Biomarkers of Environmental Benzene Exposure

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Environmental exposures to benzene result in increases in body burden that are reflected in various biomarkers of exposure, including benzene in exhaled breath, benzene in blood and urinary *trans-trans*-muconic acid and *S*-phenylmercapturic acid. A review of the literature indicates that these biomarkers can be used to distinguish populations with different levels of exposure (such as smokers from nonsmokers and occupationally exposed from environmentally exposed populations) and to determine differences in metabolism. Biomarkers in humans have shown that the percentage of benzene metabolized by the ring-opening pathway is greater at environmental exposures than that at higher occupational exposures, a trend similar to that found in animal studies. This suggests that the dose–response curve is nonlinear; that potential different metabolic mechanisms exist at high and low doses; and that the validity of a linear extrapolation of adverse effects measured at high doses to a population exposed to lower, environmental levels of benzene is uncertain. Time-series measurements of the biomarker, exhaled breath, were used to evaluate a physiologically based pharmacokinetic (PBPK) model. Biases were identified between the PBPK model predictions and experimental data that were adequately described using an empirical compartmental model. It is suggested that a mapping of the PBPK model to a compartmental model can be done to optimize the parameters in the PBPK model to provide a future framework for developing a population physiologically based pharmacokinetic model. — Environ Health Perspect 104(Suppl 6):1141–1146 (1996)

Key words: benzene, human, muconic acid, metabolism, physiologically based pharmacokinetic model

Introduction

Benzene is present in the environment, primarily the air, because of emissions from automobiles, the petrochemical industry, and combustion processes. It has been demonstrated that tobacco smoke is a primary contributor to indoor air levels of benzene, thereby increasing personal exposure to benzene above that from ambient levels (*1,2*). Benzene is metabolized by the body by two different pathways, with a number of metabolites excreted in the urine. The complete metabolism of benzene takes hours following its entry into the body via an inhalation exposure route; thus, benzene is present in the blood and expired in the breath for extended time periods. A series of potential biological markers of exposure therefore exists for benzene, including expired benzene, blood benzene, and urinary metabolites of each pathway.

Exhaled Breath Concentrations

The continuous environmental exposures to benzene have resulted in benzene being detectable in the expired breath (*2*) in the general population. A relationship between benzene exposure as measured by personal monitors and the breath concentration was identified during the Total Exposure Assessment Methodology (TEAM) study, indicating that breath concentration reflects inhalation exposure (*3*). Differences between the benzene breath concentrations of smokers (21 $\mu g/m^3$) and nonsmokers (7.8 $\mu g/m^3$) were observed in workers without occupational benzene exposure, confirming smoking as an exposure source of benzene and expired benzene as a biomarker of that exposure (*4*). A similar relationship between the breath concentration of smokers (14 $\mu g/m^3$) and nonsmokers (2 $\mu g/m^3$) was detected during the TEAM study (*2*). An association between benzene breath concentration and the time spent driving an automobile shortly before the breath sample was taken was identified using stepwise regression analysis with an $r^2 = 0.55$ at an $\alpha = 0.5$ level (*5*). The interior of an automobile has been demonstrated to have higher benzene concentrations than other microenvironments such as outdoors and residences without smokers (*6–8*). Changes in the benzene breath concentration with time following distinct environmental exposures to benzene have been empirically modeled using the sum of two exponential expressions, suggesting that a minimum of two compartments exist within the body with different biological resident times, a rapid exchange region with resident times of minutes such as the blood, and more slowly exchanging regions with biological residence times of tens of minutes to hours such as in rapidly perfused tissue (*9,10*). Higher level exposures, such as those that occur in occupational settings, have identified a third, more slowly exchanging, compartment, presumably fatty tissue, that uptakes and releases benzene with biological resident times of tens of hours (*11*).

Blood Concentrations

The general population has a log-normal distribution of blood concentrations of benzene; this has been measured in the National Health and Nutrition Examination Survey (NHANES) project, with the volatile organic compound (including benzene) blood concentrations associated with recent exposures (*12*). As was identified for breath, blood benzene concentrations of smokers were elevated compared to those of nonsmokers (*13*). A correlation was identified with blood and breath levels for benzene consistent with the expected equilibrium between these two bodily fluids (*13*). A correlation between occupational exposures and blood benzene concentrations has been identified (*14*).

