measurement. The devices were set in the hygiene mode (with STEL and TWA capability) with 30 second measurement at one minute average time intervals. All instruments were used with a 10.6 eV ionization potential (IP) lamps, appropriate to detect most VOC components. Measurements were taken mostly at the breathing zone height of the workers and at the vehicle tank opening positions.

A fifteen-minute occupational exposure measurement to benzene in gasoline vapours was tested using the UltraRae 2000 direct reading photoionization detector (PID); the device is hung on a gasoline station attendant with its air inlet extended via Tygon tubes and placed in the workers' breathing zones. The purpose of this measurement was to illustrate the fluctuation of gasoline vapour concentrations in the air during vehicle refuelling and to show how the vapour plumes behave (e.g. passing through the attendants' breathing zone) and influenced by the different factors such as the wind speed and direction (Kountouriotis, Aleiferis and Charalambides, 2014). Additionally, such concentration permutations were viewed using a forward-looking infrared (FLIR) thermal image analysis system.

The data was statistically analysed using pre-programmed MS Excel IHSTAT by John Mulhausen from the AIHA and SPSS Ver. 22 statistics program computer software, licenced via Cranfield University. The correlation coefficient interpretation adopted Evans guide (1996) for *r* value as follows:

- 0.00-0.19 "Very Weak"
- 0.20-0.39 "Weak
- 0.40-0.59 "Moderate"

- 0.60-0.79 "Strong"
- 0.80-1.0 "Very Strong"

4.3. Results

The descriptive statistics of the components of interest, BTEX and MTBE sampling results, are shown in Table 4-3. The five components were collected on the same adsorbent media. The geometric means (GM) of all tested components were within the adopted occupational exposure limits (OEL) in Saudi Arabia. The personal active sampling results for the BTEX and MTBE showed small differences in the GM values when comparing the personal passive results, except for the ethylbenzene, which had the largest variation. The GM for the active sampled toluene and benzene were 9-13% higher than those detected by the personal passives. The MTBE, xylene, and ethylbenzene results showed lower GM values of 12, 49, 77%, respectively, as shown in Table 4-3 and Table 4-4. Ethylbenzene was also the lowest detected component amongst the BTXs in the gasoline vapour exposure study conducted by CONCAWE (2002).

Table 4-3: Descriptive statistics of the personal passive sampling results

Parametre	Benzene	Toluene	Ethylbenzene	Xylene	MTBE
			Personal Passive		
N	50	50	50	50	50
Mean (ppm)	0.22	0.28	0.09	0.24	1.89
Median (ppm)	0.21	0.26	0.09	0.18	1.78
SD	0.41	0.57	0.08	1.68	4.10
GM (ppm)	0.18	0.24	0.09	0.19	1.57
GSD	2.03	1.77	1.20	1.64	1.90

The area measurement of the BTEX and MTBE compounds were measured by diffusive samples similar to those used for the personal exposure assessment. The results of these

Table 4-4: Descriptive statistics of the personal active sampling results

	Personal Active				
Parametre	Benzene	Toluene	Ethylbenzene	Xylene	MTBE
N	10	10	10	10	10
Mean (ppm)	0.37	0.46	0.03	0.14	2.22
Median (ppm)	0.19	0.24	0.016	0.09	1.41
SD	1.46	2.09	0.11	0.54	8.30
GM (ppm)	0.20	0.26	0.02	0.10	1.37
GSD	3.01	2.92	2.08	2.33	2.82

measurements are shown in Table 4-5. The results for benzene, toluene and MTBE were lower (19-29%) for the area than those measured for personal exposure. The ethylbenzene and xylene results were higher (12-13%) for the area than the personal exposure results.

Table 4-5: Descriptive statistics of the area passive sampling results

Parametre	Benzene	Toluene	Ethylbenzene	Xylene	MTBE
N	26	26	26	26	26
Mean (ppm)	0.06	0.15	0.13	0.27	0.40
Median (ppm)	0.04	0.10	0.10	0.18	0.29
SD	0.14	0.42	0.49	0.96	1.30
GM (ppm)	0.05	0.12	0.10	0.22	0.30
GSD	1.89	1.80	1.99	1.787	2.23

The direct instrumental reading of the gasoline vapour in the area (background), measured as total VOCs ranged from 0.17 to 6.8 ppm. The VOCs readings at the vehicle tank opening ranged from 300 to more than 1,300 ppm (Figure 4-2). Such levels dramatically decreases (diluted) by around 92% at a distance of 50 cm, which is the approximated distance between the tank opening and the petrol attendants' breathing zone when placing the nozzle for pumping. This photograph was taken under the conditions of 1.2 m/s wind speed, 35°C

temperature, and 24% relative humidity. Thus, the aforementioned VOC concentration range would be reduced to 20-100 ppm at 0.5 metres away from the car tank opening.

Figure 4-2: Direct reading measurement of gasoline vapour at vehicle fuel tank opening

Furthermore, the benzene exposure results were statistically analysed to test their relationships, with different factors including quantity of gasoline sold, ambient air temperatures, relative humidity, the wind speed, and the location factors. The benzene exposure results were refined by taking the GMs of the benzene results for each similar factor values. For example, the GM was taken for all of benzene exposure results at a similar temperature of 39ºC. The results of these relations are shown in Figure 4-3. The quantity of gasoline pumped (2,453-14,553L) showed a significant moderate positive correlation (r = 0.53; $p < 0.01$) with the personal exposure to benzene. A significant moderate negative (inverse) correlation (r = - 0.53; $p < 0.04$) was found for the benzene results and wind speed

(0.7-4.2 m/s). A very weak positive relationship (r = 0.08) was found for the ambient temperature (18-42ºC). A very weak negative (r = - 0.08) relationship was found for relative

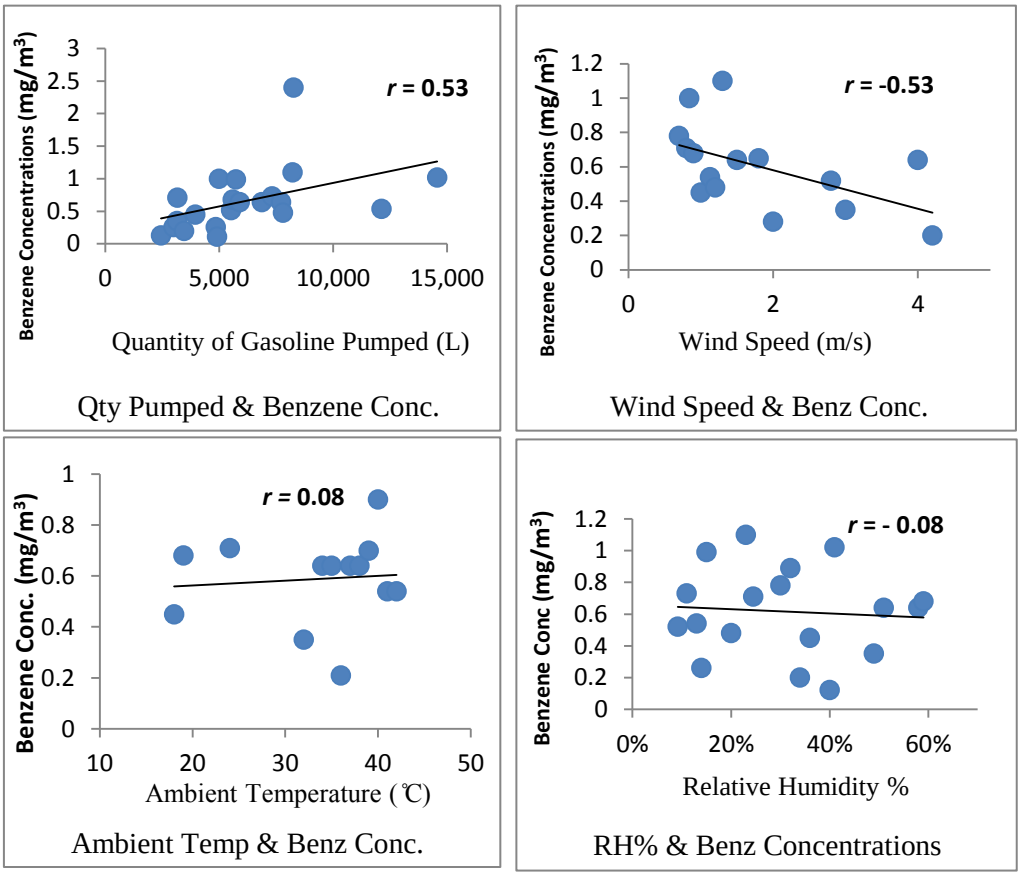


Figure 4-3: Benzene concentration and relationship with various factors

humidity (9-59%).

A clear relationship was found between the workers' exposure levels and the location of the petrol stations. The highest concentrations were recorded at the locations that are both near highways or busy streets and are surrounded by buildings, as illustrated in Figure 4-4.

For the short-term exposure testing, a weak negative relationship (r = - 0.25) was also found between the wind speed and the direct reading exposure correlation, during the fifteen-minute experimental measurement of benzene. This relationship is shown in Figure 4-5. Both wind speed and benzene concentrations responses were included in the same figure to demonstrate their symmetrical interaction. In this illustration, there is a trend showing a slight decrease in the wind speed and an opposite increase in the peak at 10:54 AM and 10:58 AM, as shown in Figure 4-6. The trend of this relationship concurs with the overall inverse relationship pattern.

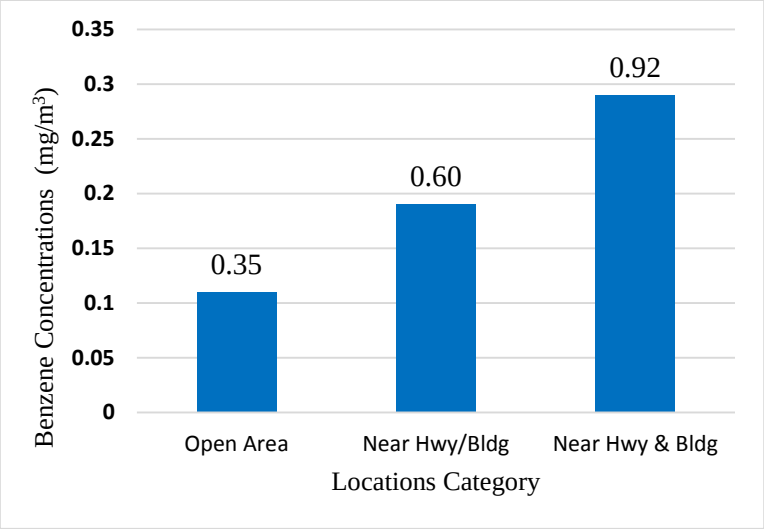


Figure 4-4: The relationship between the petrol station locations and the benzene geometric means for workers exposure levels.

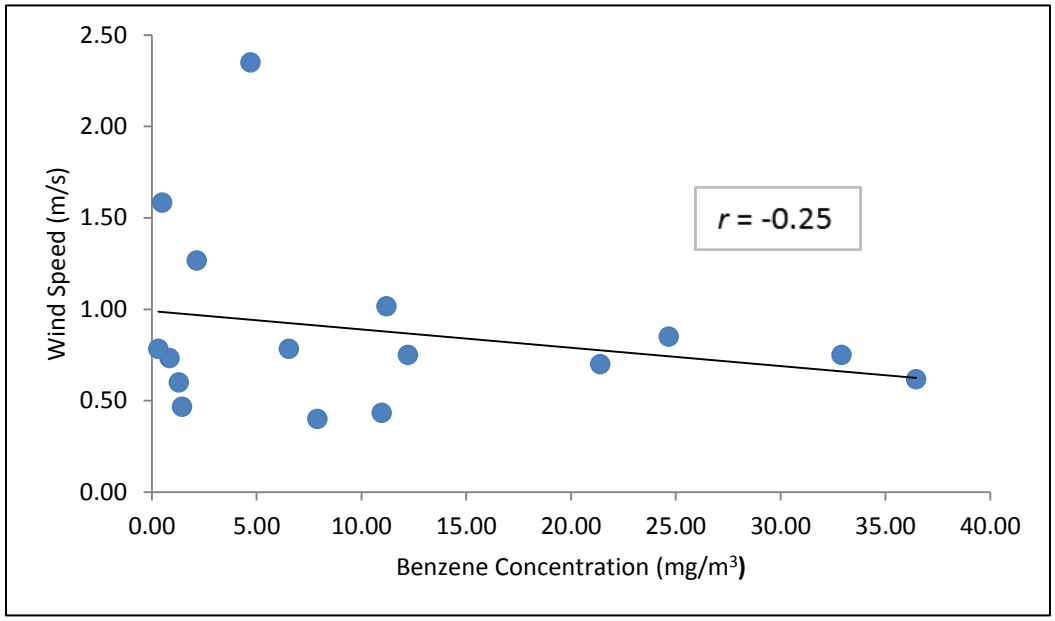


Figure 4-5: Wind speed and benzene concentration in a 15 min air monitoring

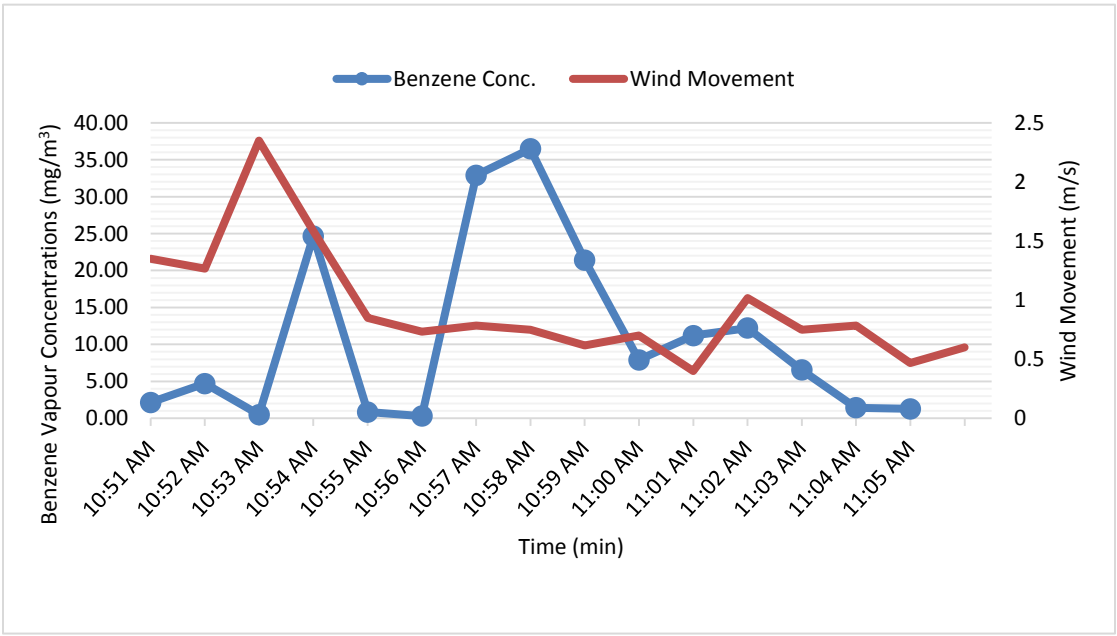


Figure 4-6: Wind speed and benzene concentration during 15 min monitoring

4.4. Discussion

The GMs of the personal passive results were below the occupational exposure limits (OEL) set by the Saudi Arabian standard and the other multiple international regulatory agencies of the US Occupational Safety and Health Administration (OSHA), the UK Health and Safety Executive (HSE), Australian Hazardous Substances Information System (HSIS), and the German DFG. All values adjusted for twelve-hour limits (Australia, 2012, DFG, 2014; HSE, 2011; OSHA, 2006), as shown in Table 4-6. Furthermore, other than for benzene, all results were below 50% of the OELs, reflecting a very low risk from exposures to the toxic gasoline vapour chemical components. These values agreed with historical studies carried out with cohort of filling station attendants' exposures by Lagorio et al., (1993).

Table 4-6: Comparison of the air sampling results to adjusted regulatory limits for 12 hrs

Chemical	GM Results (ppm)	HSE UK (ppm)	OSHA USA (ppm)	HSIS Australia (ppm)	DFG Germany (ppm)
Benzene	0.18	0.70	0.70	0.70	0.70
Toluene	0.24	33	130	34	33
Ethyl Benzene	0.09	67	67	67	13
Xylene	0.19	33	67	53	67
MTBE	1.57	33	50	61	33

Most of the GMs of the personal passive exposure results agreed with those of active results. The closest result values were for benzene, and the highest variation was in the ethylbenzene samples. A comparison between the personal and area passive results, benzene, toluene and MTBE values were 47-80% higher for the former measurements. Inversely, the personal results for ethylbenzene and xylene were 12-15% less than that in the area results. The GMs

for exposures to BTEX and MTBE for the examined petrol station attendants were found to be relatively higher than those reported for attendants' exposure levels in Europe and North America (Concawe, 2002; IARC, 2012). Such variation was attributed to the different components' concentrations in addition to the mixture of influencing factors, of the ambient temperature, a larger quantity of gasoline pumped per petrol attendant in 24 hr operating service stations and the locations of the petrol stations as explained previously.

The effect of the ambient temperature on the increase in exposure was minimal as illustrated by the very weak positive correlation between this factor and benzene exposure concentrations. This phenomenon concurs with the conclusions of the theoretical tests detailed in Chapter 3 where the effect of temperature on the increase of the benzene vapour concentration was slight. Furthermore, the exposure of petrol pump attendants to benzene increased with the quantity of gasoline pumped (Kearney and Dunham, 1986). This was shown by the significant moderate positive correlation coefficient of $r = 0.53$; $p < 0.01$ (2-tailed). The wind speed factor was found to have a significant inverse relationship with the benzene concentration levels. The wind speed most likely influences and possibly decreases the exposure concentrations (Kountouriotis, Aleiferis and Charalambides, 2014). The correlation coefficient was negative ($r = -0.53$; $p < 0.04$ and -0.25 $p < 0.30$) during the full shift and short term monitoring. The higher the wind speed, the lower the vapour concentrations measured. No significant correlation was found between the relative humidity and the concentrations in air.

In contrast, the location of the petrol stations was found to have a clear relationship with the exposure levels (Bono et al., 2003). The stations that were located adjacent to heavy

traffic, and where surrounded by buildings (and thus poor ventilation) (Kountouriotis, Aleiferis and Charalambides, 2014) had the highest exposure levels. The lowest concentrations were found for the petrol stations that were less affected by heavy traffic and surrounding buildings. This suggests that location of petrol stations plays important roles in determining the workers' exposures to volatile gasoline vapours.

The results of the tests undertaken with the FLIR to investigate gasoline vapour plumes produced during refuelling. The FLIR recordings enabled estimation of the vapour plume distance (30 cm) from vehicle fuel tank opening with high concentrations (Figure 4-7). This also has helped estimating safe distance (>50 cm) at which the vapour concentrations would be substantially reduced. The vapour distance was measured utilising VOC direct reading instrumentation previously shown in Figure 4-2.

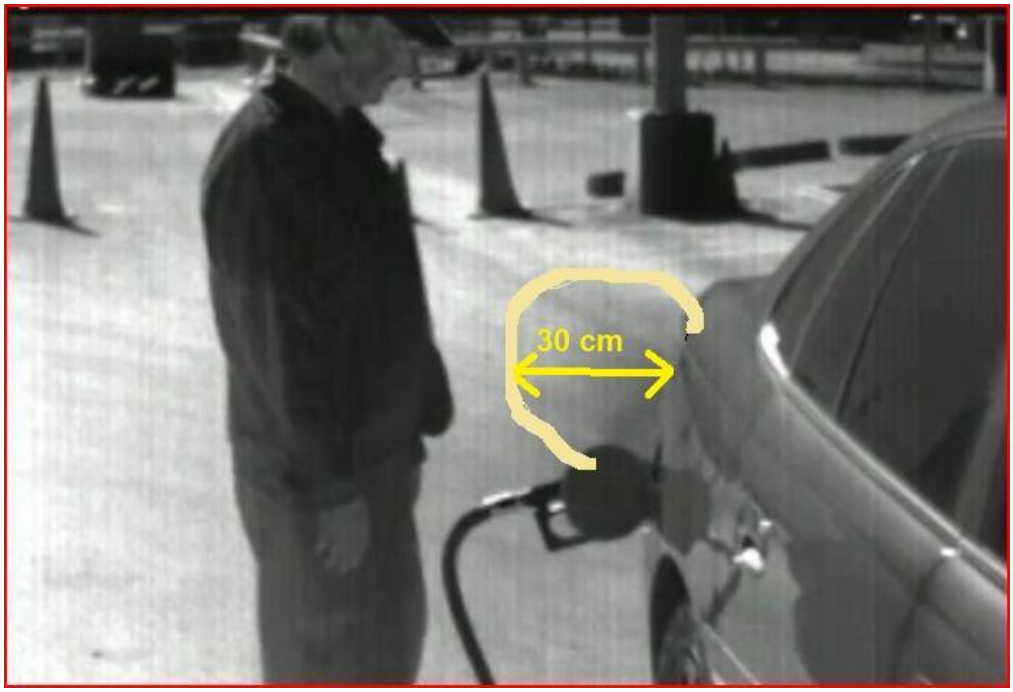


Figure 4-7: FLIR qualitative image illustrating gasoline vapour plume distance

Furthermore, it revealed (video not shown) that the highest release of a gasoline vapour plume occurs initially during the beginning of pumping and gradually decreases as the fuel tank becomes full for the tested vehicles. This is because the vapour volume inside the tank is gradually displaced with the gasoline liquid. Therefore, in this testing scenario the maximum vapour release occurs at the beginning of the filling up process. Figure 4-8 illustrates a qualitative evaluation of the plume size during the beginning, mid and end of pumping gasoline fuel into vehicle tank.

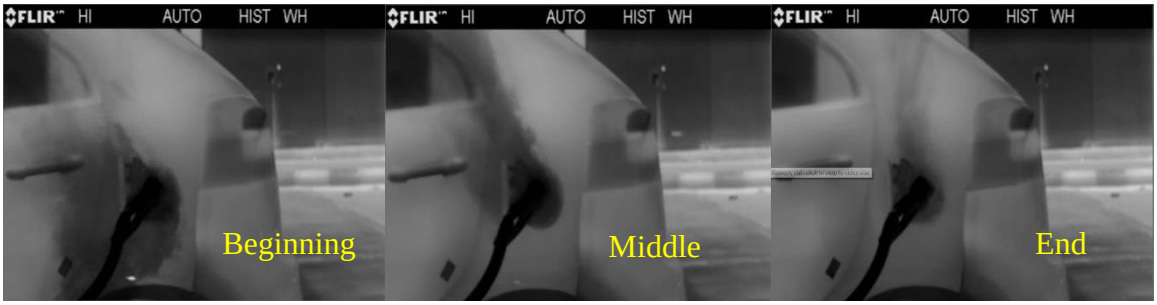


Figure 4-8: FLIR images illustrating three qualitative assessment vapour plume sizes

Reduction of exposure values of 66% by gasoline vapour recovery system has been illustrated in a study conducted by the Conservation of Clean Air and Water in Europe (CONCAWE) for petrol station attendants in European countries. Others have reported a 80% reduction in Mexico (Concawe, 2002; Cruz-Nunez, Hernandez-Solıs and Ruiz-Suarez, 2003). For similar efficiency of CONCAWE, the exposure values from this study could be

Table 4-7: Comparison of the GM for the tested exposure results and the vapour recovery retrofitted stations tested in Europe (Concawe, 2002)

	Tested Exposure Results (ppm)	Theoretical values w/VR* (ppm)
Benzene	0.18	0.06
Toluene	0.24	0.08
Ethylbenzene	0.09	0.03
Xylene	0.19	0.06

*Vapour Recovery retrofitted stations.

reduced to the levels shown in Table 4-7 by the application of the gasoline vapour recovery system.

4.5. Summary and Conclusions

All five measured toxic compounds (BTEX and MTBE) in the gasoline vapour were found to be well below a number of governmental OELs. This suggests a very minimal risk for the petrol station attendants in Saudi Arabia. The hot summer season slightly increases the exposure levels for the petrol station attendants. The location of the petrol stations was found to be an important factor that can influence the level of exposures. The relationship between the location of petrol stations near heavy traffic or being surrounded by high buildings can positively influence the exposure to benzene.

Therefore, it would be beneficial to design the petrol stations in patterns that promote natural ventilation due to its important roles in diluting/reducing VOC concentrations in the air. The wind speed and the quantity of gasoline pumped are other factors that play significant roles in effects on the exposure levels of petrol pump attendants. The volume of the released vapour from the cars' tank is most likely the highest at the beginning of the pumping process. The vapour components concentrations recorded were the highest at <30 cm from the car tank opening. Such aforementioned conditions should be considered in setting up a petrol station attendant exposure monitoring strategy. The concentrations of such released vapour dramatically decreased at 50 cm distance away from the tank opening.

The gasoline vapour exposure levels can theoretically be reduced by 66-80% by installing vapour recovery systems. Administrative controls are recommended for educating and training petrol station attendants on the exposure to hazardous gasoline components and their

associated health risks, the typical routes of exposure, improving the work practices and exposure avoidance techniques (e.g. standing upwind of the vehicles' tank opening and at a distance of more than 0.5 metre).

CHAPTER 5

5. Employing Exhaled Breath for Gasoline Vapour Exposures Assessment

5.1. Introduction

Interest in exhaled breath sampling as a method for detecting certain chemicals in the human body has increased in the recent years. It is used for two purposes; clinical applications for disease detection (endogenous) and for environmental chemical exposure assessment (exogenous). The latter application is the technique used in this study to determine exposures to volatile organic compounds (VOCs) (Cao and Duan, 2006). This chapter reports studies carried out using exhaled breath monitoring for determining the occupational exposures at petrol stations to five gasoline vapour constituents, namely; benzene, toluene, ethylbenzene, xylene, and the oxygenating additive methyl tertiary butyl ether (MTBE). These are five of the most hazardous compounds that are usually evaluated in occupational studies of exposure to gasoline vapours. The benzene exposure results in exhaled breath were further tested for the possible correlation with different influencing factors. Alveolar air samples from the petrol station attendants were collected during their work shifts to evaluate the gasoline vapour intake by using a commercial device that allows for the collection of the workers' deep breaths. The outcomes, from this evaluation of the exhaled breath exposure assessment at petrol stations, are used to investigate the effects of

various factors, including weather conditions, on the amount of exposure present; this method complements the other measures of chemical exposure assessment.

5.2. Materials and Methods

A set of ethics forms and agreements were prepared and approved by the Cranfield University Health Research Ethics Committee (CUHREC), allowing the use of personal bio-samples and related information for the purposes of the research. A questionnaire was prepared for the participants detailing their exposure habits and work practices. These forms are included in Appendix A. The required forms and documents were filled out prior to the sampling and bio-data collection of at least twenty-four hours. In several cases the forms were filled out by the researcher after discussion and explanation of the study with the workers because some of them had poor literacy skills. An authorisation letter was prepared for the management of the three companies, authorising the research to be carried out at their facilities (petrol stations).

The sampling study was carried out for the exhaled breath exposure assessment at eleven petrol stations of high and low sale categories. The sites (petrol stations) information was obtained prior to and during the monitoring work. Some of the important information needed were the total number of petrol station attendants in the shift, the total quantity of gasoline pumped by registering dispensing quantity sale readings prior to and after the shift, weather conditions (ambient temperature, wind speed, relative humidity), obtained via the mini-weather station, and general observation and work practices, such as gasoline spillage, receiving refilling bulk tanker truck. The field site information sheet is attached in Appendix B. The tested sites had a temperature range of 26-42ºC, RH% 9-49%, wind speed 0.7-4.2

m/s, and pumped gasoline quantity range of 1000-12,000 L per attendant. The sites tested were located in different locations of near heavy traffic or highways, surrounded by tall buildings, or not characterised by these two factors. The number of fuel pumps per station ranged from four (two sided nozzles), for small stations, to sixteen pumps, for the large stations. All stations are not fitted with vapour recovery systems. The attendants' age ranged from 20 to 45 years old and they were predominantly expatriates from East Asia.

The exhaled breath samples from twenty-five male petrol station attendants were collected before and after the work shifts to evaluate the gasoline vapour intake. This was performed using a commercial sampling kit Bio-VOC Breath Sampler (C-BIO01) (Figure 5-1) supplied by Markes International Limited, Llantrisant, UK. The Bio-VOC container (~0.129 L) allows for the collection of the last portion of the deep alveolar air. This

Figure 5-1: Bio-VOC sampler kit designed for exhaled breath collection

is the area in the lungs in which the air is exchanged with the blood stream. The samples' collection is detailed in Appendix C.

Eighty-three (83) breath samples were collected for non-smoker subjects of which 41 were collected before shifts start (pre-exposure) and 42 were collected after shifts end (post-exposure). The breath sample collection was often conducted in clean areas away from the gasoline vapours (e.g. inside enclosed supervisor offices), few metres away from the gasoline pumps areas. The subjects were instructed to inhale and hold their breath for 10 seconds to allow for equilibrium of the alveolar air with the blood in the pulmonary capillaries (Wilson and Monster, 1999). The subjects then exhaled their maximum volume of breath to empty the lungs into the inlet of the sampling kit to ensure capturing the alveolar air (end-tidal). The captured breath was discharged into a stainless-steel sorbent tube containing Tenax TA (200 mg 35-60 mesh with 2,6-diphenylene-oxide polymer resin)- provided by the Health and Safety Laboratory (HSL), Harpur Hill, Buxton, Derbyshire SK17 9JN- immediately by pressing the plunger slowly and steadily into the tube. The transfer process takes place over a period of about ten seconds. Two breath samples in two Tenax tubes were collected for pre- and post-exposure per subject to allow for enough volume to be captured. The tubes were closed firmly and placed in small sealed plastic bags and put in a thermal insulated cooler container so that it may be transported to the storage refrigerator in the laboratory. The tubes were then air shipped to the HSL in the UK, which took from two to three days. The solvents trapped in the Tenax tubes were analysed by thermal desorption at the laboratory. A whole array of solvents was available for analysis. Samples are either analysed for known solvents or screened and subsequently semi-quantitated. All sample results were reported in nanomol per litre (nmol/l) by first converting the mass to µg/l from the 129 ml breath tube then multiplying by 1000/MW. The laboratory's reported detection limit was 1 nmol/l. The none-

detectable limits (ND) were assigned 0 (zero) values in the results and statistical calculations except for the GMs as they would be zero. Therefore, the GMs were considered for values greater than zero (Vijay P. Singh , Sharad K. Jain, 2007). The analytical method for breath solvent determination is given in Appendix E. The data statistical analysis was carried out using MS Excel (IHSTAT by John Mulhausen from the AIHA) and SPSS Version 22 statistics program computer software, licenced via Cranfield University.

5.3. Results and Discussion

All tested subjects were non-smokers based on the questionnaire answers. The limit of detection (LOD) of the analysis was 1 nmol/l for each of the five tested compounds. Table 5-1 summarises the number of pre- and post-shift breath samples in which the target compounds were detected. The MTBE was found to be the most abundant compound detected followed by toluene and benzene. It is one of the highest percentage (15%) components in the gasoline mixture. Therefore, MTBE detection can be used as an indicator for gasoline mixture vapour exposure. The ethylbenzene was the least frequent compound similar to what was found by Chen et al., 2002. Xylene was the second lowest detected component. Some of tested compounds were unexpectedly detected in the pre-exposure samples. There were no clear or definite reasoning for this notion. Possible causes are suggested in the further work section in chapter 9. An increase in the detected VOC concentration levels from pre- to post-shift in

Table 5-1: Summary of the number of breath samples with target compound concentrations above the limit of detection

Chemical	Pre-shift	Detected %	Post-shift	Detected %
Benzene	11	38	18	58
Ethylbenzene	0	0	1	3
Toluene	14	47	20	64
Xylene	3	9	3	9
MTBE	25	81	25	79

the majority of the samples indicates an effect of exposures to the tested components during the re-fuelling activities. The highest increment (79%) in the GM value was found for MTBE followed by toluene (38%). There was a small increase (16%) in GM of benzene although. The general interpretation of the results indicated a consistent increase and decrease of the exhaled breath post-exposure results with the personal environmental air sample results. Such trend agreement is discussed in Chapter 8.

5.3.1. Descriptive Statistics

The statistical analysis was calculated for the three most abundant constituents detected in exhaled breath namely: benzene, toluene, and MTBE as shown in Table 5-2.

