Occupational Exposure and Biological Monitoring

Abstract

Personal monitoring of the service station workers in five busy petrol pumps in Kolkata was done to study their occupational exposure towards the mono-aromatics along with the ambient air quality in these sites. Toluene (ranged between 49.3 - 236.8 μg m$^{-3}$) was found to be the most abundant hydrocarbon followed by benzene (ranged between 17.4 - 81.6 μg m$^{-3}$). The levels of personal exposure for benzene and toluene were 3.9 and 5.5 fold higher respectively than the ambient air. The occupational exposure of petrol pump workers were found to be 1.5 and 3.5 fold higher for benzene and toluene respectively when compared to those of car drivers.

Apportionment of target species into their immediate sources was done by the method of chemical mass balance (CMB). Exhaust from roadway and refueling were found to be two major contributing sources towards total VOC level.

Concentration of trans,trans-Muconic Acid (t,t-MA) was measured as biomarker of benzene exposure in urine of two occupational groups namely petrol pump workers and car drivers and compared with an environmentally exposed positive control group. The mean urinary t,t-MA levels for petrol pump workers, car drivers and control groups were 145.4±55.3, 112.6±63.5 and 60.0±34.9 μg g$^{-1}$ of creatinine respectively. Attempt has been made for the first time to estimate the internal dose or body burden by measurement of urinary t,t-MA using physiologically based pharmacokinetic model as a tool. The weight adjusted total body burden of petrol pump workers and the car drivers were found to be higher than the control group by 3.52 and 2.22 fold respectively.
5.1 State of Art

Occupational exposure is a measure of the intensity and/or extent to which the human body experiences a particular hazard while during workshift in a definite occupation. For a given hazard, the greater the exposure the greater is the risk of an adverse effect on health. The importance of occupational exposure study is two fold:

1. Such study concerns the health condition of a group of workers involved in some specific profession and exposed to some specific hazardous compound or group of compounds in the course.

2. There are many toxic compounds relevant to some specific occupational environment, that may also be present in ambient air but at such a lower level that no apparent health manifestation is prominent at ambient level.

Occupational exposure study in those cases can provide valuable information which can be extrapolated to predict or assess the effects at a lower ambient level which can affect a wider population. The studies involve two simultaneous examinations complementary to each other; 1) personal exposure monitoring and 2) biological monitoring.

The former determines the level of the toxin of concern, at which the workers are being exposed while the latter helps to understand the metabolism, fate and effect of the toxins actually caused due to the exposure. Compared to large volume and varieties of studies carried out in the developed world, exposure assessment studies in developing countries are relatively scarce.

In previous chapters it has been established that the urban ambient atmosphere of Kolkata is laden with elevated levels of mono-aromatic hydrocarbons mainly BTEX. Vehicular exhaust emission is shown to be the predominant source of such VOCs. The
general public of Kolkata may be assumed to be suffocating with huge emissions of these toxic pollutants. Due to the preponderance of use of traditional fuels the conditions of the inmates of residences are no better. Traffic sector in Kolkata has been identified as the hot spot of VOC emission and people associated with traffic related occupations may be considered as the worst sufferer. Thousands of professionals like traffic policeman, car drivers, workers at the petrol pumps etc., are the persons who are exposed heavily to these pollutants during their work hours.

Among different traffic related activities, refueling of motor vehicles is a major source of benzene and other VOCs in the atmosphere. Huge numbers of vehicles in the city street require regular refueling and a large number of workers are associated with this refueling industry. Those workers at the petrol pumps are involved in handling various petroleum products during pumping and serving of fuels. Refueling station attendants are occupationally exposed to higher level of petroleum related VOCs compared to other traffic oriented occupations like drivers, traffic polices or street vendors (Bahrami et al., 2007, Han et al 2005, Romieu et. al. 1999). The ambient level of VOCs in petrol pumps as well as the level of exposure of the workers towards the VOCs is largely dependent on various activities in and around the premises such as petrol refueling procedure, exhausts from the vehicles entering the premises, fugitive emission from storage facilities, use of emission control technology like vapour recovery system and also on technical specifications of gasoline, especially its benzene content (Periago and Prado, 2005). Two worker groups in this class, namely passenger car drivers and petrol pump workers and a control group consisting of some office workers were chosen for exposure assessment towards certain VOCs.

Benzene exposure has been related in numerous occupational studies to increased risk of aplastic anaemia, myelodysplastic syndromes, and acute non-lymphocytic
leukaemia (IARC, 1982; IARC, 2002). Chronic (long-term) inhalation exposure to benzene has been reported extensively to cause various blood disorders (ATSDR, 1997; USEPA, 2002). Apart from use of benzene as additive in gasoline, it was extensively used in different industrial processes and also as solvent till 1980s. Progressive elimination, replacements, and use restrictions, when possible, or the introduction of closed cycles to protect worker health in industrial and manufacturing workplaces, and the banning from the commercial products, have ensured that health effects due to high concentration levels do not occur nowadays (Fondelli et al. 2008) in most of the developed nations. It has become one of the major threats to public health because of its acute carcinogenicity (Rinsky et al., 1981; IARC, 1982) as much that no safe exposure level could be recommended. Even the environmental benzene exposure in typical urban area is not negligible (Johnson et al., 2007).

