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ORIGINAL ARTICLE

Influence of systems biology response and environmental exposure level on between-subject variability in breath and blood biomarkers

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Abstract

To explain the underlying causes of apparently stochastic disease, current research is focusing on systems biology approaches wherein individual genetic makeup and specific 'gene–environment' interactions are considered. This is an extraordinarily complex task because both the environmental exposure profiles and the specific genetic susceptibilities presumably have large variance components. In this article, the focus is on the initial steps along the path to disease outcome namely environmental uptake, biologically available dose, and preclinical effect. The general approach is to articulate a conceptual model and identify biomarker measurements that could populate the model with hard data. Between-subject variance components from different exposure studies are used to estimate the source and magnitude of the variability of biomarker measurements. The intent is to determine the relative effects of different biological media (breath or blood), environmental compounds and their metabolites, different concentration levels, and levels of environmental exposure control. Examples are drawn from three distinct exposure biomarker studies performed by the US Environmental Protection Agency that studied aliphatic and aromatic hydrocarbons, trichloroethylene and methyl tertiary butyl ether. All results are based on empirical biomarker measurements of breath and blood from human subjects; biological specimens were collected under appropriate Institutional Review Board protocols with informed consent of the subjects. The ultimate goal of this work is to develop a framework for eventually assessing the total susceptibility ranges along the toxicological pathway from exposure to effect. The investigation showed that exposures are a greater contributor to biomarker variance than are internal biological parameters.

Keywords: *Systems biology, human biomarkers, jet fuel, trichloroethylene, methyl tertiary butyl ether, exposure reconstruction, variance*

Introduction

The concept that environmental exposures can cause human diseases is not new; however, with few exceptions (cigarettes, asbestos, lead, radon, benzene, etc.), it is difficult to demonstrate exposure linkage to a specific disease (Bruske-Hohlfeld 2009, Luster & Rosenthal 1993, Huff 2007, Bergen & Caporaso 1999). Environmental exposures are generally complex, vary spatially and temporally, depend on individual activity, and the time to disease onset can be as long as decades. Additionally, many chronic and systemic diseases are rare, and so appear to be random in the population presumably because individuals have widely varying responses to similar external stressors. In contrast to many infectious disease transmissions, the toxicological pathway from environmental vector to adverse outcome is obscured by these and possibly epigenetic factors (Jirtle & Skinner 2007, Lund & Dumeaux 2008, Schmidt et al. 2008, Smolders et al. 2009).

The scientific study of the progression from environmental pollution sources to eventual adverse health outcome may be divided into two categories: (1) 'exposure assessment' which deals with the source

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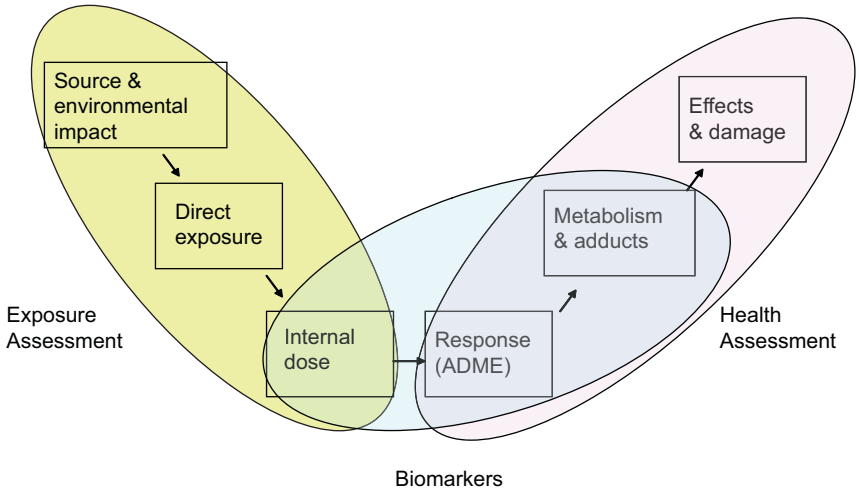

Figure 1. Diagram of path from source of contamination through exposure and then to health effect. The left and right ovals represent exposure and health assessment disciplines, respectively; the centre overlap area (biomarkers) represents the linkage between them.