Table 5-2: Descriptive analysis of the detected chemicals in the breath sample results

Parametre	Benzene		Toluene		MTBE	
	Pre-Shift	Post-Shift	Pre-Shift	Post-Shift	Pre-Shift	Post-Shift
N	18	18	21	21	27	27
Mean (nmol/l)	1.05	1.83	1.14	2.57	27.14	34.77
SD	1.11	1.46	1.45	2.76	43.29	46.00
GM (nmol/l)	1.51	1.76	1.42	1.96	9.36	16.82
GSD	1.62	1.96	1.84	2.08	5.33	4.47

There were small increases in the GMs of the benzene and toluene for the exposure results between the pre- and post-shift sampling. The MTBE showed the clearest increase in the concentrations between the pre- and post-shift results. Similarly, these were reflected by the arithmetic means. Such increases are indications for exposures to the chemicals during the working shifts. Furthermore, the GSD showed that the highest variation occurred in the pre-shift measurements of the MTBE and the lowest is for the pre-shift of benzene.

5.3.2. Paired *t* Test and Pears' Correlations Analysis

A paired *t* test was conducted to further test the hypothesis of the means of the pre- and post-exposure results. These results were considered dependant observations of two measures for each tested component. The hypothesis statements tested are as follows:

Null Hypothesis: The mean difference of pre- and post-exposures are equal to zero (no difference).

Alternative Hypothesis: The mean difference of pre- and post-exposures are not equal to zero (significantly different).

The test was applied and the results helped clarifying increasing effects of the exposure levels during the working shifts as a comparison between the pre- and post-exposure. This is illustrated in the three tested components' negative means in Table 5-3. The negative mean

Table 5-3: Paired samples test of pre- and post-exposures to three tested gasoline components

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	SD	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 Pre-Benz – Post-Benz	-0.77	1.16	0.27	-1.35	-0.19	-2.83	17	0.012
Pair 2 Pre-MTBE – Post-MTBE	-7.62	19.61	3.77	-15.38	0.13	-2.02	26	0.054
Pair 3 Pre-Tolu – Post-Tolu	-1.42	2.69	0.58	-2.65	-0.20	-2.43	20	0.025

values suggest that the post-exposures were higher than the pre-exposures for the three tested chemicals. This suggest rejection of the null hypothesis and the means of the pre- and post-exposure results is not zero. The largest SD for MTBE concurs that it is the most variable as well. The standard error mean provides an indication of the expected variability that can occur if repeated random trials were carried out similarly. As shown in Table 5-3, the benzene has the lowest standard error mean making it the lowest possible variable. Furthermore, the

significance values (<0.05) in all three tested chemicals indicated that the increase between the pre- and post-exposures is not due to chance variation, and can be attributed to the exposure to gasoline vapours during the shifts.

Significant correlation was found between the pre- and post-exposure for two chemicals benzene and MTBE similar to that found by Alonso and Sanchez (2013). This implied dependency among the pre- and post-exposure variables. As shown in Table 5-4 a strong relationship $p < 0.05$ (2-tailed) with correlation coefficient $r = 0.62$ was found for the benzene pre- and post-exposure results. Moreover, a very strong correlation $r = 0.90$; $p < 0.000$ (2-tailed) was found for the pre- and post-shift results for the MTBE. The toluene's pre- and post-shift relationship showed weak correlation $p < 0.16$ (2-tailed) with coefficient of $r = 0.31$. Results are tabulated in Appendix E.

Table 5-4: Paired sample correlations of pre- and post-exposures for the three tested chemicals

		N	Correlation	Sig.
Pair 1	PreBenz & PostBenz	18	0.621	0.006
Pair 2	PreMTBE & PostMTBE	27	0.905	0.000
Pair 3	PreToluene & PostToluene	21	0.313	0.167

The possible effects of the wind speed on the exposure to benzene concentrations tested via the exhaled breath were examined. An inverse weak, negative correlation $r = -0.29$ was found between the wind speed and the post-shift results for the benzene. The negative correlation coefficient result indicates that the higher wind speed caused a reduction of the exposure concentrations to benzene. This can be caused by dilution or dispersion of the gasoline vapours from the worker's breathing zones.

Furthermore, the relationship between the quantity of gasoline pumped and the actual exposure concentrations to benzene in exhaled breath was also tested. A weak positive

relationship (r = 0.25) was found for this correlation. This suggests that the benzene intake by the petrol attendants is slightly affected by the amounts of the petrol pumped. The more the attendants dispense petrol, the higher the risk of exposure to benzene.

The relationship between the ambient temperature and the concentrations of the benzene inhaled was also tested. A very weak positive r = 0.02 correlation coefficient was found. The higher the temperature the slightly more exposure increase to benzene levels.

The relative humidity was also tested for its relation to the actual calculated levels of benzene. The test results showed a positive, very weak correlation r = 0.02. Therefore, the relative humidity has similar effects as the temperature which may slightly contribute to the increase of the concentrations of benzene.

The location of the petrol stations was also tested for the possible effect on the actual amounts of concentrations for benzene. The results showed a positive relationship between the benzene exposures and the location category of the petrol stations as shown in Figure 5-2.

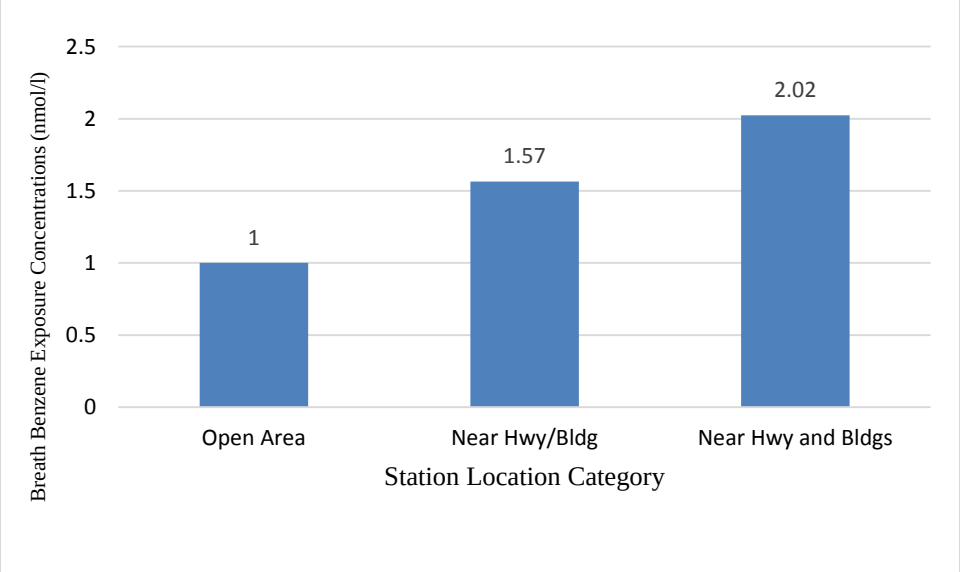


Figure 5-2: The relationship between the benzene exposure concentrations and the station location category

5.4. Summary and Conclusion

The alveolar exhaled breath monitoring was tested in this study to assess occupational exposures to five toxic chemical components (BTEX and MTBE) in the gasoline vapours for non-smoking petrol station attendants under various conditions. A commercial Bio-VOC sampling kit was used with Tenax tubes. These tubes were the most appropriate for the sampling.

The MTBE was predominantly found to be the most abundant compound detected in breaths amongst the five tested constituents, followed by toluene and benzene. It also demonstrated the highest increase between the pre- and post-shift exposure results, followed by the toluene. A number of the pre-shift results showed relatively high values, which were attributed to several possibilities, such as remaining amounts in the body from the previous shifts, bringing contaminated uniforms with gasoline, vicinity of the accommodation or downwind to the petrol stations, and sharing rooms with smokers for the increased benzene concentration cases. Therefore, a questionnaire about the living areas and work practices is important when carrying out exhaled breath exposure monitoring to clarify such effect possibilities. Positive very weak correlations were found between the benzene exposure results (dependents) and the independent factors of weather conditions. The quantity of gasoline pumped showed a positive weak correlation with the benzene exposure results. Lastly, the locations of the petrol stations suggest possible influence on the increase of the exposure to benzene. Such factors are important consideration when sampling for exhaled breath exposure assessments.

CHAPTER 6

6. Comparison of Different Urine Exposures Assessment Methods for Benzene under Different Conditions

6.1. Introduction

This chapter discusses exposure levels to benzene in gasoline vapour, regarding the petrol station attendants in the Eastern Province of Saudi Arabia using different biological metabolites in urine. This chapter follows air exposure monitoring (Chapter 4) and exhaled breath exposure assessment (Chapter 5) to provide different evaluation method comparisons summarised in Chapter 8. The weather conditions in the area vary from extremely hot long summers to moderate winters (Average 25-42ºC) (Weather Online Ltd., 2014), which might have some effects on the exposure levels. The benzene was the selected component to be evaluated among other components because of its high health hazard (carcinogen). The levels of three benzene metabolites: phenol, trans-trans-Muconic Acid (*t,t,*-MA), and S-Phenylmercapturic Acid (*S*-PMA) were assessed before and after the exposures to the gasoline vapours during full day working shifts (10-12 hours). A questionnaire (Appendix A) was prepared to understand the nature of the exposures and to support the interpretation of the results. Participants were interviewed about lifestyles, smoking habits, housing conditions (e.g. if gasoline odorous uniforms are brought inside the rooms or if gasoline odour is perceived in living places), vicinity of accommodations to the petrol stations, duration of work at the petrol station, and the health effects or symptoms that they may have

experienced. When the questionnaire was completed, identification numbers were assigned anonymously to participants and their petrol stations.

6.2. Materials and Methodologies

The biological urine samples were collected from petrol station attendants at different locations to assess exposure to benzene in gasoline vapour via its metabolites. An approval for personal sampling was obtained on February 20, 2013 from Cranfield University Health Research Ethics Committee (CUHREC). The samples were collected immediately from the workers prior to and after their working shifts. Control samples (non-exposed) were also collected from shift supervisors who spent more than 80% of their time in offices away from the gasoline vapour. The workers were given sampling plastic tubes with screw-caps, as detailed in Appendix E and instructed to collect midstream urine samples to the 20ml mark.

The three tested benzene metabolites in urine were: phenol, *S*-PMA, and *t,t*-MA. The only samples within the creatinine concentration limits of > 0.3 g/l and < 3.0 g/l were considered for data analysis as recommended by the World Health Organization (WHO), through the American Conference of Governmental Industrial Hygienists (ACGIH). This is because specimens that are highly concentrated or highly diluted are not normal and not suitable for exposure assessments (ACGIH, 2015). Furthermore, smokers were identified for the *S*-PMA and *t,t*-MA samples, through selecting sampling results, that have greater than 100 µg/l cotinine concentrations (Lee So Ryong, 2014). Some of these smokers could also be considered passive smokers, as workers share rooms in the accommodations. This is to eliminate the confounding effects of benzene in the tobacco, which ranges between 20 to 90 µg per cigarettes (Carrieri et al., 2006; Eruopean Commission, 1994).

The metabolisms levels in the urine increases with time of exposure. This was experimented in an exposure study of four volunteers to toluene (17 ppm), xylene (33 ppm) and ethylbenzene (33 ppm) during 7-hour exposure that was carried out when urine samples were collected after the first 3 hours of exposure (0-3 hr), at the end of exposure (3-7 hr), and during 17 hour following the exposure (7-24). Results are showed the following urinary metabolites excretions Table 6-1 (Tardif et al., 1997).

Table 6-1: Urinary metabolites excretion of toluene, xylene, and ethylbenzene after 7 hr experimental exposure

Chemical	Metabolites	Amount excreted (µmol)/period		
		0-3 hr	3-7 hr	7-24
Toluene	Hippuric Acid	566 ± 121	752 ± 83	2644 ± 690
Xylene	Methylhippuric Acid	476 ± 139	745 ± 188	405 ± 65
Ethylbenzene	Mandelic Acid	113 ± 55	294 ± 111	520 ± 253

This illustrates the optimum time for collecting urine after the exposure. Similarly, another study showed continuous increases of the benzene metabolites phenol, *t,t*-MA and *S*-PMA in urine throughout exposure during an eight hour shift (Boogaard, P., 1995; European Commission, 1991). Therefore, urine samples would contain representative levels of metabolites along with continuity of exposure and thus the sampling time is recommended to be at the end of the exposure duration (ACGIH, 2015).

Weather factors, such as ambient air temperature, relative humidity, and wind movement (wind speed) were measured on hourly recordings using a specially designed portable mini-weather station. The unit was always placed near attendants' working locations at a height of

1.5 metre. The unit consisted of an ambient weather sensor (Weather Hawk 5.10), 12 volt battery pack, and Campbell Scientific CR8500 data logger that were fitted into a 5 pound (lb) fiberglass box. A standardised site information record sheet (Appendix B) was prepared and used during the fieldwork to have consistent data collection and to facilitate the structuring results' data bank. The record sheet included the following: shift length, number of workers, and number of gasoline pumps, location and information of the station, and unusual events or activities

Phenol in urine assessment sampling was implemented separately in different times than the other metabolites, as it was analysed by different laboratory. This was marked as survey 1. The phenol in urine sample collection and analysis followed the American National Institute for Occupational Safety and Health (NIOSH), method 8305. Samples were analysed by the Environmental Chemistry Laboratory, Saudi Arabia. Thirty-Two (28+4 control) urine samples were collected for phenol analysis from pre- and post-shift exposures at five petrol stations. Samples were collected from eleven expatriate petrol attendants with different nationalities from East Asian countries. The workers were all males, ages (25 – 45 years). None of the participants consumed alcohol, as it is totally prohibited in Saudi Arabia. Participants are not allowed to smoke during work for safety purposes (i.e. near flammable gasoline). The collected samples were placed inside igloo thermal insulated boxes containing ice and taken immediately to the lab which was 15 minute away from most of the locations. All samples were kept inside a refrigerator at - 4°C for 3-14 days before being analysed.

For the *S*-PMA benzene metabolite collection, twenty urine samples of pre- and post-exposure were analysed by the Biosciencia Laboratory located in Ingelheim, Germany. This

was a pilot study that was conducted to test the overall sampling and analysis strategies. The samples were collected in 30 ml screw-cap polyethylene tubes provided by the laboratory. Samples were kept at - 4°C during storage until they were ready to be air-shipped from Saudi Arabia to Italy. This took anywhere from 2-4 days.

Survey 2 assessment included 118 urine samples (pre- and post-exposures), which were collected during the summer and winter 2013-2014. Twenty-two pairs (44 samples) of pre- and post-exposure samples met the criteria requirements for creatinine levels. The remaining samples were either out of the creatinine determined levels (12 samples) or had more than 100 g/l cotinine (46 samples) that was considered smokers or those possibly exposed to tobacco. The samples were analysed for both *S*-PMA and *t,t*-MA from the same single urine samples. Sampling surveys number breakdown is summarised in Table 6-2

Table 6-2: Breakdown of the number of samples in different studies with their different benzene metabolites

	Phenol		*t,t*-MA		*S*-PMA	
	Pre	Post	Pre	Post	Pre	Post
Pilot	--	--	--	--	10	10
Survey 1	13	15	--	--	--	--
Survey 2	--	--	16	16	16	16
Survey 2 (Cotinine > 100 g/l)	--	--	22	22	22	22

The samples were collected from 45 expatriate petrol station male attendants from East Asian countries (ages ranging from 25-45 years, from fourteen petrol stations, distributed in the Dammam city area of the Eastern Province of Saudi Arabia. The stations were pre-

selected based on the criteria of high (>15,000 L/day) and low (<15,000 L/day) daily sales. The samples in survey 2 were analysed by the INAIL Research Centre, located in Rome, Italy, under the supervision of Doctor Tranfo Giovanna, Department of Occupational and Environmental Medicine, Epidemiology and Hygiene. These samples were taken immediately to a storage refrigerator (15-25 minutes away) and stored frozen at below zero centigrade for 3-5 days. The samples were placed in insulated plastic boxes in an instructed certified biological sample package and then shipped DHL to Italy. The shipping time could range from three to twelve days, depending on the customs' delay. The condition of the samples after delivered to the laboratory were reported thawed, but in analysable conditions for *S*-PMA and *t,t*-MA level testing. A laboratory detailed analytical procedure is included in Appendix F.

A Pearson correlation comparison analysis was applied using SPSS Ver. 22 statistics program computer software licenced via Cranfield University. Different variables and factors of each result data set were collected for the three benzene metabolites. The personal exposure results were averaged per stations for the correlation analysis. The scattered data diagrams were utilised to validate the applicability of the correlation comparison and to verify that the data is not of curvilinear type or inappropriate for comparison (Kuzma and Bohnenblust, S., 2001). The correlation coefficient interpretation adopted Evans guide (1996) for *r* value as follows:

- 0.00-0.19 "Very Weak"
- 0.20-0.39 "Weak
- 0.40-0.59 "Moderate"

- 0.60-0.79 "Strong"
- 0.80-1.0 "Very Strong"

6.3. Results

Survey 1: Phenol

Although some of the phenol post-exposure result values were higher than the pre-exposure, 46% of the pre-exposure results were higher than their pertinent post-exposures. The results were normally distributed. All of the phenol in urine results was below the 21 mg/g phenol/creatinine. The normal range for phenol found by NIOSH and stated in its analytical method, No. 8305 for humans not exposed to benzene, phenol was 4.5 to 20.7 mg phenol/g creatinine. The pre-exposure results (n = 13) for phenol in urine showed geometric mean (GM) 3.26 mg/g and geometric standard deviation (GSD) 2.71. The GM of the phenol's post-exposure results (n = 15) was 4.36 mg/g and GSD was 1.50 as shown in Survey 1 Table 6-3. The mean of the pre-exposure controls (n = 2) was 4.36 mg/g and 3.75 mg/g for the post-exposure (n = 2).

Pilot: S-PMA

The *S*-PMA benzene metabolite results for the pilot study (n = 20) were sorted separately. The GM of the pre-exposure results (n = 10) was 10.48 µg/g with GSD 2.75. The GM of the post-exposure (n = 10) for the pilot study was 17.13 µg/g with GSD 2.55. None of the pre-exposure values exceeded their correspondent post-exposures. Three out of ten (30%) of the pre-exposure results exceeded the recommended level of 25 µg/g by the ACGIH. Similar number of results in the post-exposures exceeded the OEL. Two samples of pre- and post-

exposure from the same subject were reported non-detectable (ND). The ND results were disregarded from the calculation.

Survey 2: t,t-MA

The results of the *t,t*-MA in survey 2 for non-smokers of both pre- and post-exposure (n = 32) were recorded. The GM of the non-smoker pre-exposure samples in survey 2 (n = 16) was 231 µg/g with GSD 4.33 (Table 6-3). The GM of post-exposure (n = 16) for the same group was 500 $\mu g.g^{-1}$ with GSD 2.79. The controls' mean (n = 2) was 110 µg/g for the pre-exposure and 60 µg/g for the post-exposure (n = 2). Clear differences was found between the exposed and control of the t,t-MA results. Around 25% of the pre-exposure results exceeded 500 µg/g recommended by the ACGIH, 2015 and 50% of the post-exposure results exceeded the limit. Furthermore, 31% of the pre-exposures exceeded the post-exposure result values.

The *t,t*-MA results of pre- and post-exposure of smokers or possibly second-hand smokers with high cotinine results (>100 µg/l) were sorted out separately. The GM (n = 23) of the pre-exposure was 344 µg/g with GSD 3.20. The GM (n = 23) of *t,t*-MA for the post-exposure was 664 µg/g with GSD 2.26. Twenty-two percent (22%) of the pre-exposure results were higher than the post-exposure. Thirty percent (30%) of the pre-exposure results exceeded the exposure standard level of 500 µg/g and sixty-five percent (65%) of the post-exposures exceeded.

Survey 2: S-PMA

The results of the pre- and post-exposure for non-smokers (n = 32) showed GM of 4.44 µg/g and 5.29 µg/g with pertaining GSD of 3.42 and 3.06, respectively. Thirty-seven percent (37%) of the pre-exposure results exceeded the post-exposures. One result value (6%) of each

of the pre- and post-exposure groups exceeded the exposure limit of 25 µg/g recommended by ACGIH. The control means for the pre- and post-exposures are 0.93 µg/g and 0.77 µg/g, respectively.

The results of the pre- and post-exposure *S*-PMA samples from the smokers and those with cotinine greater than 100 µg/g (n = 46) were calculated separately. The GM of the pre-exposure results (n = 23) was 9.90 µg/g with GSD 2.93 and 12.14 µg/g for the post-exposure with GSD 2.56. It was found out that 35% of the pre-exposure results were more than the post-exposures. For the exposure limit exceedance, 26% of the pre-exposure results exceeded the recommended level of 25 µg/g when 22% of the post-exposures exceeded the standard.

Table 6-3: Descriptive statistics of the benzene urinary metabolites results

Metabolite	N	GM	GSD	>limits	Pre>Post Exposures	Mean Control
			Survey 1			
Phenol Pre-Exposure	13	3.26 mg/g	2.71	0%	46%	4.36 mg/g
Phenol Post-Exposure	15	4.36 mg/g	1.50	0%		3.73 mg/g
			Pilot			
S-PMA Pre-Exposure	**10**	**10.48 µg/g**	**2.75**	**33%**	**0%**	
S-PMA Post-Exposure	10	17.13 µg/g	2.55	33%		
			Survey 2 (Non-Smoker)			
t,t-MA Pre-Exposure	16	231 µg/g	4.33	25%	31%	110 µg/g
t,t-MA Post-Exposure	16	500 µg/g	2.79	50%		60 µg/g
S-PMA Pre-Exposure	16	4.44 µg/g	3.42	6%	37%	0.93 µg/g
S-PMA Post-Exposure	16	5.29 µg/g	3.06	6%		0.77 µg/g
			Survey 2 (Smoker)			
t,t-MA Pre-Exposure	23	344 µg/g	3.20	30%	22%	
t,t-MA Post-Exposure	23	644 µg/g	2.26	65%		
S-PMA Pre-Exposure	23	9.90 µg/g	2.93	26%	35%	
S-PMA Post-Exposure	23	12.14 µg/g	2.56	22%		

Number of Samples (N); Standard Deviation (SD); Geometric Mean (GM); Geometric Standard Deviation (GSD)

6.4. Discussion

All of the phenol in urine results was below the recommended level of 21 mg/g phenol/creatinine by the ACGIH. Almost half (46%) of the phenol in urine sample result values decreased after the exposure time during the shifts. This can be explained by taking in consideration that the phenol metabolite in urine might give better results for exposure levels greater than 10 ppm in air (Weisel, 2010). Furthermore, phenol excretion percentages can be higher at exposures below 1 ppm than for exposure to 10 ppm or more (Sungkyoon K. et al., 2006). All the associated air sampling results during this study was below the 10 ppm. Furthermore, some of the control sampling results (non-exposed supervisors) values decreased, as well as the pre- and post-exposures. There was a discrepancy between the exposed and control subjects indicating a noticeable increase in the phenol level, due to benzene exposures.

Similar to the inverse results in phenol, 25% of the *t,t*-MA in urine sampling results decreased. Also, the same decrease was found in the control sampling results. All results are shown in Appendix H. Some of the pre-exposure results were already exceeding the ACGIH recommended levels for non-exposed workers before the actual exposures (Aprea et al., 2008; Weisel, 2010). A possible reason for this is the effect of metabolite of sorbic acid, which is a common food additive that can increase in the urinary *t,t*-MA. A quantity of 47 mg sorbic acid digested can increase the averaged *t,t*-MA level by 20 times (Carbonari et al., 2016; Carrieri et al., 2006). The effects of the sorbic acid can vary from person to person and for the same person, day to day. The discrepancy between the results from the exposed and

control for the *t,t*-MA was smaller than that found in other urinary metabolites. This reflects other possible effects occurring other than the benzene in the gasoline vapour, only.

The GM of the smoker or those with high cotinine in urine results was higher than those of the other groups. Furthermore, a decrease in the measured exposure values was found between the pre- and post-exposure results. More exceedances to the OEL were found in those with high cotinine level results for both pre- and post-exposures.

The *S*-PMA in urine sampling results for the non-smokers had larger values in the pre-exposures, as noticed in other tested benzene metabolites. A discrepancy was found between the GM of the exposed pre- and post-exposure (4.44 and 5.29 µg/g). An inverse value was also found in the average pre- and post-exposure in the non-exposed controls (0.93 and 0.77 µg/g). Such differences suggested that benzene exposure in the gasoline vapour is a cause of the increased metabolite in the exposed subjects.

In comparison between the smoker and non-smoker subjects, the GM of the post-exposure results was higher than those of non-smokers. It was noticed that the difference between the pre- and post-exposure values were also higher than in the non-smokers. This suggests that tobacco exposures contributed to the increase of the *S*-PMA excretion in urine (Weisel, 2010). This also supports the smoking confounder effect assumption on the urinary *S*-PMA metabolite.

6.4.1. Relationship Comparison for Various Factor Effects on Exposures

The correlation comparison was tested to investigate the relationships between dependant (post-exposure) and independent variables (other factors). Therefore, Pearson correlation analysis was applied to the collected non-smoker result data to discover possible influences of different factors, such as the weather, quantity of gasoline sold, and the location of the

petrol stations on the post-exposure results of the urinary metabolites. All comparisons were conducted on non-curvilinear scattered data to meet the statistical testing requirements (Kuzma and Bohnenblust, S., 2001). The relationships between the variables and the post-exposure results from the three metabolites were tested for different petrol stations.

Correlation between the Pre- and Post-Exposure Levels of Benzene Urine Metabolites

A paired *t* test was conducted for the results of the three benzene metabolites to further test the hypothesis of the means of the pre- and post-exposure results. These results were considered dependant observations of two measures for each tested metabolite. The hypothesis statements tested are as follows:

Null Hypothesis: The mean difference of pre- and post-exposures are equal to zero (no effects).

Alternative Hypothesis: The mean difference of pre- and post-exposures are not equal to zero (effects occurred).

Phenol Urine Metabolite

The paired *t* test was applied and the outcomes showed increases between the pre- and post-exposure results as seen in the negative mean (Table 6-4). This suggests dependency

Table 6-4: Paired sample test of phenol in urine results of pre- and post-exposure to benzene

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	SD	Std. Error Mean	Lower	Upper			
Pair 1 Pre- and Post-Exposure	-0.60	1.86	0.53	-1.78	0.58	-1.124	11	0.28

between the pre- and post-exposure result. This also indicates that such results are most likely caused by environmental exposure. This suggest rejection of the null hypothesis and the means of the pre- and post-exposure results is not zero. The standard error mean provides an indication of the expected variability that can occur if repeated random trials were carried out similarly. It is considered small from the phenol results as shown in the table below.

Furthermore, the correlation test revealed a moderate correlation ($r = 0.50$; $p < 0.09$) as shown in Table 6-5. Such correlation level was expected because phenol biomarker would show clearer difference if exposed to higher levels of benzene (>5 ppm) as shown in other studies (Boogaard, P., 1995; Weisel, 2010).

Table 6-5: Pearson correlation analysis of phenol pre- and post-exposure results for benzene exposure

		N	Correlation	Sig.
Pair 1	Pre- and Post-Exposure	12	0.50	0.093

T,T-MA Urine Metabolite

The paired *t* test for *t,t*-MA results did not indicate good evidence of the dependency between the pre- and post-exposure results as shown by the positive mean 0.66 in Table 6-6.

Table 6-6: Paired *t* test of *t,t*-MA results for pre- and post-exposure to benzene

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	SD	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 Pre-Exposure and Post-Exposure	0.66	1455.39	363.84	-774.86	776.19	0.002	15	0.99

This indicates that the post-exposure values were not always higher than the pre-exposure values and that the gasoline vapour was not the only source of *t,t*-MA biomarker. This is explained by the high results of one petrol attendant that was recognized to have high values in the pre- and post-exposure results (2124.65 and 1705.58 µg/g). Another high reading of 6776.86 µg/g was found for pre-exposure from different petrol attendant. This agrees with the concept of other effects by nutrition (sorbic acid) on the *t,t*-MA metabolism (Carbonari et al., 2016; Carrieri et al., 2006; European Commission, 1991). Although some values in the pre-exposures were higher than those from the post-exposures the overall indication of the correlation results suggest that both scenarios' values are related and that there is a moderate correlation (r = 0.51; p < 0.04) between the pre- and post-exposure values as shown in Table 6-7. This suggest rejection of the null hypothesis and the means of the pre- and post-exposure results is not zero as changes have occurred.

Table 6-7: Pearson correlation analysis of *t,t*-MA pre- and post-exposure results for benzene exposure

		N	Correlation	Sig.
Pair 1	*t,t*-MA Pre-Exposure & Post-Exposure	16	0.51	0.04

S-PMA Urine Metabolite

The paired *t* test was applied and the outcomes showed increases between the pre- and post-exposure result values as seen in the negative mean in Table 6-8. This suggest rejection of the null hypothesis and the means of the pre- and post-exposure results is not zero. This also indicates that the increase between the pre- and post-exposures is not due to chance variation, and can be attributed to the exposure to gasoline vapours during the shifts. The standard error mean provides an indication of the expected variability that can occur if

repeated random trials were carried out similarly. It shows higher value here than the phenol making it higher variable.

Table 6-8: Paired *t* test of S-PMA results for pre- and post-exposure to benzene

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	SD	Std. Error Mean	Lower	Upper			
Pair 1 Pre-SPMA and Post-SPMA	-0.12	6.95	1.73	-3.83	3.58	-0.074	15	0.942

The relationship between the pre- and post-exposure data for the third metabolite *S*-PMA was statistically significant ($P < 0.01$) with very strong coefficient correlation factor $r = 0.80$.

Table 6-9: Pearson correlation analysis of S-PMA pre- and post-exposure results for benzene exposure

	N	Correlation	Sig.
Pair 1 Pre-SPMA and Post-SPMA	16	0.80	0.000

This means that the result values from the two scenarios are related in Table 6-9.

Correlations between the Post-Exposure Benzene Urine Metabolites and Wind Speed

Phenol Urine Metabolite

Pearson correlation test was applied to analyse relationship between the phenol post-exposure results and the wind speed values. The post-exposure results were grouped per weather wind speed values by taking the mean of the results that had the same averaged wind speed reading. The tests showed positive very weak correlation ($r = 0.009$; $p > 0.90$) as shown in Figure 6-1. This indicates that there is no significant effect of the wind speed on the concentration of the urinary phenol. This correlation was not anticipated as the wind's

movement would logically dilute the vapour concentrations in the area, which in turn reduces personal exposures. One possibility here is the low exposure levels to benzene which may not have provided good indication as phenol in urine is better indication for higher levels >5 ppm (Boogaard, P., 1995; Weisel, 2010).

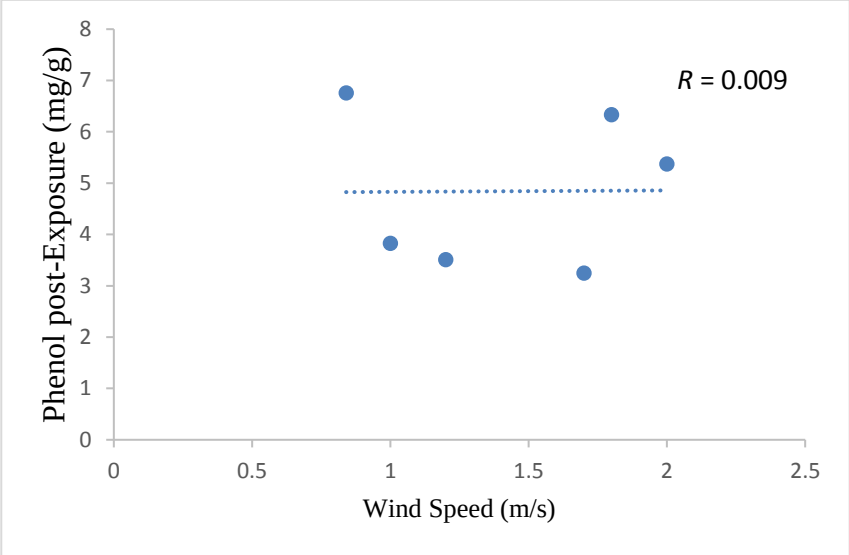


Figure 6-1: Relationship between the wind speed and the post-exposure results of phenol in urine

T,T-MA Urine Metabolite

Pearson correlation test was applied to examine the relationship between the wind speed and the concentrations of the *t,t*-MA in urine of the exposed petrol station attendants as if higher wind speed would decrease the levels of *t,t*-MA. The *t,t*-MA post-exposure results were grouped per the similar values of averaged wind speed by taking the average of them. One reading of wind speed (4.2 m/s) matched with individual post-exposure *t,t*-MA concentration of 567.05 mg/g was considered an outlier and disregarded. An inverse very

weak correlation (r = - 0.12; p < 0.63) was found for the relationship between the wind speed and the post- exposure results of the *t,t*-MA as illustrated in Figure 6-2. This indicates that the wind movement may slightly have decreased the exposure to the gasoline vapour in air and in turn reduced the level of *t,t*-MA in urine.