Urinary Metabolites

Urinary phenol, *trans-trans*-muconic acid and *S*-phenylmercapturic acid (PMA) are the two excreted metabolites that have been used most as biomarkers of benzene

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Abbreviations used: ETS, environmental tobacco smoke; GC–MS, gas chromatography–mass spectrometry; HPCL, high performance liquid chromatography; PBPK, physiologically based pharmacokinetics; TEAM, total exposure assessment methodology; VOCs, volatile organic compounds.

exposure in occupational studies. The utility of urinary phenol as a biomarker of benzene exposure is limited to inhalation exposures at air concentrations exceeding 3 mg/m^3 and has been found to be proportional to benzene concentrations at exposures as high as 620 mg/m^3 (*15–17*). Additional sources of urinary phenol due to diets and ingestion of medicine (*18*) have precluded its use as a biomarker of environmental benzene exposures. Urinary *S*-phenylmercapturic acid is a metabolite of the ring hydroxylation pathway of benzene. It was found to be increased in a dose–response fashion to benzene in the urine of coke production workers, in a similar manner as urinary phenol, and to be higher in the postshift urine compared to prework urine of two workers exposed to 1.1 and 0.15 ppm benzene when no discernable differences in the urinary phenol levels were detectable (*19*). This suggested that urinary *S*-phenylmercapturic acid is a more sensitive biomarker of benzene exposure than urinary phenol. Urinary *S*-phenylmercapturic acid was used to evaluate benzene exposure of car mechanics who were exposed to a mean concentration of 1 ppm, with a stronger correlation between exposure and than determined for urinary *trans-trans*-muconic acid (*20*) and workers at a chemical plant, who had a geometric mean benzene exposure level of 0.1 ppm, as well as smokers and nonsmokers, with a statistically significant between the urinary excretion of both *S*-phenylmercapturic acid and urinary *trans-trans*-muconic acid with the benzene exposure (*21*). Urinary *S*-phenylmercapturic acid appears to be a specific and sensitive biomarker of benzene, whose only apparent source is the ring hydroxylation pathway; thus it has the potential to be a useful biomarker of low-level benzene exposures (*22*). However, it is excreted in very low quantities (µg/g creatinine) and requires a complex analytical method to be analyzed. Urinary *trans-trans*-muconic acid has been used as a biomarker of sub-mg/m^3 exposures (*23–25*), the upper range of environmental benzene exposure; but other sources of this urinary metabolite have been identified, such as metabolism of sorbic acid—a food additive (*26,27*)—which makes it a nonspecific biomarker of low-level environmental benzene exposure. Urinary *trans-trans*-muconic acid was found to be elevated in a single individual, as was the exhaled breath concentration, following exposure to benzene-contaminated water (*28*). A study of six individuals exposed to benzene in environmental tobacco smoke (ETS) found they also had elevated urinary *trans-trans*-muconic acid compared to their urinary *trans-trans*-muconic acid on days without ETS exposure, but the use of urinary muconic acid as a biomarker in this study depended upon a knowledge of both the background excretion rate of muconic acid and the background exposure to benzene from other sources, and thus cannot be generalized to use in the general population for short-term exposures (*29*). A correlation between urinary benzene and the time-weighted 8-hr exposure among nonexposed, nonsmokers, smokers, and occupationally exposed individuals has been reported, but the exposure levels were higher than would occur for environmental exposures (*30*). Thus urinary biomarkers of benzene exposures currently have limited utility in establishing low-level, distinct environmental exposures in individuals, particularly in single urine samples.

Methodology

Research using biomarkers of environmental benzene exposure has been successful when applied in two categories: distinguishing chronic exposures on a population basis, such as smokers versus nonsmokers, and evaluating changes in a single individual after an exposure event, provided care was taken to establish the individual's background biomarker levels resulting from existing chronic environmental exposures and confounding compounds that contribute to the biomarker (such as sorbic acid to *trans-trans*-muconic acid). These latter data have also been used to determine what portion of the biomarker can be attributed to the exposure event being studied. Time-series data on changes in biomarker concentration following an exposure can be used to evaluate physiologically based pharmacokinetic (PBPK) models.

Breath Samples

The analysis of low-level benzene breath concentrations has relied on combining several breaths (either whole or alveolar) into a tedlar bag (*3*), adsorbent trap (*10*), or canister (*31,32*) to obtain sufficient quantities of benzene to be detectable. These breath samples have been analyzed by gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) in a manner analogous to the analysis of air samples. Detection capability of sub-µg/m^3 values is possible and sequential samples can be collected during the rapidly changing benzene breath concentrations to determine the biological half-life and evaluation of PBPK models.