terms, environmental transport, human exposure routes and internal dose, and (2) 'health assessment' which deals with metabolism, cell damage, DNA changes, pathology and onset of disease. The primary goal of understanding the linkage from source to health outcome is to provide the most effective and efficient environmental intervention methods to reduce health risk to the population. An important tool for deducing an individual response to a common external environmental stressor is the biomarker measurement (Albertini et al. 2006, Needham et al. 2007, Sexton & Hattis 2007). Biomarkers can be subdivided into chemical markers, exogenous metabolites, endogenous response chemicals and complex adducts (e.g. proteins, DNA). The relationships among these markers and corresponding environmental conditions can be interpreted to confirm exposure, to establish biological activity, to assess subtle damage to cells and proteins and to demonstrate early adverse effect. Biomarker levels can be interpreted retrospectively to reconstruct previous exposures (Pleil et al. 2007, Sohn et al. 2004) or prospectively as precursors to disease (Nieuwhuijsen et al. 2006, Perera et al. 1996, Perera & Weinstein 2000).

The study of environmental stressors and their relationship to health is viewed as a series of linked steps. Efforts to identify and relate these steps have been articulated in the literature as a variety of multidisciplinary or integrated approaches (Furtaw 2001, Hansen et al. 1998, Ritter et al. 2006, Rosenbaum et al. 2007, Bates et al. 2005, Simmons et al. 2005, Birnbaum & Cohen-Hubal 2006, Albertini et al. 2006). The US Environmental Protection Agency (EPA) has provided guidance through public websites (USEPA 2009a,b,c), and the author has summarized the current research approach for exhaled breath biomarkers as employed in EPA's Human Health Research Program (Pleil 2008, USEPA 2009c). For the purposes of this paper, the path from exposure to health effect is summarized in a simple six-step diagram (Figure 1).

As mentioned above, these general concepts have been expressed in a variety of different ways; for the purposes of this article, the six steps indicated in the diagram are defined as representing the following activities:

1. *Source and environmental impact*: this includes direct source measurements, fate and transport modelling, receptor measurement and modelling and ecological measurements.

2. *Direct exposure*: this includes direct environmental measurements (e.g. air, water, soil), personal monitoring, estimates of inhalation, ingestion and dermal exposures and documenting human activity patterns.

3. *Internal dose*: this includes estimating the magnitude of internal dose for individuals using chemical biomarkers, classical pharmacokinetic modelling, assessing between- and within-individual variance statistics and interpreting temporal and spatial dependences of exposures.

4. *Response ADME*: this includes assessing biological responses to internal dose by estimating rates of absorption, distribution, metabolism and elimination (ADME) of exogenous substances and their initial metabolites, modelling of physiologically based pharmacokinetic (PBPK) concentrations and deducing interactions of multiple chemicals and multiple exposure pathways.

5. *Metabolism and adducts*: this includes measurements of metabolic pathways, enzyme activity, differential protein/gene expression, formation of protein and DNA adducts, mutation markers and estimates of target organ doses.

6. *Effects and damage*: this includes assessing adverse biological effects to cells, tissues and DNA through study of tumour formation, propagation of mutations, cell death, germ-line mutation, disruption of metabolic function, impairment of repair function, biochemical enzymatic alterations and pathology.

The diagram presented in Figure 1 is informative, but can be somewhat misleading for the casual observer. The illustration of the path from source to effect is simplified and thus does not show the complex interactions within and between the basic steps of the progression. The most important pieces of information absent from the diagram are the loss and repair mechanisms between steps. For example, between no. 2 (direct exposure) and no. 3 (internal dose), there can be interindividual differences in absorption and elimination; between no. 5 (metabolism and adducts) and no. 6 (effects and damage), there can be large interindividual differences in enzyme detoxification and repair function. Such intersubject variances could conceivably cascade along the path and result in widely varying individual health outcomes from nominally identical external environmental exposures. This concept is most often referred to as individual (human) variability in susceptibility and has been discussed in context of biomarkers in the literature (e.g. Gallo et al. 2008, Lin et al. 2006, Hattis 1997, Hattis et al. 1999, Watson & Mutti 2004). More broadly, this is referred to as a 'systems biology' approach to exposure assessment (EPA 2009d).

This article addresses the concept of between-individual variance as an indicator of generic susceptibility to adverse health outcome; as the studies used do not have independent repeat measures, within-individual variance could not be assessed. A conceptual model is used to outline the possible pathways leading from environmental exposure to health effect analogous to the type of research performed in pharmacokinetics (e.g. Bauer 2008). The focus is on the early portion of the process (exposure biomarkers) where actual measurement data are available. Biomarker data are used from three distinct studies performed by EPA: (1) breath measurements of military jet fuel exposure, (2) blood and breath measurements of controlled exposure to trichloroethylene (TCE), and (3) blood and breath measurements of exposures to methyl tertiary butyl ether (MTBE). Empirical biomarker data are statistically interpreted to estimate the source of variance components that contribute to the overall variability observed in population based studies.