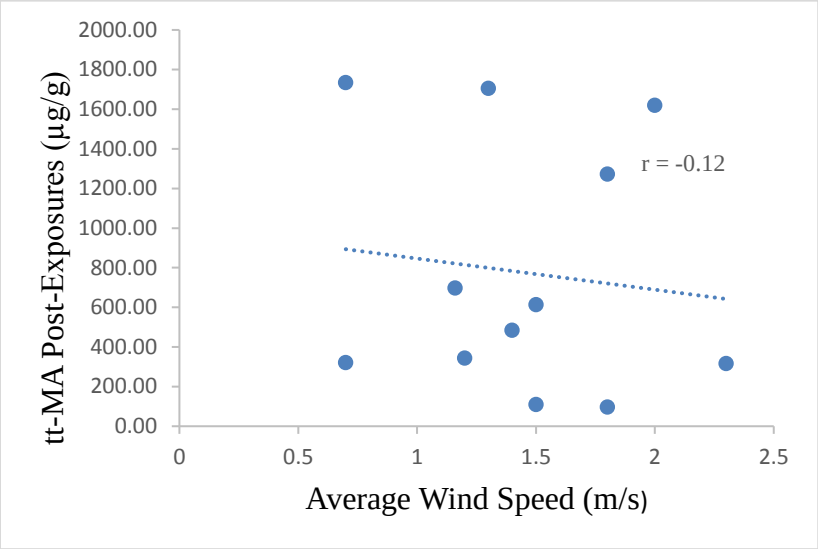


Figure 6-2: Relationship between the wind speed and the *t,t*-MA post-exposure results

S-PMA Urine Metabolite

Pearson correlation test was conducted to test if there is relationship between the wind speed and the concentration of *S*-PMA of the petrol station attendants. The results from the post-exposure of the *S*-PMA were grouped per the similar values of averaged wind speed values by taking the average of each group. An inverse weak correlation coefficient (r = - 0.32; p < 0.28) was found for the relationship between the post-exposure results of the *S*-PMA in urine and the wind speed as shown in Figure 6-3. This suggests that the wind's

movement had inversely affected the exposure to the gasoline vapour in air. It can be concluded that the *S*-PMA concentration level in urine was the highest affected benzene

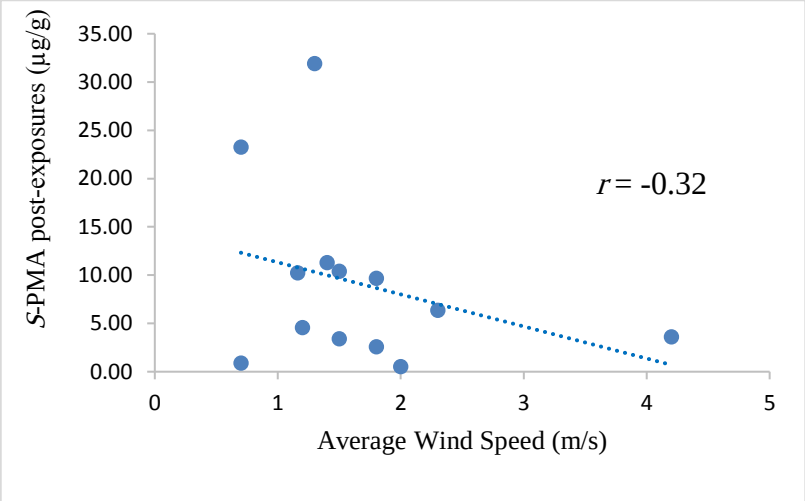


Figure 6-3: Relationship between the wind speed and the *S*-PMA post-metabolite by the wind speed.

Correlations between the Post-Exposure Benzene Urine Metabolites and Ambient Temperature

Phenol Urine Metabolite

Pearson correlation test was applied to test if there is any relationship between the ambient temperature and the concentrations of the phenol in urine of the exposed petrol station attendants as if high temperature would cause an increase in the excreted phenol. The post-exposure results were grouped per their alike averaged temperatures by taking the means. The correlation coefficient was found to be $r = 0.75$; $p < 0.25$ indicating strong positive relationship between the excreted phenol in urine of the exposed petrol station attendants and the ambient temperature as shown in Figure 6-4. Such relationship suggests that the hot weather may cause an increase of the exposure to gasoline vapour in air. This can

also be an important consideration of the weather temperature when monitoring exposures at petrol stations as it would be expected to cause higher levels of phenol metabolite for

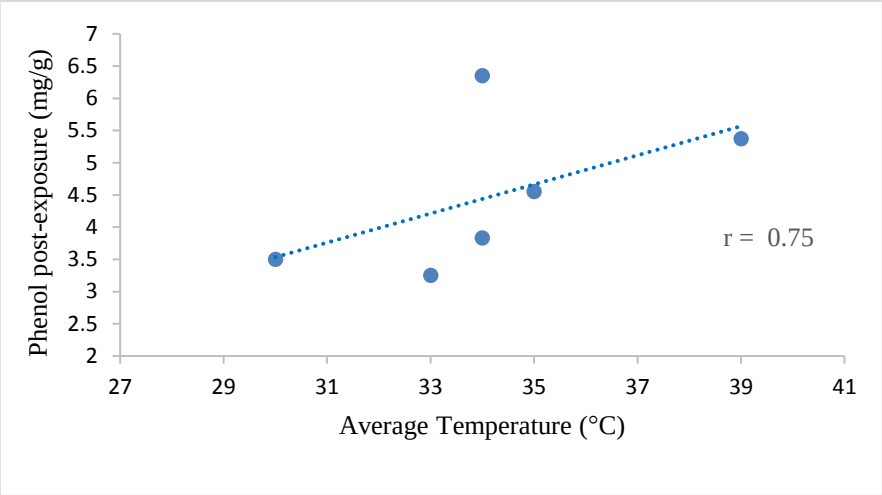


Figure 6-4: Relationship between the ambient temperature and the phenol post-exposure results

exposure to benzene in the gasoline vapour.

T,T-MA Urine Metabolite

Pearson correlation test was carried out on the excreted levels of *t,t*-MA in the post-exposure and the ambient temperature to examine any relationship or effects by the high

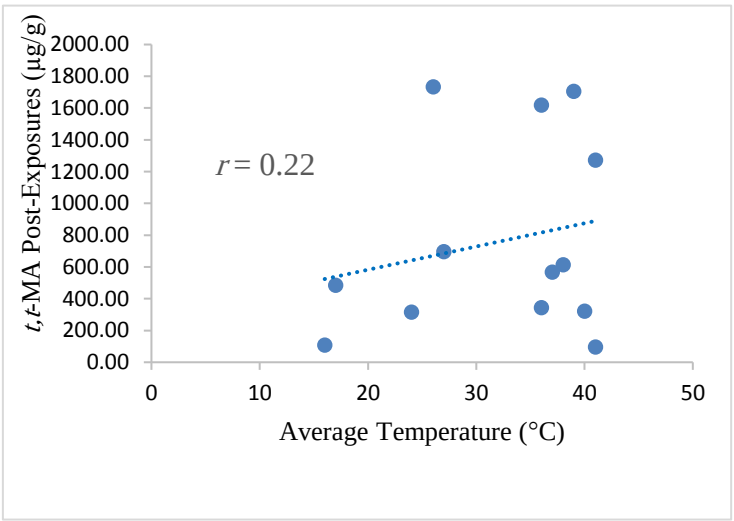


Figure 6-5: Relationship between the ambient temperature and the *t,t*-MA post-exposure results

temperature on the levels of exposure. The post-exposure results of the *t,t*-MA were grouped by their matching averaged temperature reading by taking the means of them. A weak correlation (r = 0.22) was found for the relationship between the temperature and *t,t*-MA post-exposure results as shown in Figure 6-5. This suggests slight effect by the temperature on the exposure to gasoline vapour and the excretion of *t,t*-MA in urine of the exposed petrol station attendants.

S-PMA Urine Metabolite

Pearson correlation test was conducted for the post-exposure results and the ambient temperature to see any effect of the high temperature on the excretion levels of *S*-PMA. The post-exposure results of the *S*-PMA were grouped by their matched averaged temperature readings by taking the means of them. A very weak negative correlation (r = -0.04) was found for the temperature and the *S*-PMA post-exposure relationship. This suggests slight effect by

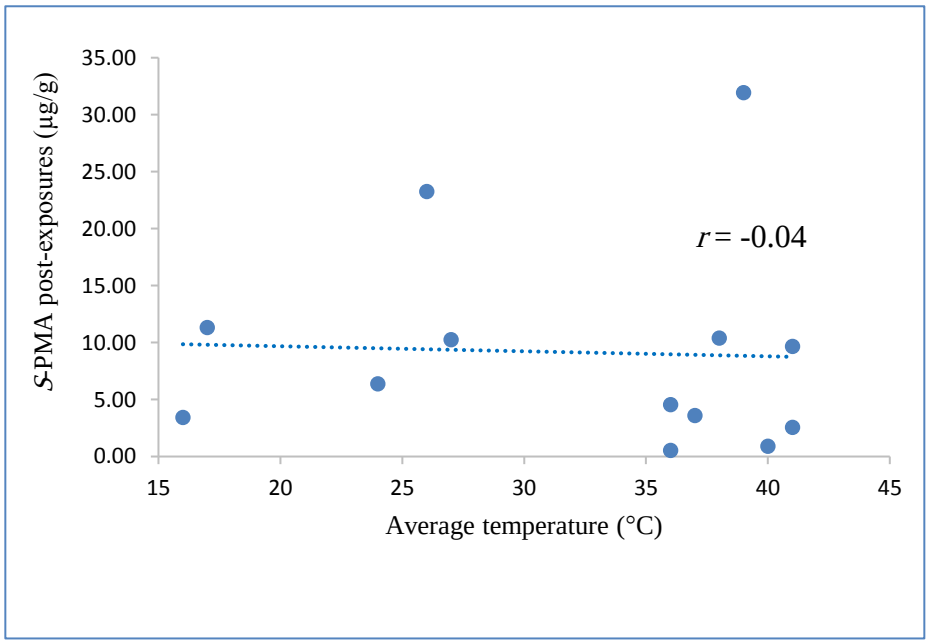


Figure 6-6: Relationship between the ambient temperature and the *S*-PMA post-exposure results

high temperature on the excretion of the *S*-PMA due to increased exposure levels of the gasoline vapour and benzene for the petrol station attendants.

Correlation between the Post-Exposure Benzene Urine Metabolites and Relative Humidity

Phenol Urine Metabolite

Pearson correlation test was applied for the post-exposure results of the phenol and the relative humidity measured during the exposure shift. This is to test if there are any effects of the humidity on the exposure level of benzene in the gasoline vapour and in turn affect the excretion levels of the phenol. The phenol post-exposure results were grouped per their matched similar averaged relative humidity readings by taking the average of such results. The test showed a very weak positive correlation ($r = 0.18$; $p < 0.01$) as shown in Figure 6-7. This suggests that the relative humidity may slightly cause an increase of the exposure to benzene in gasoline vapour and the excretion level of phenol in urine which also should be considered when monitoring exposure and interpreting results.

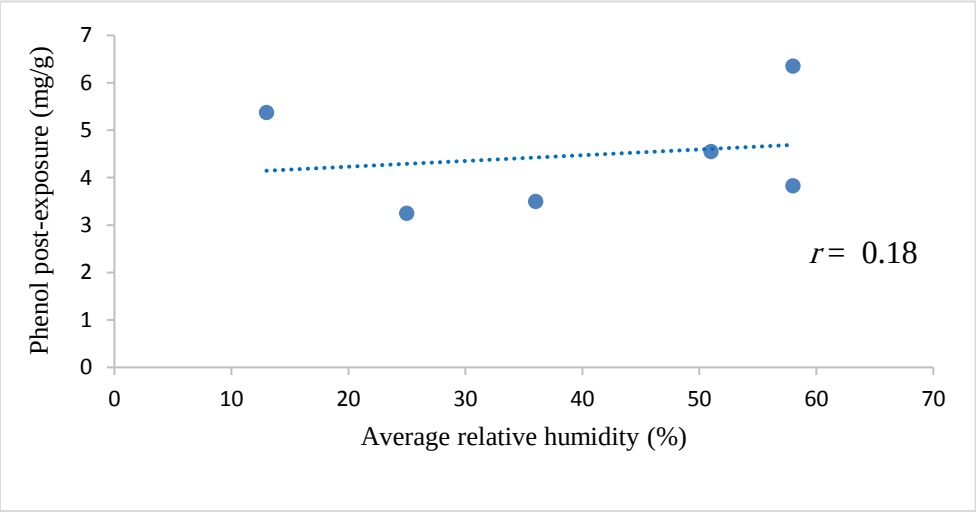


Figure 6-7: Relationship between the relative humidity and the phenol post-exposure results

T,T-MA Urine Metabolite

Pearson correlation test was applied for the post-exposure results of the *t,t*-MA and the levels of relative humidity to test any effects of the ambient humidity on the levels of excreted *t,t*-MA in urine. The *t,t*-MA post-exposure results were grouped per their matched similar averaged relative humidity readings by taking the average of such results. The test results indicated an inverse weak correlation (r = - 0.20; p > 0.50) as shown in Figure 6-8. This suggests that higher relative humidity may slightly decrease the level of *t,t*-MA excretion in urine and that the relative humidity levels should be considered during exposure monitoring and results interpretation.

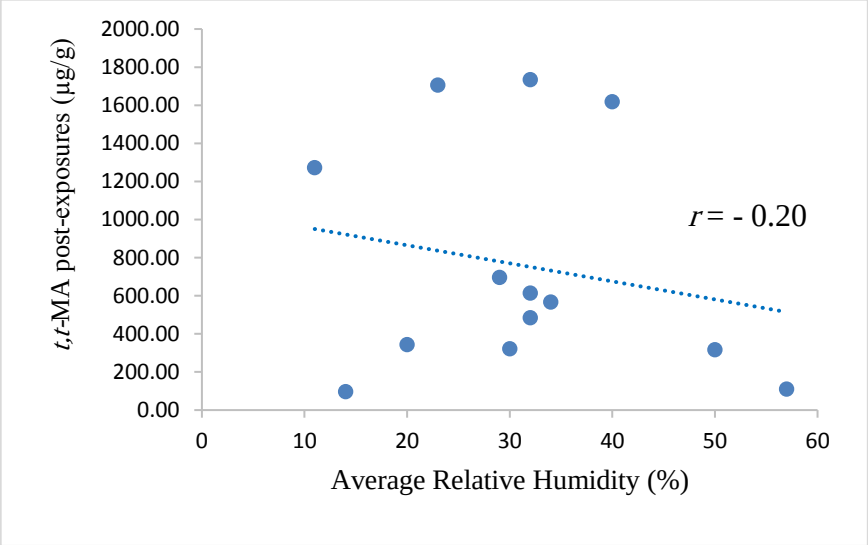


Figure 6-8: Relationship between the relative humidity and the *t,t*-MA post-exposures

S-PMA Urine Metabolite

Pearson correlation test was applied for the post-exposure results of the *S*-PMA and the relative humidity levels. This is to test any effects of the high relative humidity on the excretion of the *S*-PMA due to the exposure to benzene in gasoline vapour. The post-exposure results of the *S*-PMA were grouped against their similar averaged relative humidity readings by taking the average of such results. An inverse weak correlation (r = - 0.21 p > 0.48) was found for the relation between the *S*-PMA and the relative humidity effects. This suggests that high relative humidity may inversely affect the levels of *S*-PMA excreted in urine which should be considered for exposure monitoring and interpreting exposure results. This correlation is very similar to that was found in the relationship between the relative humidity

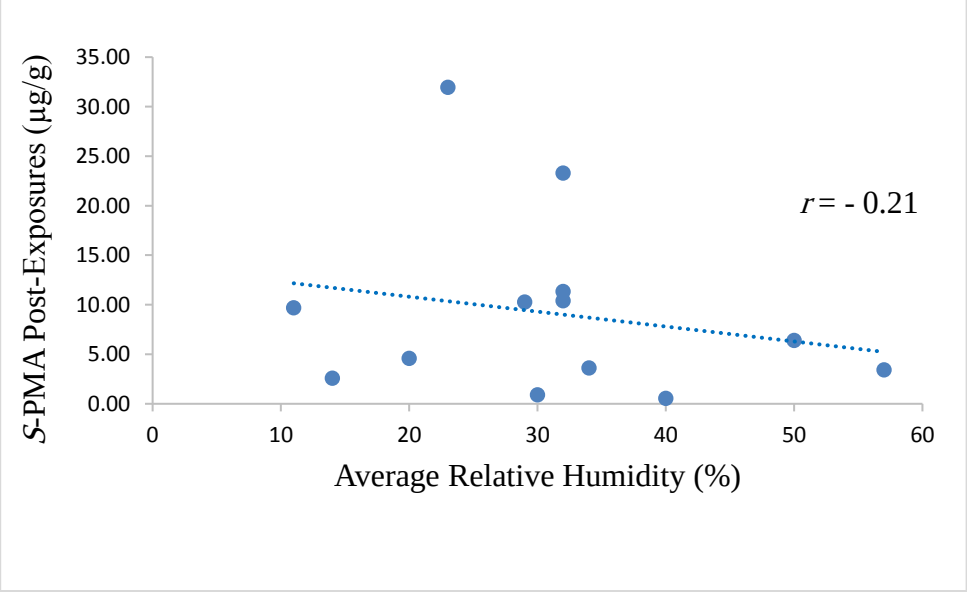


Figure 6-9: Relationship between the relative humidity and the *S*-PMA post-exposure results

level and the post-exposure to *t,t*-MA.

Correlation between Post-Exposure Benzene Metabolite Levels and Quantity of Gasoline Pumped

Phenol Urine Metabolite

Pearson correlation test was conducted for the post-exposure results of the phenol in urine and the quantity of the gasoline pumped. This is to examine any relationship between the pumped quantity of gasoline and the excretion levels of phenol in urine. The quantity of gasoline dispensed was divided on the numbers of attendants in the same shift at a petrol station to get the average pumped fuel per worker. The test results showed a weak positive correlation level ($r = 0.20$; $p < 0.47$) between the excreted post-exposure phenol in urine and the quantities of gasoline sold. This suggests a slight effect of the gasoline pumped quantities on the excreted levels of phenol in urine.

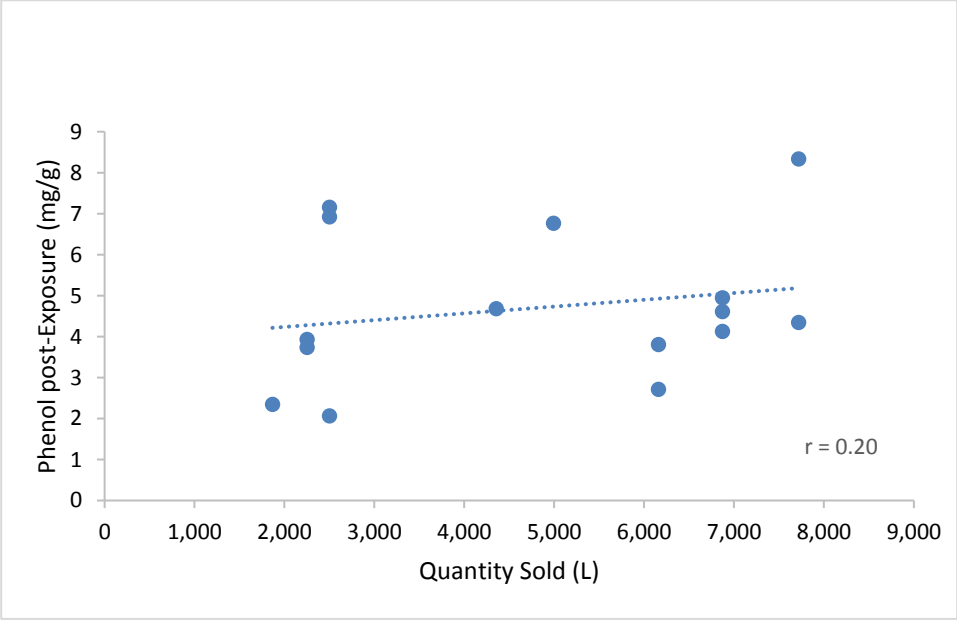


Figure 6-10: Relationship between the quantity of gasoline sold and the phenol post-exposure results

T,T-MA Urine Metabolite

Pearson correlation test was applied to the quantity of gasoline pumped and the post-exposure results of the *t,t*-MA to test any effects of the pumped gasoline quantity on the excretion levels of *t,t*-MA. The test showed very weak positive correlation (r = 0.16; p > 0.59) between the post-exposure results and the averaged quantity of gasoline pumped per worker as shown in Figure 6-11. This suggests that the quantity of gasoline pumped can increase the level of excreted *t,t*-MA due to the exposure to benzene in gasoline vapour. Therefore, the quantity of gasoline pumped should be considered when conducting gasoline vapour exposure monitoring using *t,t*-MA excreted metabolite.

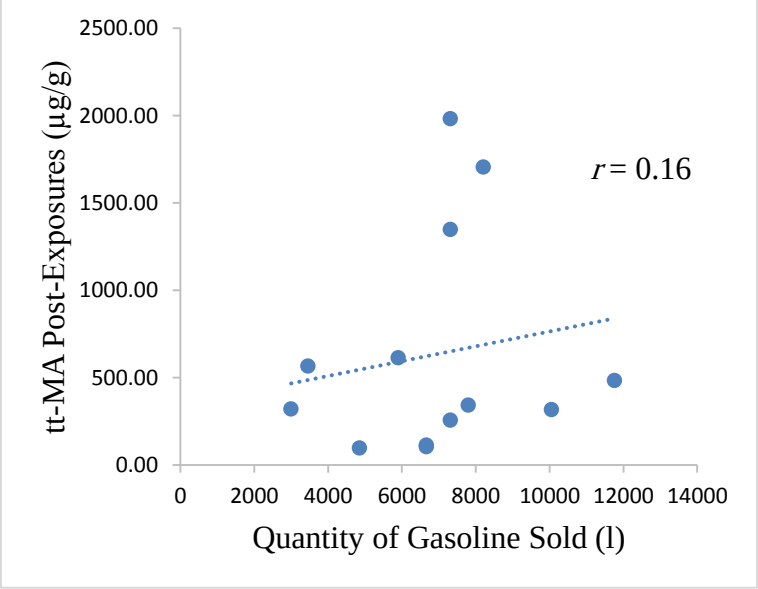


Figure 6-11: Relationship between the quantity of gasoline sold and the *t,t*-MA post-exposure results

S-PMA Urine Metabolite

Pearson correlation test was carried out for the post-exposure results of the *S*-PMA and the quantities of gasoline pumped. This is to test any effects of the quantity of gasoline sold on the excretion levels of *S*-PMA in urine. A very weak positive relationship ($r = 0.13$; $p < 0.53$) was found for the post-exposure *S*-PMA levels and the average quantity of gasoline pumped per individuals. This suggests a slight effect of the quantity of gasoline sold on the excreted *S*-PMA in urine due to exposure to benzene in gasoline vapour. This again is similar to that correlation level found for the post-exposure *t,t*-MA and the quantity of gasoline sold. Therefore, quantity of the gasoline pumped should be considered as a slight affecting factor on the exposure of benzene in the gasoline vapour and the excretion of the metabolites.

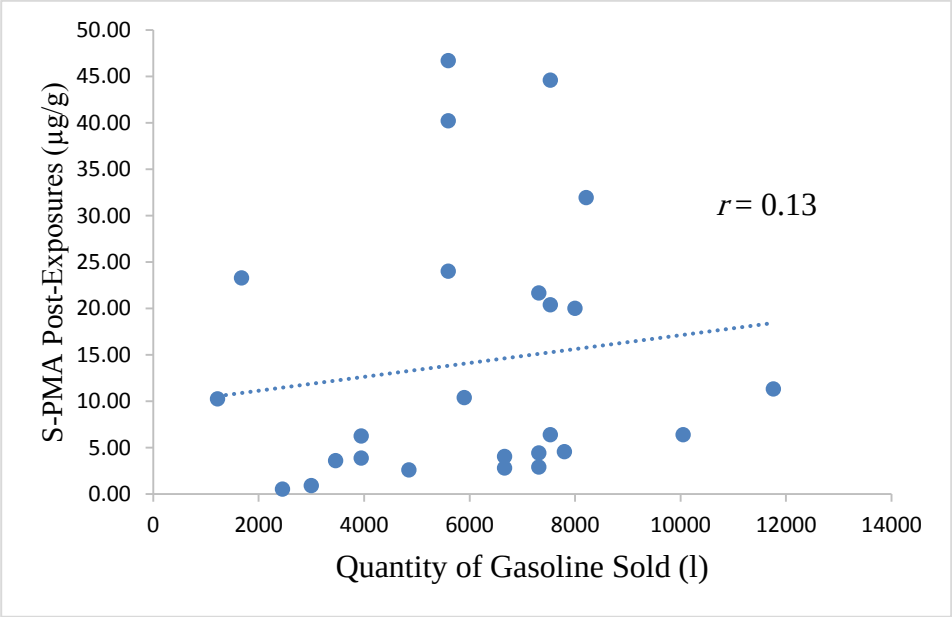


Figure 6-12: Relationship between the quantity of gasoline sold and the *S*-PMA post-exposure results

Table 6-10 below summarises the correlation coefficients for different factors and the level of urine metabolite excretions.

Table 6-10: Correlation coefficient between the post-exposure metabolites and various factors

	Phenol Correlation Coefficient Factors				
Post-Exposure	Pre-Exposure	Wind Speed	Temp.	RH%	Qty Pumped
	0.50	0.009	0.75	0.18	0.20
	***t,t*-MA Correlation Coefficient Factors**				
	0.51	- 0.12	0.22	- 0.20	0.16
	***S*-PMA Correlation Coefficient Factors**				
	0.80	- 0.32	- 0.04	- 0.21	0.13

Relationship between Post-Exposure Benzene Urine Metabolites Levels and the Locations of the Petrol Station

The post-exposure levels for the three metabolites were assessed with consideration to the locations of the petrol stations to identify any possible effects by adjacent traffic (e.g. tail exhausts) or limited wind movements, and due to surrounding building. The petrol station sites without heavy traffic in its vicinity are categorized as open area, and therefore is considered the first category. Others that are either surrounded by buildings or near heavy traffic are the second category and those that are surrounded with buildings and located in a nearby heavy traffic are the third category, with two combined factors. Based on such classifications, the relationships were illustrated.

Phenol Urine Metabolite

A clear difference in the phenol in urine concentrations was noticed for the different location categories of the petrol stations. The exposure of the petrol attendants who worked in petrol stations near highways and surrounded by buildings was the highest as shown in Figure 6-13.

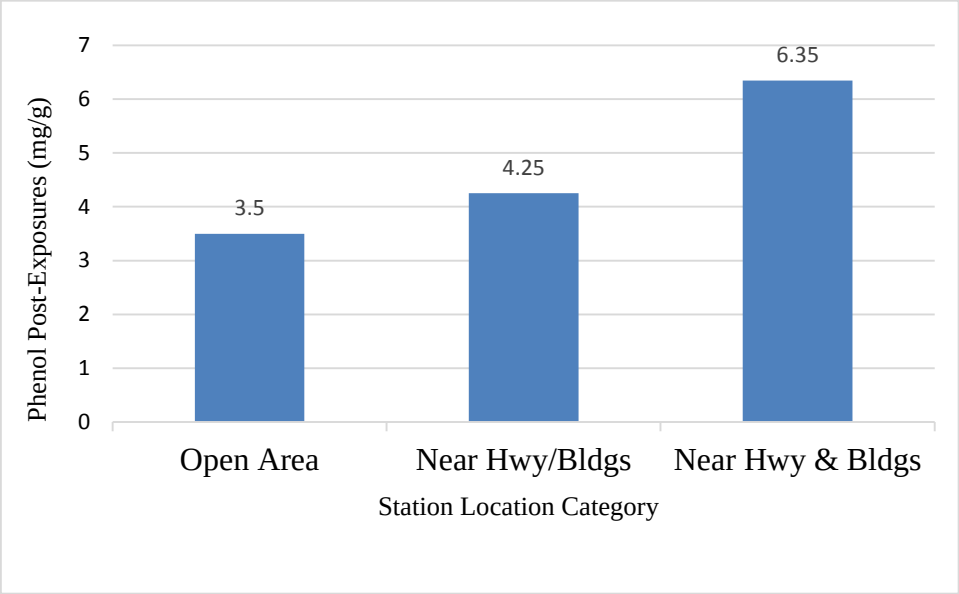


Figure 6-13: Relationship between phenol post-exposure levels and the locations of the petrol stations

T,T-MA Urine Metabolite

The location factors did not show clear effects (Figure 6-14) concerning the levels of post-exposure *t,t*-MA excreted, due to the exposure to benzene in gasoline vapour when considering one factor separately. Unexpectedly, exposure results obtained from stations located in open areas were higher than others, with at least one location factor. This indicates that there were no effects pertaining to the location of the petrol station sites on the excretion of the *t,t*-MA from exposure to benzene vapours. This unpredicted trend agrees with the

notion of confounding effects by nutrition metabolism on *t,t*-MA excretion (Carrieri et al., 2006; Euopean Commission, 1994).

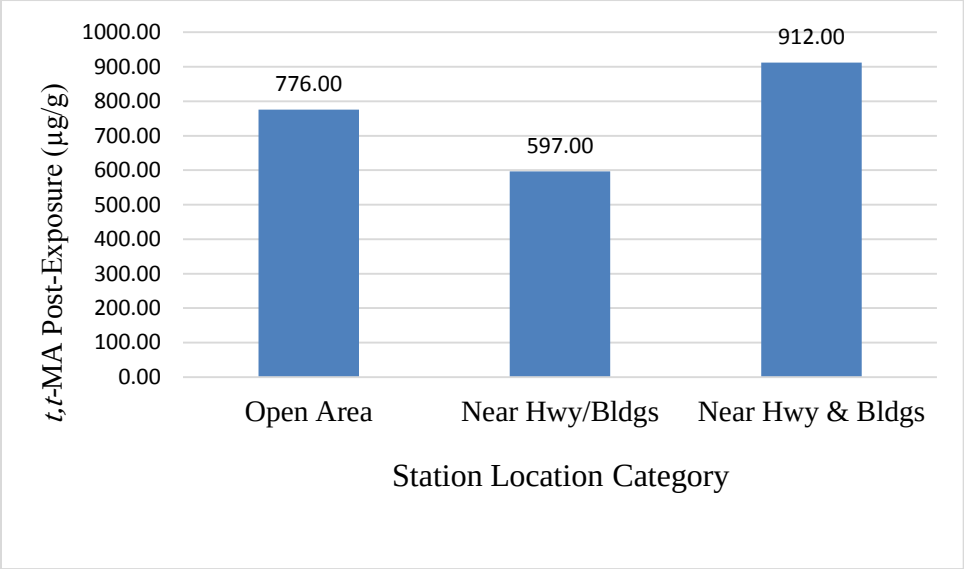


Figure 6-14: Relationship between *t,t*-MA post-exposure levels and the locations of the petrol stations

S-PMA Urine Metabolite

A strong relationship was illustrated between the excretions of the *S*-PMA with the increase levels of exposures to benzene vapours in air accumulated, due to the location factor of the petrol stations. The highest increase in the *S*-PMA concentrations was found in petrol stations containing two combined factors: those near heavy traffic and those surrounded by buildings as illustrated in Figure 6-15.

