ORTHO-CRESOL AS INDICATOR FOR TOLUENE EXPOSURE AMONG WORKERS

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A special dedication to my parents,

Said Bin Zen Umayar and Indok Umi Hani Binti Meri,

To my siblings, my very friends, laboratory assistants,

My supervisor that has always inspired me with the ideas,

A special thanks to Encik Raizul Bin Zinalibdin for his unconditional guidance,

And finally to everybody that always motivates and supports me throughout my study

I am eternally grateful…

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PREFACE

This thesis is the result of my work carried out in Department of Chemistry, Universiti Teknologi Malaysia between December 2010 and May 2013 under the supervision of Prof. Dr. Abd. Rahim Yacob. Part of my work described in this thesis has been used for exhibition participations and reported in the following publications:

1. Yacob, A. R., Zinalibdin, M. R. And Said, N. (2011). Detection of Vapour Metabolites of Glue Sniffer's Urine Using Head Space Gas Chromatogrpahy Mass Spectrometry. J. Drug Metabol Toxicol. 2(3), 1-4.

2. Abd. Rahim Yacob, Nazirah Said and Mohamad Raizul ZInalibdin. (2011). *Ortho*-Cresol Detector Kit: An In-situ Kit fot the Detection of Glue Sniffing Activity. Exhibition participation for the 13^{th} Industrial Art and Technology Exhibition (INATEX) 2011. Silver medal award. Universiti Teknologi Malaysia, Skudai, Johor 16-18 November 2011.

3. Abd. Rahim Yacob, Nazirah Said and Mohamad Raizul bin Zinalibdin. (2012). *Ortho*-Cresol Detection Kit for Detection of Glue Sniffing Activity and Toluene Exposure in Occupational Hazards. Exhibition participation for Malaysia Technology Expo (MTE) 2012. Gold medal award. Putra World Trade Centre, Kuala Lumpur 18 February 2012.

ABSTRACT

Toluene is a volatile organic solvent used by most industries and household items such as lacquers, paints, glue and nail polish. Recently, the negative health effects on exposure to toluene have attracted various studies. To combat the negative effects, American Conference of Governmental Industrial Hygienists (ACGIH) had lowered the occupational exposure limit from 100 ppm to 50 ppm of toluene vapour. Therefore, this could be translated into the need for a new, simple and more specific indicator test to monitor the degree of toluene exposure among workers. Reports showed that toluene is transformed into its metabolites once consumed or exposed to it. The qualitative detection of these metabolites therefore can be used as an indicator for toluene exposure. Presently in Malaysia, hippuric acid detected in urine is used as the indicator for the degree of toluene exposure. However, hippuric acid is also influenced by one's dietary intake like black tea, acidic food and sodium benzoate as in food preservatives. In addition, at low levels of toluene exposure, hippuric acid showed poor correlation and linearity in analysis. Thus, in this study, another metabolite of toluene, *ortho*-cresol was introduced. It was found that urinary *ortho*-cresol was more specific and sensitive in monitoring toluene exposure. In this study, the degree of toluene exposure of six workers (real samples) was analyzed using urinary *ortho*-cresol. The samples, along with standards, were characterized using UV-Vis spectrophotometer. Three samples out of six samples were found to have *ortho*-cresol concentrations between 3.00 ppm - 3.05 ppm. These concentrations are all above the permitted value of 3.00 ppm. A detector kit which establishes the presence of *ortho*-cresol in urine was also developed in this study. The kit, which exploits a simple colour test with 0.2 ppm as its lowest limit of detection is indispensible especially to monitor the health of workers that are exposed almost daily to toluene.

