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Exposure reconstruction for reducing uncertainty in risk assessment: example using MTBE biomarkers and a simple pharmacokinetic model

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Abstract

Adverse health risks from environmental agents are generally related to average (long-term) exposures. Because a given individual's contact with a pollutant is highly variable and dependent on activity patterns, local sources and exposure pathways, simple 'snapshot' measurements of surrounding environmental media may not accurately assign the exposure level. Furthermore, susceptibility to adverse effects from contaminants is considered highly variable in the population so that even similar environmental exposure levels may result in differential health outcomes in different individuals. The use of biomarker measurements coupled to knowledge of rates of uptake, metabolism and elimination has been suggested as a remedy for reducing this type of uncertainty. To demonstrate the utility of such an approach, we invoke results from a series of controlled human exposure tests and classical first-order rate kinetic calculations to estimate how well spot measurements of methyl tertiary butyl ether and the primary metabolite, tertiary butyl alcohol, can be expected to predict different hypothetical scenarios of previous exposures. We found that blood and breath biomarker measurements give similar results and that the biological damping effect of the metabolite production gives more stable estimates of previous exposure. We also explore the value of a potential urinary biomarker, 2-hydroxyisobutyrate suggested in the literature. We find that individual biomarker measurements are a valuable tool in reconstruction of previous exposures and that a simple pharmacokinetic model can identify the time frames over which an exogenous chemical and the related chemical biomarker are useful. These techniques could be applied to broader ranges of environmental contaminants to assess cumulative exposure risks if ADME (Absorption, Distribution, Metabolization and Excretion) is understood and systemic biomarkers can be measured.

Keywords: *Environmental health risk, classical pharmacokinetic model, exhaled breath sample, blood sample, exposure assessment, exposure reconstruction*

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Introduction

The conventional method for assessing human exposure to an environmental pollutant is to measure the compound in air, water, food, etc., and estimate total uptake based on typical breathing, drinking and eating volumes, and for dermal exposure, with estimates of typical contact duration and mean surface areas. As such, the exposure estimates for a given group are the same for all individuals. Because people have different behaviour patterns, their exposures cannot be expected to be uniform. Furthermore, due to differences in genetic makeup, coincident exposures, and current health state, individuals have different susceptibilities for adverse health outcome at the same exposure level; consider the examples that only some smokers get lung cancer, only some alcoholics get cirrhosis of the liver, and only some asbestos workers get mesethelioma. As such, knowledge of the distributions of exposure and of bioavailable dose is indispensable for eventual risk calculations. To determine the exposure variability within a group requires activity diaries coupled to some form of dosimetry usually in the form of microenvironmental or personal samplers. Another approach is to measure the level of the compound in the blood, urine or breath and to estimate further bioavailable dose by measurements of metabolites or adducts, collectively referred to as 'biomarker' measurements.

Biomarkers of exposure are generally defined as chemicals measured in biological media; these include the original compounds of exposure, their metabolic products, and various large molecule adducts. They may also include changes in levels or patterns of common biological compounds like ketones in urine, alcohols, alkanes and aldehydes in breath, and liver enzymes in blood. Biomarkers of current health state may additionally include some chemical or physical parameters such as blood pH, forced vital capacity, urine colour and breath odour. Biomarker measurements can be used in many ways including evaluating the time course and distribution of a chemical in the body, estimating health risk, assessing disease state, and inferring previous exposure or dose (Watson & Mutti 2004, Clewell et al. 2005). Blood and urine measurements are the primary methods employed but lately it has been recognized that collecting exhaled breath is an attractive alternative because it is less invasive (Lindstrom & Pleil, 2002). Another advantage is that the biomarker encompasses all routes of exposure, in contrast, for example, to ambient measures of air that may disregard ingestion or dermal exposure from water. Finally, metabolic biomarkers exhibit 'biological damping' where the response to an external exposure or stimulus is smoothed or slowed by the associated biochemistry and thus serves to time-integrate previous events (Rappaport 1985, Rappaport & Spear 1988, Rappaport et al. 1995).

The utility of biomarkers in exposure assessment can be demonstrated with methyl tertiary butyl ether (MTBE) exposure and environmental classical pharmacokinetics (PK). MTBE is ubiquitous in the USA and has been linked to toxicity and cancer in animal studies; it was originally introduced in the late 1970s to replace lead in gasoline as an octane enhancer. More recently (since 1990), it has become the most common 'oxygenate' added to fuel to reduce vehicular carbon monoxide emissions in cold climates and now comprises 1–15% of all automotive fuels by volume (EPA 2001, USGS 2001, J.S. Zogorsky & D.A. Bender, personal communication). MTBE is a volatile, flammable, and colourless liquid that readily evaporates and dissolves easily in water; it has a very distinctive odour and (unpleasant) taste at very low levels (Prah et al. 1994). Fuel spills, atmospheric deposition, leaking underground storage tanks

and fuel transmission pipe leaks have introduced MTBE into drinking water supplies; evaporative emissions from auto refueling add vapour-phase MTBE to the ambient air (CRS 2001, ATSDR 2006). MTBE has one major metabolite, tertiary butyl alcohol (TBA), which is easily measured in human breath, blood and urine (Buckley et al. 1997, Dekant et al. 2001). Incidental refuelling exposures to MTBE along with benzene and toluene in ambient air have been documented (Lindstrom & Pleil 1996). In a recent US Environmental Protection Agency (EPA) study of controlled human exposures, native MTBE and the metabolite TBA were measured during uptake and elimination in blood and breath. Time-dependent data were collected for inhalation, ingestion and dermal exposure routes (Prah et al. 2004).