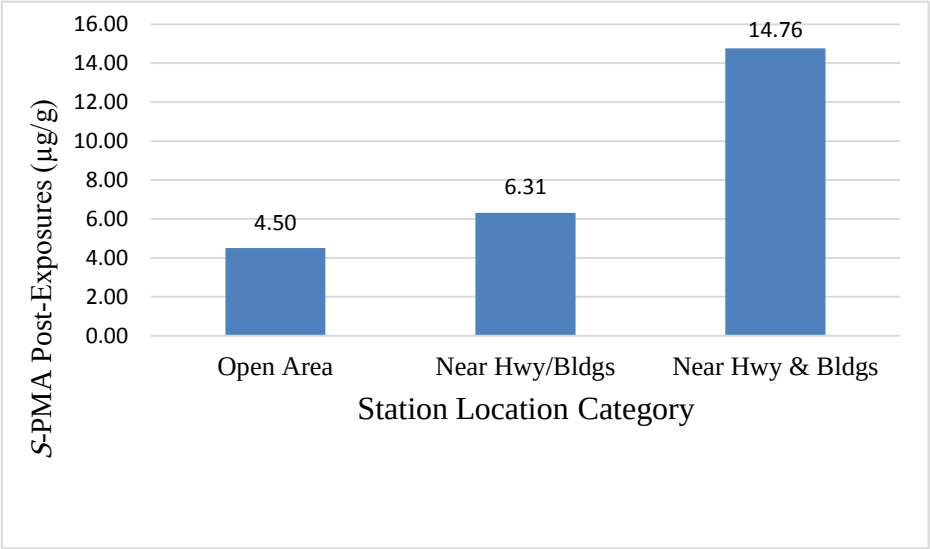


Figure 6-15: Relationship between phenol post-exposure levels and the locations of the petrol stations

6.5. Summary and Conclusion

The benzene biological exposure assessment test showed that it is possible to experience higher levels of the urinary metabolites in pre-exposures than post-exposures. This is due to various reasons as follows: diet of the individual, exposure to different chemicals, cigarette smoke or exposures, previous accumulated exposure effects, and different work practices and exposure scenarios. Sorbic acid is an example that can be a confounder, which increases the levels of the *t,t*-MA metabolite. Therefore, interpretation of the results from these metabolites should be carefully made. Furthermore, smoking habit is another confounder that can influence the results, especially for the *S*-PMA metabolite in urine, due to the effects of the tobacco. Therefore, the smoking factor should be considered for benzene exposure assessment in the assessment of petrol station attendant exposures. The high results can also be justified, accordingly.

Different factors, such as weather conditions, quantity of gasoline pumped, and the location of the petrol stations may positively and/or inversely influence the exposure results and the excretion levels of the urinary metabolites. Higher wind movement decreased the levels of exposure for the *S*-PMA and the *t,t*-MA, but not for the phenol metabolites. Therefore, it can be expected to have lower exposure results for the first two metabolites in high wind movement conditions.

Furthermore, the ambient temperatures caused inversely very weak to moderate effects on the benzene exposures. High temperatures were associated with higher excretions of phenol and *t,t*-MA. A very weak inverse effect was found for the *S*-PMA relation, with the high ambient temperature. The relative humidity did not show significant influence on the excretion of the benzene urinary metabolites. Very weak positive relationship was found for the effect on the phenol level. Inverse weak correlations were found for the effects on the *t,t*-MA and the *S*-PMA.

The relationship of the quantity of gasoline sold was very weak for the *t,t*-MA and *S*-PMA and was weak correlation for the phenol. The locations of the petrol station were found to be an important influencing factor to consider during the assessment of the benzene in gasoline vapour exposure, especially for the phenol and the *S*-PMA metabolites. The stations that were located near highways or heavy traffic, and surrounded by buildings that limited the winds' movement, showed higher gasoline vapour exposure results.

CHAPTER 7

7. Occupational Exposure to Gasoline as a Mixture

7.1. Introduction

This Chapter analyses the method of measuring occupational exposures to gasoline vapour as a mixture and accounts for all of its vapour components in air for the petrol station attendants during dispensing of motor fuel. Although, it is less commonly used than the method of assessing exposures to individual components (e.g. BTX and MTBE) it is considered a challenging trend to gradually change to mixture-oriented or what is also known as "real life-oriented" (Feron et al., 1995). As the individual component exposure assessment is a common practice for occupational health hazards exposure assessments, it is applied to estimate the exposures to the components of the gasoline vapour considered, to represent the highest health hazard (Tunsaringkarn et al., 2012). In contrast, the exposure assessment to multiple chemicals or mixture vapour is a comprehensive method that considers the effects of all components and the contributions to the health effects by each of them. Some of the gasoline components affect specific organs in the human body creating a potential additive health impact, with others that impair the same organs. For example, the butane, normal nonane, xylene, cyclopentane, and cyclohexane that exist in the gasoline mixture can individually affect the human central nervous system (CNS) (ACGIH, 2015). Therefore, the exposure assessment to the gasoline vapour, as a mixture, has been carried out and discussed in this Chapter to test its application. The Saudi Arabian gasoline fuel was used as tested fuel.

The ACGIH mixture additive dose formula was used to calculate a designated OEL for the Saudi gasoline fuel vapour exposure, as an example, via inhalation. This proposal is original in the Kingdom of Saudi Arabia and it was implemented because of the need to derive a specific OEL for the locally produced gasoline, with consideration to specific factors, including the chemical ingredients and the climatic conditions. Different OELs that were adopted by the ACGIH (TLV = 300 ppm) have been proposed in other countries (Concawe, 2002) to reflect local conditions. Similarly, this study was approved to support the development of a specific OEL for the Saudi gasoline vapour exposure by taking account of unique variables, such as longer working shifts involving only fuel refill elevated ambient temperatures, higher benzene levels in the gasoline than those in Europe and USA, and the lack of vapour recovery systems.

7.2. Materials and Methodologies

The components of the gasoline fuel mixture were separated using the vial headspace method and the gas chromatography (GC) technique, at two different temperatures. A GC analytical method was developed by the laboratory to separate gasoline vapour into its chemical components. This is the same method used for the gasoline component characterization in Chapter 3, of which the GC analyser (Model 6890N Agilent Technologies) connected to a flame ionization detector (FID), was used. A volume of 0.5 ml of gasoline vapour was utilized to extract the vapour samples and run them into the GC. The samples were preheated in a water bath (Julabo TW12) and the vapour was extracted and tested at 25°C and 45°C. This represents actual ambient and fuel car tank temperatures during the summer and winter in Saudi Arabia. The GC column capillary was used with the

isothermal temperature of 275°C. Helium gas was the carrier gas for this experiment. Results were reported by the computer software (Hydrocarbon Expert V4.2) that processed the data, with respect to chemical groups, the number of carbons, constituent by carbon, constituents by mole fraction, and by the separation time.

7.2.1. Gasoline Vapour Mixture Detection Analysis

The head space vapour experiments were repeated three times for each of the gasoline types (91 and 95 RON) at 25°C and 45°C, which completed twelve runs. Additional six repeats were conducted for the 91 RON, only for the winter mixture formulae at the two temperatures 25°C and 45°C. Out of the 18 trials, 14 were considered because they had more than 99.98% of the sample components detected. The geometric means of all vapour volumes for each chemical component were taken.

7.2.2. Setting OEL for Tested Gasoline Vapour Mixture

The ACGIH mixture additive dose equation (Equation 2) was used to calculate a designated OEL for the Saudi gasoline fuel vapour exposure via inhalation (McDermott and Killiani, 1978). In the gasoline vapour mixture calculation, all component values detected in the vapour phase are included in the OEL formula as tabulated in Appendix G. From the separation of the gasoline vapour components by the GC and head space techniques, each chemical vapour concentration value is converted from percentage to ppm (multiplying by 10,000) then divided by its pertaining OEL (Appendix G). The same repeated for all fourteen trials. The OELs for each constituent are obtained from different international governmental organizations listed below and they were based on the availability of the required OELs. The latest and applicable OELs were considered when more than one was available:

1. The United States Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PEL)
2. The United Kingdom Health and Safety Executive (HSE) EH40/2005 Workplace exposure limits.
3. The German Research Foundation (DFG)-MAK values
4. American Conference for Governmental Industrial Hygienist (ACGIH) Threshold Limit Value (TLV),
5. Exploratory Survey of Occupational Exposure Limits for Carcinogens, Mutagens and Reprotoxic substances at EU Member States Level.

All values were calculated in ppm and converted from mg/m^3 in some cases using the Equation 3.

The molecular weight of the gasoline vapour mixture was calculated using Equation 12 below, based on the mole fractions (%mol) of individual compounds obtained from the GC associated software (Hydrocarbon Expert V4.2). Examples of the %mol provided by the software are shown in Figure 7-1.

Component List

Pk#	Time	Group	Component	%Wgt	%Vol	%Mol
1	7.765	I4	i-Butane	0.670	0.826	0.944
2	8.115	P4	n-Butane	2.694	3.196	3.796
3	9.397	I5	i-Pentane	17.008	18.848	19.307
4	10.090	P5	n-Pentane	17.560	19.254	19.933
5	11.403	I6	2,2-Dimethylbutane	0.714	0.756	0.679
6	12.794	M5	Cyclopentane	0.646	0.595	0.755
7	12.895	X5	Methyl-t-butyl ether	19.957	18.505	18.543
8	13.094	I6	2-Methylpentane	6.115	6.429	5.812
9	13.905	I6	3-Methylpentane	3.798	3.925	3.609
10	14.916	P6	n-Hexane	6.333	6.595	6.019
11	16.833	M6	Methylcyclopentane	1.421	1.303	1.383
12	18.835	Q6	Benzene	2.835	2.215	2.973
13	20.612	I7	2-Methylhexane	1.785	1.807	1.459
14	21.572	I7	3-Methylhexane	1.958	1.956	1.600
15	24.637	P7	n-Heptane	1.568	1.574	1.281
16	33.188	Q7	Toluene	7.381	5.846	6.561
17	53.657	Q8	m-Xylene	2.976	2.364	2.295
18	53.947	Q8	p-Xylene	1.439	1.147	1.110
19	59.485	M9	1c,2t,4c-Trimethylcyclohexane	1.810	1.609	1.174
20	77.721	I10	5-Methylnonane	1.332	1.249	0.767

Figure 7-1: Obtained information including %mol of components via Hydrocarbon Expert V4.2 software

Each of these values was multiplied by the MW of the pertaining component (i) and summed to obtain the MW of the mixture Equation 12. The average of the molecular weight obtained from the fourteen trials was used as the $MW_{mixture}$ of the gasoline vapour mixture as shown in Appendix G.

$$MW_{mixture} = \sum_{1}^{n} MWi * xi)$$

where:
MWi = molecular weight of the chemical component i
xi = mole fraction value of the component i
MWmixture = Calculated sum of the molecular weights of the vapour mixture components

Equation 12: Total molecular weight calculation based on individual mole fractions of the components

7.3. Personal Exposure Assessment to Gasoline Vapour Mixture

The applicability of the proposed OEL for gasoline vapour exposure at petrol stations was tested using ToxiRae Pro personal PID direct reading instrumentation Figure 7-2. The

Figure 7-2: ToxiRae Pro PID personal VOC exposure monitoring instrumentation (Courtesy: Rae Corporation)

instruments were equipped with 10.6 eV lamps that were capable of detecting more than 300 VOCs (Rae, 2013) in air including the component of concern. The sampling was conducted at twelve petrol stations, with different sale rates, locations and weather conditions. The

sampling time ranged from 5-7 working hours. Two samples were used per station and the results were averaged per station. The instruments calculate the time weighted average (TWA) exposure during the sampling duration (e.g. 8-hour). This is the time identified by ACGIH, as a maximum averaged value, "under which is it believed that nearly all workers may be repeatedly exposed day after day, over working lifetime, without adverse health effects." The same TLV-TWA of the ACGIH is applied in Saudi Arabia.

7.4. Results

The means of the gasoline vapour concentrations in air divided by the pertaining OELs for each component are illustrated in Appendix G. Some of the gasoline liquid components were not detected in the vapour, therefore, only repeatedly detected components were considered. The highest toxic components of the BTEX and MTBE were within the detected compounds and included in the OEL calculation. The sums of all fourteen trials are averaged and was found to be 42,356.74 ppm as in Table 0-10 Appendix G. By applying Equation 2 to calculate OEL_{mix} the result was found to be 23.60 ppm and rounded up to 25 ppm.

The overall experimented OEL for the mixture gasoline vapour in air was found to be 25 ppm. Furthermore, an excursion limit of 75 ppm was applied, which is three times the limit that one can be exposed to, for no more than 30 minutes, provided that the OEL of full time is not exceeded during the shift (ACGIH, 2015). This is the recommended OEL value for tested Saudi gasoline vapour exposure of both RON 91 and 95 for 8-hour working shifts for petrol station attendants. For 12-hour working shifts the OEL is 16 ppm based on OSHA adjustment calculation.

The MW of the calculated gasoline vapour mixture is 85.77 g/mol using Equation 12. This is based on the fourteen trial outputs with their detected components detailed in Table 0-11 in Appendix G. The average of the sum of MW of detected components at two temperatures of 25°C and 45°C for both gasoline grade 91 and 95 RON was calculated to be 85.77 g/mol.

The results of the personal exposure assessment to gasoline vapour mixture using ToxiRae Pro direct reading instrument are shown in Table 7-1 below.

Table 7-1: Summary of gasoline vapour exposure assessment at different petrol stations using PID direct reading instruments

Station No.	Sampling Time (min)	TLV-TWA (ppm)
1	420	8.90
2	420	6.90
3	400	14.90
4	345	2.73
5	390	3.10
6	360	5.92
7	390	8.36
8	300	1.77
9	420	6.23
10	390	5.60
11	360	9.00
12	420	0.07

The recorded TWA exposures ranged from 0.07 ppm to 14.90 ppm. These were within the proposed allowable OEL of 25 ppm for 8-hour TLV-TWA working shifts. These results represented the levels of exposures per types of petrol stations. In other words, they showed higher levels of exposure, as presumed, at high level categorized stations. Furthermore, they agreed with other validated exposure assessment method used under similar conditions. All results by PID showed compliance with OSHA standards for gasoline vapour exposure as

well. Thus, indicated the validity of using direct reading PID instrumentations for personal exposure assessment with results that can be referred to the newly proposed OEL.

7.5. Discussion and Conclusion

The gasoline mixture vapour values were obtained experimentally via applied head space method associated with the GC technique. Some components in the gasoline liquid were not detected in the vapour. The overall OEL was calculated based on fourteen repeated tests. These trials had more than 99.98% detected component values in vapour for two simulated ambient simulated temperatures 25ºC and 45ºC. The generated vapour values were extrapolated based on the atmospheric pressure and ambient temperature. The calculated OEL for gasoline vapour mixture indicated that 25 ppm for eight-hour exposure and 16 ppm are reasonable and applicable for petrol station attendant exposure limits in Saudi Arabia. This was verified by the personal exposure assessment for VOCs by direct reading PID instruments (i.e. ToxiRae Pro). The recommended value can be used as a reference when monitoring workers' exposures to gasoline vapour mixture at petrol stations. The levels of exposures by the PID instruments varied depending and corresponding to the levels of vapour exposures at the different petrol stations. The low result values were measure at low sale petrol stations making it consistent with other results tested via different methods at the same stations. Similarly applies to higher results by the PID instruments which also were collected from busier stations with higher levels of exposures measured previously by other methods. This is convenient new sampling strategy and more efficient method for assessing the exposure to the gasoline vapour mixture rather than the traditional individual component measurements. Furthermore, this method counts for the health effects for all chemical

components that exist and are detected in the vapour mixture when compared against this newly proposed OEL. Excursion limit (that can be exceeded three times the OEL for maximum of 30 minutes) of 75 ppm for peaks can also be used as a recommended reference. The MW of the gasoline vapour mixture was calculated to be 85.77 g/mol which can be used in unit conversion purposes (i.e. ppm and mgm^3).

CHAPTER 8

8. Evaluation of Exposure Assessment Methods and Findings

8.1. Introduction

This thesis comprehensively investigates different aspects of the toxic petrol vapour components, their characterisations and behaviours under weather simulated conditions, and petrol station attendants' exposure assessment methodologies. These methods evaluated exposures to petrol vapour concentrations in the breathing air around the workers and the intake amounts of such contaminant concentrations into the human body system (biological exposure assessments). Such methods differ in their applications, accuracy and suitability depending on various factors, such as: the chemical of interest, duration of exposures, analytical facilities available and others. The thesis investigation focused on petrol stations in the Eastern Province of Saudi Arabia, as one of the tropical region, for the exposure assessment to the locally produced motor vehicle petrol vapours. The factors that have been investigated in this work were the ambient temperatures, relative humidity, wind movement (speed), quantity of petrol pumped per exposed worker, and the location of the petrol station. These are natural variables encountered at the Saudi petrol stations. A proposed occupational exposure limit (OEL) has been experimentally derived and designated for exposure assessment to mixture of petrol vapour. The applicability of the recommended OEL was verified in the field and under routine work conditions.

8.2. Discussion

The currently available handheld area direct reading photo ionizing detections (PID) technology can be employed to estimate mixtures of volatile organic compound (VOC) in air rather than individual components. Therefore, such methods are unspecific for particular chemicals in mixtures. However, if only one chemical exist in the air and the instrument is calibrated for that chemical then it can be assumed that the instrument reading represents that chemical merely. There is another approximating method to estimate specific chemicals in vapour mixtures, which is using correction factors (CF) determined by instrumentation manufacturers for every constituent to be used for the approximation of the chemical in air. Furthermore, a new technology is capable of measuring benzene only in a vapour mixture. This is known as benzene specific instruments. Moreover, the new personal VOCs direct reading instrument (e.g. ToxiRae) can monitor petrol station attendants' exposures to petrol vapour as VOC mixture. The newly developed and proposed OEL in this study for the Saudi gasoline vapour can be utilised to compare against results obtained by the personal VOC monitors to ensure safe exposure levels with regards to current standards.

The environmental air concentration measurements carried out in this study evaluated the amount of contaminants around the petrol attendants' breathing areas with the assumption that such amounts enter into the body through the inhalation route of exposure. Because of the large number of gasoline vapour chemical components existing in the air the highest most toxic five constituents, namely; benzene, toluene, xylene, ethylbenzene, and methyl-tertiary butyl ether (MTBE), were measured using active and passive methods. These are the methods traditionally applied to measure individual components in vapours. It was concurred that in

this study, the passive samplers were more convenient than the active samplers due to the disadvantages elaborated in Chapter 2. In addition, the environmental passive air assessment was found to be reliable in measuring exposures under different factors and conditions investigated. This was clear from the obtained results and the increase and decrease of the concentrations with response to the affecting factors, especially, the locations of the petrol stations. The responses by other methods to the assessed variables were not as clear, as it was by the environmental passive air method. Therefore, this method was considered the most applicable for the petrol station attendant exposure assessment. Thus, the efficiency of the other methods was compared against it.

A comparison was made between the environmental passive air sample results and the post-exposure exhaled breath sampling for benzene that were carried out in parallel. This means that both methods were carried out at the same time, for the same petrol station, under the same affecting factors. The non-detectable (ND) results were treated as zero number (0) and interpreted as no exposure result. The majority of the results showed similar levels trend (increased and decreased) to the air samples. Significant ($P < 0.002$) moderate correlation coefficient ($r = 0.49$, N=40) was found between the air samples and the exhaled breath for benzene (Appendix I).

In comparison to the other measured components results of toluene, xylene, ethylbenzene and MTBE, it was found that the correlation to the air passive method depends on the volatility of the measured compounds. More volatile compounds correlated better with the passive sampling. Chapter 3 showed that benzene, toluene, and MTBE were the highest volatile compounds. Therefore, they were detected mostly from the exhaled breath samples.

Similarly, the environmental passive air samples were compared against the post-exposure *S*-PMA results for benzene exposure. The two methods were carried out at the same time for the same subject and under the same conditions. The recorded numerical results are shown in Appendix I. The comparison showed significant ($P < 0.01$) strong correlation coefficient ($r = 0.75$, N = 27).

The third exposure assessment method of *t,t*-MA metabolite in urine was compared against the environmental passive air method under the same sampling conditions. The *t,t*-MA results did not show a consistent trend of increased or decreased values with the environmental passive air results variation. Furthermore, very high results were found for the *t,t*-MA against very low corresponding air exposure results making insignificant correlation implications. The result of correlation analysis showed a very weak ($p < 0.92$) correlation coefficient ($r = 0.02$, N=27). All results are also illustrated in Appendix I.

The fourth biological personal exposure method is the phenol metabolite level in urine. In comparison of the phenol in urine with the environmental passive air results, it showed a very weak ($p < 0.58$) correlation coefficient ($r = 0.17$, N = 12) shown in Appendix I. Therefore, the methods' results comparison indicated that *S*-PMA was the closest to the environmental passive air sampling method in measuring gasoline vapour. The breath and urine biological samples were found suitable for cohort exposure investigations.

Exposure to petrol vapour mixture can be confidently assessed using PID personal direct reading instruments. This is a more effective exposure assessment method that counts for all vapour components' effects. The exposure results can be compared against the newly developed OEL which is suitable for petrol station assessment.

8.3. Summary and Conclusion

All five measured toxic compounds (BTEX and MTBE) in gasoline vapour, measured by the environmental passive air sampling for the monitored petrol stations were found to be in compliance with the current governmental OELs. The passive air sampling method was found to be the most convenient in collecting personal exposure air samples. This method is less affected by the individuals' living habits and different dietary metabolisms. The biological *S*-PMA benzene metabolite was found to be the closest correlated method to the environmental passive air sampling. The exhaled breath was found to be the second method that correlated with the passive samplers. This thesis produces a new OEL available for occupational exposure assessments to gasoline vapour mixture at petrol stations in Saudi Arabia that can be utilised rather than individual components.

CHAPTER 9

9. Conclusion and Recommendations for Further Work

9.1. Summary

Investigations from this thesis are summarised into several points. The number of components in the vapour phase of the gasoline ranged between 22-23% of the liquid gasoline phase at temperatures 25°C and 45°C, respectively. This suggests that exposure risk to gasoline vapour is slightly higher in summer than in winter (Section 3.3).

It was found that there is no occupational overexposure concerns of gasoline vapour toxic components by air sampling method, during both summer and winter seasons, for the petrol station attendants monitored in Saudi Arabia, when tested under various characterizations in full service stations, 10-12 hours working shifts, different weather conditions, different locations, different volume of gasoline pumped and no vapour recovery systems (Section 4.5).

Furthermore, the factors of locations (near highways and surrounded by tall buildings), the winds' movement, and volume of the gasoline pumped per person play important roles in increasing and decreasing the exposure concentrations to the gasoline vapour. These can be influencing factors that might contribute to increase exposure risks due to limiting area dilution ventilation, contaminants from highways, and more vapour exposure from larger gasoline volume pumped. (Section 4.5).

There are no significant effects of the ambient temperatures on the increase of exposure to gasoline results, as was tested in the laboratory and in the field (Section 3.3 and 4.5). The wind speed predominantly inversely correlated with the gasoline vapour exposure results by air sampling, exhaled breath and S-PMA benzene urine metabolite (Section 4.5, 5.3.2 and 6.4.1).

The highest concentration of the gasoline vapours occurs at about 30 centimetres (1 foot) from the vehicle tank opening, making an important healthy work practice for the petrol station attendants to avoid breathing within this perimetre. (Section 4.5). The Bio-VOC sampler kit is a reliable commercially available non-invasive exhaled breath sampler, particularly for high volatile compounds (Section 5.2). Similar to the air sampling, the exhaled breath can be influenced by factors such as the following: temperature, wind speed, quantity of gasoline pumped and the location of the petrol stations. Furthermore, the exhaled breath testing responded to highest volatile compounds (i.e. benzene, toluene, and MTBE), as also was shown in the correlation with the air sampling results. The relative humidity did not make significant effects on the exposure results (Section 5.3.2).

The biological sampling methodologies of exhaled breath and benzene urine metabolize are more appropriate for cohort exposure assessment for gasoline vapour in petrol stations. This is due to the variety of personal nutrition system and different lifestyles that can influence the evaluation results (Section 6.4). The S-PMA benzene urine metabolite for non-smoker attendants had the closest exposure trend to the air exposure assessment, compared to phenol and *t*,*t*-MA, which could have been subjects of nutrition influence (e.g. sorbic acid) (Section 6.4).

S-PMA in urine testing shows higher results in smokers. Therefore, smoking is a confounder to this method and best results can be obtained from non-smoker subjects (Section 6.3). Some of the pre-shift results of the three benzene metabolites were higher than the post-shift. This was attributed by possible different reasons, such as accumulation from previous shifts, effects of certain types of nutrition, exposure to smoking (2^{nd} hand smokers) and others (Section 6.4.1).

9.2. Conclusions

Based on the literature review, it was found that petrol vapour exposure assessment for petrol station attendants have not been thoroughly covered, especially with a number of influencing factors that can contribute to the increase of exposures. Therefore, this thesis was implemented to address this gap by investigating the applicability of different assessment methodologies, including the new exhaled breath sampling kits. Based on the results obtained, the following conclusions were made.

1. The great public concern about 'health risks" associated with petrol station attendants exposures' to toxic gasoline vapour components was found to be invalid. Results obtained by air sampling in this thesis revealed that the petrol station attendants in Saudi Arabia are not overexposed to chemical components in the gasoline vapour and the exposure levels are within acceptable limits. Therefore, vapour recovery system is not warranted.
2. The factors of low wind movement, high quantity of gasoline pumped per person, and the surrounded locations of petrol stations by buildings were found to be reasons for increasing the workers exposures to the gasoline vapours. It is concluded that these

variables should be considered in the evaluation of exposures to gasoline vapours at petrol stations via environmental air or biological methods, and the exposure levels are related to them. Such factors should also be considered in the designing of petrol stations.

3. A preliminary study was conducted to study the possibility of using the exhaled breath sampling method for attendants' exposures at petrol stations. It was found that the method is suitable for evaluating exposures to gasoline vapour, especially, the high volatile compounds (e.g. benzene and MTBE). Therefore, it was concluded that the method is a good example of fast, convenient and non-invasive exposure assessment.
4. The S-PMA urine metabolite for benzene was found to be the most suitable urine biological sampling method for low levels of concentrations at the petrol stations and not affected by the low levels and nutrition as in the phenol and *t,t*-MA. It was concluded that the S-PMA is reliable method for detecting benzene exposure levels at the end of the shifts for attendants at petrol stations.

9.3. Future Work

Some results of the exposure assessment to VOC via exhaled breath and urine biological analysis showed higher levels in the pre-exposures than the post-exposures. Such phenomena was thought to be due to remaining amounts in the body from the previous shifts that might be due to slow half-life elimination (e.g. 11-29 hr for benzene) as indicated by Ernstgård et al., 2014. Other possibilities were that the subjects exposed to contaminated uniforms with petrol into the living rooms, and by contamination of the air very close to the accommodation and/or downwind of the stations. Moreover, sharing rooms with smokers (second-smokers)

was also a possible contributing factor that resulted in detectable pre-shift benzene results. These factors were verified from the questionnaire and found to be reasonable. Therefore, such possibilities can be tested via further work to be carried out on extensive control groups with different characteristics than those aforementioned (e.g. longer periods between recurrent exposures to allow contaminant elimination and no exposures to gasoline vapour or nicotine at the accommodations).

Appendix A (Questionnaire)

ID No.:

Questionnaire

We would like to ask you some information regarding your lifestyle and smoking habit to include it in our study. Please be assurred that we will not use your identity (e.g. name or employee number) in the report as it will be used as general anonymous participants' information.

1. What age group do you belong to:

☐	20-25 years	☐	40-45 years
☐	25-30 years	☐	45-50 years
☐	30-35 years	☐	50-55 years
☐	35-40 years	☐	55-60years

2. How long have you been working at this petrol station?

3. Have you worked previously in roles involving exposure to gasoline fuel?

4. Do you wear protective gloves when carrying out your work?

5. Is your living accommodation located within the vicinity of the petrol station?

☐ Yes: ☐ No.

- How far is it in metres?.....
- Is it located downwind?.....
- Can petrol be smelled inside it?.....

6. Is your living accommodation located within 25 metre of a main road?

☐ Yes ☐ No

7. Does anyone else in your living accommodation work at the petrol station? How many?

☐ Yes ☐ No

8. Do they bring coveralls/uniforms to the living accommodation?

☐ Yes ☐ No

9. Have you experienced any different health conditions since starting this job?……. What is it? ……

10. Do you currently smoke cigarettes?

☐ Yes:

- On average how many cigarettes do you smoke per day? ……..
- Approximately how long have you been smoking cigarettes? ……
- Do you currently use nicotine Patch or Gum? …….
- Do you share accommodation with someone who usually smoke inside the accommodation? ……..
- During your breaks and free time do you go to places where you are exposed to cigarette smoke? ……

☐ No.

Table 0-1: Summary of participants' survey data collected

Survey Item	Indication
Number of participants	50
Respondents per age range 20-25 years	10
25-30 years	11
30-35 years	15
35-40 years	8
40-45 years	6
Years of Service at Petrol Stations <2 years	15
2-5 years	17
> 5 years	18
Use personal protective equipment (respirator, gloves, goggles, etc.)	None
Accommodation near station (within 25metre)	24
Smokers	12

Appendix B (Field Sheet)

VOC Personal Exposure Monitoring Field Sheet

Sampling Site ID			
Participant's ID			
Smoking Participant?	Yes ☐ No ☐	Number of Cigarettes per day	
Air Monitoring			
Sample ID		Sample Media	
Start Time		Stop Time	
Flow Rate (ft/min)		Collected Vol. (Litre)	

Worker Location			
Qty of Gasoline Pumped/worker			
Breath Sampling			
Pre-Shift Sample No.		Post-Shift Sample No.	
Pre-Shift Sample Time		Post-Shift Sample Time	
Number of Exhales			
Biological Sampling			
Pre-Shift Sample No.		Post-Shift Sample No.	
Remarks			

Site Information			
Location	Enclosed by Buildings? Close to Highway or busy traffic?	Number of Pumps	

Appendix C (SOP)

Gasoline Vapour Exposure Measurement Standard Operating Procedure for Occupational Exposure in Petrol Stations

Purpose:

The main purposes of this standard operating procedure (SOP) is to produce detailed instructions of the recurring personal exposure monitoring and data collection processes during the routine work practices under various conditions of the seasons. This is to minimize variations and fulfil an emphasis on the conformance and control of the quantitative exposure assessment methodologies for the support of confident analysis and data quality.

Personal Data Collection and Protection:

Prior to the start of any collections of personal data and biological samples, clear information must be conveyed to the participants, detailing the purposes of the monitoring comprehensible to individuals that have no specific technical knowledge and disclose all relevant study information. Obtaining consent from participants that are allowing the collection of their personal data and samples should be provided in writing. These are the starting stage of the study to be introduced to participants via the following forms:

- Study information sheet (specifying the purpose and specific requirements from participants)
- Letter of invitation distributed to participants
- Consent form concurred and signed by the participants
- Questionnaire of supporting information and facts needed for the study

Sufficient time consisting of at least twenty-four hours should be allowed for the participants to review prior to signing the consent forms. Further verbal explanation must be provided by the investigator, whenever needed.

Site Information:

Prior to the beginning of the personal monitoring, the sampled site information needs to be recorded. The following data are required at minimum (refer to the attached field data sheet):

- Number of gasoline pumps and types of gasoline
- Number of workers in the shift
- Site location information (surrounded by buildings or near heavy traffic
- Quantity of gasoline dispensed (pre- and post- shift pump readings)
- Draw a sketch of the site set up (pumps, shops, street, wind direction, accommodation, locations of the underground holding tanks and their vents, roof height, etc.).

Bio-VOC Breath Sample:

The Breath Bio-VOC sampling kits were obtained from Markes International. They were provided to the participants along with the application sampling instructions, before and after the shift or exposure. The collected breath is retained in collective sorbent materials. Below is the breath Bio-VOC sampling steps:

1. Breath sampling collection should be conducted in a clean room, distant from any VOC contaminations.
2. Make sure the piston inside the sampler is pulled up to the green cap (mouthpiece) by pulling the pushrod up to the mouthpiece and unscrewing until it can be removed.
3. Place the cardboard on the inlet nozzle and pass it to the monitored participant.
4. Ask the participants to inhale a full breath, hold breath for 10 seconds, and then exhale steadily into the Bio-VOC for 5 seconds until all the alveolar air is released.
5. Prepare the sorbent material tube by opening the two sides of the tube.
6. After the participant exhales, remove the cardboard and insert the absorbent tube grove end into the opposite end side of the Bio-VOC.
7. Screw the pushrod into the breath-in side (green cap mouthpiece) and transfer the trapped breath by pushing the piston using slow and steady motion towards the end side of the Bio-VOC. The movement should take about 40-50 seconds.
8. Wait for five seconds, remove the absorbent tube from the Bio-VOC and securely cap it.
9. Clear out the Bio-VOC by pulling and pushing the rod at least three times.
10. Repeat the entire process to obtain duplicate samples for each participant.
11. Place the absorbent tube in a small plastic bag and store it in thermal insulated, cooled container.