Urinary Metabolites

Both high-power liquid chromatography (HPLC) and GC methods have been developed that are sufficiently sensitive to measure background levels of tens of ng/ml of urinary *trans-trans*-muconic acid (*24,27*) The HPLC methods require a series of preconcentration and clean-up steps, with added specificity obtained when using a scanning ultraviolet/visible (UV/VIS) spectrophotometer as the detector (*29*). A highly specific and sensitive method of GC–MS analysis has been developed using isotopically labeled *trans-trans*-muconic acid obtained by feeding labeled benzene to mice as an internal standard. A second GC method, using flame ionization detection (FID) has been developed (*5*); this method is simpler to perform and more readily adapted to most laboratories but is not as specific or sensitive as the internal standard GC–MS technique. The GC methods require derivitization prior to the GC analysis. All three approaches have been compared in the analysis of low-level urine samples and provided comparable results (*33*).

Physiologically Based Pharmacokinetic Modeling

The PBPK model developed by Travis (*34*) describing benzene was evaluated using time-series exhaled-breath concentration data, and the body weight of the subject was taken into account in determining parameter values used in the model.

Results and Discussion

Breath Samples

The exhaled benzene breath concentration during and following environmental benzene exposure in ETS increased exponentially during exposure to benzene and then decreased in an exponential fashion once the exposure ceased (Figure 1). The ratio of the inspired air to the exhaled breath concentration during the exposure represents the absorption efficiency across the lung–blood capillary boundary. The absorption efficiency was greatest at the initial part of the exposure and declined as the exposure continued, reflecting a shift in equilibrium as the benzene blood concentration increased. An average value of 64% has been determined for these environmental exposures (*10*) that exceeds the absorption efficiencies of 30% calculated during higher and more prolonged

exposures of >100 mg/m^3 and several hours (*35*). The absorption efficiencies for Sprague-Dawley rats and B6C3F1 mice are 31 and 50%, respectively (*36*). These differences indicate that when calculating the internal dose (the amount of the exposure that enters the body), the exposure level and duration must be considered to evaluate whether a steady-state condition has been reached. Further, extrapolation of absorption efficiency calculated from high-exposure studies to environmental conditions will tend to underestimate the inhalation dose.

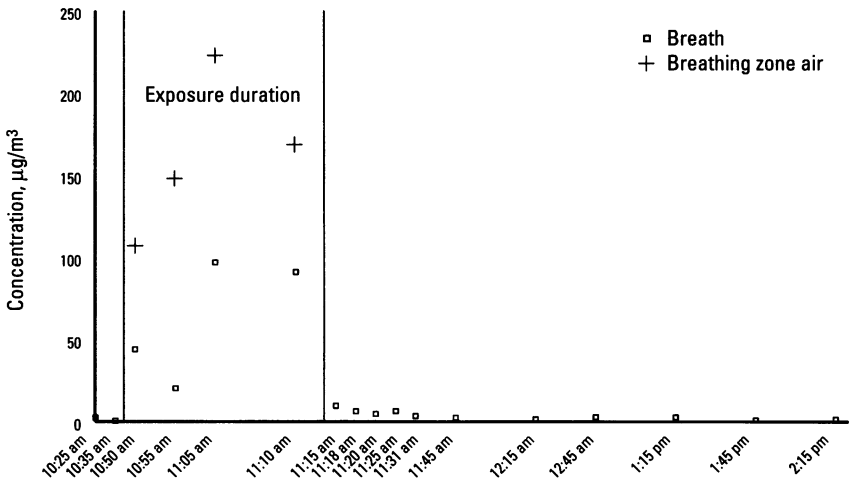

Figure 1. Benzene concentration in breath as a function of time, during and postexposure (Subject no. 5, 8/26/93, 30 min, 218 µg/m^3).