After uptake via inhalation pathway, benzene is distributed in various body compartments and a portion is subsequently metabolized in the liver which is excreted through urine. Such distributions are based on the underlying mechanisms by which a toxic substance is absorbed, transported, and metabolized in the primary target organs and can be effectively expressed using Physiologically Based Pharmaco-Kinetic (PBPK) models (EOHSI, 1997; Sherwood et. al., 1999; Thomas et. al., 1996. Bois et al., 1991; Travis et al., 1990)

The metabolism of benzene in human physiology is complex. Benzene is initially converted to benzene oxide by oxidation with cytochrome P4502E1 (CYP2E1), which may form ring-hydroxylated metabolites such as phenol, which is subsequently converted to hydroquinone and catechol, or reacts with glutathione (GSH) to form premereapturic acid which ultimately converted to S-phenylmercapturic acid (SPMA). Alternatively, benzene oxide is converted to epoxepin via oxepin, which is metabolized, by ring
opening, to muconaldehyde and ultimately to \textit{trans-trans} muconic acid (t,t-MA) which is discarded from the body through urine (ATSDR, 1997; Karam et al., 1989; Kim et al., 2006; Mark et al., 2001; Rothman et al., 1998; USEPA, 2002; Witz et al., 1989). Figure 5.1 describes the metabolic pathways of benzene in human physiology.

![Figure 5.1 Metabolic activation pathways of benzene](image)

**Figure 5.1 Metabolic activation pathways of benzene**

Urinary \(t,t\)-MA is widely recognized as a biomarker of benzene (Paula et al., 2003; Raghavan, and Basavaiah, 2005; Weisel et al., 1996; Wiwanitkit et al., 2003; Yu, and Weisel, 1996). The American Conference of governmental Industrial Hygienists (ACGIH) introduced \(t,t\)-MA as biological exposure index for benzene exposure (ACGIH, 2003).

The total amount of a chemical substance present in an organism at a given time is known as body burden (Herber et al., 2001). The Agency for Toxic Substances and
Disease Registry (ATSDR) considers data regarding human body burden of chemicals to be valuable and essential in tracking levels of chemicals in the environment and in human populations and also for the derivation of health-based guidance values (Pohl, 2007). The potential health risks (both carcinogenic and non-carcinogenic) from pollutant can be estimated more efficiently from measurement of body burden than exposure. (Bailer and Hoel, 1989; Herber et al., 2001). Lifestyle of a person (smoking, drinking and eating habits, exercise etc.) can influence the effective dose of a chemical. Many organic substances, drugs, including prescribed medicines may change the body’s ability to metabolize toxic chemicals (Herber et al., 2001).

To determine the body burden of benzene, its concentration is directly measured either in the arterial blood (Berlin, 1985; Brugnone et. al., 1999, Giardino and Wireman, 1998) or in the exhaled breath (Wallace and Pellizzari, 1995, Wallace et. al 1996) of an exposed person. No attempt has so far been made to estimate the body burden by measurement of urinary metabolites. Excretion of metabolites through urine and residence of benzene in other body compartments can be related by simple equations using PBPK models. Urinary metabolite concentration is easy to measure and may be used for indirect estimation of benzene in different body compartment as well as its total body burden. Collection of urine sample is far less insidious than blood sampling; moreover urine samples are less sensitive to storage than blood samples.

The present study deals with the measurement of ambient levels of some mono-aromatic hydrocarbons and carbonyls in five refueling stations of Kolkata. The occupational exposures of the filling workers towards mono-aromatics were also studied. Chemical mass balance (CMB) receptor models have been used to apportion different fractions of VOCs according to immediate source-contributions.
Urinary \( t,t \)-MA has been measured in two occupational groups, namely petrol pump workers and car drivers as well as in a positive control group exposed through inhalation to different level of benzene. An attempt has been made for the first time to estimate the total body burden resulting from the exposure, utilising urinary measurement of \( t,t \)-MA based on the understanding of the distribution, metabolism and elimination of benzene in human physiology using PBPK model.

This study will provide information about internal dose resulted due to inhalation exposure from measurement of urinary metabolite that can be useful in verifying exposures and dose estimated from blood benzene or exhaled benzene concentration following conventional method. Moreover, this method may facilitate in reducing uncertainty in exposure and health risk assessed than the same assessed from environmental concentrations (EOHSI, 1997).

5.2 Experimental Methodology

The study of occupational exposure of petrol pump workers was conducted in five busy petrol pumps in Kolkata, representing North, South, Central, East and West part of the city during December 2005 to February 2006. All the service stations are situated on roads with high traffic volume and are considered busy refueling \(-50\) vehicles per hour during peak time.

Static sampling was performed for both mono-aromatic hydrocarbons and the carbonyls \( (n = 27, \text{ for each group of pollutants}) \) within 5 to 10 m radius of the fuel outlets at a height of about 1.5 m from ground level. Sampling of mono-aromatic hydrocarbons was done similarly as personal exposure monitoring keeping the pump at a fixed position for the whole sampling period of 8 hrs.
5.2.1 Subject Selection

Petrol pump workers and drivers were selected as two exposed groups and office workers, mostly engaged in desk job, were selected as positive control group (non-smoker and only exposed environmentally) for biological monitoring purpose.

The study group from petrol pump workers was non-smoker subjects chosen from the workers in the service stations engaged only in refueling work. 6-8 persons in each station were selected for personal exposure assessment.

Among the drivers (non-smoker), personnel driving either petrol driven cars fitted with catalytic converter or diesel driven cars were selected for this study.