Estimating uptake (or production), distribution, target organ dose, metabolism and elimination of an endogenous or exogenous chemical generally requires detailed knowledge of many physical, chemical and biological parameters. These parameters are measured or estimated from *in vitro* laboratory studies, animal models and controlled human studies. Subsequently, the results are interpreted to deduce potential mechanisms, diffusion constants and tissue transfer rates that are then incorporated into a complex physiologically based pharmacokinetic (PBPK) model. Furtaw (2001) has published a historical overview of the EPA's efforts in human exposure modelling. PBPK models may incorporate 40 or more distinct accumulation points for individual organs, body fluids and tissues. If, however, we are interested only in assessing average exposures, bioavailable dose, basic rates of uptake, metabolism and elimination, then simple classical PK models suffice. PK models are empirical; the body is treated as a few theoretical compartments without a detailed physiological relationship (e.g. highly, moderately and poorly perfused tissues, rather than brain, lung, fat, blood, kidney, muscle, etc.). PK models can be developed and validated using simple measurements of a few compounds in blood, urine and breath. For this work, we rely on the classical PK model and follow the approach and the general principles invoked in standard texts (e.g. Boroujerdi 2002). A number of PBPK models have been developed based on animal and human data to assess target organ estimates and have been published (for example) by Dekant et al. (2001) and Licata et al. (2001). Recently, a detailed PBPK model focusing on the dermal exposure pathway has been developed (D. Kim, personal communication).

The primary goal of this work is to demonstrate the value of spot measurements of breath or blood for reconstructing an individual's recent average exposure to MTBE. We start with a PK model developed using laboratory experiments and generalize to hypothetical exposure scenarios including random intermittent exposures as may be encountered in the real world. For simplicity, we focus on the inhalation exposure pathway only. Random sampling times are chosen for spot measurements and used to back-calculate the expected average exposure values. We demonstrate the utility of single measurements per subject as a potential replacement for numerous ambient and microenvironmental measurements. Urinary monitoring is also considered a valuable resource for assessing exposure (Imbriani & Ghittori 2005) and may be preferable for specific situations (Jones & Cocker 2003); therefore, we also consider the value of a potential urinary biomarker that would exhibit more biological damping than the primary metabolite TBA.

Methods

Available data

From our previous work, we have available sets of data 'building blocks' of MTBE and TBA in blood and breath from separate controlled ingestion, inhalation and dermal exposures. These time series data follow the uptake and elimination for 24 h from short term exposures (1 h dermal, 1 h inhalation, or bolus ingestion exposure). This work was performed under University of North Carolina Institutional Review Board approval with informed consent of the subjects. The methods and results of these experiments have been described in detail and published elsewhere (Prah et al. 2004); for clarity, we provide a brief description of the inhalation experiments.

Inhalation exposure was studied through collection of blood samples from 14 individual male subjects; breath samples were co-collected for a subset of seven subjects due to resource constraints. Volunteers were exposed to 3 ppmv (10.93 μg l^{-1}) MTBE in air for 1 h. Blood and breath samples were collected periodically before, during and after the exposures on a timing pattern chosen to describe accurately the temporal behaviour ending about 23 h postexposure (Pleil & Lindstrom 1998). About 16 samples per subject were collected for each experiment; in addition to pre-exposure controls, samples were collected at 0, 15, 30, 45 and 60 min during the exposures and at 0, 5, 15, 30, 60, 120, 180, 360 and ~1320 min postexposure. Blood samples were assayed for MTBE and TBA by David Ashley's group at the Centers for Disease Control Laboratories in Atlanta, Georgia, using their specific gas chromatography/mass spectrometry methods (Ashley et al. 1992); breath samples were assayed for MTBE and TBA at the EPA Laboratories in Research Triangle Park, North Carolina with 1-l 'single-breath canisters' collection (Pleil & Lindstrom 1995a) and analytical methods developed by Pleil and Lindstrom (1995b). To demonstrate the potential value of other biomarkers, in particular 2-hydroxyisobutyrate (2-HIBA), we estimate relevant parameters with calculations derived from biomarker studies by Amberg et al. (1999, 2001).

Classical pharmacokinetic model

Figure 1 shows a simple PK model for the disposition of MTBE and TBA in the body. The central compartment represents the circulating blood and other fluids in equilibrium with the blood. We consider only one peripheral compartment to serve as an empirical representation of the moderately and poorly perfused tissues. This model assumes that once MTBE is absorbed into the central compartment (blood), the rate constants for exchange with the peripheral compartment, elimination via breath and conversion to metabolite are first order. We also allow for direct conversion of MTBE to TBA in the lung via cytochrome P450 enzymes as indicated by the dashed path and the rate constant K_{oT} in Figure 1. Furthermore, we assume that MTBE is entirely eliminated via breath or metabolized to TBA; we do not consider any other loss mechanisms. The breath compartments for MTBE and TBA are not considered as standard accumulation points and are thus represented with dashed lines. This approach and the mathematical development have been published (Wallace et al. 1997, Pleil & Lindstrom 1998). We note that the removal of central compartment TBA with time constant K_{TL} is considered to be via metabolism and eventual urinary elimination.

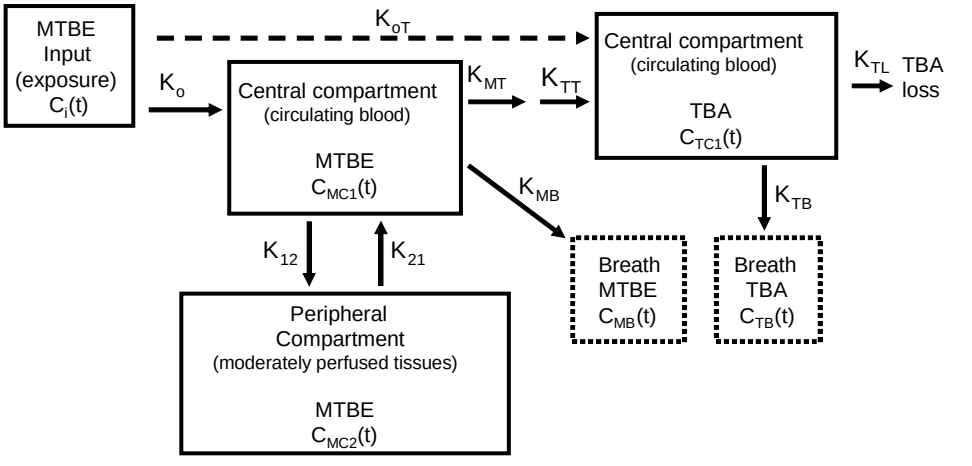

Figure 1. Simple classical pharmacokinetic diagram of deposition of absorbed methyl tertiary butyl ether (MTBE) among compartments, metabolite and excretion; the 'Ks' are first-order rate constants except for K_0 which is zero order for inhalation and dermal exposures. K_{0T} represents direct conversion of MTBE to tertiary butyl alcohol (TBA).