12. Conduct the Breath VOC collection for both exposed and unexposed (control) participants.
13. Attached previously prepared matching numbering labels on both the sample container and corresponding data sheet (refer to the attached field data sheet).

Biological Urine Samples:

The biological urine sampling method is adopted from the American National Institute for Occupational Safety and Health (NIOSH), method number 8305. This method is designed for the detection of exposure to phenol, benzene, and P_Cresol.

1. Two urine samples from for each participant is needed and collected before and after the exposure/shift.
2. Pass polyethylene screw-cap vials to the participants to provide urine sample.
3. The urine sample should be collected from the mid-stream.
4. Always handle the entire collection process with the required personal protective equipment, using at least the goggles and latex gloves.
5. Make sure the container's cap is on tight, then place container in a small plastic bag.
6. Place the urine containers in a thermal insulated, cooled container with ice/dry ice.
7. The samples should be stable for 4 days in 25°C and can be stored for 3 months at temperature of -4°C.
8. Collect urine samples from exposed and unexposed (control) participants.
9. Attach previously prepared matching numbering labels on each sample container and corresponding data sheet (refer to the attached field data sheet).

Personal Exposure Air Samples:

This personal exposure air sampling is for diffusive badges (e.g. SKC 575-001). The badges can be used in temperature ranges of 7-37˚C, up to 80% humidity as estimated by the US OSHA. The sampling instructions and the lab analytical method are adopted from the American National Institute for Occupational Safety and Health (NIOSH) method number 1501 for aromatic hydrocarbons.

1. To begin sampling, tear off the air-tight bag.
2. Attach the previously prepared numbered labels on both the badge and corresponding data sheet.
3. Uncap the sampling badge and log in the following information on the data sheet:
    a. Start and stop time of sampling
    b. Participant's assigned identification number
    c. Working location of the participant
    d. Names of the location and the site (petrol station)
    e. Note down any unusual events/observation occurred during the shift (e.g. spill).
4. Attach the badge via the clip on the participant's lapel in the breathing zone (1 ft surrounding the nose).
5. Inform the participant to perform normal and routine work practice and observe the badge every two hour to ensure that it is remained clipped in-place and not covered by any materials (e.g. jacket, scarf, etc.).
6. Place the mini-weather station near the attendants' location at a height approximately 1.5 m.
7. At the end of the shift, collect the badge, cap it and return to its preserving bag.
8. Log the end time (refer to the attached field data sheet).
9. Store badges in a chilled temperature (4˚C).
10. Ship to lab in a chilled condition using dry ice.
11. Turn off the mini-weather station. Transfer collected weather data to the laptop computer.
12. At the lab, the collected contaminants in the charcoal granulates are desorbed via solvents such as carbon disulphide. The solvent with the collected contaminants mixture is injected into the Gas Chromatography (GC) which gives the profiles of the chemical components in the mixture per their boiling points.

Direct Reading of VOC and Other Pollutants:

VOCs of concern, namely; benzene, toluene, xylene, and ethylbenzene are instantly measured via photoionization detector (PID) direct reading instrumentations and estimating their percentages in the VOC mixture. Benzene, which is the most toxic VOC, is measured by a benzene specific instrument Ultra Rae 2000 is to be used to assess benzene levels at the pumping area. Other VOCs component levels are estimated by using correction factors for each component to approximate its level in the VOC mixture. Ultra-Rae 2000 without the benzene

filter or MiniRae 3000 can be used to measure the general VOCs in air. The Drager CMS colourimetric direct reading instrument can also be used to estimate different VOC components (e.g. benzene). Readings are to be taken at head height at about two metres near the gasoline pumping and around the site. Several readings should be taken at consecutive measurements throughout the shifts/the sampling time.

(Version 2) 20/02/2013

Letter of Invitation

Participant's Full Name:

Participant's Job Title:

Petrol Station Name:

Petrol Station Location:

Date:

Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations in Saudi Arabia

Dear Mr. <Surname>

We are conducting a research study on exposure to gasoline vapours at petrol stations in Saudi Arabia. Our main purpose is to evaluate exposures of individual workers to gasoline vapours during the filling up of vehicles. The study will involve measuring vapours in air and collecting urine and/or breath samples from randomly selected petrol station attendants and staff undertaking other duties. A number of petrol stations with high and low gasoline sales have been determined to evaluate their workers' exposures.

Study Benefits:

The study will benefit petrol station staff in Saudi Arabia by assessing exposures to gasoline vapours and identifying any need for actions to reduce exposure in order to minimize any risks to health.

Station Personnel Participation:

We invite you to participate in this study because you meet our study criteria. Taking part in the study will involve a personal discussion with a researcher who will ask you questions about your lifestyle such as whether you smoke, any health symptoms and you will be asked to provide urine and breath samples. These samples will be sent to a laboratory for analysis of chemical components and metabolites of gasoline contents. Air samples will also be collected via

attaching samplers to participants' lapels during their working shifts. All participants' information and identities (e.g. names, employee numbers, and station location) will be coded and kept strictly confidential and secured under the custody of the researcher Ahmed Alyami. Analysis results will be anonymously used for statistical analysis of the data.

We have attached an Information Sheet, which tells you more about the study. If you are able to take part in this study, please complete one copy of the Consent form for return to the researcher. If you would prefer not to take part, please return the consent form anyway to let us know. The researcher will contact you to collect in 1 – 2 days and in the meantime please read the information provided before filling out the forms. If you have any questions about the project, please feel free to call the researcher.

Thank you for your help.

Researcher: Ahmed Alyami
Office: 03 880-0394
e-mail: a.alyami@cranfield.ac.uk

(Version 2) 20/02/2013

Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations in Saudi Arabia

Participation Information Sheet

We are asking for your participation in our study of occupational exposure to gasoline vapour at petrol stations in Saudi Arabia. This is to generally quantify gasoline vapours released from vehicle refuelling at the patrol stations in Saudi Arabia. Participation involves a short discussion with a researcher about lifestyle and smoking habit, and provision of urine and breath samples once before and after your working shift. This is very much all that is needed from you to participate. There are no potential risks for you involved in participation.

It is absolutely your choice to participate in the study but before you decide to participate or not it is important that you understand the purpose of the study, what we need you to do, and what we are going to do with the collected information and results. The information below will clarify these questions and more. Please read the information about the study carefully and take the time you need to decide on your participation. As well as this information the researcher will meet you prior to the study to further clarify all you need to do and to answer any questions that you may have.

Please feel free to contact the study researcher, Ahmed Alyami, by telephone (0554440134) or e-mail a.alyami@cranfield.ac.uk if you have any question.

Assessment of Exposure to Gasoline Vapour at Petrol Stations in Saudi Arabia

Why are we doing this study?

Gasoline contains potentially hazardous chemical components on humans' health if they are exposed to more than allowable limits. Petrol station attendants in Saudi Arabia spend long periods near to the gasoline vapours during their everyday working shifts, therefore, the main purpose of the study is to evaluate the exposure and compare with limit values. The exposure is assessed by measuring vapours in air and also by measuring chemicals in urine and in breath if possible.

Why have I been selected to Participate?

The study involves a random selection of a number of participants who conduct gasoline fuel pumping (exposed) and others who do not (control). Such participants are asked to provide a urine sample and/or exhale breath to complement assessment of personal exposure by air sampling. Therefore, we are asking for your participation because you

meet our criteria of being either petrol station attendant or work at a nearby location as 'control' for comparison of exposure.

How long and how many times am I needed to participate?

You can participate once or twice during both summer and winter study seasons to give urine samples and exhale breath to be used for gasoline components exposure analyses. If you chose to participate for one time only please let us know so that we will not include you in the second random selection.

Do I have to take part?

No. It is up to you to choose to participate in our study by giving your urine and breath sample or not. If you decide to give urine and/or breath once only you will not be selected for a second time. Your station is one of the selected for the study and arranged with its manager.

What happens if I take part?

Once you decide to participate in our study, we will shortly discuss with you general questions of smoking habit and lifestyle and ask you to provide urine and/or exhale breath sample in a provided hygienic container and special kit that will be collected back from you. This will be provided to you at your workplace. The total amount of time required as a participant will be about 30 minutes.

Collected urine and breath samples will be used to measure gasoline component exposures that might occur when refuelling cars and compare the results with collected air samples and applicable occupational exposure standard limits. We will not test for anything else. The results will help us clarify the overall levels of exposures to the gasoline and its components.

What are the possible benefits of taking part?

The study will benefit all petrol station attendants in Saudi Arabia and other similarly set up stations in the Arabian Gulf Countries by providing better information about exposure to gasoline components and recommending any appropriate actions required to achieve a safe work environment according to current best practice.

Will my taking part in this study and collected information about myself be kept confidential?

Yes. All personal collected information and test results will be kept strictly confidential and secured under the custody and responsibility of the researcher. All personal information will be kept only for the period needed for the study to complete then will be securely destroyed. No personal identities will be used in the study and collected results will be anonymous and the subject of statistical evaluation.

Why has this study been carried out and who recommended it?

The study is a unique with respect to investigating petrol stations in Saudi Arabia. The study will address many of the public inquiries concerning whether station workers are at risk of health effects due to exposure to gasoline vapour. Related studies have been carried out in other countries but under different practice conditions and set ups. Therefore, the study was proposed to be carried out as a thorough PhD research project.

Who has reviewed the study?

Before any research goes ahead it has to be checked by a Research Ethics Committee. They make sure that adequate safeguards for participant's well-being and privacy are included in the planned research. The project has been checked by the Cranfield University Health Research Ethics Committee (CUHREC).

Contact for further information

If you would like any more information about this study, please feel free to contact the researcher:

Address:
Ahmed Alyami
P.O. Box 20
Najmah, Ras Tanura 31311
Saudi Arabia
Telephone:
Mobile: +966554440134

E-mail:
a.alyami@cranfield.ac.uk

If you are not happy with any aspect of this study you should contact either the researcher (details above) or Professor Joe Lunec, Head of Cranfield Health (see below).

Joseph Lunec
Head of School of Health
Cranfield Vincent Building
Cranfield University
Tel.: +441234 758348
E-mail: j.lunec@cranfield.ac.uk

Thank you for reading the information sheet – please do not hesitate to ask if you have any further questions.

Centre Number:

Study Number:

Participant Identification Number:

CONSENT FORM

Title of Project: Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations in Saudi Arabia

Name of Researcher: Ahmed Alyami

If you would like to participate in the study of Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations in Saudi Arabia please put a check mark in the appropriate box and then complete the consent form below.

I would like to participate in the study and thereby provide lifestyle information as well as urine samples.
(Please complete questionnaire blow) ☐

I would prefer not to participate in the study ☐

If you would like to participate in the study of Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations in Saudi Arabia, please read every statement below and mark in each correspond box.

Please initial box

1. I confirm that I have read and understand the information sheet dated …….. (version ……..) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐

3. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person Taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher.

Appendix D Gasoline Chemical Components

Table 0-2: GC detected components in liquid gasoline

Group	Chemical Components	Gasoline Liquid @ 25°C (RON 95)	Gasoline Liquid @ 25°C (RON 95)	Avg	Gasoline Liquid @ 25°C (RON 91)	Gasoline Liquid @ 25°C (RON 91)	Avg
		%Vol	%Vol	%Vol	%Vol	%Vol	%Vol
Paraffin	Propane	0.11	0.11	0.11	0.10	0.09	0.09
	n-Butane	2.35	3.89	3.12	1.79	3.69	2.74
	n-Pentane	8.35	7.12	7.74	11.62	9.51	10.57
	n-Hexane	4.11	4.11	4.11	5.79	5.31	5.55
	n-Heptane	1.90	1.85	1.87	2.10	1.89	1.99
	n-Octane	0.67	0.58	0.62	0.84	0.80	0.82
	n-Nonane	ND	ND	ND	ND	ND	ND
	n-Decane	ND	ND	ND	ND	ND	ND
I-Paraffins	i-Butane	0.50	1.37	0.93	0.44	1.23	0.83
	2,2-Dimethylpopane	ND	0.05	0.05	ND	ND	ND
	i-Pentane	10.08	9.89	9.99	10.75	10.66	10.71
	2,2-Dimethylbutane	0.47	0.57	0.52	0.51	0.33	0.42
	2,3-Dimethylbutane	ND	4.08	4.08		4.62	4.62
	2-Methylpentane	4.00	2.78	3.39	4.93	3.12	4.03
	3-Methylpentane	2.70	0.14	1.42	3.19	0.07	1.63
	2,2-Dimethylpentane	ND	0.22	0.22	0.24	0.21	0.23
	2,4-Dimethylpentane	0.44	0.49	0.46	0.39	0.44	0.41
	2,2,3-Trimethylbutane	ND	ND	ND	ND	ND	ND
	3,3-Dimethylpentane	0.24	0.22	0.23	0.22	0.19	0.20
	2-Methylhexane	2.05	1.92	1.99	2.02	1.99	2.01
	2,3-Dimethylpentane	0.75	0.72	0.74	0.68	0.69	0.69
	3-Methylhexane	2.39	2.25	2.32	2.34	2.28	2.31
	5-Methylnonane	ND	ND	ND	ND	ND	ND
	3-Ethylpentane	0.25	0.24	0.25	0.23	0.23	0.23
	2,2,4-Trimethylpentane	0.51	1.05	0.78	ND	0.80	0.80
	2,2-Dimethylhexane	ND	ND	ND	ND	ND	ND
	2,5-Dimethylhexane	0.21	0.25	0.23	0.18	0.24	0.21
	2,4-Dimethylhexane	0.33	0.35	0.34	0.25	0.31	0.28
	3,3-Dimethylhexane	ND	ND	ND	ND	ND	ND
	2,3,4-Trimethylpentane	0.23	0.49	0.36	ND	0.36	0.36
	2-Methyl-3-ethylpentane	ND	ND	ND	ND	ND	ND
	2,3-Dimethylhexane	0.18	0.22	0.20	ND	0.23	0.23

	2-Methylheptane	0.58	0.54	0.56	ND	0.63	0.63
	4-Methylheptane	0.23	0.23	0.23	0.65	0.27	0.46
	3-Methyl-3-ethylpentane	ND	ND	ND	0.24	ND	0.24
	3,4-Dimethylhexane	ND	ND	ND	ND	ND	ND
	3-Methylheptane	0.63	0.60	0.62	0.68	0.67	0.68
	3-Ethylhexane	0.26	0.22	0.24	0.35	0.38	0.37
	2,3-Dimethyloctance	ND	ND	ND	ND	ND	ND
	Heptane,2,4,6-trimethyl-	0.67	0.67	0.67	0.59	0.60	0.60
	C10 - IsoParafin -2	ND	ND	ND	ND	ND	ND
	2,6-Dimethyloctane	ND	ND	ND	ND	ND	ND
	5-Methylnonane	ND	ND	ND	ND	ND	ND
	Benzene	2.27	2.10	2.18	2.27	1.94	2.11
Aromatics	Toluene	10.43	10.32	10.37	8.57	9.53	9.05
	Ethylbenzene	1.72	1.56	1.64	1.67	1.39	1.53
	m-Xylene	5.03	4.53	4.78	5.65	3.89	4.77
	p-Xylene	2.25	1.99	2.12	2.10	1.72	1.91
	o-Xylene	2.80	2.57	2.69	2.72	2.21	2.47
	i-Propylbenzene	0.18	0.16	0.17	0.16	0.14	0.15
	n-Propylbenzene	ND	ND	ND	ND	ND	ND
	1-Methyl-3-ethylbenzene	1.91	1.94	1.92	1.58	1.69	1.63
	1-Methyl-4-ethylbenzene	0.89	0.89	0.89	0.73	0.78	0.75
	1,3,5-Trimethylbenzene	0.82	0.84	0.83	0.66	0.70	0.68
	1-Methyl-2-ethylbenzene	0.83	0.83	0.83	0.72	0.73	0.72
	1,2,4-Trimethylbenzene	3.22	3.22	3.22	2.63	2.76	2.70
	1,2,3-Trimethylbenzene	0.75	0.74	0.74	0.63	0.64	0.63
	1,3-Diethylbenzene	0.12	0.11	0.12	0.23	ND	0.23
	1-Methyl-3-n-propylbenzene	0.29	0.26	0.27	0.19	0.20	0.19
	1-Methyl-4-n-propylbenzene	0.17	0.19	0.18	0.21	0.15	0.18
	1,3-Dimethyl-5-ethylbenzene	0.27	0.25	0.26	ND	0.18	0.18
	1-Methyl-2-n-propylbenzene	0.13	0.10	0.12	0.12	ND	0.12
	1,4-Dimethyl-2-ethylbenzene	0.23	0.20	0.21	0.19	0.16	0.17
	1,3-Dimethyl-4-ethylbenzene	0.22	0.19	0.21	0.18	0.14	0.16
	1,2-Dimethyl-4-ethylbenzene	0.41	0.34	0.37	0.30	0.27	0.28
	1,2-Dimethyl-3-ethylbenzene	0.11	0.09	0.10	ND	ND	ND
	1-Ethyl-4-i-propylbenzene	0.19	0.18	0.19	0.15	0.15	0.15
	1-Methyl-1-n-butylbenzene	0.29	0.26	0.27	0.23	0.21	0.22
Naphthalenes	Naphthalene	0.17	0.12	0.14	0.12	0.09	0.11
	2-Methylnaphthalene	0.09	ND	0.09	0.09	ND	0.09
	2-Methylindan	0.11	ND	0.11	ND	ND	ND
Naphtheno/Olfin	2,3-Dihdroindene	ND	0.15	0.15	0.10	0.10	0.10

	Cyclopentane	0.27	0.43	0.35	0.41	0.35	0.38
Naphthenes	Methylcyclopentane	0.91	1.09	1.00	1.18	1.31	1.24
	1c, 2t, 4c-Trimethylcyclohexane	ND	ND	ND	ND	ND	ND
	Cyclohexane	0.24	0.26	0.25	0.46	0.43	0.44
	1,1-Dimethylcyclopentane	ND	0.58	0.58	ND	ND	ND
	1t,3-Dimethylcyclopentane	0.20	0.20	0.20	0.25	0.27	0.26
	1C,3-Dimethylcyclopentane	0.17	0.18	0.18	0.22	0.23	0.23
	1t,2-Dimethylcyclopentane	0.19	0.19	0.19	0.27	0.26	0.26
	Methylcyclohexane	0.35	0.28	0.31	0.73	0.64	0.69
	1,1-Methylethylcyclohexane	ND	ND	ND	0.18	ND	0.18
	C9 - MonoNaph - 8	ND	ND	ND	ND	ND	ND
	Pentene-1	0.11	0.09	0.10	ND	0.05	0.05
n-Olefins	t-Pentene-2	0.29	0.41	0.35	ND	0.16	0.16
	c-Pentene-2	0.16	0.18	0.17	ND	0.08	0.08
	t-Hexene-2	0.14	0.20	0.17	ND	0.11	0.11
	C10-iso-olefin-10	ND	ND	ND	ND	ND	ND
	Decene - 1	ND	ND	ND	ND	ND	ND
	2-Methylbutene-1	0.32	0.21	0.26	ND	0.15	0.15
Iso-Olefins	2-Methylbutene-2	0.64	ND	0.64	0.13	ND	0.13
	2-Methylpentene-1	0.08	ND	0.08	ND	ND	ND
	3-methyl-c-pentene-2	0.13	0.20	0.16	ND	0.11	0.11
	Methyl-t-butyl ether	14.25	13.06	13.66	13.75	13.34	13.54

ND: None Detected Compounds.

Table 0-3: Detected gasoline vapour components via GC head space method

Gasoline Vapour Components	25°C (95)	25°C (91)	25°C (91)	25°C (91)	25°C (91)	25°C (91)	45°C (91)	45°C (91)	45°C (91)	45°C (91)	45°C (91)	45°C (95)	45°C (95)	45°C (95)
	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol	%Vol
Propane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Butane	3.246	3.196	4.797	1.259	2.696	2.804	2.408	2.96	3.359	2.431	2.862	1.585	2.203	3.001
n-Pentane	16.128	19.254	12.314	11.292	9.883	9.318	14.535	14.522	9.206	9.043	9.18	7.779	7.869	10.629
n-Hexane	8.834	6.595	7.334	8.357	6.806	7.567	9.083	8.937	7.093	7.249	7.38	5.869	6.285	5.755
n-Heptane	ND	1.574	ND	2.723	2.443	2.483	3.388	ND	2.872	2.68	2.718	2.799	ND	ND
n-Octane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Nonane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Decane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
i-Butane	ND	0.826	ND	ND	0.785	1.003	ND	ND	1.241	0.874	0.968	ND	ND	ND
2,2-Dimethylpopane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
i-Pentane	15.711	18.848	16.579	9.276	10.19	9.393	13.539	14.07	9.715	9.13	9.574	8.503	9.522	12.977
2,2-Dimethylbutane	ND	0.756	ND	0.648	0.477	ND	ND	ND	ND	0.527	ND	ND	ND	ND
2,3-Dimethylbutane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylpentane	8.466	6.429	7.001	6.845	5.922	6.464	7.885	7.254	5.97	6.427	6.395	5.534	5.817	5.698
3-Methylpentane	5.095	3.925	ND	4.377	3.822	4.144	4.895	4.769	3.969	4.041	4.057	3.693	3.903	3.848
2,2-Dimethylpentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-Dimethylpentane	ND	ND	ND	ND	0.618	ND	ND	ND	ND	0.566	ND	ND	ND	ND
2,2,3-Trimethylbutane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-Dimethylpentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylhexane	ND	1.807	ND	2.855	2.633	2.809	3.202	ND	2.821	3.046	2.858	3.172	4.571	ND
2,3-Dimethylpentane	ND	ND	ND	ND	0.895	1.065	ND	ND	0.977	0.979	0.977	1.156	ND	ND
3-Methylhexane	ND	1.956	ND	3.101	2.977	3.176	4.164	5.343	3.325	3.24	3.246	3.648	4.69	ND
5-Methylnonane	ND	1.249	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Ethylpentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

2,2,4-Trimethylpentane	ND	ND	ND	ND	1.206	ND	ND	ND	1.492	1.261	1.402	ND	ND	ND
2,2-Dimethylhexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-Dimethylhexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-Dimethylhexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-Dimethylhexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-Trimethylpentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methyl-3-ethylpentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-Dimethylhexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylheptane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Methylheptane	ND	ND	ND	ND	0.767	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Methyl-3-ethylpentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-Dimethylhexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Methylheptane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Ethylhexane	ND	ND	ND	ND	0.687	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-Dimethyloctance	ND	ND	ND	ND	0.816	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptane,2,4,6-trimethyl-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C10 - IsoParafin -2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-Dimethyloctane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-Methylnonane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzene	4.823	2.215	4.647	3.485	2.45	2.946	4.068	4.472	2.787	2.709	2.832	3.436	4.473	4.174
Toluene	19.657	5.846	25.639	10.074	11.018	11.985	12.248	18.943	12.886	12.165	12.456	14.015	22.186	20.577
Ethylbenzene	ND	ND	ND	1.638	1.212	1.856	ND	ND	1.389	1.459	1.461	1.91	ND	ND
m-Xylene	ND	2.364	ND	4.542	3.507	4.361	ND	ND	4.285	4.06	4.152	5.786	6.938	7.326
p-Xylene	ND	1.147	ND	2.22	1.57	1.965	ND	ND	2.153	1.965	1.744	2.556	ND	ND
o-Xylene	ND	ND	ND	2.467	1.971	2.614	ND	ND	2.27	2.204	2.226	2.845	ND	ND
i-Propylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Propylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methyl-3-ethylbenzene	ND	ND	ND	ND	1.265	1.556	ND	ND	ND	ND	ND	1.634	ND	ND

1-Methyl-4-ethylbenzene	ND	ND	ND	ND	0.572	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-Trimethylbenzene	ND	ND	ND	ND	0.541	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methyl-2-ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	1.404	1.417	1.585	ND	ND	ND
1,2,4-Trimethylbenzene	ND	ND	ND	2.362	1.961	2.454	ND	ND	2.327	ND	2.425	2.769	ND	5.437
1,2,3-Trimethylbenzene	ND	ND	ND	ND	0.442	ND	ND	ND	ND	ND	ND	ND	3.694	ND
1,3-Diethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methyl-3-n-propylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methyl-4-n-propylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-Dimethyl-5-ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methyl-2-n-propylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-Dimethyl-2-ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-Dimethyl-4-ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-Dimethyl-4-ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-Dimethyl-3-ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Ethyl-4-i-propylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methyl-1-n-butylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Naphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylnaphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylindan	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-Dihdroindene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyclopentane	ND	0.595	ND	ND	0.437	ND	ND	ND	ND	0.437	ND	ND	ND	ND
Methylcyclopentane	ND	1.303	ND	1.77	1.72	2.002	1.96		1.798	1.818	2.003	1.39		
1c, 2t, 4c-Trimethylcyclohexane	ND	1.609	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyclohexane	ND	ND	ND	ND	0.552	ND	ND	ND	ND	0.615	ND	ND	ND	ND
1,1-Dimethylcyclopentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1t,3-Dimethylcyclopentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1C,3-Dimethylcyclopentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

1t,2-Dimethylcyclopentane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methylcyclohexane	ND	ND	ND	ND	0.697	ND	ND	ND	ND	0.765	ND	ND	ND	ND
1,1-Methylethylcyclohexane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C9 - MonoNaph - 8	ND	ND	ND	1.706	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pentene-1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
t-Pentene-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c-Pentene-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
t-Hexene-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C10-iso-olefin-10	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.878	ND	ND	ND	ND
Decene - 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylbutene-1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylbutene-2	ND	ND	ND	ND	0.358	ND	ND	ND	ND	ND	ND	0.628	ND	ND
2-Methylpentene-1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-methyl-c-pentene-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methyl-t-butyl ether	18.038	18.505	21.689	19.002	16.104	18.036	18.625	18.73	16.663	17.013	17.479	19.286	17.848	20.578
Sum	99.998	99.999	100	99.999	100	100.001	100	100	100.002	99.999	99.98	99.993	99.999	100

ND: None Detected compounds.

Appendix E Breath Analysis

Analysis of Breath Solvents

1.1 This section operating procedure describes an analytical method using the Bio-VOC breath sampler for the determination of solvents in breath.

1.2 This method can be used to assess solvent exposure in the workplace. The sampler works by capturing the final portion of an exhalation, which is then transferred onto a stainless steel tube packed with adsorbent material (Tenax, 200 mg 35-60 mesh). Any solvents present are trapped in the tube and can be analysed by thermal desorption. A whole array of solvents can be analysed. Samples are either analysed for known, named, solvents or screened and subsequently semi-quantitated.

1.3 Analytical procedure

Prepare a stock solution by adding 50 µl (using Gilson Multipette) of each solvent to a 5 ml volumetric flask (10 µl/ml). Make up to the mark with methanol.

1.4 Prepare working solutions as follows, using 5 ml volumetrics. Prepare the standards by adding 1 µl volumes of spiked working solutions to labelled breath tubes.

Table 0-4: Working standard solutions preparation

Standard Number	Volumetric flask (ml)	Volume of stock solution (µl)	Vol. Injected (µl)	Conc. of solvent (µl/100 ml)
S1	MeOH		1	0
S2	5	50	1	10
S3	5	100	1	20
S4	5	200	1	40
S5	5	500	1	100
S6	5	1000	1	200

Prepare standards from the S1 up to the S6. Prepare 3 tubes at S4 level (one standard and two QCs). Also, take an unused tube from the box and label as 'lab blank'. Field blanks should be supplied with the samples.

A system is set up for the spiking of the breath tubes (L.2.13). A supply of nitrogen is fed into a brace on a clamp; tubes are then placed into the brace and injected with the standards from the hole at the end of the brace.

Ensure that the nitrogen supply is turned on and affix the end nut onto a tube awaiting spiking. Then place the tube into the brace, with the groove to the rear. Rinse the syringe a couple of times with methanol and ensure the stopper (that ensures 1 µl is added to the tube) is firmly in place. Then inject a 1 µl aliquot of the appropriate standard in to the tube. Set the timer for three minutes and begin the countdown. After three minutes is up remove the tube and place end nuts on both ends and hand tighten securely. Place in the appropriate box. Repeat this procedure as many times as necessary.

1.5 Calculations

An Excel spreadsheet template, 'BREATH', is available from Management\Reactive\Templates for the processing of data. The concentration of solvent in the standards depends on the solvent used. In the template there is a Calibration sheet - this lists commonly used solvents, their density and MW and their calibration values.

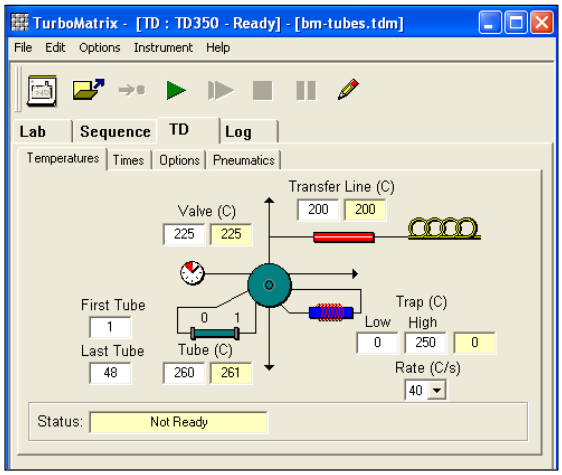
The concentration of the standard (in µl/ml, see 4.3.2) is converted to µg/µl by multiplying by the solvent density and dividing by 100. As 1 µl of this standard is injected onto a tube, this translates as µgs per tube.

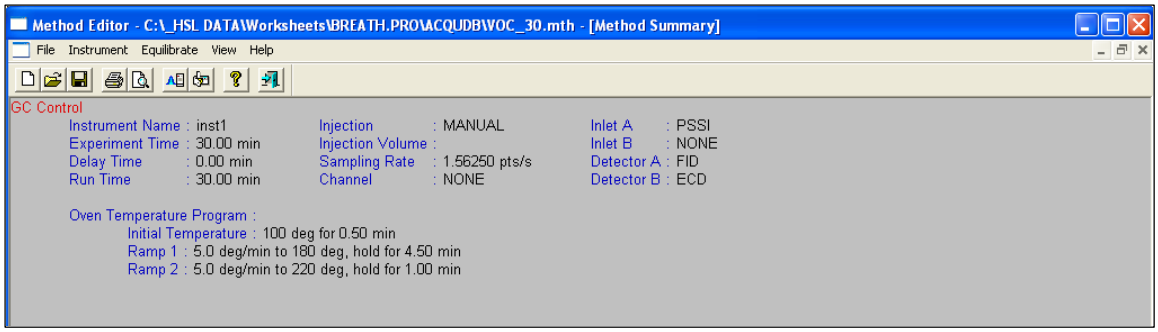
As the standards are surrogates for a breath sample, the µg/tube is assumed to represent µg/sampler. The sampler has a mean volume of 129 ml. The calculated µg/sampler for the sample tubes is converted to µg/l by multiplying by 1000/129. This is converted to nmol/l by multiplying by 1000/MW.

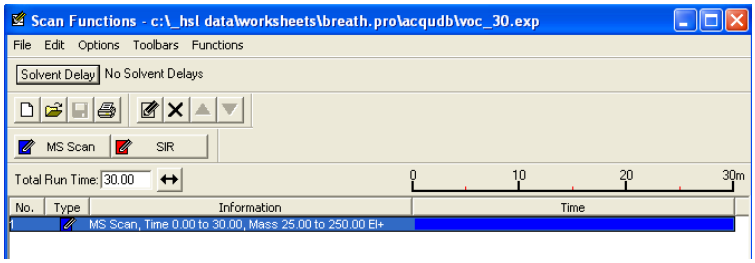
1.6 Interpretation of results

Breath sampling is only semi-quantitative and this should be remembered when interpreting results.