5.2.2 Exposure monitoring

For personal exposure monitoring of mono-aromatic hydrocarbons, air was drawn at a rate of 100 ml min\(^{-1}\) from 6 am to 2 pm (for a full work shift of 8 hrs) through two charcoal sorbent tubes each of four hours duration with a low volume personal sampler (SKC Inc, USA) attached to the waist of the petrol pump worker. The tube held in a low flow holder was clipped to the attire of the worker close to the personal breathing zone as far as possible (NIOSH, 1997. A total of 70 samples (two for each worker) were collected from 35 petrol pump workers for personal exposure measurement during a 8 hr work shift (\(n = 70\)).

The detailed procedure of personal exposure assessment of car drivers is described in details in Chapter 4 Section 4.2.1. A total of 35 samples were collected (\(n=35\)).

Personal exposure measurement of control group was done during working hours, similarly as done for petrol pump workers. Altogether 27 samples were collected (\(n=27\)).
5.2.3 Biological sample collection:

25 non-smoker volunteers from each exposed and control group were recruited with informed consent for biological monitoring purpose. Individual data such as age, body weight, height etc., were obtained by questionnaires (Appendix II) at the time of recruitment. Spot urine samples were collected in sterilized polyethylene bottles in the second half of the eight hour work shift at free will during the last two working days of the week and put in a refrigerator at -20°C immediately after collection.

5.3 Result and Discussion

5.3.1 Ambient levels and personal exposure of VOCs

Figure 5.1 and Figure 5.2 represent the mean static level concentrations of mono-aromatic and oxygenated hydrocarbons respectively in ambient air at the petrol pumps in Kolkata. Among the mono-aromatics, toluene was the most abundant (49.3 - 236.8 µg m⁻³) followed by benzene (17.4 - 81.6 µg m⁻³).

Personal exposure measurement was made for only Benzene, Toluene, Ethylbenzene and Xylenes (BTEX), which showed that the workers were exposed to toluene level ranging from 210.4 to 1536.0 µg m⁻³ while benzene varied between 58.2 - 253.3 µg m⁻³. The mean personal exposure values for BTEX obtained in the present study (137.5 µg m⁻³ for benzene, 643.6 µg m⁻³ for toluene, 118.0 µg m⁻³ for ethylbenzene, 209.7 µg m⁻³ for m-p-xylene and 68.2 µg m⁻³ for o-xylene) were slightly more than those reported by Han et al, 2005 in Trujillo, Peru (111, 254, 43 and 214 µg m⁻³ for BTEX respectively) but comparable to the winter exposure levels reported by Bono et al., 2003 in Northwestern Italy (geometric mean for BTX were 161, 568 and 285 µg m⁻³ respectively). Higher reactivity of toluene resulted in the decrease in average
toluene/benzene (T/B) ratio from 4.7 in personal exposure measurement to 3.5 in static measurement.

Figure 5.1: Static level of mono-aromatic

Figure 5.2: Static level of oxygenated hydrocarbons

*Personal Communication (C. Dutta), Department of Chemistry, University of Calcutta

Figure 5.2: Static level of oxygenated hydrocarbons

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5.3.2 Comparison of exposure between Drivers and Refueling Station attendants:

In Chapter 4, the BTEX exposure levels of drivers of different types of passenger cars running with different fuels in Kolkata have been discussed in detail. The relatively enhanced BTEX exposure level of the drivers of the passenger cars in Kolkata may be due to penetration of roadway air, enriched with VOCs, into the cabin. Exposure of the refueling station attendants were compared (Figure 5.3) with those of the drivers as both the groups are exposed to similar kind of transport related pollutants though to different extents. The exposure levels of the attendants were found to be greater than those of the drivers by a factor of 1.5 for benzene and 3.5 for toluene. In similar studies, Han et al (2005) in Peru and Bahrami et al (2007) in Iran reported exposure of the service station workers were 10 and 5 times higher respectively than those of the drivers of passenger cars.

![Figure 5.3: Personal exposure levels for petrol pump attendants and car drivers](image)

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All the petrol pumps were situated within the heart of the traffic and it was found that the roadway exhaust emission was the highest contributor towards exposure of the workers in the pumps (Section 5.3.3).

5.3.3 Source Apportionment using Chemical Mass Balance

The immediate sources and their contribution to the VOCs at the petrol pump ambient air were estimated using EPA’s CMB 8.2. The details of CMB calculation has already been discussed in Chapter 3 Section 3.1.3.

The source profiles of the VOCs were taken from US EPA Speciate 3.2 database. For modeling, these profiles were normalized according to the 18 target VOC species (seven monoaromatic hydrocarbons, namely benzene, toluene, ethylbenzene, m&p-xylene (combined) and o-xylene, chlorobenzene, mesitylene, and eleven oxygenated hydrocarbons, namely formaldehyde, acetaldehyde, acetone, propanal, crotonaldehyde, methylethylketone, butanal, benzaldehyde, hexanal, octanal, decanal). All the species were observed in appreciable concentrations in all the collected samples.

Percentage source contributions to total VOCs observed at petrol stations are presented as an average of all samples in Figure 5.4. It was observed that vehicular exhaust from adjacent roadways was the highest contributor (39.0±15.7%). The hourly fleet volume on the neighbouring roads of all the petrol pumps was ~1500 - 2500 with an average traffic speed of less than 20 km hr$^{-1}$. 