ABSTRAK

Toluena adalah sejenis pelarut mudah meruap yang digunakan oleh kebanyakan industri dan barangan rumah seperti laker, cat, gam dan pengilat kuku. Baru-baru ini, kesan negatif akibat terdedah kepada toluena telah menarik pelbagai kajian. Bagi memerangi kesan negatif ini, *American Conference of Governmental Industrial Hygienists* (ACGIH) telah menurunkan had pendedahan pekerjaan dari 100 ppm ke 50 ppm wap toluena. Maka, ini boleh diterjemahkan sebagai keperluan kepada ujian penunjuk baharu yang mudah dan lebih khusus bagi pemantauan tahap pendedahan toluena di kalangan pekerja. Laporan kajian menunjukkan bahawa toluena terubah kepada metabolitnya apabila dimakan atau terdedah kepadanya. Pengesanan secara kualitatif metabolit ini boleh digunakan sebagai penunjuk kepada pendedahan toluena. Di Malaysia kini pengesanan asid hipurik dalam air kencing digunakan sebagai penunjuk kepada tahap pendedahan toluena. Bagaimanapun asid hipurik juga boleh dipengaruhi oleh bahan makanan seseorang seperti teh hitam, makanan berasid dan natrium benzoate yang digunakan sebagai bahan pengawet makanan. Di samping itu, pada pendedahan tahap rendah toluena, pengesanan asid hipurik menunjukkan korelasi dan kelinearan yang rendah. Oleh itu, dalam kajian ini, satu lagi metabolit daripada toluena iaitu *orto*-kresol telah diperkenalkan. Didapati *orto*-kresol dalam air kencing adalah lebih spesifik dan sensitif dalam pemantauan tahap pendedahan kepada toluena. Dalam kajian ini, tahap pendedahan enam pekerja (sampel sebenar) telah dianalisis dengan menggunakan pengesanan *orto*-kresol dalam air kencing. Sampel, bersama-sama dengan larutan piawai, telah dicirikan menggunakan spektrofotomoter UV-Vis. Tiga sampel daripada enam sampel didapati mempunyai kepekatan *orto*-kresol antara 3.00 ppm – 3.05 ppm. Kepekatan ini melebihi nilai yang dibenarkan 3.00 ppm. Satu kit pengesanan *orto*-kresol dalam air kencing juga telah dibangunkan dalam kajian ini. Kit ini, yang mengeksploitasi ujian warna mudah dengan 0.2 ppm sebagai had pengesanan, adalah penting terutamanya untuk memantau kesihatan pekerja yang terdedah hampir setiap hari dengan toluena.

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LIST OF SYMBOLS AND ABBREVIATIONS

ACGIH	America Conference of Governmental Industrial Hygienists
ASTDR	Agency for Toxic Substances and Diseases Registry
TLV	Threshold Limit Value
MAK	Maximum Allowable Concentration
UV-Vis	Ultra Violet- Visible
ppm	parts per million
mg/l	milli gram/litre
nm	nanometer
$0^{\circ}C$	degree Celcius

CHAPTER 1

INTRODUCTION

This first chapter describes the negative impacts that toluene has as the result of exposure to the solvent. The myriad of negative health effects that exposure to toluene has on the human body are also included. This chapter also illustrates the threat toluene brings to individuals and the society as the most prevalent abused inhalant. Next, the chapter includes information on the various markers that were used by concerned agencies in order to determine the level of exposure to toluene in work places so that the workers' health condition could be monitored and maintained at its optimum level. Also this chapter provides the study's problem statement, the objectives that the study aspires to achieve, the scope or area that the study intends to include and lastly the significance of the study conducted.

1.1 Negative Health Effects of Toluene Exposure

A large amount of organic solvents' vapour is present in the atmospheric air. This is true for both working and home environment. Dry-cleaning fluids, nail polishers, paint and adhesives are some of daily products that are used at home that contain volatile organic solvents. These solvents' vapours are more often than not harmful to the human body (Qliverin and Curry, 1984). Normally, the vapours of these volatile liquids, once inhaled, are readily absorbed in the lungs through the alveoli. The solubility of the

vapours is the only determining factor for the vapour to pass through the blood and into the human system. Toxicants that are low in solubility depend on the blood-flow rate of the body. Solvent vapours of high solubility on the other hand, are dependent upon the respiratory process of the individual (Klassen, 1980).

Materials or chemicals that have psychoactive properties are most commonly volatile substances (Beauvais, 1992). The documentation of cases of mass exposure to these substances have brought upon the toxic effects associated (Rosenberg and Sharp, 1992; Snyder and Andrews, 1996). The target sites of inhalants are lipid-rich organs and the nervous system (Hormes *et al*., 1986; Schaumburg and Spencer 1987). Neurophysiologic, magnetic resonance imaging (MRI) and autopsy studies have shown that acute high-level exposures are reversible but prolonged exposure to these inhalants leave eternal harm (Rosenberg *et al*., 1986).