Appendix 1. INSTRUMENT conditions







Results and Correlations of Air Samples and Exhaled Breath Results

Table 0-5: Correlations of air samples and exhaled breath results for the tested BTEX

Chemical	Air Sample Results (mg/m^3)	Breath Preshift Results (nmol/l)	Breath Postshift Results (nmol/l)
Benzene	1	ND	1
Benzene	0.63	ND	1
Benzene	2.4	ND	4
Benzene	1.1	2	4
Benzene	1.1	4	6
Benzene	0.33	ND	1
Benzene	0.26	1	2
Benzene	0.89	2	3
Benzene	0.75	ND	1
Benzene	0.64	ND	1
Benzene	0.37	1	1
Benzene	2.2	2	2
Benzene	0.81	2	1
Benzene	0.39	2	1
Benzene	1	1	1
Benzene	0.45	1	1
Benzene	0.29	ND	1

Benzene	0.93	1	1
GM	**0.70**	**1.55**	**1.47**
Correlation	**0.49**		**0.62**
Chemical	**Air Sample Results (mg/m^3)**	**Breath Preshift Results (nmol/l)**	**Breath Postshift Results (nmol/l)**
MTBE	9	6	2
MTBE	10	4	2
MTBE	2.6	3	ND
MTBE	9	1	11
MTBE	4.9	1	2
MTBE	5.8	ND	7
MTBE	7.1	1	4
MTBE	7.9	ND	3
MTBE	2.8	2	ND
MTBE	22	2	46
MTBE	3.4	124	141
MTBE	14	162	190
MTBE	1.4	5	3
MTBE	3.9	3	25
MTBE	2.7	15	45
MTBE	9.6	29	81
MTBE	8.2	4	27

MTBE	7	6	38
MTBE	1.9	1	1
MTBE	2.2	36	19
MTBE	19	104	95
MTBE	6.5	88	59
MTBE	3	40	16
MTBE	7.6	19	45
MTBE	4.1	11	16
MTBE	1.6	4	12
MTBE	7.3	62	49
GM	**5.34**	**8.94**	**16.24**
Correlation	**0.38**		**0.90**
Chemical	**Air Sample Results (mg/m^3)**	**Breath Preshift Results (nmol/l)**	**Breath Postshift Results (nmol/l)**
Toluene	0.34	1	ND
Toluene	1.5	1	2
Toluene	0.8	ND	3
Toluene	1	ND	1
Toluene	2.9	ND	12
Toluene	1.7	4	5

Toluene	2.2	6	7
Toluene	0.3	ND	1
Toluene	0.49	1	1
Toluene	0.48	1	3
Toluene	1.3	2	3
Toluene	1.1	ND	2
Toluene	0.91	ND	1
Toluene	1.2	2	1
Toluene	3	1	1
Toluene	1.3	1	1
Toluene	1.3	1	1
Toluene	1.9	1	2
Toluene	0.99	1	5
Toluene	0.96	ND	1
Toluene	1.5	1	1
GM	**1.09**	**1.38**	**1.91**
Correlation	**0.52**		**0.31**

Table 0-6: Benzene post-exposure correlation with various factors

Temp (°C)	Benz Post GM	RH %	Benz Post GM	Wind Speed (m/s)	Benz Post GM	Qty (Litre)	Benz Post GM
26	2.45	9	ND	0.7	1	1,000	1
27	1.00	11	1	1.13	ND	1,173	1
29	1.41	13	ND	1.16	1	1,220	1
32	ND	14	ND	1.2	1.41	1,675	2.45
36	2.45	15	1	1.3	1	2,453	ND
37	1.00	20	2.45	1.5	1	2,664	1.00
38	1.00	23	4.90	1.8	1	2,738	2.00
39	4.90	25	1.41	2	ND	3,000	ND
40	2.00	29	1	2.8	ND	3,150	ND
41	1.00	30	4	3	ND	3,460	1.00
42	ND	32	1.82	4.2	1	4,850	ND
		33	1	**Correlation**	**-0.29**	4,906	ND
Correlation	**0.02**	34	1			5,520	ND
		40	ND			5,727	1.00
		49	ND			5,900	1.00
		Correlation	**0.02**			7,312	1.00
						7,800	2.45
						8,211	4.90

						8,260	4.00
						12,114	ND
						Correlation	**0.25**

Appendix F Urine Analysis

HPLC-MS/MS analytical method for the simultaneous determination of *S*-PMA, *t,t*-MA and cotinine in urine samples

Chemicals and supplies

The analytical reference standard of DL-*S*-PMA, *t,t*-MA and cotinine were purchased from Spectra 200 s.r.l. Rome, Italy) . The deuterium labeled internal standards, DL-SPMA-3,3-d_2, t,tMA-d_4 and Cotinine-d_3, were obtained from CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). 6N hydrochloric acid, Glacial acetic acid, 30% NH3 and Ammonium Acetate were obtained from Sigma Aldrich (Saint Louis, MO,USA). Purified water was obtained from a Milli-Q Plus system (Millipore, Milford, MA, USA). CHROMASOLV® gradient grade, ≥99.9% Methanol and Acetonitrile for LC/MS were provided by Sigma-Aldrich (Saint Louis, MO,USA). SPE Vacuum Manifold and Sep-Pak Plus C18 (500 mg) cartridges were supplied by Waters (Milford, MA, USA). Anotop 10 LC syringe filter devices (0.2 mm pore size, 10 mm diametre) were from Whatman Inc. (Maidstone, UK). A chromatographic column Sinergi Fusion RP 80A, (150 x 4.6 mm) (Chemtek Analitica, Anzola, BO) was used. The urinary creatinine concentration of the samples has been determined by the method of Jaffè (Henry, 1974), using alkaline picrate test with UV/VIS detection at 490 nm.

Sample collection and purification

Urine samples were collected and stored frozen at -20° C until analysis; after thawing, an aliquot of 3 ml of each sample was acidified to pH 2 with 30 µl of 6N hydrochloric acid in order to perform partial hydrolysis of pre-SPMA (*Paci E, Pigini D, Cialdella AM, Faranda P, Tranfo G. Determination of free and total S phenylmercapturic acid by HPLC/MS/MS in the biological monitoring of benzene exposure. Biomarkers 2007; 12 (2): 111- 122*), and, after 10 minutes, deuterium labelled isotopes of the analytes, to be used as internal standards were added, in suitable amounts to obtain the following final concentrations:

SPMA-^{2}D = 5 µg/l

t,t-MA-^{4}D = 100 µg/l

cotinine-^{3}D = 500 µg/l

In order to achieve alternate retention and elution of both the acidic (S-PMA and t,t-MA) and basic metabolites (cotinine) using the same cartridge, the pH of the washing and eluting solvents was varied: Sep-pak C18 cartridges were conditioned with 3 mL of methanol and then 3 ml of 2% v/v acetic acid in water; after loading the sample, the SPE cartridges were washed with further 3 ml of 2% v/v acetic acid in water, and the 6 ml solution resulting from sample loading and cartridge washing were added with 1 ml of 0.1 M ammonium acetate buffer at pH 7.6 and 160 µl of concentrated ammonia solution in water (30% v/v) to lead the sample pH to 7.5 – 8 and stored for further purification. The cartridges were then eluted with 1.5 ml of methanol, obtaining the fraction which contains the acidic metabolites SPMA and t,t-MA.

Afterwards, the SPE cartridges were reconditioned with 3 ml of methanol and 3 ml of water, and the previously stored 6 ml solution was loaded, and, after a 3 ml of water washing, with 1.5 ml of methanol the fraction containing the cotinine was eluted. Both fractions were filtered on 0.2 µm Anotop 10 LC syringe filter and injected separately into the HPLC-MS/MS system.

Standard solutions and calibration curves

Stock standard solutions were prepared by weighing and dissolving the pure standard in methyl alcohol at the concentrations of 1 mg/l for S-PMA and SPMA-d_2, 10 mg/l for t,tMA and t,t-MA-d_4 and 100 mg/l for Cotinine and Cotinine-d_3. Calibration standards are then obtained by further dilutions with urine in the concentration range 0 -25 µg/l for S-PMA, 0-500 µg/l for t,t-MA and 0-2500 µg/l for Cotinine; internal standard concentrations were 5 µg/l for S-PMA-d_2, 100 µg/l for t,t-MA-d_4 and 500 µg/l for cotinine-d_3. The analytes concentration in urine samples were determined using calibration curves obtained by analyzing urine samples of nonsmoking subjects, non-occupationally exposed to benzene, spiked with known concentrations of pure standards; such samples were then subjected to the overall SPE purification procedure, and analyzed with HPLC-MS/MS.

HPLC-MS/MS analytical procedure

A Perkin Elmer series 200 HPLC, coupled with an API/4000 MS/MS spectrometre (Applied-Biosystem) and a Turbo Ion Spray source were used.

For all the analytes HPLC separation was performed on a Sinergi Fusion RP 80A, 150 x 4.6 mm chromatographic column (Chemtek Analitica, Anzola, Bologna), using a gradient of acetonitrile (A phase) and acetic acid 1% v/v in water (B phase), but the two fractions were analyzed in separate runs. Cotinine analysis was performed with a isocratic 60% A and 40% B, 800 µl/min flow, with a duration of 4 minutes, while S-PMA and t,t-MA analysis required a gradient from 10% to 77 % A for 9 minutes, and return to initial conditions in 1 minute, for a total time of 10 minutes, with a flow of 600 µl/min.

The mass spectrometric detector parametres were optimized for each analyte with the automated procedure "Infusion Quantitative Optimization" and subsequently refined with the FIA analysis "flow injection analysis" using pure standards. 10 µl of each sample was injected onto the HPLC-MS/MS system for analysis, and tested in triplicate.

SPMA and t,t-MA were ionized by negative-ion and Cotinine by positive ESI and detection was performed in selected reaction monitoring (SRM) mode following the transitions characteristic of the analytes. The (*m/z)* values used both for identification and quantification are reported in Table 0-7:

Table 0-7: Precursor ion → Product ion

t,t-MA	m/z	**-141.0 → -53.00**
SPMA	m/z	**-240.1 → -109.1**
t,t-MAd$_4$	m/z	**-145.0 → -100.0**
SPMAd$_2$	m/z	**-238.1 → -109.1**
Cotinine	m/z	**+177.3 → +80.10**
Cotinine-d$_3$	m/z	**+180.3 → +80.10**

The instrumental, optimized, parametres are reported in Table 0-8:

Table 0-8: API 4000 optimized parametres:

Metabolite	**CAD**	**CXP**	**CE**	**DP**	**EP**
(arbitrary units)		**ΔV**	**ΔV**	**ΔV**	**ΔV**
tt-MA	6.00	-7.00	-16.00	-40.00	-10.00
S-PMA	6.00	-7.00	-20.00	-25.00	-10.00
tt-MAd$_4$	6.00	-14.00	-14.00	-20.00	-15.00
S-PMAd$_2$	6.00	-7.00	-20.00	-25.00	-10.00
Cotinine	8.00	6.00	29.00	70.00	10.00
Cotinine-d$_3$	8.00	9.00	39.00	61.00	10.00

Data processing

The peak areas generated by the samples were integrated by the 1.5 Analyst® software.

For each sample tested the arithmetic mean value of the peak areas of the three replicate injections was used. For each analyte the area of peak a blank urine sample was subtracted from the areas of the corresponding urine calibration standards. The calibration curves were generated using linear regression analysis according to the equation y = ax + b, where y is the ratio between the area of the analyte calibration standards (after subtraction of the blank) to that of the internal standard, is the slope of the regression line, x is the concentration of the analyte, and b is the intercept. The concentrations of the analyte in the unknown or quality control samples were calculated from the regression equation of the calibration curve and

expressed as µg/l of urine. Concentrations of urinary metabolites were expressed as a function of creatinine concentration in order to normalize values respect to urine dilution variability.

Validation of the analytical method

Three quality control samples were prepared at low, median and high concentrations and analyzed on five different days, three of them not consecutive.

The quality control concentrations for SPMA were 0.8, 5 and 25 µg/l , for tt,-MA 17,100 and 500 µg/l and for the Cotinine 83, 500 and 2500 µg/l. The results were used to establish the performance of the method in terms of interday and intraday variability, accuracy, LOD, calculated as the ratio S/N > 3, and LOQ calculated as the ratio S/N > 10. Validation results are reported in Table 0-9:

Table 0-9: Method validation results

Analyte	**S-PMA**		**t,t-MA**		**Cotinine**	
Calibration range µg/l	**0-25**		**0-500**		**0-2500**	
R^2	**0.9969**		**0.9980**		**0.9996**	
LOD µg/l	**0.01**		**0.50**		**0.80**	
LOQ µg/l	**0.03**		**2.00**		**2.00**	
intra day	**%CV**	**%Accuracy**	**%CV**	**% Accuracy**	**%CV**	**%Accuracy**
low concentration	**8**	**60.3**	**7**	**66.5**	**2**	**99.1**

median concentration	**2**	**109.0**	**10**	**99.2**	**7**	**94.5**
high concentration	**2**	**99.3**	**1**	**98.6**	**10**	**92.4**
inter day	**%CV**	**%Accuracy**	**%CV**	**% Accuracy**	**%CV**	**%Accuracy**
low concentration	**5**	**74.5**	**7**	**97.8**	**4**	**112.4**
median concentration	**21**	**105.3**	**10**	**107.5**	**6**	**99.1**
high concentration	**11**	**99.5**	**15**	**109.3**	**9**	**100.7**

General Guidelines for Collection of Urine Specimens

1. All urine specimens should be clearly labeled and kept cold inside the designated thermal sample collection container.

2. Collect clean mid-stream urine by allowing first urinary flow to escape. First portion of urine washes out urethra and contains debris.

3. Stop collection when container is about half full.

4. Screw cap on container

5. Wash hands with soap.

6. Give sample to the researcher in sterile container. Do not touch inside of container or lid.

Table 0-10: OELs mixture results and calculations

C/T	28.35	91.89	144.55
ppm 14	28620	91800	73800
Vol% @ 45C (91)	2.86	9.18	7.38
C/T	24.08	90.52	141.98
ppm 13	24310	90430	72490
Vol% @ 45C (91)	2.43	9.04	7.25
C/T	33.27	92.15	138.93
ppm 12	33590	92060	70930
Vol% @ 45C (91)	3.36	9.21	7.09
C/T	29.32	145.37	175.04
ppm 11	29600	145220	89370
Vol% @ 45C (91)	2.96	14.52	8.94
C/T	23.85	145.5	177.9
ppm 10	24080	145350	90830
Vol% @ 45C (91)	2.41	14.54	9.08
C/T	27.77	93.27	148.21
ppm 9	28040	93180	75670
Vol% @ 25C (91)	2.8	9.32	7.57
C/T	26.7	98.93	133.31
ppm 8	26960	98830	68060
Vol% @ 25C (91)	2.7	9.88	6.81
C/T	12.47	113.03	163.68
ppm 7	12590	112920	83570
Vol% @ 25C (91)	1.26	11.29	8.36
C/T	47.51	123.26	143.65
ppm 6	47970	123140	73340
Vol% @ 25C (91)	4.8	12.31	7.33
C/T	31.65	192.73	129.17
ppm 5	31960	192540	65950
Vol% @ 25C (91)	3.2	19.25	6.6
C/T	29.72	106.4	112.72
ppm 4	30010	106290	57550
Vol% @ 45C (95)	3	10.63	5.76
C/T	21.82	78.77	123.1
ppm 3	22030	78690	62850
Vol% @ 45C (95)	2.2	7.87	6.29
C/T	15.7	77.87	114.95
ppm 2	15850	77790	58690
Vol% @ 45C (95)	1.59	7.78	5.87
C/T	32.15	161.44	173.03
ppm 1	32460	161280	88340
Vol% @ 25C (95)	3.25	16.13	8.83
OEL (ppm)	1009.64	999	510.56
Component	n-Butane	n-Pentane	n-Hexane

C/T	55.69	9.59	ND
ppm 14	27180	9680	95740
Vol% @ 45C (91)	2.72	0.97	9.57
C/T	54.92	8.66	91.33
ppm 13	26800	8740	91300
Vol% @ 45C (91)	2.68	0.87	9.13
C/T	58.85	12.29	97.18
ppm 12	28720	12410	97150
Vol% @ 45C (91)	2.87	1.24	9.72
C/T	ND	ND	140.74
ppm 11	ND	ND	140700
Vol% @ 45C (91)	ND	ND	14.07
C/T	69.42	ND	135.43
ppm 10	33880	ND	135390
Vol% @ 45C (91)	3.39	ND	13.54
C/T	50.88	9.93	93.96
ppm 9	24830	10030	93930
Vol% @ 25C (91)	2.48	1	9.39
C/T	50.06	7.78	101.93
ppm 8	24430	7850	101900
Vol% @ 25C (91)	2.44	0.79	10.19
C/T	55.8	ND	92.79
ppm 7	27230	ND	92760
Vol% @ 25C (91)	2.72	ND	9.28
C/T	ND	ND	165.84
ppm 6	ND	ND	165790
Vol% @ 25C (91)	ND	ND	16.58
C/T	32.25	8.18	188.54
ppm 5	15740	8260	188480
Vol% @ 25C (91)	1.57	0.83	18.85
C/T	ND	ND	129.81
ppm 4	ND	ND	129770
Vol% @ 45C (95)	ND	ND	12.98
C/T	ND	ND	95.25
ppm 3	ND	ND	95220
Vol% @ 45C (95)	ND	ND	9.52
C/T	57.35	ND	85.06
ppm 2	27990	ND	85030
Vol% @ 45C (95)	2.8	ND	8.5
C/T	ND	ND	157.16
ppm 1	ND	ND	157110
Vol% @ 25C (95)	ND	ND	15.71
OEL (ppm)	488.02	1009.64	999.7
Component	n-Heptane	i-Butane	i-Pentane

C/T	ND	128.06	81.25
ppm 14	ND	63950	40570
Vol% @ 45C (91)	ND	6.4	4.06
C/T	10.55	128.71	80.93
ppm 13	5270	64270	40410
Vol% @ 45C (91)	0.53	6.43	4.04
C/T	ND	119.55	79.49
ppm 12	ND	59700	39690
Vol% @ 45C (91)	ND	5.97	3.97
C/T	ND	145.27	95.51
ppm 11	ND	72540	47690
Vol% @ 45C (91)	ND	7.25	4.77
C/T	ND	157.9	98.03
ppm 10	ND	78850	48950
Vol% @ 45C (91)	ND	7.89	4.9
C/T	ND	129.45	82.99
ppm 9	ND	64640	41440
Vol% @ 25C (91)	ND	6.46	4.14
C/T	9.55	118.59	76.54
ppm 8	4770	59220	38220
Vol% @ 25C (91)	0.48	5.92	3.82
C/T	12.98	137.08	87.66
ppm 7	6480	68450	43770
Vol% @ 25C (91)	0.65	6.85	4.38
C/T	ND	140.2	ND
ppm 6	ND	70010	ND
Vol% @ 25C (91)	ND	7	ND
C/T	15.14	128.75	78.61
ppm 5	7560	64290	39250
Vol% @ 25C (91)	0.76	6.43	3.93
C/T	ND	114.11	77.06
ppm 4	ND	56980	38480
Vol% @ 45C (95)	ND	5.7	3.85
C/T	ND	116.49	78.17
ppm 3	ND	58170	39030
Vol% @ 45C (95)	ND	5.82	3.9
C/T	ND	110.82	73.96
ppm 2	ND	55340	36930
Vol% @ 45C (95)	ND	5.53	3.69
C/T	ND	169.54	102.04
ppm 1	ND	84660	50950
Vol% @ 25C (95)	ND	8.47	5.1
OEL (ppm)	499.33	499.36	499.33
Component	2,2-Dimethylbutane	2-Methylpentane	3-Methylpentane

C/T	ND	57.13	24.42
ppm 14	ND	28580	9770
Vol% @ 45C (91)	ND	2.86	0.98
C/T	14.15	60.89	24.47
ppm 13	5660	30460	9790
Vol% @ 45C (91)	0.57	3.05	0.98
C/T	ND	56.39	24.42
ppm 12	ND	28210	9770
Vol% @ 45C (91)	ND	2.82	0.98
C/T	ND	ND	ND
ppm 11	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	64.01	ND
ppm 10	ND	32020	ND
Vol% @ 45C (91)	ND	3.2	ND
C/T	ND	56.15	26.62
ppm 9	ND	28090	10650
Vol% @ 25C (91)	ND	2.81	1.07
C/T	15.44	52.64	22.37
ppm 8	6180	26330	8950
Vol% @ 25C (91)	0.62	2.63	0.9
C/T	ND	57.07	ND
ppm 7	ND	28550	ND
Vol% @ 25C (91)	ND	2.86	ND
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	36.12	ND
ppm 5	ND	18070	ND
Vol% @ 25C (91)	ND	1.81	ND
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	91.38	ND
ppm 3	ND	45710	ND
Vol% @ 45C (95)	ND	4.57	ND
C/T	ND	63.41	28.89
ppm 2	ND	31720	11560
Vol% @ 45C (95)	ND	3.17	1.16
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	400.14	500.22	400.14
Component	2,4-Dimethylpentane	2-Methylhexane	2,3-Dimethylpentane

C/T	73.9	ND	46.8
ppm 14	32460	ND	14020
Vol% @ 45C (91)	3.25	ND	1.4
C/T	73.77	ND	42.09
ppm 13	32400	ND	12610
Vol% @ 45C (91)	3.24	ND	1.26
C/T	75.7	ND	49.8
ppm 12	33250	ND	14920
Vol% @ 45C (91)	3.33	ND	1.49
C/T	121.65	ND	ND
ppm 11	53430	ND	ND
Vol% @ 45C (91)	5.34	ND	ND
C/T	94.8	ND	ND
ppm 10	41640	ND	ND
Vol% @ 45C (91)	4.16	ND	ND
C/T	72.31	ND	ND
ppm 9	31760	ND	ND
Vol% @ 25C (91)	3.18	ND	ND
C/T	67.78	ND	40.26
ppm 8	29770	ND	12060
Vol% @ 25C (91)	2.98	ND	1.21
C/T	70.6	ND	ND
ppm 7	31010	ND	ND
Vol% @ 25C (91)	3.1	ND	ND
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	44.53	40.38	ND
ppm 5	19560	12490	ND
Vol% @ 25C (91)	1.96	1.25	ND
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	106.78	ND	ND
ppm 3	46900	ND	ND
Vol% @ 45C (95)	4.69	ND	ND
C/T	83.06	ND	ND
ppm 2	36480	ND	ND
Vol% @ 45C (95)	3.65	ND	ND
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	439.22	309.32	299.58
Component	3-Methylhexane	5-Methylnonane	2,2,4-Trimethylpentane

C/T	ND	ND	ND
ppm 14	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 13	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 12	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 11	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 10	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 9	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	25.6	13.37	26.98
ppm 8	7670	6870	8160
Vol% @ 25C (91)	0.77	0.69	0.82
C/T	ND	ND	ND
ppm 7	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 5	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 3	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 2	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	299.66	513.7	302.45
Component	4-Methylheptane	3-Ethylhexane	2,3-Dimethyloctance

C/T	28357.94	623.31	145.83	414.43
ppm 14	28320	124560	14610	41520
Vol% @ 45C (91)	2.83	12.46	1.46	4.15
C/T	27126.3	608.75	145.63	405.25
ppm 13	27090	121650	14590	40600
Vol% @ 45C (91)	2.71	12.17	1.46	4.06
C/T	27907.34	644.83	138.64	427.7
ppm 12	27870	128860	13890	42850
Vol% @ 45C (91)	2.79	12.89	1.39	4.29
C/T	44779.92	947.93	ND	ND
ppm 11	44720	189430	ND	ND
Vol% @ 45C (91)	4.47	18.94	ND	ND
C/T	40734.5	612.9	ND	ND
ppm 10	40680	122480	ND	ND
Vol% @ 45C (91)	4.07	12.25	ND	ND
C/T	29499.47	599.74	185.26	435.29
ppm 9	29460	119850	18560	43610
Vol% @ 25C (91)	2.95	11.99	1.86	4.36
C/T	24532.83	551.35	120.97	350.05
ppm 8	24500	110180	12120	35070
Vol% @ 25C (91)	2.45	11.02	1.21	3.51
C/T	34896.69	504.11	163.5	453.36
ppm 7	34850	100740	16380	45420
Vol% @ 25C (91)	3.49	10.07	1.64	4.54
C/T	46532.26	1283	ND	ND
ppm 6	46470	256390	ND	ND
Vol% @ 25C (91)	4.65	25.64	ND	ND
C/T	22179.68	292.54	ND	235.96
ppm 5	22150	58460	ND	23640
Vol% @ 25C (91)	2.22	5.85	ND	2.36
C/T	41795.92	1029.7	ND	731.24
ppm 4	41740	205770	ND	73260
Vol% @ 45C (95)	4.17	20.58	ND	7.33
C/T	44789.93	1110.21	ND	692.51
ppm 3	44730	221860	ND	69380
Vol% @ 45C (95)	4.47	22.19	ND	6.94
C/T	34406.04	701.33	190.65	577.53
ppm 2	34360	140150	19100	57860
Vol% @ 45C (95)	3.44	14.02	1.91	5.79
C/T	48294.62	983.66	ND	ND
ppm 1	48230	196570	ND	ND
Vol% @ 25C (95)	4.82	19.66	ND	ND
OEL (ppm)	1	199.84	100.19	100.19
Component	Benzene	Toluene	Ethylbenzene	m-Xylene

C/T	174.08	222.19	ND
ppm 14	17440	22260	ND
Vol% @ 45C (91)	1.74	2.23	ND
C/T	196.14	219.99	ND
ppm 13	19650	22040	ND
Vol% @ 45C (91)	1.97	2.2	ND
C/T	214.9	226.58	ND
ppm 12	21530	22700	ND
Vol% @ 45C (91)	2.15	2.27	ND
C/T	ND	ND	ND
ppm 11	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 10	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	196.14	260.91	764.95
ppm 9	19650	26140	15560
Vol% @ 25C (91)	1.97	2.61	1.56
C/T	156.71	196.73	621.89
ppm 8	15700	19710	12650
Vol% @ 25C (91)	1.57	1.97	1.27
C/T	221.59	246.24	ND
ppm 7	22200	24670	ND
Vol% @ 25C (91)	2.22	2.47	ND
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	114.49	ND	ND
ppm 5	11470	ND	ND
Vol% @ 25C (91)	1.15	ND	ND
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 3	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	255.13	283.97	803.3
ppm 2	25560	28450	16340
Vol% @ 45C (95)	2.56	2.85	1.63
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	100.19	100.19	20.34
Component	p-Xylene	o-Xylene	1-Methyl-3-ethylbenzene

C/T	ND	ND
ppm 14	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	ND
ppm 13	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	ND
ppm 12	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	ND
ppm 11	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	ND
ppm 10	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	ND
ppm 9	ND	ND
Vol% @ 25C (91)	ND	ND
C/T	281.18	132.97
ppm 8	5720	5410
Vol% @ 25C (91)	0.57	0.54
C/T	ND	ND
ppm 7	ND	ND
Vol% @ 25C (91)	ND	ND
C/T	ND	ND
ppm 6	ND	ND
Vol% @ 25C (91)	ND	ND
C/T	ND	ND
ppm 5	ND	ND
Vol% @ 25C (91)	ND	ND
C/T	ND	ND
ppm 4	ND	ND
Vol% @ 45C (95)	ND	ND
C/T	ND	ND
ppm 3	ND	ND
Vol% @ 45C (95)	ND	ND
C/T	ND	ND
ppm 2	ND	ND
Vol% @ 45C (95)	ND	ND
C/T	ND	ND
ppm 1	ND	ND
Vol% @ 25C (95)	ND	ND
OEL (ppm)	20.34	40.69
Component	1-Methyl-4-ethylbenzene	1,3,5-Trimethylbenzene

C/T	779.15	953.73
ppm 14	15850	24250
Vol% @ 45C (91)	1.59	2.43
C/T	696.56	ND
ppm 13	14170	ND
Vol% @ 45C (91)	1.42	ND
C/T	690.17	915.19
ppm 12	14040	23270
Vol% @ 45C (91)	1.4	2.33
C/T	ND	ND
ppm 11	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	ND
ppm 10	ND	ND
Vol% @ 45C (91)	ND	ND
C/T	ND	965.14
ppm 9	ND	24540
Vol% @ 25C (91)	ND	2.45
C/T	ND	771.25
ppm 8	ND	19610
Vol% @ 25C (91)	ND	1.96
C/T	ND	928.96
ppm 7	ND	23620
Vol% @ 25C (91)	ND	2.36
C/T	ND	ND
ppm 6	ND	ND
Vol% @ 25C (91)	ND	ND
C/T	ND	ND
ppm 5	ND	ND
Vol% @ 25C (91)	ND	ND
C/T	ND	2138.33
ppm 4	ND	54370
Vol% @ 45C (95)	ND	5.44
C/T	ND	ND
ppm 3	ND	ND
Vol% @ 45C (95)	ND	ND
C/T	ND	1089.03
ppm 2	ND	27690
Vol% @ 45C (95)	ND	2.77
C/T	ND	ND
ppm 1	ND	ND
Vol% @ 25C (95)	ND	ND
OEL (ppm)	20.34	25.43
Component	1-Methyl-2-ethylbenzene	1,2,4-Trimethylbenzene

C/T	ND	ND	50.11
ppm 14	ND	ND	20030
Vol% @ 45C (91)	ND	ND	2
C/T	ND	7.29	45.48
ppm 13	ND	4370	18180
Vol% @ 45C (91)	ND	0.44	1.82
C/T	ND	ND	44.98
ppm 12	ND	ND	17980
Vol% @ 45C (91)	ND	ND	1.8
C/T	ND	ND	ND
ppm 11	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	49.03
ppm 10	ND	ND	19600
Vol% @ 45C (91)	ND	ND	1.96
C/T	ND	ND	50.08
ppm 9	ND	ND	20020
Vol% @ 25C (91)	ND	ND	2
C/T	173.82	7.29	43.03
ppm 8	4420	4370	17200
Vol% @ 25C (91)	0.44	0.44	1.72
C/T	ND	ND	44.28
ppm 7	ND	ND	17700
Vol% @ 25C (91)	ND	ND	1.77
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	9.92	32.6
ppm 5	ND	5950	13030
Vol% @ 25C (91)	ND	0.6	1.3
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	1452.7	ND	ND
ppm 3	36940	ND	ND
Vol% @ 45C (95)	3.69	ND	ND
C/T	ND	ND	34.77
ppm 2	ND	ND	13900
Vol% @ 45C (95)	ND	ND	1.39
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	25.43	599.57	399.75
Component	1,2,3-Trimethylbenzene	Cyclopentane	Methylcyclopent

C/T	ND	ND	ND
ppm 14	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	20.17	15.36
ppm 13	ND	6150	7650
Vol% @ 45C (91)	ND	0.62	0.77
C/T	ND	ND	ND
ppm 12	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 11	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 10	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 9	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	18.1	13.99
ppm 8	ND	5520	6970
Vol% @ 25C (91)	ND	0.55	0.7
C/T	ND	ND	ND
ppm 7	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	79.12	ND	ND
ppm 5	16090	ND	ND
Vol% @ 25C (91)	1.61	ND	ND
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 3	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 2	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	203.36	304.97	498.06
Component	1c, 2t, 4c-Trimethylcyclohexane	Cyclohexane	Methylcyclohexa

C/T	ND	ND	ND
ppm 14	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	150.19	ND
ppm 13	ND	18780	ND
Vol% @ 45C (91)	ND	1.88	ND
C/T	ND	ND	ND
ppm 12	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 11	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 10	ND	ND	ND
Vol% @ 45C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 9	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	14.34
ppm 8	ND	ND	3580
Vol% @ 25C (91)	ND	ND	0.36
C/T	83.89	ND	ND
ppm 7	17060	ND	ND
Vol% @ 25C (91)	1.71	ND	ND
C/T	ND	ND	ND
ppm 6	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 5	ND	ND	ND
Vol% @ 25C (91)	ND	ND	ND
C/T	ND	ND	ND
ppm 4	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	ND
ppm 3	ND	ND	ND
Vol% @ 45C (95)	ND	ND	ND
C/T	ND	ND	25.16
ppm 2	ND	ND	6280
Vol% @ 45C (95)	ND	ND	0.63
C/T	ND	ND	ND
ppm 1	ND	ND	ND
Vol% @ 25C (95)	ND	ND	ND
OEL (ppm)	203.36	125.04	249.62
Component	C9 - MonoNaph - 8	C10-iso-olefin-10	2-Methylbutene-2