Figure 5.4: Source contribution of total VOCs in refueling stations

The fleet consisted of 21% heavy vehicles, 59% medium vehicle and 20% light vehicles. The average age of passenger cars, buses and trucks in Kolkata are 10 years or above, 50% of passenger cars are more than 10 years old and 42% of 2-wheelers have 2-stroke engines. Most of the 3-wheelers (71%) are pre-1986 manufactured (ADB, 2005). The major fractions of old vehicles are poorly maintained and without emission control system like catalytic converter. The roadside exhaust emission is thus a major factor in determining the VOC level at the petrol pump ambient air. Han et al (2005) in Peru attributed traffic to be the main source of BTEX exposure though qualitatively. In this study, refueling was found to be another major contributor (36.4±17.9%). In India (including Kolkata), there is still no practice of emission control system at the petrol pumps during vending of fuels in the storage tanks. Periago and Prado (2005) reported a significant decrease in benzene level in service stations after installation of vapour recovery system. Bahrami et al. (2007) attributed refueling as the source of elevated
benzene exposure of refueling station attendants. VOC concentrations at petrol pumps in two other mega-cities of India were monitored (though no personal exposure was studied). Diesel engine exhaust and evaporative emission were found to be the highest contributor towards VOCs in Delhi and Mumbai respectively (Srivastava et. al. 2005).

On an average, about 50 vehicles per hour entered the petrol pump premises for refueling amongst which more than 80% was light duty vehicle (passenger cars, three- and two wheelers). Contributions of exhaust from light duty diesel driven as well as petrol driven vehicles, (majority of the petrol driven light duty vehicles were without catalytic converter), were also significant (10.3±5.2% and 6.5±6.4% respectively) towards the VOC level in the petrol pumps. The petrol pumps monitored in this study had automotive refinishing; repairing and washing service facilities in the adjoining garages inside the petrol pump premises. Automotive painting, repairing works (3.0±2.6%) and degreasing (4.0±4.2%) were small source contributors of the target VOCs. Fugitive emissions from the petrol storage facilities constituted a minor (0.8±1.3) source. Other sources like evaporative emissions (hot-soak and diurnal), automotive consumer products, automotive tyres, diesel internal combustion engine, although initially considered for the modeling calculation as prospective contributors, were not included in the final source composition as their contribution were found to be insignificant. Percent mass (percent ratio of the sum of the model-calculated source contribution estimates to the measured mass concentration) was found to be 80.7±18.5 % with $R^2$ value of 0.83±0.04.

The species wise source contribution as obtained from CMB model run is given in Table 5.1 for a representative sample.
Table 5.1: Source contribution by species as obtained from CMB result
(Representative Sample)

<table>
<thead>
<tr>
<th>Species</th>
<th>Calculated</th>
<th>Measured</th>
<th>AutoPnt</th>
<th>FugEm</th>
<th>Degreasing</th>
<th>Refuel</th>
<th>Exh_LDDV</th>
<th>Exh_Roadway</th>
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<td>*******</td>
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<td>0.01</td>
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<td>*******</td>
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<td>0.02</td>
<td>0.01</td>
<td>0.39</td>
<td>0.09</td>
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</table>

[NMHC - non methane hydrocarbon; BENZE - benzene; TOLUE - toluene; CLBEN - chlorobenzene; ETBZ - ethylbenzene; MP_XYL - m,p-xylene; O_XYL - o-xylene; BZ135M - 1,3,5 - trimethylbenzene; FORMAL - formaldehyde; ACETAL - acetaldehyde; ACETO - acetone; PROPAL - propanal; CROTON - crotonaldehyde; MEK - methylethylketone; BUAL - butanal; BENZAL - benzaldehyde; HEXAL - hexanal; OCTAL - octanal; DECAL - decanal]
Among the individual pollutants, the common and principal source of BTEX was vehicular exhaust coming from adjacent road. Significant amount of benzene was also contributed by fugitive emission and refueling. 1,3,5-trimethylbenzene mostly released during refueling. Automobile painting and repairing works contributed for toluene, isomers of xylene, most of acetone and some methylethylketone (MEK). Degreasing operations in the garages released significant amount of MEK along with some toluene and 1,3,5-trimethylbenzene. Automobiles entering the refueling station premises emit exhausts, which had contribution towards VOC emission level. Among these, gasoline driven vehicles that are not fitted with catalytic converter acted as significant point source towards BTEX, 1,3,5-trimethylbenzene and some oxygenated VOCs like formaldehyde, acetaldehyde and benzaldehyde. It was discussed (Chapter 4, Section 4.2.3), that gasoline driven passenger cars without catalytic converters had higher level of BTEX both inside and immediate outside the car than cars with catalytic converters. Exhaust from diesel driven light duty vehicles that enter the refueling station premises was the principal contributor of formaldehyde, acetaldehyde, propanal and benzaldehyde. Some oxygenated VOCs like acetaldehyde, benzaldehyde and hexanal etc., are poorly described by the above-mentioned sources in most cases. This may be due to the fact that CMB considers only the primary sources whereas these oxygenated VOCs are also produced as secondary pollutants in the lower atmosphere under the influence of tropospheric oxidants like ozone and sunlight.

5.3.4 Assessment of Body Burden from benzene exposure using PBPK model:

Physiologically based pharmacokinetic (PBPK) models are mathematical constructs that are used to calculate the concentrations or amounts of a chemical in body tissues and fluids as a function of time. In these models, the body is subdivided into a
series of physiological compartments that correspond to specific organs (e.g., liver, kidney, lung) or lumped tissue and organ groups (viz., fat, richly perfused, and slowly perfused tissues). The compartments are connected through blood or lymphatic circulation and chemical transfers between compartments are described by mass balance differential equations.