Differential equations

From Figure 1, the concentrations (mass/volume) in the various parts of the models are defined as follows:

$C_i(t)$ = exposure input concentration of MTBE at time = t;
$C_{MC1}(t)$ = blood concentration of MTBE in central compartment at time = t;
$C_{MC2}(t)$ = blood concentration of MTBE in peripheral compartment at time = t;
$C_{TC1}(t)$ = blood concentration of TBA in central compartment at time = t;
$C_{MB}(t)$ = breath concentration of MTBE at time = t;
$C_{TB}(t)$ = breath concentration of TBA at time = t;

where Ko is in units of mass/time and the other various 'Ks' are all first-order rate constants in units of 1/time. The conversion of MTBE to TBA is adjusted by a change from K_{MT} to K_{TT}($K_{TT} = K_{MT} \times k_d$). For the accumulation compartments for blood levels, the differential equations are:

$$dC_{MC1}(t)/dt = (K_o \times k_a)\, C_i(t) + (K_{21} \times kb)\, C_{MC2}(t) - (K_{12})\, C_{MC1}(t) - (K_{MT} + K_{MB})\, C_{MC1}(t) \quad (1)$$

$$dC_{MC2}(t)/dt = (K_{12} \times k_c)\, C_{MC1}(t) - (K_{21})\, C_{MC2}(t) \quad (2)$$

$$dC_{TC1}(t)/dt = (K_{MT} \times k_d)\, C_{MC1}(t) - (K_{TL} + K_{TB})\, C_{TC1}(t) + (K_{oT} \times k_e)\, C_i(t) \quad (3)$$

where the lower case k_{a-e}'s represent adjustment for concentration units, volume of distribution, and analyte molecular weight. Breath concentrations of MTBE and TBA are approximately proportional to the blood concentrations; we adjust slightly during the times of exposure to account for the delay between the venous blood collected from the antecubital vein (arm) and the arterial blood represented by the exhaled breath:

$$C_{MB}(t) = [A_{pMBo} + A_{pMB1}\ C_i(t)]\ C_{MC1}(t) \quad (4)$$

$$C_{TB}(t) = [A_{pTBo} + A_{pTB1}\ C_i(t)]\ C_{TC1}(t) \quad (5)$$

This approach represents the best (and simplest) of a number of different methods that were considered for these empirical adjustments. Furthermore, the use of the correction term $A_{pTB1}\ C_i(t)$ for TBA in equation 5 is useful primarily for 'fine tuning' the model across a major step function such as in the original experiment. Under smoothly varying conditions, or for fast random conditions as would be more likely in reality, it can be removed from the calculation without loss of accuracy.

Calculated values for classical PK constants

As detailed in a previous publication (Pleil et al. 2005) the basic PK model (Figure 1) was implemented using equations 1–5 with estimates from empirical data used as initial conditions. The model was processed through the acslXreme software package and adjustable parameters were optimized to fit means of empirical blood and breath data. The final optimized constants for the PK model as represented in $\mu g\ l^{-1}$ units concentrations and 1 h^{-1} time constants, as appropriate, are:

$K_o \times k_a = 6.85$
$K_{21} = 0.938$
$K_{21} \times k_b = 5.12$
$K_{12} = 3.12$
$K_{12} \times k_c = 0.530$
$K_{MT} = 0.820$
$K_{MB} = 0.380$
$K_{TT} = K_{MT} \times k_d = 0.165$
$K_{oT} \times k_e = 0.901$
$K_{TL} = 0.0594$
$K_{TB} = 0.000317$
$A_{pMBo} = 0.0592$
$A_{pTBo} = 0.00120$
$A_{pMB1} = 0.0101$
$A_{pTB1} = 0.0000200$

The functional form of the simple PK model and the associated differential equations reproduce the character of the measured data well suggesting that the model assumptions are reasonable and that the uptake and elimination kinetics are linear (Figures 2 and 3). It also reinforces the conjecture that the model could be generalized to multiple and intermittent exposures.

Potential urinary metabolite

Although further metabolites were not measured in this study, the literature suggests urinary metabolites of TBA may be useful as well. According to Amberg et al. (1999, 2001), TBA in blood is further metabolized through a single pathway to 2-methyl-1,2-propanediol and then to 2-HIBA for eventual excretion in urine. In a

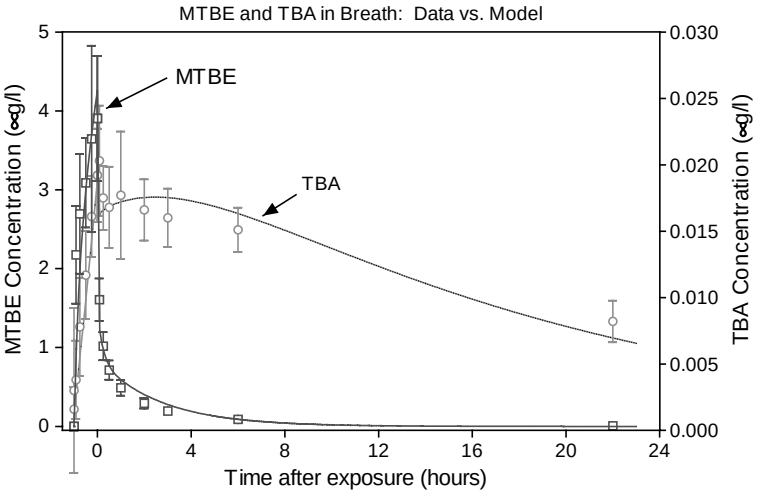

Figure 2. Breath measurements and classical pharmacokinetic model calculations for native compound and biomarker from inhalation exposure to methyl tertiary butyl ether (MTBE). TBA, tertiary butyl alcohol.

human exposure study, they measured the half-life of 2-HIBA excretion as 17.8 h (time constant $K_{HU} = 0.039\ 1\ h^{-1}$). In a single-step input/output PK model under linear kinetics, the empirical differential equation relating blood-borne TBA concentration (C_{TC1}) in ng ml^{-1} to urinary 2-HIBA concentration (C_{HU}) in μg ml^{-1} urine) would be:

$$dC_{HU}(t)/dt = (K_{TT} \times k_h)\ C_{TC1}(t) - (K_{HU})\ C_{HU}(t) \quad (6)$$

where k_h is an empirical adjustment for the difference in volume of distribution, molecular weight and biological media partitioning for the two compounds. By invoking average urine production as 350 ml in 6 h and extracting some limited data

