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Inhalation exposure or body burden? Better way of estimating risk – An application of PBPK model

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ABSTRACT

We aim to establish a new way for estimating the risk from internal dose or body burden due to exposure of benzene in human subject utilizing physiologically based pharmacokinetic (PBPK) model. We also intend to verify its applicability on human subjects exposed to different levels of benzene. We estimated personal inhalation exposure of benzene for two occupational groups namely petrol pump workers and car drivers with respect to a control group, only environmentally exposed.

Benzene in personal air was pre-concentrated on charcoal followed by chemical desorption and analysis by gas chromatography equipped with flame ionization detector (GC-FID). We selected urinary *trans*,*trans*-muconic acid (*t*,*t*-MA) as biomarker of benzene exposure and measured its concentration using solid phase extraction followed by high performance liquid chromatography (HPLC).

Our estimated inhalation exposure of benzene was 137.5, 97.9 and 38.7 $\mu g/m^3$ for petrol pump workers, car drivers and environmentally exposed control groups respectively which resulted in urinary *t*,*t*-MA levels of 145.4 ± 55.3, 112.6 ± 63.5 and 60.0 ± 34.9 $\mu g\, g^{-1}$ of creatinine, for the groups in the same order.

We deduced a derivation for estimation of body burden from urinary metabolite concentration using PBPK model. Estimation of the internal dose or body burden of benzene in human subject has been made for the first time by the measurement of *t*,*t*-MA as a urinary metabolite using physiologically based pharmacokinetic (PBPK) model as a tool. The weight adjusted total body burden of benzene was estimated to be 17.6, 11.1 and 5.0 $\mu g\, kg^{-1}$ of body weight for petrol pump workers, drivers and the environmentally exposed control group, respectively using this method. We computed the carcinogenic risk using both the estimated internal benzene body burden and external exposure values using conventional method. Our study result shows that internal dose or body burden is not proportional to level of exposure rather have a non-linear relationship. At a higher exposure level such as for occupational exposure of petrol pump workers and drivers, the conventionally estimated risk is higher than risk estimated from internal body burden. Likewise, for environmental exposure the conventional risk estimation predict lower level than estimated in our study. This emphasizes the importance of body burden and to consider it as a key parameter while estimating health risk at varying level of exposure.

1. Introduction

Benzene exposure has been related in numerous occupational studies to increased risk of aplastic anaemia, myelodysplastic syndromes, and acute non-lymphocytic leukaemia (IARC, 1982, 2012). Chronic (long-term) inhalation exposure to benzene has been reported extensively to cause various blood disorders (ATSDR, 1997; USEPA, 2002).

The metabolism of benzene in human physiology is complex. Benzene is initially converted to benzene oxide in equilibrium with oxepin by oxidation with cytochrome P4502E1, which may form ring-hydroxylated metabolites such as phenol. Phenol is either excreted as it is or metabolized. The metabolism of phenol may follow two pathways, either to hydroquinone, catechol, and benzoquinone or reacts with glutathione to form mercapturic acid. Alternatively, benzene oxide is converted to benzene glycol via epoxide hydrolase, which is oxidized to trans, trans muconic aldehyde and finally to *trans*,*trans*-muconic acid (*t*,*t*-MA) and is excreted from body through urine. The carcinogenicity of benzene is largely attributed to its various metabolites. Benzeneoxide, benzoquinone, etc. are electrophiles and react with peptides, proteins and DNA. Formation of benzoquinone from hydroquinone in the bone marrow may also be responsible for the carcinogenicity. However, it

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is still not clear the role played by different metabolites in the carcinogenic nature of benzene (Snyder and Hedli, 1996; ATSDR, 1997; USEPA, 2002; IARC, 2012).

Urinary *t,t*-MA is widely recognized as a biomarker of benzene (Tunsaringkarn et al., 2011; Raghavan and Basavaiah, 2005; Paula et al., 2003; Yu and Weisel, 1996). Good correlation has been obtained between benzene exposure and urinary *t,t*-MA level (Scherer et al., 1998). The American Conference of governmental Industrial Hygienists (ACGIH) introduced *t,t*-MA as biological exposure index for benzene exposure (ACGIH, 2003).