A four compartmental physiologically based pharmacokinetic (PBPK) model for benzene absorption, distribution and transportation was used in this study (Bernillon and Bois, 2000). As described in Figure 5.5 the human body is divided into four compartments namely, poorly perfused tissues, well-perfused tissues, fat, and liver.

![Figure 5.5: PBPK Model](image-url)
These compartments are assumed to be homogeneous and the distribution limited by blood flow. Pulmonary exchanges are assumed to be attained at instantaneous equilibrium between alveolar air, venous blood, and arterial blood. Differential equations of the form

\[ V_i \left( \frac{dC_i}{dt} \right) = Q_i (C_{\text{art}} - \frac{C_i}{P_i}) \] ............ (5-1)

describes the concentration of benzene (C_i) in each body compartment i as a function of time (t), volumetric blood flow (Q_i), volume of body compartment (V_i), arterial blood concentration (C_{\text{art}}) and partition coefficient (P_i) of benzene between body compartment i and blood. It is assumed that under a given physiological condition, C_i is dependent only on C_{\text{art}}.

Out of four body compartments considered above, there will be metabolic clearance of benzene in liver. In this case, the equation becomes non-linear and takes the form:

\[ V_i \left( \frac{dC_i}{dt} \right) = Q_i (C_{\text{art}} - \frac{C_i}{P_i}) - \frac{V_{\text{max}}C_i}{K_m+C_i} \] ............ (5-2)

where, V_i, P_i, Q_i and C_i are volume, partition coefficient, blood flow and concentration of benzene in liver respectively, V_{\text{max}} is the maximum rate of metabolism and K_m, the Michaelis-Menten constant. (EOHSI, 1997; Travis et al., 1990; Bois et al. 1996). The benzene metabolized in liver will eventually be excreted through urine and the metabolite in urine will correspond to the extent of benzene metabolism in liver which in turn is dependent on C_{\text{art}}. Arterial blood concentration, C_{\text{art}} can be estimated by solving equation (5-2).

Derivation of body burden from urinary metabolite concentration:

Metabolites are supposed to be excreted in urine from the liver of volume (V_i) by a first-order process (with excretion rate constant K_e). Excretion of metabolite in urine
may be expressed as a function of metabolite concentration in liver (Cmet\(_l\)). The amount of excreted metabolite (Amet\(_E\)) in time \(t\) is given by,

\[
Amet_E = V_l \int_0^t \left( \frac{dCmet_E}{dt} \right) = K_e V_u Cmet_l t
\]

\[\text{...... (5-3)}\]

where, \(V_u\) is the volume of urine and \(Cmet_E\) is the concentration of excreted metabolite. The metabolite excreted from liver is equal to the metabolite obtained in urine with urinary metabolite concentration of \(Cmet_u\).

Hence,

\[
K_e V_u Cmet_l t = Cmet_u V_u
\]

\[\text{...... (5-4)}\]

It is reported that in exposure level below \(< 100\) ppm, the amount of metabolite excreted as \(t\,t\)-MA (\(f_{tt\text{-MA}}\)) is only 21% of the total metabolite (Weisel et al., 1996). From equation 5-4, total metabolite concentration in liver can thus be calculated from the urinary concentration of \(t\,t\)-MA (\(C_{tt\text{-MAu}}\)).

\[
Cmet_l = \frac{C_{tt\text{-MAu}}}{0.21 K_e t}
\]

\[\text{...... (5-5)}\]

The formation of metabolite in liver follows the first order rate equation. At equilibrium, the formation of metabolite in liver will be at endogeneous metabolite formation rate \(K_f\) and the amount of metabolite formed in liver (Amet\(_F\)) will be given by,

\[
\frac{dAmet_F}{dt} = K_f
\]

\[\text{...... (5-6)}\]

The metabolite present in liver at any given time \(t\) is given by:

\[
Cmet_l V_l = Amet_F - Amet_F
\]

\[\text{...... (5-7)}\]

\(K_f\) can be derived from equations 5-3, 5-6 and 5-7 as,

\[
K_f = Cmet_l \frac{V_l + (V_u K_e t)}{t}
\]

\[\text{...... (5-8)}\]

Now, the rate of formation of metabolite in liver is equal to the rate of clearance of benzene from liver as described by the Michaelis-Menten term in equation (5-2),
\[ \frac{dA_{\text{met}}}{dt} = -\frac{dA_{\text{benz}}}{dt} = \frac{V_{\text{max}}C_i}{(K_{m_i} + C_i)} \]  \hspace{1cm} (5-9)

where, \( A_{\text{benz}} \) is the amount of benzene in liver. From equations 6 and 9, the value of \( C_i \) is found to be:

\[ C_i = \frac{K_fK_{m_i}}{(V_{\text{max}i} - K_f)} \]  \hspace{1cm} (5-10)

It is assumed that the benzene PBPK model will be approximately linear as long as inhalation exposure concentration \( << 100 \) ppm (EOHSI, 1997) and thus in the metabolite excretion term in equation 2, \( (K_{m_i} + C_i) \) is replaced by \( K_{m_i} \).