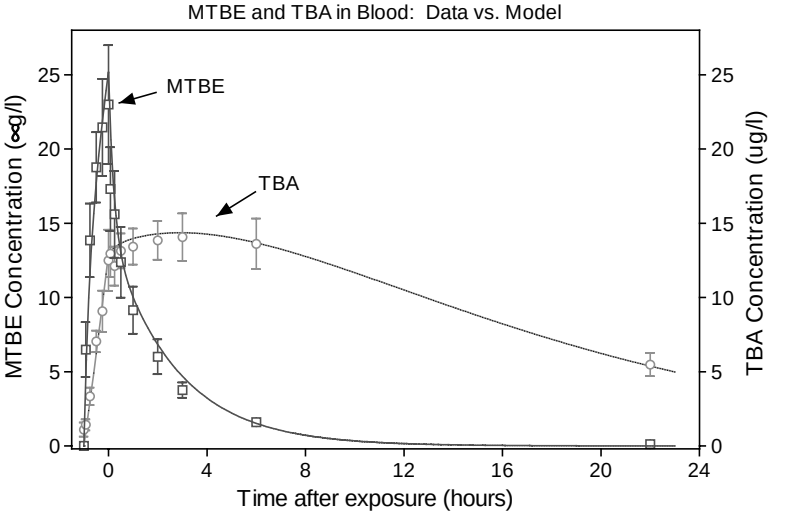

Figure 3. Blood measurements and classical pharmacokinetic model calculations for native compound and biomarker from inhalation exposure to methyl tertiary butyl ether (MTBE). TBA, tertiary butyl alcohol.

from the Amberg study, we estimate k_h from the slowly changing portion of curve where $dC_{HU}(t)/dt \approx 0$ in μg/ml urine/hr, and find that $k_h \approx [(K_{HU})\ C_{HU}(t)]/\ [(K_{TT})\ C_{TC1}(t)] \approx 0.052$.

Application to various exposure scenarios

To illustrate the biological damping aspect of the biomarker in contrast to the environmental exposure and the native compound level, we construct a completely theoretical exposure sequence of the original experiment wherein the 1-h exposure is repeated every 8 h. This is not meant to be realistic but serves to demonstrate the accumulation of body burden. Two additional hypothetical (yet plausible) exposure scenarios were created to demonstrate the prediction approach. They are an occupational exposure profile based on a fictitious gas station attendant and a typical environmental exposure profile for a fictitious resident of a community with water-borne MTBE.

The occupational scenario assumes a random intermittent exposure for five consecutive 8-h days inferred for a gasoline filling station worker. We assume that the worker fills on average two cars per hour for 6 min per car (including interaction with the customer), and has other duties with some background exposure. The worker has no exposure outside of work. According to limited literature information, gas station average exposures have been measured at 0.3 ppmv and 0.9 ppmv (Tsai et al. 2002). Refueller breathing space exposures have been measured at 6.4 ppmv (range 0.5–20.3 ppmv), 1.5 ppmv (range 0.05–4.4 ppmv) (Hakkola & Saarinen 2000), 4.6 ppmv (Lindstrom & Pleil 1996), and ranging from 0.3 to 10 ppmv (HEI 2004). Based on these data, we constructed a normally distributed background exposure (mean 0.6 ppmv, SD ± 0.2 ppmv), random intermittent exposure spikes (6-min duration) with lognormal distribution (g.m. 4.5 and g.s.d. 2.1 ppmv) for an 8-h work period, and zero exposure for the remaining 16 hours per day. These concentrations were mathematically smoothed via a running three-point average to mimic realistic mixing in air.

The environmental exposure scenario assumes varying incidental inhalation exposures primarily from evaporative emissions from contaminated water in micro-environments but also including outdoor exposure from automobiles. In California, for example, the interim drinking water action level is 35 $\mu g\ l^{-1}$; this corresponds to estimated air concentrations of 82 ppbv during a shower, 33 ppbv in bathroom air and 2.7 ppbv in whole house air (McKone 1987, CalEPA 1998). MTBE exposure from commuting in a car has been estimated at 16.7 ppbv (Tsai et al. 2002), and typical ambient exposures in Los Angeles range from 0.4 to 13.2 ppbv (CARB 1997). Based on these estimates, we constructed a 24-h background exposure with random lognormal distribution (g.m. 5 ppbv and gsd 4 pbbv); this is reduced and smoothed between 11 p.m. and 7 a.m. to represent sleep inactivity. To represent early morning shower exposure, we overlay 12 min at 82 ppbv, 24 min at 33 ppbv and 60 min at 2.7 ppbv. To represent commuter exposure, we further overlay 1 h each in the morning and in the evening at 16.7 ppbv. To investigate a more diffuse exposure, we added a fourth scenario wherein we further smoothed the environmental exposure with a 3-h running average. Figure 4a–d shows the hypothetical exposure scenarios each for 5-day periods.