Component	OEL (ppm)	Vol% @ 25C (95)	ppm 1	C/T	Vol% @ 45C (95)	ppm 2	C/T	Vol% @ 45C (95)	ppm 3	C/T	Vol% @ 45C (95)	ppm 4	C/T	Vol% @ 25C (95)	ppm 5	C/T	Vol% @ 25C (91)	ppm 6	C/T	Vol% @ 25C (91)	ppm 7	C/T	Vol% @ 25C (91)	ppm 8	C/T	Vol% @ 25C (91)	ppm 9	C/T	Vol% @ 45C (91)	ppm 10	C/T	Vol% @ 45C (91)	ppm 11	C/T	Vol% @ 45C (91)	ppm 12	C/T	Vol% @ 45C (91)	ppm 13	C/T	Vol% @ 45C (91)	ppm 14	C/T
Methyl-t-butyl ether	49.91	18.04	180380	3613.75	19.29	192860	3863.77	17.85	178480	3575.68	20.58	205780	4122.61	18.51	185050	3707.31	21.69	216890	4345.19	19	190020	3806.88	16.1	161040	3226.29	18.04	180360	3613.35	18.63	186250	3731.35	18.73	187300	3752.38	16.66	166630	3338.28	17.01	170130	3408.4	17.48	174790	3501.76
Total C/T				53687.39			42941.75			52332.79			50387.62			27577.67			52780.91			42152.66			32100.62			37361.87			46094.62			50333.13			35386.63			33892.56			35964.16

Average = 42356.74

$$OEL_{mix} = \frac{1}{\frac{C1}{T1}+\frac{C2}{T2}+\cdots\frac{Cn}{Tn}} = \frac{1}{42356.74}\times 10^6 = 23.60 \text{ ppm or } 83 \text{ mg/m}^3$$

Table 0-11: Gasoline vapour mixture MW results and calculations:

Component	n-Butane	n-Pentane	n-Hexane	n-Heptane	i-Butane	i-Pentane
MW	58.12	72.2	86.2	100.2	58.12	72.149
%Mol@ 25C (95)	3.72	16.11	7.78	ND	ND	15.53
MW@ 25C (95)	2.16	11.63	6.71	ND	ND	11.20
%Mol@ 45C (95)	1.89	8.09	5.38	2.29	ND	8.74
MW@ 45C (95)	1.10	5.84	4.63	2.29	ND	6.31
%Mol@ 45C (95)	2.58	8.04	5.66	ND	ND	9.62
MW@ 45C (95)	1.50	5.80	4.88	ND	ND	6.94
%Mol@ 45C (95)	3.46	10.69	5.10	ND	ND	12.91
MW@ 45C (95)	2.01	7.72	4.40	ND	ND	9.32
%Mol@ 25C (91)	3.80	19.93	6.02	1.28	0.94	19.31
MW@ 25C (91)	2.21	14.39	5.19	1.28	0.55	13.93
%Mol@ 25C (91)	5.42	12.12	6.36	ND	ND	16.14
MW@ 25C (91)	3.15	8.75	5.48	ND	ND	11.65
%Mol@ 25C (91)	1.51	11.81	7.70	2.24	ND	9.60
MW@ 25C (91)	0.88	8.52	6.64	2.24	ND	6.92
%Mol@ 25C (91)	3.25	10.38	6.30	2.02	0.91	10.59
MW@ 25C (91)	1.89	7.50	5.43	2.02	0.53	7.64
%Mol@ 25C (91)	3.34	9.68	6.93	2.03	1.15	9.65
MW@ 25C (91)	1.94	6.99	5.97	2.03	0.67	6.96
%Mol@ 45C (91)	2.85	14.99	8.26	2.75	ND	13.82
MW@ 45C (91)	1.66	10.82	7.12	2.75	ND	9.97
%Mol@ 45C (91)	3.43	14.67	7.96	ND	ND	14.07
MW@ 45C (91)	1.99	10.59	6.86	ND	ND	10.15
%Mol@ 45C (91)	4.01	9.58	6.50	2.35	1.43	10.00
MW@ 45C (91)	2.33	6.91	5.61	2.35	0.83	7.21
%Mol@ 45C (91)	2.91	9.44	6.67	2.20	1.01	9.43
MW@ 45C (91)	1.69	6.82	5.75	2.20	0.59	6.80
%Mol@ 45C (91)	3.42	9.57	6.78	2.23	1.11	9.87
MW@ 45C (91)	1.99	6.91	5.85	2.23	0.65	7.12

MW@ 45C (91)	ND	5.02	3.25
%Mol@ 45C (91)	ND	5.82	3.77
MW@ 45C (91)	0.41	4.81	3.23
%Mol@ 45C (91)	0.48	5.59	3.75
MW@ 45C (91)	ND	4.67	3.16
%Mol@ 45C (91)	ND	5.42	3.67
MW@ 45C (91)	ND	5.52	3.69
%Mol@ 45C (91)	ND	6.40	4.28
MW@ 45C (91)	ND	6.12	3.86
%Mol@ 45C (91)	ND	7.10	4.48
MW@ 25C (91)	ND	5.05	3.29
%Mol@ 25C (91)	ND	5.86	3.82
MW@ 25C (91)	0.37	4.68	3.07
%Mol@ 25C (91)	0.44	5.43	3.57
MW@ 25C (91)	0.51	5.39	3.50
%Mol@ 25C (91)	0.59	6.25	4.07
MW@ 25C (91)	ND	5.18	ND
%Mol@ 25C (91)	ND	6.02	ND
MW@ 25C (91)	0.59	5.01	3.11
%Mol@ 25C (91)	0.68	5.81	3.61
MW@ 45C (95)	ND	4.31	2.96
%Mol@ 45C (95)	ND	5.00	3.44
MW@ 45C (95)	ND	4.47	3.05
%Mol@ 45C (95)	ND	5.19	3.54
MW@ 45C (95)	ND	4.33	2.94
%Mol@ 45C (95)	ND	5.02	3.41
MW@ 25C (95)	ND	6.36	3.90
%Mol@ 25C (95)	ND	7.38	4.52
MW	86.18	86.175	86.18
Component	2,2-Dimethylbutane	2-Methylpentane	3-Methylpentane

MW@ 45C (91)	ND	2.33	0.82
%Mol@ 45C (91)	ND	2.32	0.81
MW@ 45C (91)	0.46	2.49	0.82
%Mol@ 45C (91)	0.46	2.48	0.82
MW@ 45C (91)	ND	2.29	0.81
%Mol@ 45C (91)	ND	2.29	0.81
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 45C (91)	ND	2.58	ND
%Mol@ 45C (91)	ND	2.58	ND
MW@ 25C (91)	ND	2.28	0.89
%Mol@ 25C (91)	ND	2.28	0.88
MW@ 25C (91)	0.50	2.16	0.75
%Mol@ 25C (91)	0.50	2.16	0.75
MW@ 25C (91)	ND	2.33	ND
%Mol@ 25C (91)	ND	2.33	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 25C (91)	ND	1.46	ND
%Mol@ 25C (91)	ND	1.46	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 45C (95)	ND	3.65	ND
%Mol@ 45C (95)	ND	3.64	ND
MW@ 45C (95)	ND	2.58	0.96
%Mol@ 45C (95)	ND	2.57	0.96
MW@ 25C (95)	ND	ND	ND
%Mol@ 25C (95)	ND	ND	ND
MW	100.21	100.2	100.21
Component	2,4-Dimethylpentane	2-Methylhexane	2,3-Dimethylpentane

MW@ 45C (91)	2.68	ND	1.16
%Mol@ 45C (91)	2.67	ND	1.02
MW@ 45C (91)	2.68	ND	1.05
%Mol@ 45C (91)	2.67	ND	0.92
MW@ 45C (91)	2.74	ND	1.24
%Mol@ 45C (91)	2.73	ND	1.08
MW@ 45C (91)	4.27	ND	ND
%Mol@ 45C (91)	4.27	ND	ND
MW@ 45C (91)	3.40	ND	ND
%Mol@ 45C (91)	3.39	ND	ND
MW@ 25C (91)	2.61	ND	ND
%Mol@ 25C (91)	2.61	ND	ND
MW@ 25C (91)	2.48	ND	1.01
%Mol@ 25C (91)	2.47	ND	0.88
MW@ 25C (91)	2.57	ND	ND
%Mol@ 25C (91)	2.56	ND	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 25C (91)	1.60	1.09	ND
%Mol@ 25C (91)	1.60	0.77	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 45C (95)	3.79	ND	ND
%Mol@ 45C (95)	3.78	ND	ND
MW@ 45C (95)	3.00	ND	ND
%Mol@ 45C (95)	3.00	ND	ND
MW@ 25C (95)	ND	ND	ND
%Mol@ 25C (95)	ND	ND	ND
MW	100.2	142.28	114.26
Component	3-Methylhexane	5-Methylnonane	2,2,4-Trimethylpentane

MW@ 45C (91)	ND	ND	2.99	12.97
%Mol@ 45C (91)	ND	ND	3.83	14.07
MW@ 45C (91)	ND	ND	2.86	12.68
%Mol@ 45C (91)	ND	ND	3.67	13.77
MW@ 45C (91)	ND	ND	2.93	13.39
%Mol@ 45C (91)	ND	ND	3.76	14.53
MW@ 45C (91)	ND	ND	4.58	19.12
%Mol@ 45C (91)	ND	ND	5.86	20.75
MW@ 45C (91)	ND	ND	4.25	12.62
%Mol@ 45C (91)	ND	ND	5.44	13.69
MW@ 25C (91)	ND	ND	3.10	12.43
%Mol@ 25C (91)	ND	ND	3.97	13.50
MW@ 25C (91)	0.59	0.73	2.61	11.56
%Mol@ 25C (91)	0.52	0.51	3.34	12.55
MW@ 25C (91)	ND	ND	3.69	10.52
%Mol@ 25C (91)	ND	ND	4.72	11.42
MW@ 25C (91)	ND	ND	4.63	25.20
%Mol@ 25C (91)	ND	ND	5.93	27.35
MW@ 25C (91)	ND	ND	3.10	6.04
%Mol@ 25C (91)	ND	ND	3.97	6.56
MW@ 45C (95)	ND	ND	4.25	20.67
%Mol@ 45C (95)	ND	ND	5.44	22.43
MW@ 45C (95)	ND	ND	4.63	22.63
%Mol@ 45C (95)	ND	ND	5.92	24.57
MW@ 45C (95)	ND	ND	3.62	14.55
%Mol@ 45C (95)	ND	ND	4.63	15.79
MW@ 25C (95)	ND	ND	4.88	19.61
%Mol@ 25C (95)	ND	ND	6.24	21.28
MW	114.23	142.28	78.1	92.13
Component	3-Ethylhexane	2,3-Dimethyloctance	Benzene	Toluene

MW@ 45C (91)	1.52	4.31	1.80	2.35
%Mol@ 45C (91)	1.43	4.06	1.70	2.22
MW@ 45C (91)	1.52	4.22	2.04	2.33
%Mol@ 45C (91)	1.43	3.98	1.92	2.20
MW@ 45C (91)	1.44	4.44	2.22	2.39
%Mol@ 45C (91)	1.36	4.18	2.09	2.26
MW@ 45C (91)	ND	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND	ND
MW@ 45C (91)	ND	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND	ND
MW@ 25C (91)	1.93	4.51	2.02	2.75
%Mol@ 25C (91)	1.81	4.25	1.91	2.59
MW@ 25C (91)	1.27	3.67	1.64	2.10
%Mol@ 25C (91)	1.20	3.46	1.54	1.98
MW@ 25C (91)	1.71	4.73	2.30	2.62
%Mol@ 25C (91)	1.61	4.46	2.17	2.46
MW@ 25C (91)	ND	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND	ND
MW@ 25C (91)	ND	2.44	1.18	ND
%Mol@ 25C (91)	ND	2.30	1.11	ND
MW@ 45C (95)	ND	7.33	ND	ND
%Mol@ 45C (95)	ND	6.91	ND	ND
MW@ 45C (95)	ND	7.06	ND	ND
%Mol@ 45C (95)	ND	6.65	ND	ND
MW@ 45C (95)	1.98	5.99	2.63	3.00
%Mol@ 45C (95)	1.87	5.64	2.48	2.82
MW@ 25C (95)	ND	ND	ND	ND
%Mol@ 25C (95)	ND	ND	ND	ND
MW	106.16	106.16	106.16	106.16
Component	Ethylbenzene	m-Xylene	p-Xylene	o-Xylene

MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 25C (91)	1.61	ND
%Mol@ 25C (91)	1.34	ND
MW@ 25C (91)	1.32	0.60
%Mol@ 25C (91)	1.10	0.50
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 45C (95)	ND	ND
%Mol@ 45C (95)	ND	ND
MW@ 45C (95)	ND	ND
%Mol@ 45C (95)	ND	ND
MW@ 45C (95)	1.70	ND
%Mol@ 45C (95)	1.42	ND
MW@ 25C (95)	ND	ND
%Mol@ 25C (95)	ND	ND
MW	120.2	120.19
Component	1-Methyl-3-ethylbenzene	1-Methyl-4-ethylbenzene

MW@ 45C (91)	ND	1.68
%Mol@ 45C (91)	ND	1.40
MW@ 45C (91)	ND	1.50
%Mol@ 45C (91)	ND	1.25
MW@ 45C (91)	ND	1.48
%Mol@ 45C (91)	ND	1.23
MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 45C (91)	ND	ND
%Mol@ 45C (91)	ND	ND
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 25C (91)	0.57	ND
%Mol@ 25C (91)	0.47	ND
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 25C (91)	ND	ND
%Mol@ 25C (91)	ND	ND
MW@ 45C (95)	ND	ND
%Mol@ 45C (95)	ND	ND
MW@ 45C (95)	ND	ND
%Mol@ 45C (95)	ND	ND
MW@ 45C (95)	ND	ND
%Mol@ 45C (95)	ND	ND
MW@ 25C (95)	ND	ND
%Mol@ 25C (95)	ND	ND
MW	120.19	120.19
Component	1,3,5-Trimethylbenzene	1-Methyl-2-ethylbenzene

MW@ 45C (91)	2.55	ND	ND
%Mol@ 45C (91)	2.12	ND	ND
MW@ 45C (91)	ND	ND	0.39
%Mol@ 45C (91)	ND	ND	0.56
MW@ 45C (91)	2.44	ND	ND
%Mol@ 45C (91)	2.03	ND	ND
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 25C (91)	2.57	ND	ND
%Mol@ 25C (91)	2.14	ND	ND
MW@ 25C (91)	2.08	0.48	0.39
%Mol@ 25C (91)	1.73	0.40	0.56
MW@ 25C (91)	2.49	ND	ND
%Mol@ 25C (91)	2.07	ND	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 25C (91)	ND	ND	0.53
%Mol@ 25C (91)	ND	ND	0.76
MW@ 45C (95)	5.52	ND	ND
%Mol@ 45C (95)	4.59	ND	ND
MW@ 45C (95)	ND	3.81	ND
%Mol@ 45C (95)	ND	3.17	ND
MW@ 45C (95)	2.90	ND	ND
%Mol@ 45C (95)	2.42	ND	ND
MW@ 25C (95)	ND	ND	ND
%Mol@ 25C (95)	ND	ND	ND
MW	120.2	120.19	70.14
Component	1,2,4-Trimethylbenzene	1,2,3-Trimethylbenzene	Cyclopentane

MW@ 45C (91)	1.80	ND	ND
%Mol@ 45C (91)	2.14	ND	ND
MW@ 45C (91)	1.64	ND	0.58
%Mol@ 45C (91)	1.95	ND	0.68
MW@ 45C (91)	1.61	ND	ND
%Mol@ 45C (91)	1.92	ND	ND
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 45C (91)	1.74	ND	ND
%Mol@ 45C (91)	2.07	ND	ND
MW@ 25C (91)	1.79	ND	ND
%Mol@ 25C (91)	2.13	ND	ND
MW@ 25C (91)	1.56	ND	0.52
%Mol@ 25C (91)	1.85	ND	0.62
MW@ 25C (91)	1.60	ND	ND
%Mol@ 25C (91)	1.90	ND	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 25C (91)	1.16	1.48	ND
%Mol@ 25C (91)	1.38	1.17	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 45C (95)	1.25	ND	ND
%Mol@ 45C (95)	1.48	ND	ND
MW@ 25C (95)	ND	ND	ND
%Mol@ 25C (95)	ND	ND	ND
MW	84.16	126.24	84.18
Component	Methylcyclopentane	1c, 2t, 4c-Trimethylcyclohexane	Cyclohexane

MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 45C (91)	0.71	ND	2.26
%Mol@ 45C (91)	0.72	ND	1.61
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 45C (91)	ND	ND	ND
%Mol@ 45C (91)	ND	ND	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 25C (91)	0.65	ND	ND
%Mol@ 25C (91)	0.66	ND	ND
MW@ 25C (91)	ND	1.64	ND
%Mol@ 25C (91)	ND	1.30	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 25C (91)	ND	ND	ND
%Mol@ 25C (91)	ND	ND	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 45C (95)	ND	ND	ND
%Mol@ 45C (95)	ND	ND	ND
MW@ 25C (95)	ND	ND	ND
%Mol@ 25C (95)	ND	ND	ND
MW	98.18	126.24	140
Component	Methylcyclohexane	C9 - MonoNaph - 8	C10-iso-olefin-10

Component	2-Methylbutene-2	MTBE	∑MWi*xi
MW	70.13	88.17	
%Mol@ 25C (95)	ND	17.44	100
MW@ 25C (95)	ND	15.37	81.82
%Mol@ 45C (95)	0.71	19.40	100.01
MW@ 45C (95)	0.50	17.11	89.21
%Mol@ 45C (95)	ND	17.64	100
MW@ 45C (95)	ND	15.56	87.77
%Mol@ 45C (95)	ND	20.03	100
MW@ 25C (95)	ND	17.66	86.15
%Mol@ 25C (91)	ND	18.54	101
MW@ 25C (91)	ND	16.35	82.69
%Mol@ 25C (91)	ND	20.66	100
MW@ 25C (91)	ND	18.22	82.26
%Mol@ 25C (91)	ND	19.23	100
MW@ 25C (91)	0.29	14.44	87.1
%Mol@ 25C (91)	0.41	16.38	99.43
MW@ 25C (91)	ND	16.96	87.76
%Mol@ 25C (91)	ND	18.13	100
MW@ 25C (91)	ND	15.99	87.38
%Mol@ 45C (91)	ND	18.59	100.01
MW@ 45C (91)	ND	16.39	83.28
%Mol@ 45C (91)	ND	18.32	100.01
MW@ 45C (91)	ND	16.15	82.92
%Mol@ 45C (91)	ND	16.78	100.01
MW@ 45C (91)	ND	14.79	87.28
%Mol@ 45C (91)	ND	17.19	99.76
MW@ 45C (91)	ND	15.15	87.68
%Mol@ 45C (91)	ND	17.63	99.99
MW@ 45C (91)	ND	15.55	87.53

$$\mathbf{MW_{mixture}} = \sum_1^n MWi * xi$$

Average ∑ MWi*xi = 85.77 g/mol

Appendix H Benzene Metabolites

Table 0-12: Survey 1 phenol in urine sampling data

Subject ID	Date	Avg Temp. (°C)	Avg Relative Humidity %	Wind Speed (m/s)	QTY Pumped Lit/person	Pre-shift (mg/g)	Post-shift (mg/g)
N7F1	11/11/2012	30	36	1.2	4,354	4.976	4.68
N7F2	11/11/2012	30	36	1.2	1,866	3.888	2.34
T1F1	11/12/2012	33	25	1.7	6,159	1.15	3.8
T1F2	11/12/2012	33	25	1.7	6,159	4.8	2.71
T1F1	7/31/2013	39	13	2	2,500	1.9	2.06
T1F2	7/31/2013	39	13	2	2,500	7.46	6.92
T1F3	7/31/2013	39	13	2	2,500	5.38	7.15
T1F4	9/9/2013	35	51	4	6,870	4.31	4.94
T1F1	9/9/2013	35	51	4	6,870	0.28	4.61
T1F3	9/9/2013	35	51	4	6,870	4.85	4.12
T2F2	9/10/2013	34	58	1	2,250	1.65	3.93
N3F1	9/11/2013	37	32	0.84	4,990	16.14	6.76
N4F1	9/12/2013	34	58	1.8	7,717	3.7	4.34
T2F1	9/10/2013	34	58	1	2,250	---	3.73
N4F4	9/12/2013	34	58	1.8	7,717	---	8.33
N3F4 (Control)	9/11/2013	---	---	---	---	1.34	0.4
N4F3 (Control)	9/12/2013	---	---	---	---	7.39	7.07

Table 0-13: Pilot S-PMA in urine sampling data

Subject ID	Date	Avg Temp. (°C)	Avg Relative Humidity %	Wind Speed (m/s)	QTY Pumped Lit/person	Pre-shift (µg/g) Creatinine	Post-shift (µg/g) Creatinine
N6F2	9/24/2013	32	36	1	8000	15.20	20.00
N6F3	9/24/2013	37	24	1	7533	5.00	6.40
N6F5	9/24/2013	37	24	1	7533	29.10	44.60
N6F6	9/24/2013	37	24	1	7533	8.70	20.40
N6F1	9/24/2013	32	36	1	8000	ND	ND
D10F1	12/29/2013	18	36	1	3948	2.97	6.25
D10F2	12/29/2013	18	36	1	3948	2.07	3.87
D11F1	12/30/2013	19	59	0.9	5596	15.40	24.00
D11F2	12/30/2013	19	59	0.9	5596	31.60	46.70
D11F3	12/30/2013	19	59	0.9	5596	26.50	40.20

Table 0-14: Survey 2 ttMA in urine sampling data (non-smoker)

Subject ID	Date	Avg Temp. (°C)	Avg Relative Humidity %	Wind Speed (m/s)	QTY Pumped Lit/person	Pre-shift (µg/g) Creatinine	Post-shift (µg/g) Creatinine
D9F1	1/15/2014	16	57	1.5	6666.25	41.27	104.43
D9F2	1/15/2014	16	57	1.5	6666.25	52.65	114.06
N6F5	2/11/2014	17	32	1.4	11760	68.64	484.47
N4F1	2/26/2014	24	50	2.3	10052.5	100.01	316.64
T1F6	7/3/2014	41	11	1.8	7312	467.31	256.70
T1F4	7/3/2014	41	11	1.8	7312	579.98	1982.56
T1F10	7/3/2014	41	11	1.8	7312	271.59	1348.56
T8F4	7/7/2014	40	30	0.7	3000	376.55	321.06
D12F1	7/8/2014	41	14	1.8	4850	38.53	96.97
T13F1	8/7/2014	39	23	1.3	8211	2124.65	1705.58
D10F2	8/10/2014	37	34	4.2	3460	59.07	567.05
N3F1	8/11/2014	36	20	1.2	7800	124.42	343.61
N7F5	8/13/2014	38	32	1.5	5900	180.09	613.74
T14F1	8/23/2014	36	40	2	2453	6776.86	1618.69
D11F10	11/10/2014	27	29	1.16	1220	275.48	696.64
T13F1	11/13/2014	26	32	0.7	1675	778.91	1734.65
N6F4 (cntl)	2/11/2014	---	---	---	---	86.78	52.27
N4F3 (cntl)	2/26/2014	---	---	---	---	133.04	69.05

Table 0-15: Survey 2 S-PMA in urine sampling data (non-smoker)

Subject ID	Date	Avg Temp. (°C)	Avg Relative Humidity %	Wind Speed (m/s)	QTY Pumped Lit/person	Pre-shift (µg/g) Creatinine	Post-shift (µg/g) Creatinine
D9F1	1/15/2014	16	57	1.5	6666	2.68	4.06
D9F2	1/15/2014	16	57	1.5	6666	1.61	2.79
N6F5	2/11/2014	17	32	1.4	11760	5.61	11.32
N4F1	2/26/2014	24	50	2.3	10053	3.85	6.38
T1F6	7/3/2014	41	11	1.8	7312	19.81	2.92
T1F10	7/3/2014	41	11	1.8	7312	2.97	4.42
T1F4	7/3/2014	41	11	1.8	7312	15.79	21.67
T8F4	7/7/2014	40	30	0.7	3000	0.92	0.91
D12F1	7/8/2014	41	14	1.8	4850	4.82	2.58
T13F1	8/7/2014	39	23	1.3	8211	46.96	31.93
D10F2	8/10/2014	37	34	4.2	3460	0.74	3.61
N3F1	8/11/2014	36	20	1.2	7800	3.69	4.56
N7F5	8/13/2014	38	32	1.5	5900	10.79	10.39
T14F1	8/23/2014	36	40	2	2453	0.60	0.54
D11F10	11/10/2014	27	29	1.16	1220	5.38	10.26
T13F1	11/13/2014	26	32	0.7	1675	13.34	23.27
N6F4 (cntl)	2/11/2014	---	---	---	---	0.82	0.91

N4F3 (cntl)	2/26/2014	---	---	---	---	1.03	0.62

Table 0-16: Survey 2 tt-MA in urine sampling data (smoker/possibly exposed to tobacco)

Subject ID	Date	Avg Temp. (°C)	Avg Relative Humidity %	Wind Speed (m/s)	QTY Pumped Lit/person	Pre-shift (µg/g) Creatinine	Post-shift (µg/g) Creatinine
T1F7	7-Feb-2014	17	42	6	7917	5.72	23.93
T1F1	7-Feb-2014	17	42	6	7917	5.27	14.79
N6F7	11-Feb-2014	17	32	1.4	11760	2.96	1.93
D9F7	2-Jul-2014	39	9	2.8	5520	35.11	24.07
T1F9	3-Jul-2014	41	11	1.8	7312	41.73	15.50
N4F6	6-Jul-2014	37	41	2	14554	2.15	10.03
T8F2	7-Jul-2014	40	30	0.7	8260	17.86	9.17
N6F1	9-Jul-2014	42	13	1.13	12114	2.79	11.15
N6F2	9-Jul-2014	42	13	1.13	12114	11.08	2.11
T13F2	7-Aug-2014	39	23	1.3	8211	26.26	20.96
D10F3	10-Aug-2014	37	34	4.2	3460	4.22	4.81
N3F6	11-Aug-2014	36	20	1.2	7800	16.30	19.86
N3F5	11-Aug-2014	36	20	1.2	2000	3.70	6.58
D11F7	12-Aug-2014	40	15	1.8	5727	5.97	11.64
D11F9	12-Aug-2014	40	15	1.8	5727	20.66	38.74
N7F3	13-Aug-2014	38	32	1.5	5900	3.89	2.59
T8F4	9-Nov-2014	29	25	0.7	1173	1.02	3.14
T8F2	9-Nov-2014	29	25	0.7	2738	47.42	33.06
D11F12	10-Nov-2014	27	29	1.16	1220	14.18	26.05
D11F11	10-Nov-2014	27	29	1.16	1220	20.02	15.85
N6F2	12-Nov-2014	27	33	1.22	2664	31.75	46.89

N6F1	12-Nov-2014	27	33	1.22	2664	12.55	14.46
T13F2	13-Nov-2014	26	32	0.7	1675	28.61	35.50

Table 0-17: Survey 2 S-PMA in urine sampling data (smoker/possibly exposed to tobacco)

Subject ID	Date	Avg Temp. (°C)	Avg Relative Humidity %	Wind Speed (m/s)	QTY Pumped Lit/person	Pre-shift (µg/g) Creatinine	Post-shift (µg/g) Creatinine
T1F7	7-Feb-2014	17	42	6	7917	172.10	342.72
T1F1	7-Feb-2014	17	42	6	7917	79.74	424.65
N6F7	11-Feb-2014	17	32	1.4	11760	60.92	69.54
D9F7	2-Jul-2014	39	9	2.8	5520	3728.83	2569.80
T1F9	3-Jul-2014	41	11	1.8	7312	326.10	717.30
N4F6	6-Jul-2014	37	41	2	14554	147.58	729.96
T8F2	7-Jul-2014	40	30	0.7	8260	733.95	606.60
N6F1	9-Jul-2014	42	13	1.13	12114	103.85	423.59
N6F2	9-Jul-2014	42	13	1.13	12114	126.84	511.90
T13F2	7-Aug-2014	39	23	1.3	8211	1905.72	1326.16
D10F3	10-Aug-2014	37	34	4.2	3460	98.35	440.00
N3F6	11-Aug-2014	36	20	1.2	7800	595.69	1019.75
N3F5	11-Aug-2014	36	20	1.2	2000	461.68	519.50
D11F7	12-Aug-2014	40	15	1.8	5727	232.91	471.93
D11F9	12-Aug-2014	40	15	1.8	5727	269.52	1067.73
N7F3	13-Aug-2014	38	32	1.5	5900	125.03	164.47
T8F4	9-Nov-2014	29	25	0.7	1173	385.83	497.05
T8F2	9-Nov-2014	29	25	0.7	2738	2015.45	1550.78
D11F12	10-Nov-2014	27	29	1.16	1220	337.53	655.32
D11F11	10-Nov-2014	27	29	1.16	1220	1442.63	847.04
N6F2	12-Nov-2014	27	33	1.22	2664	1866.42	2528.80
N6F1	12-Nov-2014	27	33	1.22	2664	165.11	1587.56
T13F2	13-Nov-2014	26	32	0.7	1675	327.93	1024.99

Appendix I Exposure sampling methods results comparison

Table 0-18: Comparison between environmental passive air results and breath post-exposures for benzene

Worker ID	Date	Air Samples (mg/m^3)	Benzene Breath Post-Exposure (nmol/l)
D9F1	15-Jan-2014	0.26	2.43
D9F2	15-Jan-2014	0.21	2.43
D9F7	2-Jul-2014	0.76	ND
D9F8	2-Jul-2014	0.91	ND
D9F1	2-Jul-2014	0.21	ND
T1F11	3-Jul-2014	1	1
T1F6	3-Jul-2014	0.53	ND
T1F9	3-Jul-2014	0.63	1
T1F4	3-Jul-2014	0.75	ND
T1F10	3-Jul-2014	0.86	ND
N4F2	6-Jul-2014	0.81	ND
N4F6	6-Jul-2014	1.3	1
T8F4	7-Jul-2014	0.26	ND
T8F2	7-Jul-2014	2.4	4
D12F1	8-Jul-2014	0.34	ND
D12F2	8-Jul-2014	0.2	ND
N6F1	9-Jul-2014	0.39	ND
N6F2	9-Jul-2014	0.75	ND
T13F2	7-Aug-2014	1.1	4
T13F1	7-Aug-2014	1.1	6
D10F3	10-Aug-2014	0.13	ND
D10F2	10-Aug-2014	0.33	1
N3F1	11-Aug-2014	0.26	2
N3F6	11-Aug-2014	0.89	3
D11F7	12-Aug-2014	0.75	1
D11F8	12-Aug-2014	1.2	1
D11F9	12-Aug-2014	1.1	2
N7F5	13-Aug-2014	0.64	1
T14F1	23-Aug-2014	0.13	ND
T14F1	18-Oct-2014	0.34	ND
T14F1	18-Oct-2014	0.37	ND
T8F4	9-Nov-2014	0.37	1
T8F2	9-Nov-2014	2.2	2

D11F12	10-Nov-2014	0.81	1
D11F11	10-Nov-2014	0.63	2
D11F10	10-Nov-2014	0.39	1
N6F2	12-Nov-2014	1	1
N6F1	12-Nov-2014	0.45	1
N6F3	12-Nov-2014	0.29	1
T13F2	13-Nov-2014	0.93	1
Correlation Coefficient (*r*)			0.49

Table 0-19: Comparison between environmental passive air results and the *S*-PMA post-exposures for benzene

Worker ID	Date	Air Samples (mg/m^3)	Post-Exposure S-PMA (µg/g) Creatinine
D9F1	15-Jan-2014	0.26	4.06
D9F2	15-Jan-2014	0.21	2.79
D9F4	15-Jan-2014	0.37	6.01
T1F8	7-Feb-2014	0.8	6.58
T1F4	7-Feb-2014	0.4	4.70
N6F7	11-Feb-2014	0.2	1.93
N6F5	11-Feb-2014	0.5	11.32
N6F3	11-Feb-2014	0.2	1.31
N3F3	12-Feb-2014	0.8	9.08
N3F1	12-Feb-2014	0.2	2.31
N4F1	26-Feb-2014	0.6	6.38
D9F8	2-Jul-2014	0.91	7.75
T1F11	3-Jul-2014	1	20.36
T1F6	3-Jul-2014	0.53	2.92
T1F4	3-Jul-2014	0.75	21.67
T1F10	3-Jul-2014	0.86	4.42
T8F4	7-Jul-2014	0.26	0.91
D12F1	8-Jul-2014	0.34	2.58
T13F1	7-Aug-2014	1.1	31.93
D10F2	10-Aug-2014	0.33	3.61
N3F1	11-Aug-2014	0.26	4.56
N3F6	11-Aug-2014	0.89	19.86
N7F5	13-Aug-2014	0.64	10.39
T14F1	23-Aug-2014	0.13	0.54
T14F1	23-Aug-2014	0.13	0.85

D11F10	10-Nov-2014	0.39	10.26
N6F3	12-Nov-2014	0.29	10.71
Correlation Coefficient (*r*)			0.75

Table 0-20: Comparison between environmental passive air results and the *t*,*t*-MA post-exposures for benzene

Worker ID	Date	Air Samples (mg/m^3)	Post-Exposure *t*,*t*-MA (µg/g) Creatinine
D9F1	15-Jan-2014	0.26	104.43
D9F2	15-Jan-2014	0.21	114.06
D9F4	15-Jan-2014	0.37	161.72
T1F8	7-Feb-2014	0.8	1171.18
T1F4	7-Feb-2014	0.4	230.35
N6F7	11-Feb-2014	0.2	69.54
N6F5	11-Feb-2014	0.5	484.47
N6F3	11-Feb-2014	0.2	189.49
N3F3	12-Feb-2014	0.8	1532.66
N3F1	12-Feb-2014	0.2	147.09
N4F1	26-Feb-2014	0.6	316.64
D9F8	2-Jul-2014	0.91	1029.69
T1F11	3-Jul-2014	1	1237.82
T1F6	3-Jul-2014	0.53	256.70
T1F4	3-Jul-2014	0.75	1982.56
T1F10	3-Jul-2014	0.86	1348.56
T8F4	7-Jul-2014	0.26	321.06
D12F1	8-Jul-2014	0.34	96.97
T13F1	7-Aug-2014	1.1	1705.58
D10F2	10-Aug-2014	0.33	567.05
N3F1	11-Aug-2014	0.26	343.61
N3F6	11-Aug-2014	0.89	1019.75
N7F5	13-Aug-2014	0.64	613.74
T14F1	23-Aug-2014	0.13	1618.69
T14F1	23-Aug-2014	0.13	4455.33
D11F10	10-Nov-2014	0.39	696.64
N6F3	12-Nov-2014	0.29	7119.89
Correlation Coefficient (r)			0.02

Table 0-21: Comparison between environmental passive air results and the phenol in urine for benzene post-exposure results

Worker ID	Date	Air Samples (mg/m^3)	Post-Exposure Phenol (mg/g) Creatinine
N7F1	11-Nov-2012	0.52	4.68
N7F2	11-Nov-2012	0.25	2.34
T1F1	12-Nov-2012	1.44	3.80
T1F2	12-Nov-2012	0.73	2.71
T1F4	9-Sep-2013	0.69	4.94
T1F5	9-Sep-2013	0.89	10.00
T1F1	9-Sep-2013	0.63	4.61
T1F3	9-Sep-2013	0.38	4.12
T2F2	10-Sep-2013	0.38	3.93
N3F1	11-Sep-2013	0.55	6.76
N4F1	12-Sep-2013	0.64	4.34
N4F4	12-Sep-2013	0.59	8.33
Correlation Coefficient (r)			0.17

Appendix J Laboratory Analysis Methods

NAPHTHAS NIOSH 1550

Table 1 MW: Table 1 CAS: Table 1 RTECS: Table 1

METHOD: 1550, Issue 2 **EVALUATION: FULL** **Issue 1: 15 February 1984 Issue 2: 15 August 1994**

OSHA : Table 1
NIOSH: Table 1
ACGIH: Table 1

PROPERTIES: Table 1

SYNONYMS: Petroleum ether (benzin), rubber solvent, petroleum naphtha, VM&P naphtha, mineral spirits, Stoddard solvent, kerosene (kerosine), coal tar naphtha.