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 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 (Knutsen et al., 2013; Haddad et al., 2001; Sherwood and Sinclair, 1999; Bois et al., 1991).

Exposure assessment and dose estimation for any toxic pollutant is usually based upon environmental monitoring and human time–activity data. The Agency for Toxic Substances and Disease Registry (ATSDR) considers data regarding 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 et al., 2007). The potential health risks (both carcinogenic and non-carcinogenic) from benzene exposure through inhalation can effectively be estimated from internal dose or body burden (Lovreglio et al., 2011; Bailer and Hoel, 1989). Determination of body burden of benzene exposure is conventionally done by directly measuring benzene either in the exhaled breath (Wallace and Pellizzari, 1995; Wallace and Buckley, 1996) or sometimes in the arterial blood (Berlin, 1985; Brugnone et al., 1999; Giardino and Wireman, 1998) of an exposed person.

In this study we attempted for the first time to estimate the total body burden resulting from the exposure, utilizing urinary measurement of *t,t*-MA. We tried to estimate the body burden base on the understanding of the distribution, metabolism and elimination of benzene in human physiology using physiologically based pharmacokinetic (PBPK) model. PBPK models are mathematical constructs that are widely 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 etc.) 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.

The study involved two occupational groups, namely petrol pump workers and car drivers as well as in a positive control environmentally exposed group exposed through inhalation to varying levels of benzene.

Urinary metabolite concentration is easy to measure and can be used for indirect estimation of benzene in different body compartments as well as its total body burden. Collection of urine sample is relatively less insidious than blood sampling; moreover urine samples are less sensitive to storage than blood samples. Excretion of metabolites through urine and residence of benzene in other body compartments can be related by simple equations using PBPK models.

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

2. Methods

2.1. Subject selection

For this study we have chosen petrol pump workers and passenger car drivers as two occupationally exposed groups and office workers, mostly engaged in desk job, as positive control group (non-smoker and only exposed environmentally).

The study group from petrol pump workers consisted non-smoker subjects chosen from the workers in the service stations engaged only in refuelling work. Non-smoker personnel driving either petrol driven cars fitted with catalytic converter or diesel driven cars were selected as car drivers for this study. All recruited subjects were male in the age group of 25–55 years. Mean age of petrol pump workers, drivers and control groups were 34, 42 and 36 years respectively. Subjects with heart disease or suffering from other chronic illness were excluded.

2.2. Exposure assessment

We have monitored the personal exposure of benzene for all three selected groups as follows. Air was drawn at a rate of 100 ml min^{-1} from 6 am to 2 pm (for a full work shift of 8 h) 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 35 samples (in duplicate) were collected from 35 petrol pump workers for personal exposure measurement during an 8 h work shift ($n = 35$). The detailed procedure of personal exposure assessment of car drivers is described in previous publications (Som et al., 2007; Majumdar et al., 2008). A total of 35 samples were collected in duplicate from 35 drivers ($n = 35$).

Personal exposure measurement of control group was done during working hours, similarly as done for petrol pump workers. Altogether 27 samples were collected in duplicate ($n = 27$) from 27 office workers.

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 at the time of recruitment. Spot urine samples were collected in sterilized polyethylene bottles in the second half of the eight hour work shift for occupational groups and at middle of the day for control group at their free will during the last two working days of the week and put in a freezer at −20 °C immediately after collection.

2.4. Estimation of urinary t,t-MA

Creatinine was estimated following Jaffe's method (Jaffe, 1886).

We determined *t,t*-MA in urine as follows; 5 ml of urine was mixed with 5 ml of phosphate buffer (pH 6.8) and passed through an SAX cartridge (quaternary ammonium ion exchange resin, Whatman Make), preconditioned with 3 ml of methanol and 3 ml of water. The cartridge was washed consecutively with 5 ml of water, 3 ml of 5 mM phosphate buffer and 3 ml of 1% aqueous acetic acid. It was then eluted with 4 ml of 10% aqueous acetic acid. Quantification was done on a HPLC (Shimadzu model LC10AVP) equipped with