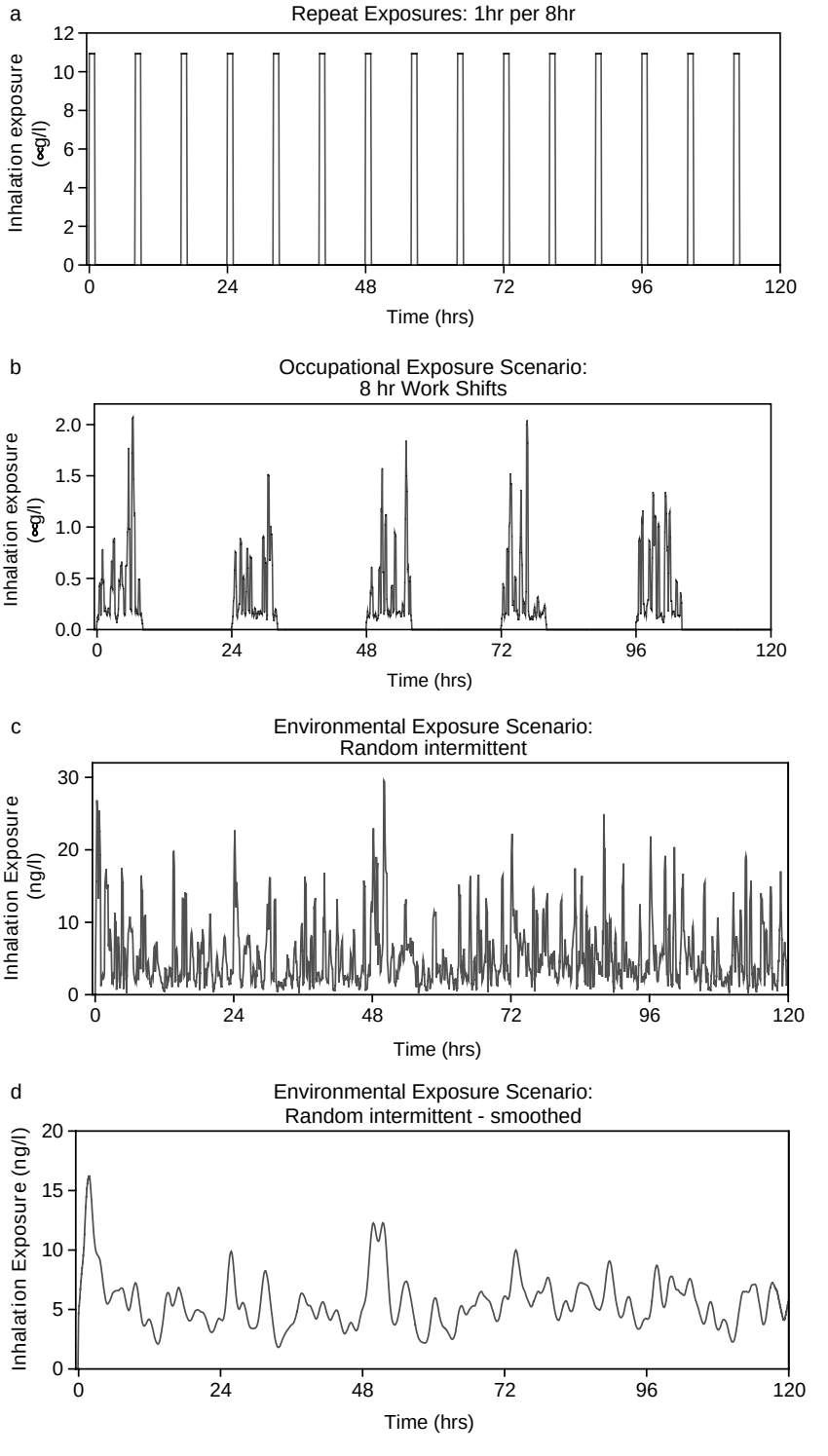

Figure 4 (Continued)

Results and discussions

As discussed earlier, the model developed here considers only the inhalation exposure route for simplicity; this is reasonable because inhalation is the dominant pathway and represents about 70% of the total exposure in the general public (Johnson 1998). More complex scenarios involving additional ingestion and dermal absorption events would not affect the underlying concepts presented here, but would require a more complicated model.

Equilibrium ratios relating blood, breath and inhalation exposure levels

Given a stable, known inhalation exposure, the expected steady state MTBE and TBA levels in the circulating blood can be calculated using the established classical PK model; we assume a brief (minimum) period of $\sim$6 min to accomplish sampling without additional exposure. Under the assumption that environmental exposures are in the range of linear kinetics and after optimizing for different averaging periods, the model predicts relative response factors with respect to actual blood or breath measurements (in same mass/volume units in respective media) to infer previous mean exposures as follows:

$$\begin{aligned}\text{Mean MTBE exposure (air)} &= (\text{MTBE in breath}) \times 4.11 \\ &= (\text{TBA in breath}) \times 25.8 \\ &= (\text{MTBE in blood}) \times 0.243 \\ &= (\text{TBA in blood}) \times 0.0355\end{aligned}$$

Although this represents the simplest scenario of a stable exposure, these constants are the best available estimates for assessing previous mean exposures because they are based on actual empirical measurements of humans and thus it should be possible to generalize to real-world exposures. We note that actual exposure scenarios are probably variable and so the calculated mean estimates must be interpreted with the understanding that they may be affected by the previous exposure pattern. The expected error between the true and the calculated mean exposures is discussed below.

Biomarker response predictions from the classical PK model

Using the 1-h repeated exposures as shown in Figure 4a, we calculated the expected levels of MTBE and TBA for a 5-day cycle; the initial cycle is identical to the first 8 h of models presented in Figures 2 or 3, depending upon the choice of blood or breath measurement. We further calculated the expected biomarker levels (blood and breath MTBE and TBA) for the hypothetical exposures presented in Figure 4b–d. To this point, all calculations are based on data from our own studies. For comparison, we also used equation 5 based on calculations from Amberg et al. (2001) to predict urinary levels of 2-HIBA. We estimated the conversion multiplier from urinary measurement to airborne concentration to be $0.162 \times$2-HIBA

Figure 4. Four hypothetical methyl tertiary butyl ether (MTBE) inhalation exposure scenarios as input for pharmacokinetic models: (a) theoretical 'repeat chamber' experiment with 1-h exposure every 8 h and average exposure of 1.37 $\mu g\, l^{-1}$; (b) hypothetical gas station attendant exposure with average exposure of 0.143 $\mu g\, l^{-1}$; (c) hypothetical environmental exposure in a location contaminated with MTBE in groundwater and in gasoline with average exposure of 5.60 $ng\, l^{-1}$; and (d) running average smoothing of exposure C to represent diffusion of local sources with average exposure of 5.60 $ng\, l^{-1}$.