Toluene is an organic solvent that is present in various products including industrial and household items. It is also abused in glue sniffing cases for the psychoactive or the euphoric effects that it offers. Paints and glues are some household items that are abused (Williams and Storck, 2007). Occupational, Safety and Health as reported by Hazardous Data Bank, also listed toluene as one of pollutants in the atmosphere as well as in working environments (http://toxnet.nlm.nih.gov accessed on 10th December 2012). Toluene is one of the solvents used in the manufacture of industrial paints, adhesives, coatings, inks, and cleaning products as well as used during the process of producing pharmaceuticals, dyes, cosmetic nail products, and in the creation of organic chemicals including benzene. The octane rating of aviation fuel is increased via the addition of toluene and toluene is also one of the raw materials used in the making of nylon, plastic soda, bottles and polyurethanes (Alyward *et al*., 2008). In addition, during gasoline's production, transport and combustion, toluene is also released to the atmosphere (Filley *et al*., 2004).

Inhalation from ambient and indoor air is the chief administration route of toluene vapour into the human body though trace amounts in food and water may also be ingested (US Environmental Protection Agency (USEPA), 1999). However, the highest concentration of toluene that could be administered into the body is during inhalant abuse or the intentional inhalation of toluene vapours. Both chronic and acute exposures to toluene have negative impact to the central nervous system (CNS). Administration of large amounts of toluene during inhalant abuse or after ingestion of food or water contaminated with toluene has also been shown to have adverse effects on liver and kidneys as well as other organs (Alyward *et al*., 2008).

Reports from across the world including Scandinavia (Lundberg, 1985; World Health Organization, 1985; Cranmer and Goldberg 1986), British and America (Cherry *et al*., 1985; Maizlish *et al*., 1985; Fidler *et al*., 1987; Baker *et al*., 1988) as well as from China (Ng and Lam, 1989) have illustrated neurobehavioural and neuropsychological changes in workers as a result being exposed to solvent vapours during working hours. Clinical cross-sectional reports that spanned over 10 years have consistently demonstrated chronic toxic encephalopathy together with slight or subclinical changes in behaviour which are ascribable to exposure to industrial solvents in Scandinavian workers who appeared healthy (Lundberg, 1985; World Health Organization, 1985; Cranmer and Goldberg 1986). However, some researches (Cherry *et al*., 1985; Maizlish *et al*., 1985) had shown results that are not coherent to the studies in Scandinavia though in more recent studies (Fidler *et al*., 1987; Baker *et al*., 1988) the results were in chorus with the Scandinavian reports.

In an experiment by Ng *et al*. (1990), the neurobehavioural effects of exposure to mixed solvents on printing and paint workers in China were studied. The group that was exposed to the solvents stated more accounts of fatigue, irritability, depression, poor memory, orthostatic giddiness and sweaty palms and soles. The participants of the study were evaluated on two neurobehavioural functions, namely psycho-motor and auditory memory test. The psycho-motor test included reaction time and digit symbol test while the auditory memory test included digit span and associate learning assessments. The

group that was exposed to the solvents showed inferior performance in comparison to the group that was not exposed to the solvents (Ng *et al.,* 1990).

Akin to mixed solvent exposure effects, the neurobehavioural effects of exposure to toluene in occupational settings on workers have been a debatable issue (Meyer-Baron, 2005) although the Danish Environment Protection Agency (DEPA, 2001) has recognized the neuropsychological effects that follow such exposures. Different results have been demonstrated by various studies that are conflicting in nature. A research by Deschamps *et al*. (2001) compared a group of workers who were not exposed to toluene with another group that were exposed to 50 ppm of toluene in the air and the results showed no difference on performance level between the two groups while Chouani'ere *et al*. (2002) illustrated that a group of workers who were exposed to a level of 22 ppm toluene showed substandard performance in comparison to a non-exposed group of workers. In a different study (Seeber *et al*., 2004) the group of worker who were exposed to a mean of 26 ppm toluene showed better performance level in contrast to the group that was not exposed. However, numerous studies (Iregren, 1982; Haenninen *et al*., 1987; Boey *et al*., 1997; Kang *et al*., 2003) had shown coherent results that reported significant effects on cognitive performance resulting from toluene exposure.

Different levels of exposure to toluene both acute and chronic have different effects on human especially in the central nervous system (CNS) which is the primary target of toluene in toxicity events. Acute inhalation of low or moderate level of toluene have reversible impairment to the CNS, narcosis and cardiac arrhythmia whilst higher level of toluene inhalation has caused CNS depression and death. In cases of toluene oral intake, severe depression of the CNS, constriction and necrosis of myocardial fibers, swollen liver, congestion and hemorrhage of the lungs, and tubular kidney necrosis have been accounted. In animal tests, toluene exposure resulted in decreased resistance towards respiratory infection (Agency for Toxic Substances and Disease Registry, 2011). Chronic exposure to toluene similar to occupational settings and in the cases of chronic inhalant abuse have been shown to cause drowsiness, ataxia, tremors, cerebral atrophy, nystagmus, impaired speech, hearing, and vision, irritation of the upper

respiratory tract and eyes, sore throat, dizziness, headache, and difficulty with sleep (U.S. Environmental Protection Agency, 1999; Agency for Toxic Substances and Disease Registry, 2011).