Integrating equation 2 for the interval \( t = 0 \rightarrow t \), the value of \( C_{\text{art}} \) is obtained as:

\[ C_{\text{art}} = C_i \frac{V_i}{Q_i} (1 + \frac{Q_i t}{V_i P_i} + \frac{V_{\text{max}i} t}{K_{m_i} V_i}) \]  \hspace{1cm} (5-11)

Once the value of \( C_{\text{art}} \) is known, the benzene concentration in other compartments at time \( t \) can be calculated by integrating equation 1 for the interval \( t = 0 \rightarrow t \),

\[ C_i = \frac{Q_i P_i C_{\text{art}}}{(V_i P_i) + (Q_i t)} \]  \hspace{1cm} (5-12)

The Total Body Burden (TB) at any time \( t \) is defined by:

\[ TB = \sum_{i}^{n} C_i V_i \]  \hspace{1cm} (5-13)

The weight adjusted body burden of an individual with a body weight, \( BW \) is given by,

\[ TB_{\text{WA}} = \frac{TB}{BW} \]  \hspace{1cm} (5-14)
Table 5.2: Physiological Parameters used in PBPK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Abbreviation</th>
<th>Scaling Factor (SC)</th>
<th>Function/Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean Body Mass</td>
<td>kg</td>
<td>LM</td>
<td>---</td>
<td>BW(^1) - (V_f \times D_f)</td>
</tr>
<tr>
<td>Density of Fat tissues</td>
<td></td>
<td>D_f</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>Volume of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well perfused tissue</td>
<td>litre</td>
<td>V_{wp}</td>
<td>0.23</td>
<td>SC \times LM</td>
</tr>
<tr>
<td>Poorly perfused tissues</td>
<td>litre</td>
<td>V_{pp}</td>
<td>adjusted(^2)</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>litre</td>
<td>V_f</td>
<td>0.25</td>
<td>SC \times BW/0.92</td>
</tr>
<tr>
<td>Liver</td>
<td>litre</td>
<td>V_l</td>
<td>0.031</td>
<td>SC \times LM</td>
</tr>
<tr>
<td>Blood</td>
<td>litre</td>
<td>V_B</td>
<td>5.5 (^3)</td>
<td></td>
</tr>
<tr>
<td>Blood Flow in</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well perfused tissue</td>
<td>litre min(^{-1})</td>
<td>Q_{wp}</td>
<td>0.34</td>
<td>SC \times V_{wp}</td>
</tr>
<tr>
<td>Poorly perfused tissues</td>
<td>litre min(^{-1})</td>
<td>Q_{pp}</td>
<td>0.041</td>
<td>SC \times V_{pp}</td>
</tr>
<tr>
<td>Fat</td>
<td>litre min(^{-1})</td>
<td>Q_f</td>
<td>0.029</td>
<td>SC \times V_f</td>
</tr>
<tr>
<td>Liver</td>
<td>litre min(^{-1})</td>
<td>Q_l</td>
<td>1.0</td>
<td>SC \times V_l</td>
</tr>
<tr>
<td>Partition Coefficients of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well perfused tissue</td>
<td></td>
<td>P_{wp}</td>
<td>---</td>
<td>1.0</td>
</tr>
<tr>
<td>Poorly perfused tissues</td>
<td></td>
<td>P_{pp}</td>
<td>---</td>
<td>1.7</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td>P_f</td>
<td>---</td>
<td>19.0</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>P_l</td>
<td>---</td>
<td>1.8</td>
</tr>
<tr>
<td>Maximal rates of metabolism in Liver</td>
<td>mg benzene min(^{-1})</td>
<td>V_{max}</td>
<td>5.8</td>
<td>SC \times LM(^{0.7})</td>
</tr>
<tr>
<td>Michaelis-Menton constant(^4)</td>
<td>mg benzene litre(^{-1})</td>
<td>K_{m}</td>
<td>---</td>
<td>0.35</td>
</tr>
<tr>
<td>Metabolites excretion rate constant(^5)</td>
<td>litre min(^{-1})</td>
<td>K_e</td>
<td>---</td>
<td>0.0023</td>
</tr>
<tr>
<td>(t,t)-MA fraction of the excreted metabolites(^6)</td>
<td></td>
<td>f_{AMA}</td>
<td>---</td>
<td>0.21</td>
</tr>
</tbody>
</table>

\(^1\)BW - Body Weight. \(^2\)The volume of the poorly perfused tissue is adjusted so that the sum weight of all the organs, blood and skeleton (17% of LM equivalent) equals the BW; densities of all organs are taken as 1 except for fat. \(^3\)Vander et al., 1997. \(^4\)Teavis et al., 1990. \(^5\)K_e is calculated from \(t,t\)-MA excretion half life (\(t_{1/2}\)) of 5 hr (Boogaard and Sittert, 1996) using the equation, K_e=ln2/t_{1/2}. \(^6\)Weisel et al., 1996.
Assumptions:

- The volume of urine at time \( t' \) in min (\( V_u \)) is calculated from the equation,

\[
V_u = \frac{1500}{C_u^{\text{Cr}}1400} t
\]

where, \( C_u^{\text{Cr}} \) is the measured concentration of creatinine in urine in mg liter\(^{-1}\) assuming daily creatinine excretion of 1500 mg for an adult human.

- The average time between successive micturition (emptying of bladder) is three hours.

- The concentration of \( t,t-\text{MA} \) and creatinine in urine measured in urine samples collected at free-will during the second half of the work shift corresponds to an average of 3 hours of metabolism at equilibrium.

Physiological parameters:

The calculation using PBPK model is largely dependent on physiological parameters. Considering the variability among the subjects, many of those parameters were linked to lean body mass or other parameters using scaling function (Bois et al., 1996; Mordenti, 1986; Watanabe et al., 1994). The scaling factors in the present study have been adapted from the posterior distribution of the parameter values presented by Bois et al. (1996), who has estimated such scaling factors using sophisticated population toxicokinetics technique. The physiological parameters, their scaling factors and scaling functions are described in Table 5.2.