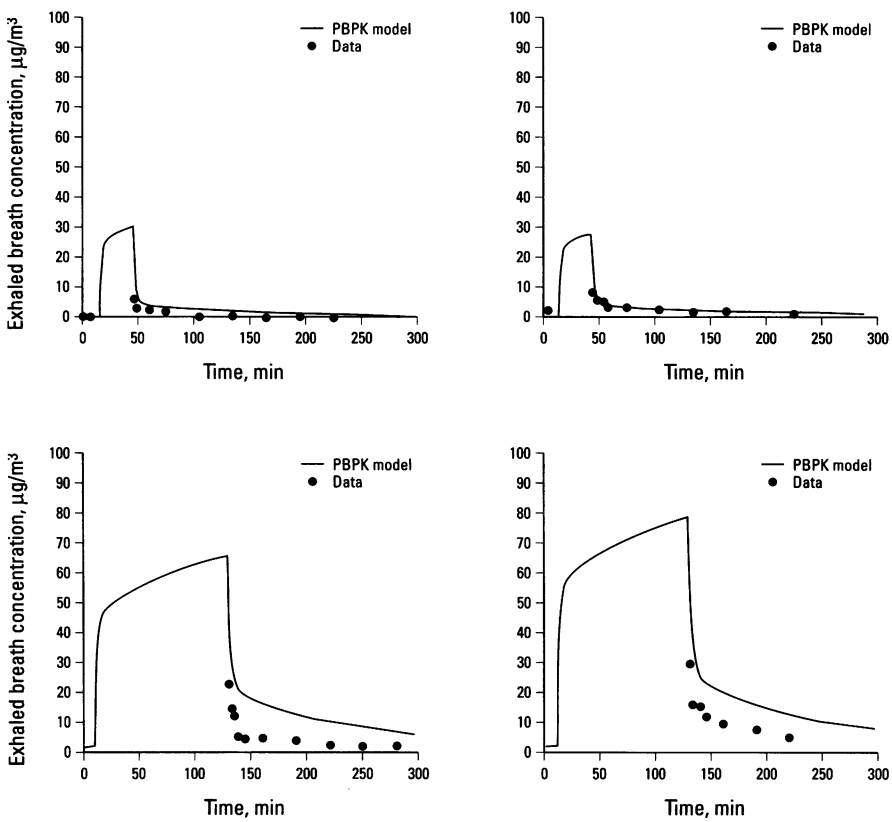

Figure 2. Comparison of measured exhaled breath concentrations with PBPK model-simulated profiles.

PBPK Model Evaluation

The series of breath concentrations measured during and following single exposures reported by Yu and Weisel (*10*) were used to evaluate the PBPK benzene model as described by Travis et al. (*34*). A consistent bias in the prediction of the breath concentration by the PBPK model compared to the experimental data following a 2-hr exposure was found (Figure 2). These data were adequately modeled using an empirically based two-compartment model assuming exponential decline (Figure 3). The systematic deviations observed with the PBPK model are to be expected, since a number of parameters in that model were derived using interspecies extrapolation and because the model is meant to represent the pharmacokinetics of an average individual; furthermore, the pharmacokinetics of a specific individual may differ significantly due to interindividual variability. To expand the utility of pharmacokinetic modeling, it is desirable to develop a population PBPK model that will provide estimates of the distribution in tissue dose (or other dose surrogate, such as amount metabolized) over a population, rather than to obtain point estimates of dose.

One approach to developing population PBPK models is to map a PBPK model onto a compartmental model and thereby establish a relationship between the parameters in the two models (this can be accomplished by adopting system reduction methods (*37*) and lumping theory (*38*). The population pharmacokinetic model can then be developed based on the compartmental model using standard population pharmacokinetic software such as the nonlinear mixed effects model (NONMEM) developed at the University of California at San Francisco (*39*). The population pharmacokinetic parameters are then mapped back into the PBPK model to obtain a population PBPK model.

The PBPK model parameters will not be uniquely determined by the compartmental model parameters, since the compartmental model is a lower-order system having fewer parameters. Instead, the PBPK model parameters will be specified by a range of values and by constraints between the parameters.

The feasibility of describing the data with a simple two-compartment pharmacokinetic model has been demonstrated by the good fit to the data obtained by that model. If such a mapping approach is successful, it will be possible to develop physiologically based population pharmacokinetic models.

A population pharmacokinetics model will enable probability-based risk estimates rather than point estimates. This is particularly important when risk calculation for the general population rather than a homogeneous subpopulation in a single setting is attempted, as there is a wide distribution of physical and biological attributes in the general population that affect the physiological parameters in a PBPK model.

Urinary *trans-trans*-Muconic Acid

Urinary *trans-trans*-muconic acid is one of the metabolites excreted from the ring-opening metabolism pathway, that may form heptatoxic benzene metabolite *trans-trans*-muconaldehyde (*40*). The percentage of the benzene dose excreted as this metabolite may therefore be indicative of a potential adverse health outcome. It has been reported in both mice and rats that the percentage of the benzene dose metabolized via the ring-opening pathway increases as the dose decreases at concentrations below the saturation value of enzyme systems (*41,42*). If a similar variation in the proportion of benzene metabolized by the ring-opening pathway occurs in humans, estimates of the cancer potency factor based on high-dose animal studies and ppm occupational data, calculated using a no-threshold model, underestimates the risk at environmental exposures in the tens of parts per billion (*23,37*). The percentages of benzene metabolized to and excreted as *trans-trans*-muconic acid for different exposures and in different species are given in Table 1. These results suggest that the percentage of a dose metabolized by the ring-opening pathway is greater at low doses in humans and animals. These variations in the percentage of the benzene dose excreted as different metabolites suggest that one or more of the elimination pathways is nonlinear and may have different metabolic mechanisms active at different concentrations; therefore the use of a linear, no-threshold model to predict cancer risk from low-level benzene exposure may not be valid. Further, if the ring-opening pathway produces toxic metabolites, an increase in their production rate as the dose decreases would lead to an underestimation of the health risk at environmental exposure if a no-threshold mechanism is correct.