Toluene exposure has also been reported to cause developmental and reproductive defects. Pregnant women that abused inhalants have been shown to bear children with CNS dysfunction, attention deficits, minor craniofacial and limb anomalies, and developmental delay in addition to dysmorphism and growth retardation. Spontaneous abortion risk has been observed in families where only either one of the parents (paternal or maternal) is exposed to toluene. However, there was no significance in risk of cancer in individuals exposed to toluene (U.S. Environmental Protection Agency, 1999; Agency for Toxic Substances and Disease Registry, 2011).

Long-term or chronic exposure to toluene, though in low-levels as in the case of workers working in industries involving toluene, has been shown to illustrate negative effects to the human body (Karabulut *et al*., 2009; Shih *et al*., 2011). In the Proposed Acute Exposure Guideline Levels (AEGLs, 2000), it is stated that toluene vapour is "*is readily absorbed from the lungs and distributed all over the cellular components of body and accumulated within lipophilic areas*". In addition, Klassen (1980) reported that other than lungs, the human skin may also play an important role of being another route of administration of these vapours into our body. Moreover, Fuke *et al*. (1996) added that these hazardous chemicals, once have entered the body, may also spread into bodily fluids, the intestines, liver, spleen and the brain while in a report by Halifeoglu *et al*. (2000), toluene in the body intensifies the formation of oxygen radicals in the body which is a major cause of cellular damage (Klassen, 1980).

The adverse effects of toluene exposure to the human body was shown to not stop only at cellular level but has also been found to cause negative impact at chromosomal level as well. In an experiment by Manikantan *et al*. (2010), the association between toluene exposure and genetic damage were assessed. The results

showed agreeable relationship between the risk of developing genetic damage and the exposure to toluene. The study showed that compared to the group that is not exposed to toluene, the group that was exposed to toluene showed more DNA damage. In addition, the group of rotogravure workers who were above 51 years old showed the most damage to their DNA. Smoking habits of the individuals who were exposed to toluene also lead to an amplified level of damage to their DNA (Manikantan *et al*., 2010).

Other than being naturally present in crude oil, the colourless liquid that has a characteristic smell, toluene also occurs in *tolu* tree. Toluene is also a product during the production of gasoline and other fuels from crude oil as well as during the process of coal production from coke. As aforementioned, toluene is used as one of the starting or raw materials in the making of numerous household products, used during the synthesis of various polymers, pharmaceuticals, dyes, organic chemicals and as a solvent in some printing and leather tanning processes. Therefore, workers in occupational settings that utilize toluene in product formation such as industries that uses toluene as a solvent are exposed to toluene. However, according to Agency for Toxic Substances and Disease Registry (ATSDR, 2011), the highest concentrations of toluene frequently happen indoor arising from the use of common household products such as paints, adhesives, synthetic fragrance, nail polish and in cigarette smoke, not taking account the concentration level of toluene in inhalant abuse events (Karabulut *et al*., 2009; Agency for Toxic Substances and Disease Registry, 2011; Shih *et al*., 2011).

Inhalants are abused for their pharmacological effects on the abuser's body. The misuse of volatile substances has in general, the same outcome as its alcohol and central nervous system depressant drugs counterparts (Bowen *et al*., 2006).Going back in time, volatile substances were originally abused by the adult population during late nineteenth and early twentieth century. However recently, the trend shifted to the younger generation with children and teenagers most frequently reported being the abuser (Cairney *et al.*, 2002; Lubman *et al.*, 2006). Misuse of volatile substances has a dangerous after effects on the central nervous system and the normal developments of

psychological, emotional and neurobiological of the individual abusing it (Balster, 1998; Kurtzman *et al.*, 2001; Bowen *et al.*, 2006; Lubman *et al.*, 2006).