Urinary Metabolite of benzene:

The mean inhalation benzene exposure in the present study was found to be 38.6 \( \mu g \) m\(^{-3}\) (0.011 ppm) for control group, 137.5 \( \mu g \) m\(^{-3}\) (0.043 ppm) for petrol pump workers and 97.9 \( \mu g \) m\(^{-3}\) (0.031 ppm) for car drivers (summarized in Table 5.3).
Table 5.3: Statistical data of exposure and Urinary \( t,t \)-MA of the occupational and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Exposure (( \mu g , m^{-3} ))</th>
<th>Urinary ( t,t )-MA (( \mu g , gm^{-1} , of , creatinine ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petrol Pump Worker</td>
<td>Max 253.3</td>
<td>237.5</td>
</tr>
<tr>
<td></td>
<td>Min 58.2</td>
<td>62.9</td>
</tr>
<tr>
<td></td>
<td>Mean 137.5</td>
<td>145.4</td>
</tr>
<tr>
<td></td>
<td>SD 57.2</td>
<td>55.3</td>
</tr>
<tr>
<td>Car Drivers</td>
<td>Max 273.0</td>
<td>212.4</td>
</tr>
<tr>
<td></td>
<td>Min 29.0</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>Mean 97.9</td>
<td>112.6</td>
</tr>
<tr>
<td></td>
<td>SD 62.0</td>
<td>63.5</td>
</tr>
<tr>
<td>Control</td>
<td>Max 150.9</td>
<td>133.9</td>
</tr>
<tr>
<td></td>
<td>Min 6.6</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Mean 38.6</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>SD 28.1</td>
<td>34.9</td>
</tr>
</tbody>
</table>

The urinary concentrations of \( t,t \)-MA, as shown in Figure 5.6, varied from 62.9 to 237.5 \( \mu g \, g^{-1} \) of creatinine for petrol pump workers, 28.8 - 212.4 \( \mu g \, g^{-1} \) of creatinine for car drivers and 16.7 - 133.9 \( \mu g \, g^{-1} \) of creatinine for occupationally unexposed positive control group. Both the exposed groups showed significantly elevated urinary \( t,t \)-MA level than control group (60.0±34.9 \( \mu g \, g^{-1} \) of creatinine). The level of urinary \( t,t \)-MA of control group in the present study corroborates with the level reported by Melikian et al. (1999) for non-smokers. The mean urinary \( t,t \)-MA level for petrol pump workers (145.4±55.3 \( \mu g \, g^{-1} \) of creatinine) was higher than that of car drivers (112.6±63.5 \( \mu g \, g^{-1} \) of creatinine) though not significantly.
In a similar study done in Iran (Baharami et al., 2007), urinary \( t,t \)-MA concentrations were shown to be 2.64 mg g\(^{-1}\) creatinine for petrol pump workers and 0.31 mg g\(^{-1}\) creatinine for bus drivers resulting from an exposure of 1.4 and 0.31 ppm of benzene respectively. Both were significantly higher than the control group. Another study conducted by Kim et al. (2006) reported 12.3 pmol l\(^{-1}\) \( t,t \)-MA (creatinine non-adjusted) in a Chinese population (non-smoker) exposed to 1.18 ppm of air-benzene compared to the non-smoking control group with 1.06 pmol l\(^{-1}\) urinary \( t,t \)-MA level corresponding to air benzene level of 0.003 ppm. The mean creatinine non-adjusted levels of \( t,t \)-MA in this study are 1.85, 1.17 and 0.52 pmol l\(^{-1}\) for petrol pump workers, car drivers and control group respectively.

![Figure 5.6: Urinary t,t-MA level in different exposure groups](image-url)
Distribution and body burden of benzene in human physiology:

The concentration of benzene in each body compartment is given in Table 5.4. Body fat shows the maximum concentration and burden (~58% of TB\textsubscript{WA}) of benzene owing to its high partition coefficient followed by poorly perfused tissue (~27% of TB\textsubscript{WA}) and well perfused tissue (~10% of TB\textsubscript{WA}). Arterial blood retains only ~5% of TB\textsubscript{WA} of benzene. Liver showed a minimum of 0.3% of TB\textsubscript{WA} owing to the continuous metabolic clearance.

Table 5.4: Exposure, urinary \(t,\text{t}-\text{MA}\) and body burden of benzene exposure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Petrol pump attendants</th>
<th>Drivers</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolite formation rate at equilibrium (K(f))</td>
<td>(\mu g) benzene min(^{-1})</td>
<td>12.2(± 5.2)</td>
<td>7.8(± 3.7)</td>
<td>3.8(± 1.8)</td>
</tr>
<tr>
<td>Concentration of benzene in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liver</td>
<td>(\mu g) l(^{-1})</td>
<td>1.9 (± 0.8)</td>
<td>1.2(± 0.6)</td>
<td>0.5(± 0.2)</td>
</tr>
<tr>
<td>Arterial Blood</td>
<td>(\mu g) l(^{-1})</td>
<td>9.9(± 4.3)</td>
<td>6.2(± 3.0)</td>
<td>2.8(± 1.1)</td>
</tr>
<tr>
<td>Well perfused tissue</td>
<td>(\mu g) l(^{-1})</td>
<td>9.7(± 4.3)</td>
<td>6.1(± 3.0)</td>
<td>2.8(± 1.1)</td>
</tr>
<tr>
<td>Poorly perfused tissue</td>
<td>(\mu g) l(^{-1})</td>
<td>13.6(± 6.0)</td>
<td>8.6(± 4.2)</td>
<td>3.9(± 1.5)</td>
</tr>
<tr>
<td>Fat</td>
<td>(\mu g) l(^{-1})</td>
<td>37.8(± 16.7)</td>
<td>24.0(± 11.5)</td>
<td>10.8(± 4.1)</td>
</tr>
<tr>
<td>TB\textsubscript{WA}</td>
<td>(\mu g) kg(^{-1})</td>
<td>17.6(± 7.7)</td>
<td>11.1(± 5.4)</td>
<td>5.0(± 1.9)</td>
</tr>
</tbody>
</table>