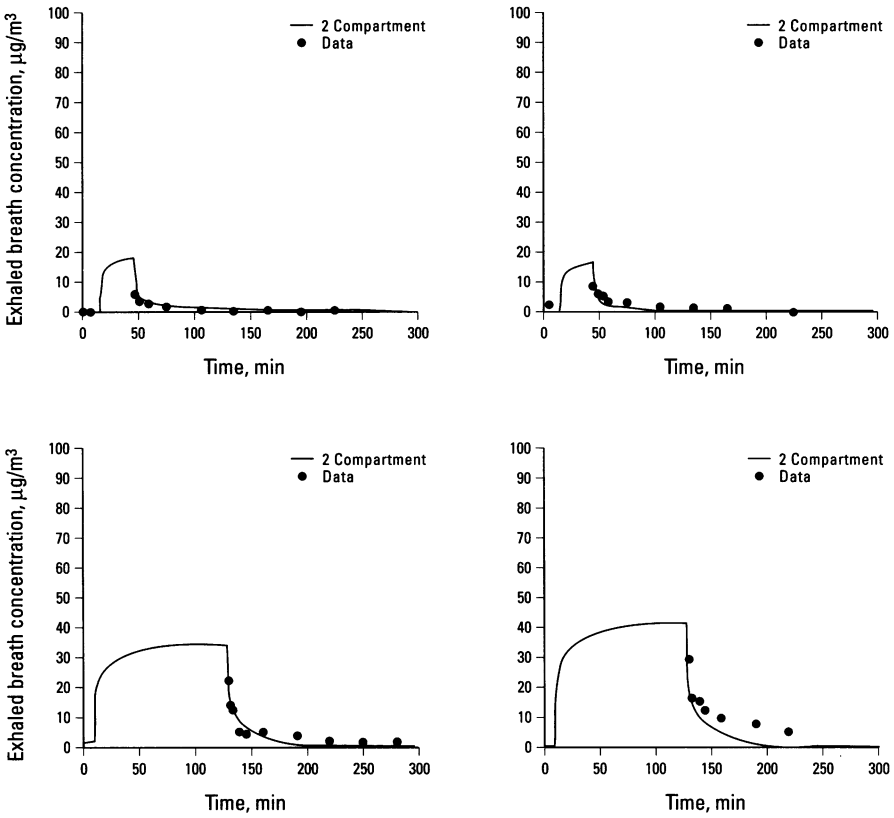

Figure 3. Two-compartmental models of post-breath concentration and experimental data.

Table 1. Relationship between dose and proportion of benzene excreted as *trans-trans*-muconic acid.

Exposure/Dose	Subject	Exposure route	Proportion	Reference
0.02–0.3 µg/kg (19–159 ppb/hr)	Human, 70 kg	Inhalation	21% (7.2–33%)	(*28*)
0.01–1 mg/kg (7–539) ppm/hr)	Human	Inhalation	2%	(*43*)
0.5 mg/kg 880 mg/kg	DBA/2N mice	Intraperitoneal	9.8% 0.4%	(*41*)
0.5 mg/kg	C57BL/6 mice		17.6% 0.2%	
1 mg/kg 10 mg/kg	Mice	Gavage	18% 14%	(*42*)
1 mg/kg 10 mg/kg	Rats		11% 9%	
0.34–0.5 g/kg	Rabbits	Oral	0.45%	(*44*)

Conclusion

Exhaled breath and urinary muconic acid are biomarkers of environment exposure, but each must be used within narrowly prescribed conditions that define background levels in the individuals being characterized, or to establish group differences. The amount of metabolism by the ring-opening pathway is greater at low exposure in humans than at high exposure, as found in animals; thus, care is needed when extrapolating potential health risk from high to low dose. The benzene PBPK model needs further evaluation using human data; optimization of parameters by mapping it to a compartmental model holds promise as a step in producing a population pharmacokinetic model.

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