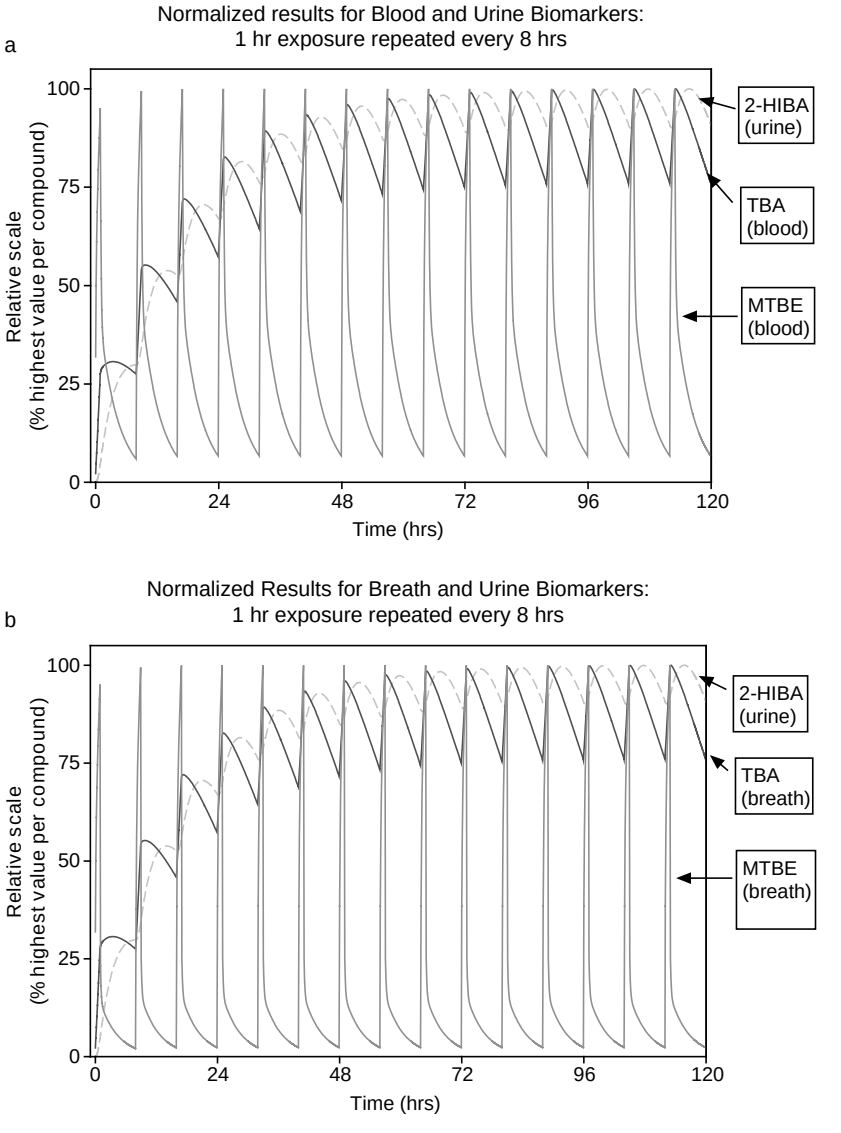

Figure 5. Comparison of normalized biomarkers response to exposure scenario from Figure 4A of methyl tertiary butyl ether (MTBE) and tertiary butyl alcohol (TBA) in blood (a) and breath (b), with respect to 2-hydroxyisobutyrate (2-HIBA) in urine. Dashed trace indicates urinary biomarker 2-HIBA.

($\mu g\ l^{-1}$) = air conc ($\mu g\ l^{-1}$). To allow comparison of the relative responses of the biomarkers within the same graph, the biomarker levels were normalized to the highest value in the 5-day period and plotted as percentage levels. Figures 5–8 show these results for the four exposure scenarios; each Figure has an 'a' and 'b' section that compares blood and breath biomarkers, respectively, with the urine 2-HIBA overlayed.

Throughout the 5-day exposures, the calculated native MTBE in blood and breath follows the exposure, and the metabolite biomarkers (TBA and 2-HIBA) are damped in response and settle into an oscillation about an elevated level. Furthermore, the blood

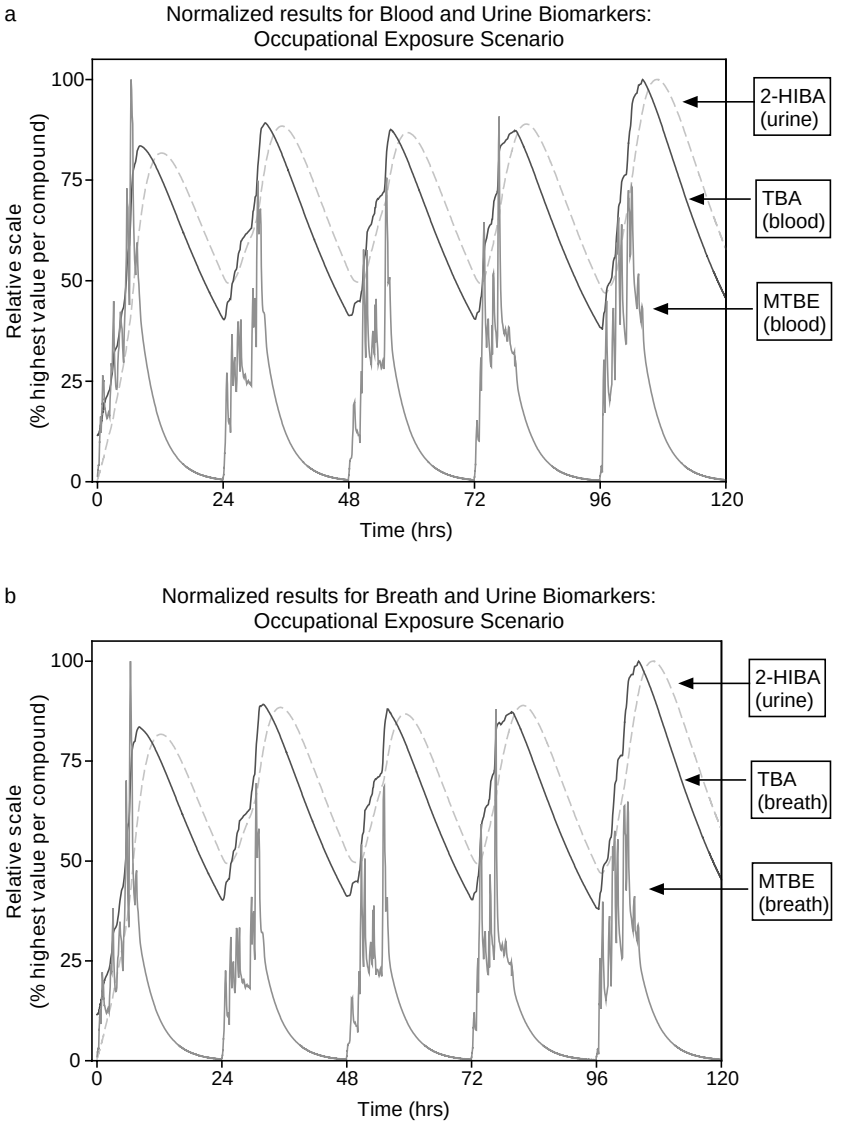

Figure 6. Comparison of normalized biomarkers response to occupational exposure scenario from Figure 4B of methyl tertiary butyl ether (MTBE) and tertiary butyl alcohol (TBA) in blood (a) and breath (b), with respect to 2-hydroxyisobutyrate (2-HIBA) in urine. Dashed trace indicates urinary biomarker 2-HIBA.