SAMPLING

SAMPLER: SOLID SORBENT TUBE (coconut shell charcoal, 100 mg/50 mg)

FLOW RATE: 0.01 to 0.2 L/min

VOL-MIN: 1.3 L @ 400 mg/m ^{3}; 0.2 L @ 2500 mg/m ^{3} 20 L
-MAX:

SHIPMENT: @ 400 mg/m ^{3}; 3.2 L @ 2500 mg/m ^{3} routine

SAMPLE STABILITY: at least 1 week @ 25 °C 2 to 10

BLANKS: field blanks per set required; 5 to 10 mL

BULK SAMPLE:

ACCURACY

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY, FID

ANALYTE: naphtha hydrocarbons

DESORPTION: 1 mL CS_2; stand 30 min

INJECTION VOLUME: 5 µL (packed column); 0.1 to 1 µL (capillary column)

TEMPERATURE-INJEC 200 to 250 °C
-
-
ETECTOR: 250 to 300 °C

CARRIER GAS: **COLUMN:** 50 to 250 °C @ 8 °/min N_2 or He, 30 mL/min

COLUMN: glass, 3 m x 6-mm, 10% SP-2100 on Supelcoport 80/100 or 30-m fused silica capillary, 0.325-mm ID, 1.0-µm DB-1 or

CALIBRATION:

RANGE STUDIED:	see EVALUATION OF METHOD	**RANGE:**	equivalent solutions of bulk naphtha
BIAS:	see EVALUATION OF METHOD	**ESTIMATED LOD:**	in CS_2
OVERALL PRECISION (S_{rT}):	0.05 [1]	**PRECISION (S_r):**	0.5 to 10 mg per sample [2,3,4]
			0.1 mg per sample
ACCURACY:	see EVALUATION OF METHOD		0.01 [1]

APPLICABILITY: The working range is 100 to 2000 mg/m^3 for a 5-L air sample. This is a general procedure for analysis of various types of hydrocarbon mixtures called "naphthas" which are used as thinners in paints and varnishes and as genera l purpose solvents.

INTERFERENCES: Most naphthas are quite complex. The components elute over a wide temperature range by gas chromatography, making interferences from other substances possible. Columns and conditions must be chosen to obtain the desired degree of separation for a given mixture.

OTHER METHODS: This method combines and replaces Methods S86 [2], S380 [3] and S382 [4]. A similar method appears in the criteria document [5].

REAGENTS:

1. Eluent: Carbon disulfide*, chromatographic quality, containing 0.1% (v/v) octane, 0.5% (v/v) hexadecane or other suitable internal standard.
   NOTE 1: Use an internal standard which is not a major constituent of the sample.
   NOTE 2: Use toluene in place of carbon disulfide for low-boiling analytes [5].
2. Naphtha bulk sample.
3. Nitrogen or helium, purified.
4. Hydrogen, prepurified.
5. Air, filtered.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: glass tube, 7 cm long, 6-mm OD, 4mm ID, flame-sealed ends, containing two sections of activated (600 °C) coconut shell charcoal (front = 100 mg; back = 50 mg) separated by a 2-mm urethane foam plug. A silylated glass wool plug precedes the front section and a 3-mm urethane foam plug follows the back section. Pressure drop across the tube at 1 L/min airflow must be less than 3.4 kPa. Tubes are commercially available.
2. Personal sampling pump, 0.01 to 0.2 L/min, with flexible connecting tubing.
3. Gas chromatograph, FID, integrator and column (page 1550-1).
4. Vials, glass, 2-mL, PTFE-lined crimp caps.
5. Syringe, 10-µL (1-µL syringe for capillary columns) and other convenient sizes for preparing standards, readable to 0.1 µL.
6. Volumetric flasks, 10-mL.
7. Pipet, delivery, 1.0-mL, with pipet bulb.

SPECIAL PRECAUTIONS: Carbon disulfide is toxic and an acute fire and explosion hazard (flash point = -30 °C); work with it only in a hood.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Break the ends of the sampler immediately before sampling. Attach sampler to personal sampling pump with flexible tubing.
3. Sample at an accurately known flow rate between 0.01 and 0.2 L/min for a total sample size which contains between 0.5 and 8 mg naphtha.
4. Cap the samplers with plastic (not rubber) caps and pack securely for shipment. Ship a bulk sample (5 to 10 mL) in a separate container from the sorbent tubes.

SAMPLE PREPARATION:

5. Place the front and back sorbent sections of the sampler tube in separate vials. Discard the glass wool and foam plugs.
6. Add 1.0 mL eluent to each vial. Attach crimp cap to each vial.
7. Allow to stand 30 min with occasional agitation.

CALIBRATION AND QUALITY CONTROL:

8. Calibrate daily with at least six working standards over the range 0.1 to 10 mg naphtha per sample.
    a. Add known amounts of naphtha bulk sample to eluent in 10-mL volumetric flasks and dilute to the mark.
    b. Analyze together with samples and blanks (steps 11 and 12).
    c. Prepare calibration graph (ratio of peak area of analyte to peak area of internal standard vs. mg naphtha).
9. Determine desorption efficiency (DE) at least once for each batch of charcoal used for sampling in the calibration range (step 8). Prepare three tubes at each of five levels plus three media blanks.
    a. Remove and discard back sorbent section of a media blank sampler.

b. Inject a known amount of naphtha bulk sample directly onto front sorbent section with a microliter syringe.
c. Cap the tube. Allow to stand overnight.
d. Desorb (steps 5 through 7) and analyze together with working standards (steps 11 and 12).
e. Prepare a graph of DE vs. mg naphtha recovered.

10. Analyze three quality control blind spikes and three analyst spikes to insure that the calibration graph and DE graph are in control.

MEASUREMENT:

11. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 1550-1. Inject sample aliquot manually using solvent flush technique or with autosampler.

NOTE 1: The columns and conditions given provide moderate to good separation of components. If less resolution is needed, use shorter, less efficient columns as were used in validation of Methods S86 [2], S380 [3] and S382 [4].

NOTE 2: If peak area is above the linear range of the working standards, dilute with eluent, reanalyze and apply the appropriate dilution factor in calculations.

12. Measure peak area. Divide the peak area of analyte by the peak area of internal standard on the same chromatogram.

CALCULATIONS:

13. Determine the mass, mg (corrected for DE) of naphtha found in the sample front (W_f) and back (W_b) sorbent sections, and in the average media blank front (B_f) and back (B_b) sorbent sections. NOTE: If $W_b > W_f/10$, report breakthrough and possible sample loss.

14. Calculate concentration, C, of naphtha in the air volume sampled, V (L):

$$C = \frac{(W_f + W_b - B_f - B_b) \cdot 10^3}{V}, \text{ mg/m}^3.$$

EVALUATION OF METHOD:

Methods S86 (Naphtha, Coal Tar), S380 (Petroleum distillate) and S382 (Stoddard Solvent) were issued on March 14, 1975 [2,3,4]. They were validated at 24 °C and approximately 755 mm Hg using 10-, 4and 3-L air samples, respectively, of 2-50-W Hi-Flash Solvent (Neville Chemical Co.; BP 154 to 195 °C; d 0.893 g/mL), VM&P Naphtha (Amsco Product 1101; BP 120 to 147 °C; d 0.743 g/mL) and Stoddard Solvent (Fisher Scientific Co.; BP 159 to 176 °C; d 0.774 g/mL) [1]. Overall precision and recovery were as shown below, representing a non-significant bias in each method. Breakthrough tests in dry air showed a capacity of 20 to 25 mg of each solvent tested. Capacity at high relative humidity was not determined.

	Overall			Range Studied		Avg.	
Method	Precision ($S\hat{}_{rT}$)	Bias	Accuracy	mg/m^3	mg per sample	DE	Ref.
S86[b]	0.051	5.99%	±15.0%	193 to 809	2 to 8	0.88	[1,2]
S380[a]	0.052	-4.37%	±12.5%	937 to 3930	4 to 16	0.96	[1,3]
S382[b]	0.052	-3.10%	±11.4%	1417 to 5940	4.5 to 18	0.95	[1,4]

NOTES: (a) Data based on experiments using an internal standard method with a 10 ft., 1/8" stainless steel column packed with 10% OV-101 on 100/120 mesh Supelcoport.

(b) Data based on experiments using an internal standard method with a 6 ft., 1/8" stainless steel column packed with 1.5% OV-101 on 100/120 mesh Chromosorb W.

REFERENCES:

[1] Documentation of the NIOSH Validation Tests, S86, S380, S382, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-185 (1977).

[2] NIOSH Manual of Analytical Methods, 2nd ed., V. 2, Method S86, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-B (1977).

[3] Ibid, V. 3, S380, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-B (1977).

[4] Ibid, S382.

[5] Criteria for a Recommended Standard...Occupational Exposure to Refined Petroleum Solvents, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-192 (July, 1977).

METHOD REVISED BY:

Ardith A. Grote, NIOSH/DPSE; S86, S380 and S382 originally validated under NIOSH Contract CDC-99-74-55.

Table 1. General information.

Exposure Limits, mg/m³ NAME OSHA CAS # Predominant RETECS Hydrocarbon Species	Boiling Range (°C) NIOSH ACGIH	Vapour Pressure, kPa (mm Hg) @ 20 °C	Liquid Density (g/mL @ 15 °C)
Petroleum ether (a)	30 to 60 C_5-C_6	13 (100) --	0.63 to 0.66
8032-32-4 OI6180000		(13 °C)	aliphatic 350; C 1800 --
Rubber solvent (a)	45 to 125 C_5-C_8	(c) 2000 (500 ppm)	0.67 to 0.85
SE7449000 8030-30-6			aliphatic 350; C 1800 1590 (400 ppm)
Petroleum naphtha (b)	30 to 238	5 (40)	0.6 to 0.8

	C_6-C_8	2000 (500 ppm)	
(Petroleum distillates mixture)			
			350; C 1800
8002-05-9 SE7449000			--
VM&P naphtha (a)	95 to 160	0.3 to 3 (2 to 20)	0.72 to 0.76
	C_7-C_{11}	--	
8032-32-4			<20% aromatic350, C 1800
OI6180000			1370 (300 ppm)
Mineral spirits (a)	150 to 200	0.2758(2)	0.77 to 0.81
	C_9-C_{12}	2900 (500 ppm)	
8052-41-3			<20% aromatic
			350; C 1800
WJ89250000			525 (100 ppm)
Stoddard solvent (a)	150 to 210	0.2758 (2)	0.75 to 0.80
	C_9-C_{12}	2900 (500 ppm)	
8052-41-3			<20% aromatic
			350; C 1800
WJ8925000			525 (100 ppm)
Kerosene (a)	175 to 325	(c)	0.8
	C_9-C_{16}	--	
8008-20-6			<20% aromatic
			100
OA5500000			--
Coal tar naphtha (b)	110 to 190	<0.7 (<5)	0.86 to 0.89
	C_8-C_{10}	400 (100 ppm)	
8030-30-6			aromatic
			400 (100 ppm)
DE3030000 (NIOSH)			--

(a) As defined by NIOSH Criteria Document [5].
(b) As defined for OSHA PEL.(c) Not available.

PHENOL and p-CRESOL in urine NIOSH 8305

(1) C_6H_5OH MW: 94.11 CAS: 108-95-2 RTECS: SJ3325000
(2) $CH_3C_6H_4OH$ MW: 108.14 CAS: 106-44-5 RTECS: GO6475000

METHOD: 8305, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 May 1985**
Issue 2: 15 August 1994

BIOLOGICAL INDICATOR OF: exposure to phenol, benzene, and p-cresol.

SYNONYMS: (1) phenol: carbolic acid (2) p-cresol: 4-methylphenol

SAMPLING MEASUREMENT

SPECIMEN: two spot urine samples (before and after exposure)

VOLUME: 50 to 100 mL in polyethylene screw-cap bottle

PRESERVATIVE: containing preservative few crystals of thymol

SHIPMENT: freeze urine; ship in dry ice in an insulated container

SAMPLE STABILITY: stable for 4 days @ 25 °C and for 3 months @ -4 °C

CONTROLS: collect urine from unexposed workers; pool and freeze the control urine

METHOD: GAS CHROMATOGRAPHY, FID

ANALYTE: phenol and p-cresol

TREATMENT: acid hydrolysis; extraction

INJECTION VOLUME: 5 µL

TEMPERATURE-INJECTION: 180 °C
-DETECTOR: 200 °C
-COLUMN: 4 min @ 120 °C; 16 °C/min; 4 min @ 190 °C

COLUMN: 3 m x 2-mm ID glass, 2% diethylene glycol adipate/Anakrom Q, 60/80 mesh

CARRIER GAS: N_2, 25 mL/min

CALIBRATION: analyte in control urine; nitrobenzene internal standard

RANGE: 2 to 300 µg phenol/mL urine;
2 to 500 µg p-cresol/mL urine

ESTIMATED LOD: 0.5 µg/mL urine

RECOVERY: (1) 94% @ 15 mg/mL; (2) 95% @ 50 µg/mL

PRECISION (S_r): (1) 0.128; (2) 0.091

ACCURACY: (1) ± 31.0%; (2) ± 22.8%

APPLICABILITY: Phenol and p-cresol occur normally in urine. This method is useful in screening workers exposed to phenol, p-cresol, and benzene. The chief metabolite of benzene is phenol [1]. Workers exposed 8 h to 25 ppm benzene excreted abou t 150 mg phenol/L urine [2].

INTERFERENCES: o-Phenylphenol has a GC retention time similar to that of phenol. A careful work history/questionnaire is suggested.

OTHER METHODS: This method replaces P&CAM 330 [3]. A nonspecific colorimetric method yields 50% higher phenol concentrations with normal urine than does this method [4].

REAGENTS:

1. Phenol calibration stock solution, 2 mg/mL. Accurately weigh 200 mg phenol* and dissolve in distilled water. Dilute to 100 mL. Stable 14 days at 25 °C.
2. p-Cresol calibration stock solution, 5 mg/mL. Accurately weigh 500 mg p-cresol* and dissolve in methanol.* Dilute to 100 mL. Stable 14 days at 25 °C.
3. Diethyl ether.*
4. HCl, concentrated, or perchloric acid, 70%.*
5. Sodium sulfate, granular, anhydrous.
6. Thymol, USP.
7. Internal standard, 0.6 mg/mL. Dissolve 30 mg nitrobenzene* in 50 mL methanol.
8. Methanol.*
9. Nitrogen, purified.
10. Hydrogen, purified.
11. Air, filtered.
12. Dry ice.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Bottles, polyethylene, screw-top, 125-mL.
2. Gas chromatograph with FID, integrator and column (page 8305-1).
3. Centrifuge tubes, 15-mL, graduated, glassstopper.
4. Syringe, 10-µL, readable to 0.1 µL.
5. Volumetric flasks, 100-mL.
6. Pipets, Pasteur.
7. Pipets, 1-, 2- and 5-mL.
8. Mixer, vibration.
9. Culture tubes, disposable, 10 x 75-mm.
10. Water bath, 95 °C.
11. Ice bath or freezer.

SPECIAL PRECAUTIONS: Samples of urine collected from humans pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this is much less. Those who handle urine specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided. Diethyl ether and methanol are fire risks. Phenol, p-cresol, methanol and nitrobenzene are toxic and can be absorbed through the skin. Hydrochloric acid and perchloric acid can damage the skin. Wear gloves and eye protection.

Work in a fume hood. Handle perchloric acid only in a perchloric acid hood.

SAMPLING:

1. Collect 50 to 100 mL urine in a 125-mL polyethylene bottle containing a few crystals of thymol.
   NOTE: Collect two urine samples for each worker, one prior to exposure and one after exposure. Also, collect and pool control urine samples from unexposed workers.
2. Close the bottle immediately after sample collection and swirl gently to mix.
3. Freeze the urine and ship in dry ice in an insulated container.

SAMPLE PREPARATION:

4. Thaw urine sample.
5. Determine creatinine (g/L urine) in an aliquot of the urine [5].
6. Pipet 5.0 mL urine into a 15-mL centrifuge tube.
7. Add 1 mL conc. HCl or 5 drops 70% perchloric acid. Mix well.
8. Stopper loosely. Heat in a water bath at 95 °C for 1.5 h.
9. Remove from water bath. Add 10 µL internal standard. Adjust volume in the centrifuge tube to 10 mL with distilled water.
10. Pipet 2 mL diethyl ether into the tube. Stopper and shake vigorously for 1 min. Cool the tube to 0 °C and allow the phases to separate.

11. Transfer ca. 0.5 mL of the clear ether layer to a culture tube. Add a few milligrams of Na_2SO_4 and mix. Cap the tube and keep it at 0 °C prior to measurement to avoid evaporation.

CALIBRATION AND QUALITY CONTROL:

12. Calibrate daily with combined working standards containing 0.5 to 300 µg phenol/mL solution and 0.5 to 500 µg p-cresol/mL solution.
    a. Add known amounts of phenol and p-cresol calibration stock solutions to pooled control urine in 100-mL volumetric flasks and dilute to the mark with pooled control urine.
    b. Process 5 mL of each working standard using the same procedure as for the samples (steps 6 through 11).
    c. Analyze working standards with urine samples and pooled control urines.
    d. Plot separate calibration graphs for phenol and p-cresol (ratio of peak area of analyte to peak area of nitrobenzene vs. µg analyte/mL solution).

MEASUREMENT:

13. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 8305-1. Inject an aliquot of the extract from step 11 using solvent flush technique.
14. Measure peak areas. Divide the peak areas of phenol and p-cresol by the peak area of nitrobenzene on the same chromatogram.

CALCULATIONS:

15. Determine the phenol and p-cresol concentrations (µg/mL) in the urine sample from the calibration graphs.
16. Calculate the concentrations of phenol and p-cresol per gram of creatinine in the urine sample by dividing by the creatinine value obtained in step 5. Compare the results obtained on the pre- and post-shift samples for each worker.

GUIDES TO INTERPRETATION:

The normal range for phenol found in this laboratory for human controls not exposed to benzene, phenol, or p-cresol was 4.5 to 20.7 mg phenol/g creatinine. The normal range found for p-cresol was 5.5 to 65 mg/g creatinine. It must be emphasized that laboratories should establish their own normal ranges using urine specimens from personnel not exposed

to benzene, phenol, p-cresol or excessive amounts of dietary sodium benzoate (used as a preservative in some foods). Lauwerys [6] reported "tentative maximum permissible values" of 45 mg phenol/g creatinine for benzene exposures and 300 mg phenol/g creatinine for phenol exposures. No values were reported for p-cresol. The ACGIH Biological Exposure Index is 250 mg phenol/g creatinine [7].

EVALUATION OF METHOD:

Ten spiked urine specimens containing phenol and p-cresol at concentrations of 10 and 50 µg/mL urine, respectively, were analyzed for each analyte. Precision (S_r) for the ten spiked replicate urine samples was 0.128 for phenol and 0.091 for p-cresol.

REFERENCES:

[1] Rainsford, S. G. and T. A. Lloyd Davies. Urinary Excretion of Phenol by Men Exposed to Vapour of Benzene: Screening Test, British J. Ind. Med., 22, 21-26 (1965).

[2] Docter, H. J. and R. L. Zielkuis. Phenol Excretion as a Measure of Benzene Exposure, Ann. Occup. Hyg., 10, 317-326 (1967).

[3] NIOSH Manual of Analytical Methods, 2nd ed., Vol. 6, P&CAM 330, U.S. Department of Health and Human Services, Publ. (NIOSH) 80-125 (1980).

[4] Buchwald, H. The Colorimetric Determination of Phenol in Air and Urine with a Stabilized Diazonium Salt, Ann. Occup. Hyg., 9, 7-14 (1966).

[5] Tietz, N. W. Fundamentals of Clinical Chemistry, 2nd ed., 994-999, W. B. Saunders Co., Philadelphia, PA (1976).

[6] Lauwerys, Robert R. Industrial Chemical Exposure: Guidelines for Biological Monitoring, 136-140, Biomedical Publications, Davis, CA (1983).

[7] 1993-1994 Threshold Limit Values and Biological Exposure Indices, American Conference of Governmental Industrial Hygienists, Cincinnati, OH (1993).

METHOD REVISED BY:

William P. Tolos, NIOSH/DBBS

Appendix K Thesis Dissemination

Table 0-1: Thesis Dissemination

Event	Organiser	Title	Remarks
2nd BOHS Worker Health Protection Conference, Abu Dhabi, 25-29 October 2015	British Occupational Health Society (BOHS)	occupational exposure assessment to gasoline vapour at local petrol station	Conference was attended and paper was presented
4th Annual International Conference on Health & Medical Sciences, 2-5 May 2016, Athens, Greece	Athens Institute for Education and Research (AIER)	Quantitative Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations	Conference was attended and paper was presented
2015 International Society of Exposure Science Annual Meeting in Henderson, NV, USA, October 21, 2015	International Society of Exposure Science (ISES)	Assessment of Petrol Station Attendant Exposures to Gasoline Vapors in Saudi Arabia	Paper was accepted but could not be attended due to time conflict with other event.
The 10th Middle Easter Refining and Petrochemicals Conference and Exhibitions	Middle East PetroTech	Occupational Exposures to Gasoline Vapour at Public Petrol Stations	Could not be attended.
Athens Institute for Education and Research	The Athens Journal of Sciences	Occupational Exposure Assessment to Gasoline Vapours Under Various Conditions at Public Gasoline Stations	Paper has been peer-reviewed and accepted to be published in the journal.

Athens Institute for Education and Research **Abstract Submitting Form**	
Conference	4th Annual International Conference on Health & Medical Sciences, 2-5 May 2016, Athens, Greece
Title of Paper	Quantitative Assessment of Occupational Exposure to Gasoline Vapour at Petrol Stations
For more than one author, please copy and paste the following eight rows for each additional author.	
Title	Dr X Mr Ms Other Specify:
First Name	Ahmed
Family Name	Al-Yami
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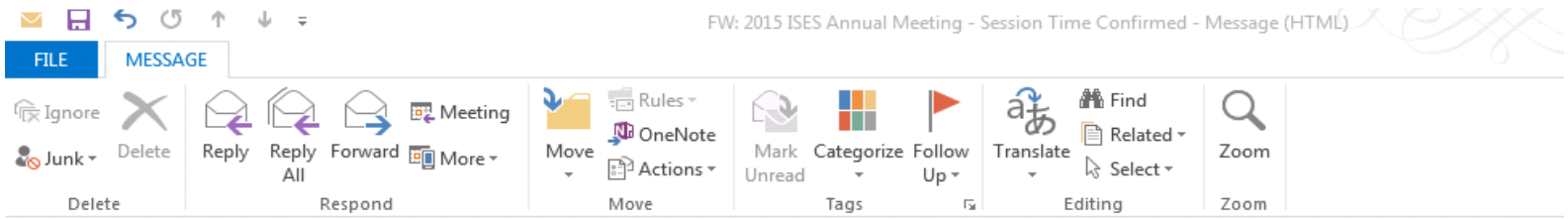
Abstract	Petrol station attendants' exposure to gasoline vapours while refuelling vehicles has raised health concerns, especially in tropical countries like Saudi Arabia. This is due to the increase of gasoline vaporisation by the predominantly hot temperatures and the increase risk of inhaling more vapours than its counterpart temperate countries. Furthermore, exposure during extended working hours (12 hr shifts), with no vapour recovery system and the handling of gasoline containing high percentage volumes of toxic substances (e.g. BTEX) have not been adequately addressed in the literature. Therefore, this work was designed and carried out to investigate the validity of this concern by assessing and

	quantifying full shift exposures to gasoline vapours during the refuelling process. Different exposure assessment methodologies were employed and evaluated for their suitability. The study assessed the exposures of 41 attendants via passive, active, and direct reading methods at twelve petrol stations of both high and low sales in the Eastern Province of Saudi Arabia. The study was conducted during the winter and summer months to test the seasonal variation of the exposure pattern: The effects of the quantity of gasoline sold, the locations of the stations, weather variations (e.g. wind speed, temperature, and humidity) were tested. Specially designed mini-weather stations and modified thermometres were utilized to accurately monitor sampling weather conditions. Forward-looking infrared (FLIR) thermal image cameras were utilised to visualise the size and movement behaviour of the vapour plumes during gasoline refuelling. The geometric means of the personal passive results for the BTEX were found to be relatively higher than those reported by the IARC, 2012 and Concawe, 2002 for Europe and North America.
Keywords	Occupational health, gasoline exposure, petrol station, BTEX, petrol station attendants, gasoline vapours,



Mon 2/1/2016 3:15 PM

Yami, Ahmed R <ahmed.yami.2@aramco.com>

FW: 2015 ISES Annual Meeting - Session Time Confirmed

To Alyami, Ahmed

From: abstracts@ises2015.org [mailto:abstracts@ises2015.org]
Sent: Tuesday, May 05, 2015 6:46 PM
To: Yami, Ahmed R <ahmed.yami.2@aramco.com>
Subject: 2015 ISES Annual Meeting - Session Time Confirmed

Dear Mr. Ahmed Alyami,

We are pleased to announce that your presentation session time(s) has been confirmed for the 2015 International Society of Exposure Science Annual Meeting in Henderson, NV, USA.

Abstract #(s): We-O-C1-04
Abstract Title: Assessment of Petrol Station Attendant Exposures to Gasoline Vapors in Saudi Arabia
Session(s): Occupational Exposures to Diesel, Gasoline, and Combustion Products
Session Date(s): October 21, 2015
Session Time(s): 8:30 AM - 10:00 AM

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Sincerely,

Judy LaKind
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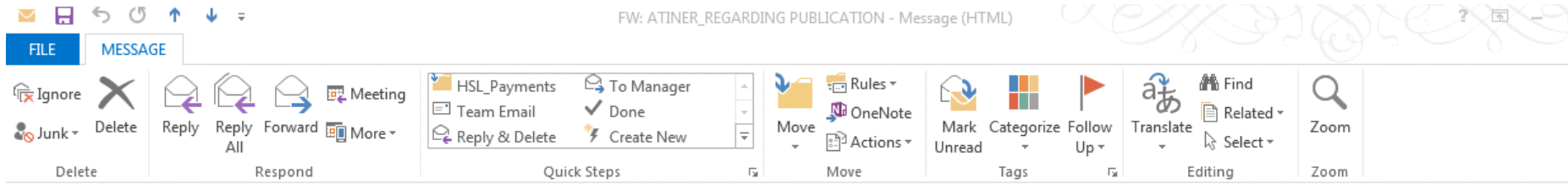
Co-Chairs, ISES 2014



Wed 10/26/2016 2:29 PM

Yami, Ahmed R <ahmed.yami.2@aramco.com>

FW: ATINER_REGARDING PUBLICATION

To 'alyamia@hotmail.com'

Dear Dr. Alyami,

I hope you are keeping well. I wish to inform you that your paper entitled

Occupational Exposure Assessment to Gasoline Vapors Under Various Conditions at Public Gasoline Stations

has been reviewed and accepted with minor revisions for publication in the Athens Journal of Sciences. Please find the reviewers comments below and kindly revise your paper in the light of these comments and please provide an author/response word file.

- In the Materials and Methodology section, further explanation on custom made mini-weather station calibration is needed.
- Figure 6 page 15, the title of the graph needs to be modified.
- Figure 7 page 17 (7), the Value (graph) needs to be modified.
- Figure 8 page 18 (1) the Value (graph) needs to be modified.

Please also follow the **Harvard Referencing System** (Author, Year) and make sure that all references that are cited in the main body of the paper, are cited as well in the references section, and vice versa (eg. Bono et al. 2003 is not cited at the end).

For your information, please find attached the originality report. Please also complete the Author's Declaration Form, which can be found here: http://www.athensjournals.gr/declaration.

Please consider the above, and respond to me at your earliest convenience. We will appreciate if you can return your revised paper by **19th of September 2016**.

Should you have any queries, or should you require any further information, please do not hesitate to contact me.

Thank you very much in advance for your time and effort.
Best Regards

Ms Olga Gkounta
Researcher, Athens Institute for Education & Research
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