A national annually held survey called Monitoring the Future Study of the 8th, 10th and 12th graders by the University of Michigan that began in the year 1992 found that the youngest participant, the 8th graders reported of 20.5% inhalant abuse during their lifetime while the 10th graders reported 18.3% and the 12th with 15.2% in the year 1998. The inhalant abuse is so popular amongst this young generation that inhalant abused substances ranked the most commonly abused substance along with drugs such as nicotine, alcohol and marijuana (University of Michigan, 1998). A more recent review of a lifetime prevalence by the study demonstrated similar results (University of Michigan, 2011). In another review of inhalant abuse trend among teenagers that are between the ages 12 and 17 years old also follow the same tune. The results illustrated an increment in each year on the risk of starting the misuse of inhalants across all age and gender (Neumark *et al*., 1998).

Amongst all drug abuse problems, inhalant abuse is the one problem that mankind has most little knowledge about and far less solutions on how to go about it. This is also true on the little knowledge available about the nature and level of severity the problem had reached even though it is widely known that the chemicals found in common and legal household products are mostly abused (Balster *et al.*, 2009). There are a number of words that define inhalant abuse in accordance to the different ways of the administration of vapours into the abuser's body. The terms most frequently used are 'sniffing', 'snorting', 'huffing', 'bagging' and 'spraying' (Balster *et. al*, 2009). All of the terms generally refer to the act of repeatedly inhaling chemical vapours to get 'high' (euphoric state) or altered mental states (Lubman et. al, 2006). Whereas according to the definition by DSM-IV (American Psychiatric Association, 2000), inhalant abuse is categorized in the same classification characteristics as other substance abuse but without the withdrawal syndrome that usually accompanies other substance abuse. International Classification of Diseases 10th Edition (ICD-10) the 2007 version also

classifies inhalant abuse in the same manner as the DSM-IV, which is the dangerous and dependence on volatile substances (World Health Organization, 2005).

There is countless number of chemicals that make up any particular inhalant product leading to the diverse types of what the abusers call as the 'high' experience resulting from abusing them (Takagi *et al.*, 2010). Generally, the respondents in a study (MacLean, 2005; MacLean, 2008) frequently stated that inhaling volatile substances as a very gratifying experience. Some studies (Brust, 1993; Dinwiddie, 1994; Broutte, 2001) have portrayed the motivation behind inhalant abuse as to reach the same intoxication experience as alcohol could offer. However, MacLean (2008) reported in his findings that the intoxication experiences stated by inhalant abusers are noticeably different from those usually reported from intoxication of alcohol.

During the misuse of inhalants, the abusers typically inhale vapours repeatedly up to 20 times over a short time span usually within 10 to 15 minutes. Depending upon the types of compounds inhaled, the intentional inhaling of volatile substances over a very brief period will results in very high concentrations of the substances entering the body, up to 4600 ppm (Bowen *et al.*, 2006). Much like the abuse of most drugs, inhalant abuse also depicts the need for higher concentration to reach the same 'high' or euphoria each time. 'Sniffing' is generally the first step in inhalant misuse where the abuser directly inhales the vapour from its container or sniffing it from a piece of clothing smeared or sprayed with the substance. When the need for higher concentrations arises, the abuser would hold a piece of fabric drenched with the substance over their mouth to breath in more vapours, known as 'huffing'. Finally, even higher concentrations of vapour is made available via breathing vapours from a plastic or paper bag that holds the substance of abuse, called 'bagging' (Henretig, 1996).

Most regularly reported abused inhalants such as toluene and xylene have been shown to be neurotoxic which is connected to cognitive deficits such as impulsive decision making and executive control deficits (Yucel *et al.*, 2008). More alarmingly,

reports have shown that inhalants are among the first drugs the young abuse (Dinwiddie, 1994; Young *et al*., 1999) and at the top for abusing it throughout their lives are 12 year olds (Lubman *et al.,* 2006; White and Hayman, 2006) during which time a very important stage of neurodevelopment is taking place. Thus, when inhalants are abused at the young age, most likely the negative cognitive impacts will be very big (Paus, 2005).

The negative consequences related to inhalant abuse are wide and it ranges from respiratory effects, functional alterations of the central nervous system, mucous and dermal inflammations, to abnormality of the chromosomes (Harkonen *et al.,* 1978; King *et al.*, 1981; Otto *et al.,* 1992; Uchida *et al.,* 1993; Chen *et al.,* 1994; Molhave *et al.*, 1997). Despite this, inhalants abuse is a worldwide problem that has 12 year olds adolescents as the most avid user (White and Hayman, 2006; Wu and Ringwalt, 2006; Medina-Mora, 2008). Moreover, a small fraction of them will continue abusing inhalants through to their young adulthood (Lubman *et al*., 2006; White and Hayman, 2006).