and breath profiles are almost indistinguishable with the exception that the blood levels of MTBE tend to be slightly more damped. We attribute this to the venous mixing in the body. The higher variability in the breath TBA over the blood-borne TBA could be explained by a small amount of rapid metabolism of MTBE to TBA in the lung during the inhalation cycle as suggested by the dotted arrow designated with 'K_{oT}' in Figure 1. This is not unreasonable as cytochromes P450 2A6 and 2E1 correlate with hepatic metabolism of MTBE in humans (Licata et al. 2001) and these isoforms are also

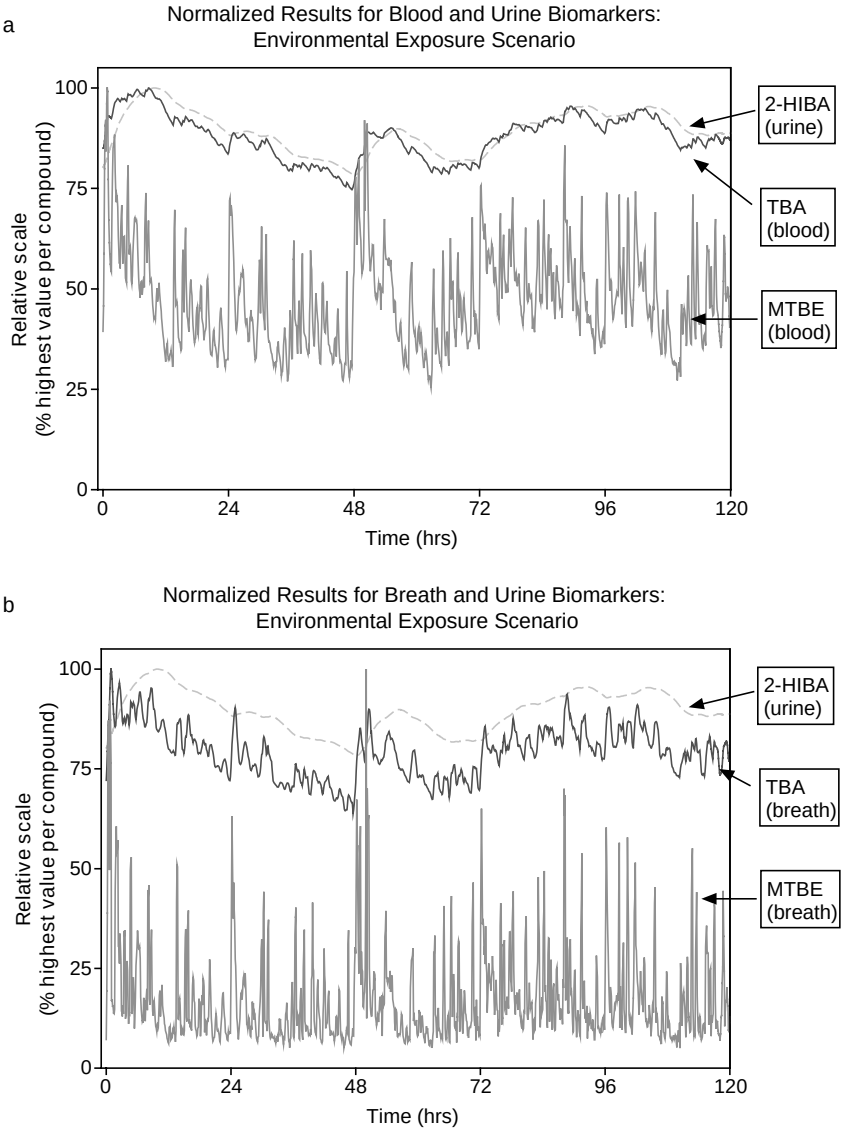

Figure 7. Comparison of normalized biomarker response to environmental exposure scenario from Figure 4C of methyl tertiary butyl ether (MTBE) and tertiary butyl alcohol (TBA) in blood (a) and breath (b), with respect to 2-hydroxyisobutyrate (2-HIBA) in urine. Dashed trace indicates urinary biomarker 2-HIBA.

associated with extrahepatic metabolism in the respiratory tract (Ding & Kaminski 2003, Castell et al. 2005). Further examination of this pathway is beyond the scope of the data presented here.

As expected, when the exposure profile is more regular or smoothed over time, the TBA and 2-HIBA metabolites exhibit less variability. From inspection of Figures 5–8 we observe that the urinary biomarker variability is indeed more damped than the TBA in breath or blood, but does not present an overwhelming statistical advantage despite having almost twice the biological (terminal) half-life.