Fat acts as a sink for benzene in the body. In the National Human Adipose Tissue Survey study by the US Environmental Protection Agency (EPA), up to 0.02 ppm of benzene levels were reported in composite samples of adipose tissue derived from biopsy and autopsy specimens, routinely collected from samples from hospitals nationwide (USEPA, 1982). The study indicated that the individual susceptibility towards benzene toxicity and metabolism capacity largely depend upon physiological factors such as body
fat content. Benzene metabolism in liver is critical (Snyder and Hedli, 1996; Witz et al., 1996) for its carcinogenic and characteristic hematotoxic or other non-cancer toxic effects. The production of benzene metabolites, largely in the liver, is followed by their transport to the bone marrow and other organs (USEPA, 2002). Benzene oxide, the primary metabolite of benzene is known to form adduct with hemoglobin and serum albumin (Rappaport et al., 2002a; 2002b). Long-term chronic exposure to benzene may cause non-lymphocytic leukemia (or myeloid leukemia). The mechanism of benzene-induced leukemia in humans is not yet fully understood.

**Statistical Analysis:**

The results of one-way analysis of variance (ANOVA) test for different parameters are given in Table 5.5. The $K_f$ value increased significantly with increasing level of exposure along the study groups but the increase dose not seem to be linear. Neither the exposure level nor the urinary $t,t$-MA level of petrol-pump workers are significantly higher than car drivers while $TB_{WA}$ has statistically significant difference. This indicates that with increasing exposure level, the metabolism capacity does not increase linearly. Such non-linearity suggests that in the sub-ppm range of benzene exposure, the total body-burden is not proportional to the exposure but points to an excess health risk at higher exposure. Also, the total body burden well represents the actual internal dose through all possible exposure routes, nature of exposure and individual physiological variability due to chronic occupational and environmental exposure at varying level. Assessment of health risk from benzene exposure using body burden is discussed in Chapter 6, Section 6.4.4.
Table 5.5: Result of one way ANOVA result among groups

<table>
<thead>
<tr>
<th></th>
<th>Petrol pump attendants</th>
<th>Drivers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drivers</strong></td>
<td>2.33(&gt;0.1)(^1)</td>
<td>6.99 (0.013)(^2)</td>
</tr>
<tr>
<td></td>
<td>6.96 (0.013)(^3)</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>19.9 (&lt;0.001)(^1)</td>
<td>6.08(0.021)(^1)</td>
</tr>
<tr>
<td></td>
<td>24.45 (&lt; 0.001)(^2)</td>
<td>10.95 (0.003)(^2)</td>
</tr>
<tr>
<td></td>
<td>25.67 (&lt; 0.001)(^3)</td>
<td>13.08 (0.001)(^3)</td>
</tr>
</tbody>
</table>

F value (level of significance)

\(^1\) Urinary t-t-MA concentration (C<sub>e-t-MA</sub>)

\(^2\) Endogenous Metabolite Formation Rate at equilibrium (K<sub>e</sub>)

\(^3\) Weight adjusted total body-burden (TB<sub>WA</sub>)

5.5 Conclusion

The concentrations of the target VOCs at the petrol pumps in Kolkata were found to be sufficiently high and their occupational exposure could be a real threat to the health of the workers there. Source apportionment reveals that exhaust dispersed from adjacent roadways and refueling together contributed more than 75% towards the total VOC concentration. The emission of volatile organic compounds from both vehicles and petrol distribution systems is therefore required to be controlled in Kolkata.

Several preventive measures such as reduction of benzene content in gasoline (up to 3%) and implementation of Bharat Stage III norms (equivalent to European Union Emission Standard, EURO III) have been adopted in India during the last few years which have a positive impact to control the vehicular emission of VOCs. The implementation of technical regulations, such as introduction of Stage I and II vapour recovery systems, which are designed to reduce the vapour emissions during transfer of
gasoline at the storage tank and the refueling of small vehicles respectively is recommended.

In the present study, a new pathway has been developed to estimate the internal dose or body burden from urinary metabolite measurement using physiologically based pharmacokinetic model as a tool. The application of the method has been elucidated by indirect measurement of internal dose or body burden in human due to inhalation exposure of benzene from urinary $t,t$-MA concentration.

This method offers many advantages over the traditional method of body burden assessment involving blood or exhaled breath measurement. It is often difficult to measure the level of benzene in different organs or in tissue samples from human being. The present method offers an effective alternative for estimating the level of benzene in different physiological component. Other than simplicity and speed this method can also be extended for exposure to other compounds with known metabolic pathway.
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Computational Chemodynamics Laboratory; Environmental and Occupational Health Sciences Institute (EOHSI). a joint project of Robert Wood Johnson Medical School, and Rutgers, The State University of New Jersey.


methods, National Institute of Occupational safety and Health, Cincinnati.