In a study by Uzun and Kendiril (2005), the solvents in thinners and glues were the inhalants of choice among inhalant abusers. The study's aim was to show the negative consequences volatile abuse especially toluene on the central and peripheral system. A series of test was conducted on two groups of adolescents, one of which being the control group while the other being inhalant (glue and thinner sniffers) abusers. The final results showed distressing impact on the abusers. In almost the entire test, the group that was abusing toluene statistically significant different in performance compared to the control group and in some cases showed scores under the limit value. Also, declining orientation, attention, learning, calculation, abstraction, information, constriction and memory functions were also observed. Furthermore, it was shown that toluene has negative impact on brainstem connection even in early stages of toluene abuse (Uzun and Kendiril, 2005).

In an experiment to ascertain the relationship between neurological behaviour and exposure to toluene, Shelton (2007) exposed mice to toluene. As a result, in a time-

dependent manner, the mice could distinguish between toluene and isoflurane and ethylbenzene after being trained. In a different experiment, abused inhalants depicted discriminative stimulus effects that are also shown by ethanol, oxazepam and pentobarbital which are all under the category of CNS (central nervous system) depressants. Cross-sensitization between inhalants of abuse and other types of drugs were shown to be most likely due to the fact that inhalants has similar characteristics to a vast amount of drugs including phencyclidine (Bowen *et al*., 1999) and amphetamine (Bowen, 2006) and toluene may someday replace them as drug of abuse.

Other than neurotoxic and cardiotoxic alterations, administrations of toluene in long or short term also have other negative effects on the body. Von *et al*. (1993) showed via an experiment that exposure to toluene even at low levels showed major alterations in the autonomic nervous system that is specifically related to the production of catecholamine and 5- hydroxytryptamine in brainstem and the hypothalamus (Soulage *et al*., 2004; Berenguera *et al*., 2003). In addition, the amendments were shown to be gender dependent. Moreover, Murata *et al*. (1999) illustrated that carcinogenesis and reproductive dysfunction may arise from administration of toluene in short and long-term exposure cases.

In an effort to monitor the ever rising number of inhalant abusers in Malaysia, a colour test to detect the presence of hippuric acid as a metabolite in urines of inhalant abusers was developed in a study by Zainalibdin and Yacob (2009). Via the research, a rapid colour test and resulted in the production of Glue Sniffing Kit or G.S Kit. The test utilizes pyridine and benzenesulfonyl chloride as the reagents to be added directly to the urine samples. Red colour of different hues in accordance to the different concentrations of hippuric acid will develop upon positive reaction (Zainalibdin and Yacob, 2009). The following figure was the suggested chemical equation of a positive equation from the study.

The study by Zinalibdin and Yacob (2009) is a major step forward in the battle between human and inhalant abuse but the reagents used for the colour indicator are both harmful to humans. Pyridine is not found naturally except in the leaves and roots of belladonna (*Atropa belladonna*) (Taufel *et al*., 2005) and in marshmallow (*Althaea officinalis*) (Tang *et al*., 1983) thus it is not plentiful. Moreover, pyridine has negative consequences to the human body if it is inhaled, swallowed or absorbed through the skin (Aylward, 2008). Acute intoxication of pyridine presents itself as dizziness, headache, lack of coordination, nausea, salivation and loss of appetite which then may develop into abdominal pain, pulmonary congestion and unconsciousness in the patient (IARC, 2000).

Along with hippuric acid as its major metabolite, toluene is also converted into three other metabolites which are *ortho*-, *para*- and *meta*-cresol (Pierce *et al*., 2002). The American Conference of Governmental Industrial Hygienists listed unmetabolized toluene in blood and hippuric acid and *ortho*-cresol in urine as the biological markers of toluene exposure (ACGIH, 2003). According to Stutaro *et al*. (1989), toluene is conjugated with glycine to hippuric acid before it is excreted via urine and only a very small amount of unmetabolized toluene. Thus, monitoring of exposure to toluene is done most commonly using hippuric acid's amount present in urine (Toftgard and Gustafsson, 1980).

Better understanding about the importance of hygiene in occupational settings and the lowering of toluene contents in solvents has instigated the revision of safe exposure limit. The safe exposure limit has been reduced to half of the original set concentration, from 100 ppm to 50 ppm (ACGIH, 2006; Kawai *et al*., 2007). Most countries in the world have also followed the 50 ppm safety limit including Malaysia (ACGIH, 2007). Hence, more specific biological indicator is needed to reflect the lower concentration of toluene limit to be detected (Kawai *et al*., 2007).