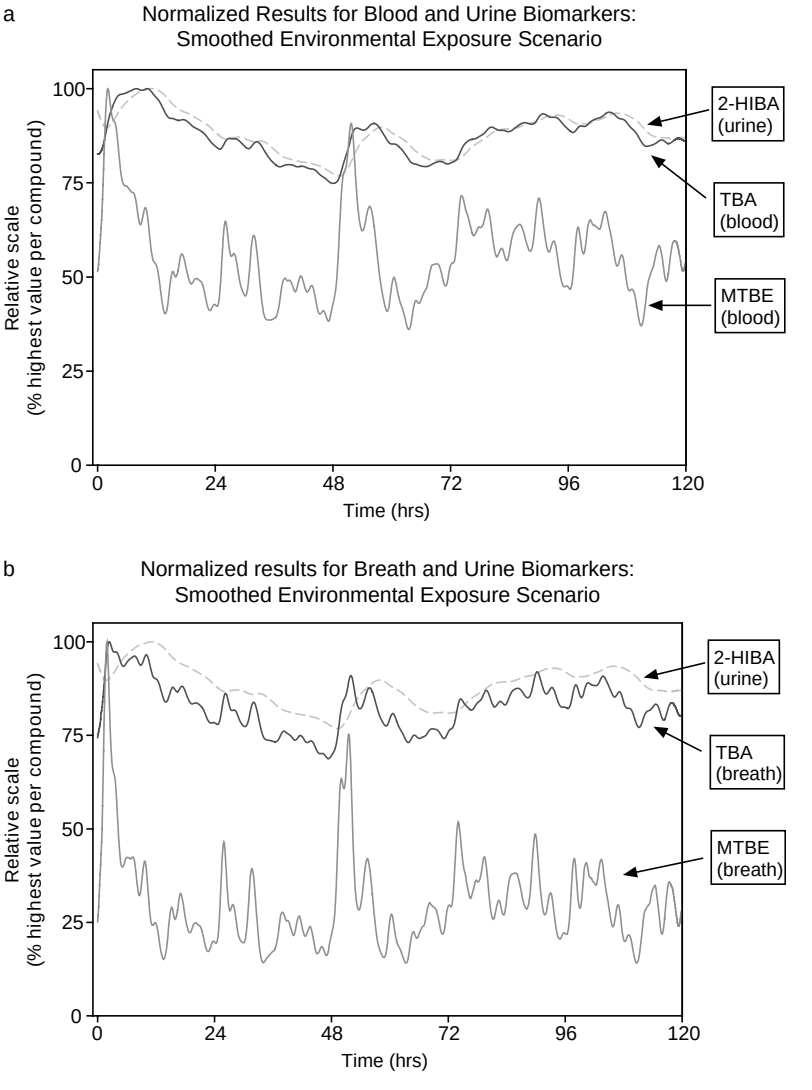

Figure 8. Comparison of normalized biomarkers response to smoothed environmental exposure scenario from Figure 4D of methyl tertiary butyl ether (MTBE) and tertiary butyl alcohol (TBA) in blood (a) and breath (b), with respect to 2-hydroxyisobutyrate (2-HIBA) in urine. Dashed trace indicates urinary biomarker 2-HIBA.

Reconstruction of previous mean exposures

Given the four exposure scenarios and the relative response factors developed from the classical PK model, we investigated the relationship among previous mean exposures and the various biomarker levels based on a random sampling of hypothetical subjects. Specifically, using the data presented in Figure 4a–d, we calculated the mean exposure for each scenario for the last 4 days to allow for equilibration of the models. We calculated only the median and range for each biomarker over the same time frame because the underlying distributions were not amenable to more detailed analysis. We used the conversion factors developed above to predict the airborne

Table I. Comparison of biomarkers for predicting expected inhalation exposures.

	Biomarkers				
	MTBE blood	MTBE breath	TBA blood	TBA breath	2-HIBA urine
Scenario 1: repeat chamber – expected 1.36 μg l^{-1}					
median	1.11	1.11	1.32	1.14	1.33
min	0.37	0.37	0.91	0.78	0.95
max	5.72	16.38	1.54	1.32	1.42
Scenario 2: occupational – expected 0.139 μg l^{-1}					
median	0.09	0.09	0.14	0.12	0.14
min	0.00	0.00	0.08	0.07	0.09
max	0.87	1.15	0.21	0.18	0.19
Scenario 3: environmental – expected 5.44 μg l^{-1}					
median	6.04	9.90	5.41	5.07	5.37
min	3.40	3.76	4.63	4.11	4.75
max	12.32	74.43	5.92	6.04	5.80
Scenario 4: environmental (smooth) – expected 5.50 μg l^{-1}					
median	6.32	12.18	5.52	5.22	5.51
min	4.42	6.12	4.80	4.37	4.86
max	10.66	32.61	6.00	5.85	5.92

MTBE, methyl tertiary butyl ether; TBA, tertiary butyl alcohol; 2-HIBA, 2-hydroxyisobutyrate.

concentrations that would give rise to the biomarker levels. In Table I we present the summary statistics for reconstructing previous mean exposures from blood, breath and urinary biomarkers. As we observe in Figures 5–8 and in the Table, the native compound MTBE follows the exposure profile with very little damping and so does not demonstrate an advantage over ambient snapshot samples. However, even under highly variable occupational and environmental exposures, we found that the metabolic biomarkers (blood/breath TBA and urinary 2-HIBA) are sufficiently biologically damped to allow us to reconstruct previous average exposure within a range of better than $\pm 20\%$.

Conclusions

Based on actual human measurements from controlled chamber exposures, we could develop a simple PK model to generalize the biomarker response to inhalation exposure as expressed in blood and breath. We further found that the model could be extrapolated to infer a major urinary biomarker based on literature PK values. By developing a series of exposure scenarios mimicking either repeat chamber experiments or reasonable occupational and environmental profiles, we found that averages of previous exposures could be reconstructed from spot biomarker measurements with increasing success as the biological damping of the biomarker increases. Furthermore, the biomarker reconstruction becomes more stable as the exposure profile is smoothed suggesting that this approach is probably more accurate for environmental exposures than for more periodic occupational exposures. We found that the exhaled breath biomarkers behave very similarly to those in the blood and give similarly valuable exposure estimates without an invasive sampling procedure. The mathematics further demonstrate that a

urinary biomarker is an equivalently useful estimator. We caution that biomarkers with half-lives on the order of a day rely on assumptions that the overall exposure scenarios do not change violently when attempting to generalize over longer time periods. It would be prudent to test such assumptions with a subset of repeat measures.

This approach demonstrates the efficiency of biomarker measurements especially for assessing personal exposures to large groups of subjects in that only single measurements are necessary. From this work, we conclude that a simple 'input–output' PK model is sufficient to generalize to complex exposures as long as we can assume that the general population is not appreciably different from the studied subjects. This issue needs to be addressed in future work wherein we would assess the within and between individual variances in response to a stressor. Given knowledge about such variance components, we suggest that our approach could be broadened to a variety of exposures and classes of compounds as long as some preliminary pharmacokinetic information is available and that an appropriate biomarker of the exposure can be measured. We also suggest that biomarkers of exposure could be metabolic products (aldehydes, ketones, cytokines, etc.) not directly chemically related to the exposure stressor, but only associated with the metabolism of the exposure. In such a case, the biomarkers could be used to address more broadly cumulative (multiple compounds and multiple routes) exposures.

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