The choice of biological indicator has been shown to be associated to the concentration of toluene in question. For instance, Ukai *et al*. (2007) demonstrated that urinary hippuric acid level reflects the level of toluene in linear fashion only when exposure to toluene in high concentrations. This is also the case as shown in an earlier study by Duydu *et al*. (1999) that proved validity of hippuric acid as biomonitoring marker only in cases of exposure to high levels of toluene. However, the poor correlation between hippuric acid and level of toluene exposure when the individual was exposed to low concentrations of toluene was found rendering the suitability of hippuric acid as an indicator to be inferior to *ortho*-cresol (Ukai *et al*., 2007). Numerous reports have also shown that *ortho*-cresol has more specificity compared to hippuric acid because it is not easily influenced by ingested food (Bazzano *et al*., 1994; Villanueva *et al*., 1994; Kawamoto *et al*., 1996; Hotz *et al*., 1997). For example, acidic foods such as berries, plums, prunes and sodium benzoate which are used as preservatives in some food may render analytical value of hippuric acid as toluene exposure indicator not reliable (Sturaro *et al*., 1989).These foods along with aspirin will produce positive result in hippuric acid test in urine (Frederick, 1994).

1.2 Statement of problem

Toluene has been shown to have adverse effects to individuals exposed to its vapour including in infants, anomalies during the development of their central nervous system (CNS) which then lead to abnormalities in the CNS itself (Chen *et al*., 1994) and it is also may be associated with cancer (Persson *et al*., 1993). Thus, monitoring the degree of exposure to toluene of these individuals particularly workers working under conditions that need them to be exposed to toluene vapour almost every day is essential for the evaluation of their health.

According to the World Health Organization (WHO) (2000), following inhalation, toluene is predominantly excreted in the urine as, hippuric acid.

Approximately 7 – 20 % of absorbed toluene is eliminated in exhaled air unchanged. However, in the case of a single acute exposure, toluene and its metabolites are almost completely eliminated within 24 hours. Like most of other solvents, toluene is also metabolized in the liver before leaving the body via urine. Hence, the levels of toluene metabolites present in the urine reveal the amount of toluene inhaled (Imbriani and Ghittori, 2005).

According to Lee *et al*. (2009), toluene will undergo either oxidation and conjugation or hydroxylation before leaving the body with urine. Conjugation and oxidation of toluene in the human body will produce hippuric acid while hydoxilation will produce *ortho*-, *meta*- and *para*-cresol (Lee *et al*., 2009). Figure 1.1 shows toluene and its possible metabolites in urine.

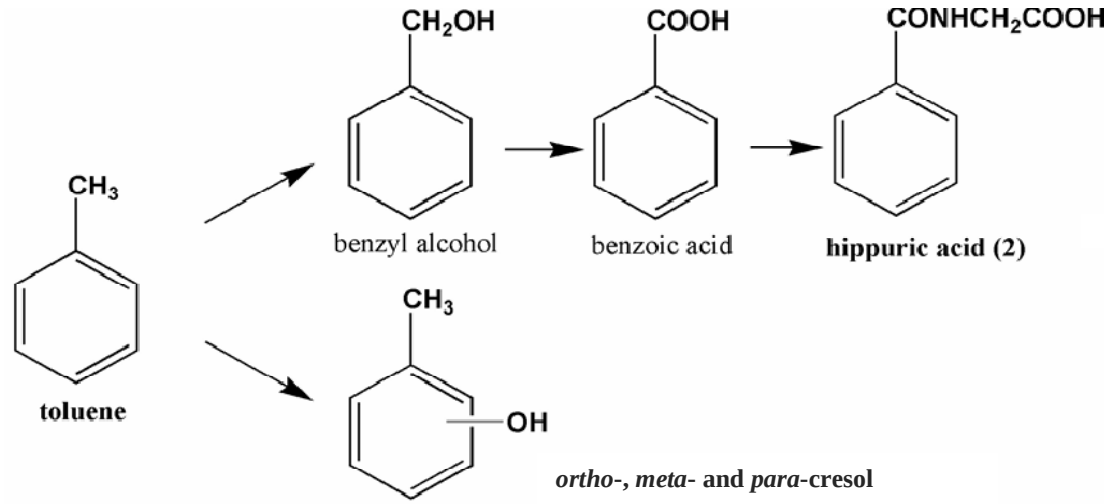

Figure 1.1: Toluene and its metabolites via two possible metabolism routes in the human body (Source: Lee *et al*., 2009)

One of this study's aims is to develop a colour test that utilizes *ortho*-cresol in urine as the determining factor of exposure to toluene in occupational settings. Numerous studies have shown the superiority of *ortho*-cresol as the marker for toleuene exposure over hippuric acid, the traditional indicator most commonly used (Sturaro *et*

al., 1989; Bazzano *et al*., 1994; Frederick, 1994; Villanueva *et al*., 1994; Kawamoto *et al*., 1996; Hotz *et al*., 1997; Ukai *et al*., 2007). The researcher also intends to construct a simple in-situ test kit which could be used as a direct on-spot urine test for evaluating exposure to toluene in work places. Other than that, the resulting kit may also provide assistance to anti-drug agencies in screening for individuals suspected to be abusing toluene.

Occupational exposure to toluene has gained the attention of health monitoring agencies that are concerned of the health of the workers exposed to industrial toluene recently. Around the world, the limit of exposure to toluene is being revised as extensive researches on the negative impacts to the health of individuals exposed to toluene in occupational settings have been demonstrated. As the exposure limit has been revised, the need for a more specific biological indicator is needed and many studies have illustrated *ortho*-cresol to be one of the best candidates. There are factories in Malaysia that produce and use toluene during manufacture and therefore the need for a study on the biological monitoring of toluene exposure in Malaysian setting. Thus, the colour test developed in the study will then be a step toward that direction. The workers will only need to provide urine samples and the results will be known immediately as the result of the colour test could be established instantly therefore creating a rapid biological monitoring system.

The planning of strategies for drug abuse deterrence, education, treatment or management is hampered due to fact that the act of abusing inhalants is not categorized in a specific class of drug (Balster *et al*., 2009). Malaysia is also currently facing the same scenario. Here in Malaysia, although recognized as having the same after effects as other drugs of abuse, PEMADAM does not classify inhalants as one of drugs of abuse (www.pemadam.org.my, obtained on June 2nd, 2011). Due to this fact, it is hard for the local authority to take further act on the abusers as there is no specific law against them. The current study focuses on the production of a method to detect a metabolite of toluene in urine; *ortho*-cresol. *Ortho*-cresol has been shown in numerous previous studies to be superior to hippuric acid in terms of its stability and is not influenced by

dietary intake as hippuric acid. The results from the research will be an advantage to many agencies especially the police and anti-drug agencies. Other than that, the study will also benefit schools and the Malaysian government in the long-term as it will help in the effort of reducing the number of toluene abusers and if possible, put an end to the misuse of inhalants.

1.3 Objectives of the study

The followings are this study's objectives:

i. To chemicalcharacterize *ortho*-cresol standards and spiked samples as in control urine using UV-Vis spectrophotometry.
ii. To assemble a test kit for a colour test that uses chemical reaction specific to *ortho*-cresol.
iii. To apply the test kit prepared to real urine samples obtained from workers exposed to toluene.

1.4 Scope of the Study

The first stage of the study is to determine and consequently develop a colour test that utilizes chemicals that are non-carcinogenic and are usually found in laboratories. Chemicals such as acids and salts that are essential to any laboratories will be reacted with *ortho*-cresol to observe if there is any colour change. Next, the reagents that produce colour change upon reaction with *ortho*-cresol will then be reacted with different concentrations of *ortho*-cresol and finally, the colour change will be evaluated, if any, during reaction between the chemicals and *ortho*-cresol in urine.

The second stage of the study will commence with the optimization of the colour test determined in the first stage. The optimization will include the establishment of detection limit using instruments including UV-Vis and observation using naked eyes to observe the colour produced as the end result. Following optimization, urine samples of in a toluene producing factory will be tested with the newly developed colour test. Confirmation test will follow suit using Ultra-Violet Visible Spectrophotometry (UV-Vis).

1.5 Significance of Study

The development of a colour test for the detection of *ortho*-cresol in the urine of workers exposed to toluene during their working hours will be a big step forward in the development of a biological indicator for toluene exposure in Malaysia especially and around the world generally. *Ortho*-cresol has been shown in numerous studies to be more reliable as a metabolite in comparison to previously used marker for toluene exposure - hippuric acid. Thus, at the end of this research, an essential kit will be produced which could be prepared in the laboratory and could be carried to the place where the test need to be done, or in other words, mobile. In addition to using reagents that are easily found and non-carcinogenic, the test also will produce result rapidly.

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