Methods

Conceptual model

Figure 1 illustrates the broad strokes of the progression from an environmental exposure source to an eventual health outcome. The actual pathway for any specific stressor is certainly more complicated, and is dependent on the chemical, biological and genetic interaction between exposure chemicals and the human systems biology. Based on the literature cited in the introduction and the author's own observations, a more detailed conceptual model is proposed that allows for multiple routes, and also provides recovery mechanisms in the form of elimination, sequestration, detoxification, repair and removal. Figure 2 presents some possible pathways from exposure to effect; the arrows can be interpreted as rate constants that would be dependent on an individual's genotype, current health state, phenotype, age, gender, activity, etc., all of which contribute to the between-subject variance represented as an individual's systems biology.

The conceptual model illustrates the potential for the propagation of uncertainty from individual exposure to effect. For example, an individual who encounters high exposures but is very efficient in eliminating the resulting chemical body burden may be at lower risk than one who experiences lower exposures but quickly metabolizes the chemical to a reactive metabolite that in turn creates harmful DNA adducts. Inversely, individuals with similar biochemical efficiencies in elimination and repair would be affected primarily by the magnitude of the external exposure. In this paper, the issues of uptake, body burden and metabolism are explored in the context of populating the respective boxes in Figure 2 with relative variance components. In the long run, such conceptual models will need to be modified and augmented to accommodate empirical data that is developed. For now, Figure 2 indicates only the potential for the complexity of the systems biology and serves as a road map for future research.

Jet fuel exposure studies

The US Air Force organized and conducted a series of studies in conjunction with US EPA and US National Institute of Occupational Safety and Health (NIOSH) regarding environmental and occupational exposure to the military jet fuel designated JP-8 (USAF 1996, Zeiger

& Smith 1998, Egeghy et al. 2003, Zeiger 2000, Serdar 2003). The author collected and analysed hundreds of exhaled breath samples from Air Force personnel before and after engaging in routine base activities. Although JP-8 is a performance rather than chemical-based mixture composed of thousands of compounds (USAF 1996), the focus was on major JP-8 constituents (C9 to C12 n-alkanes and single-ring aromatic compounds). Over 20 US Air Force and National Guard Bases were studied representing over 300 individuals in total with special emphasis on seven bases that were part of a more detailed study described in the National Academy of Sciences report (NAS 2003). For this paper, only the speciated results measured in the author's laboratory for exhaled breath measurements are used. Briefly, single alveolar breaths were collected using 1-l stainless steel canisters and analysed by gas chromatography–mass spectrometry (Pleil & Lindstrom 1995a,b, Pleil et al. 2000). Subjects were assessed before and after a 4-h activity period classified by exposure type. The total variance component was calculated including individual 'micro-activities' differences as well as individual differences in biological uptake and elimination. All samples were collected and analysed under Institutional Review Protocols approved by the US Air Force with informed consent of the subjects.

Trichloroethylene exposure study

US EPA and US Air Force collaborated on a controlled exposure study with human subjects to assess uptake and elimination of TCE, a commonly used degreasing agent and consumer product constituent. In addition to preliminary sampling and methods development tests (aggregate of 12 subjects), the detailed portion of this study observed three pairs of subjects in a 'live-in' exposure chamber over a 24-h period. During the first 4 h, the subject pairs were exposed to 100 parts per million by volume (ppmv) TCE atmosphere; this was followed by 20 h of purified air exposure. Throughout the total time, periodic breath and venous blood samples were collected in tandem to assess the kinetic response to the exposure and to compare the parameters with other halogenated organic compounds (Pleil & Lindstrom 1997). Breath samples were collected and analysed by the author (Pleil et al. 1998); blood analyses were performed under the direction of Jeffrey Fisher, Armstrong Laboratories, Wright Patterson Air Force Base, Ohio (Bishop et al. 1995). In contrast to the jet fuel work described above, in this study the exposure level and duration were accurately known and controlled. As such, intersubject variance in blood and breath assessment of body burden is due exclusively to biological factors. All samples were collected

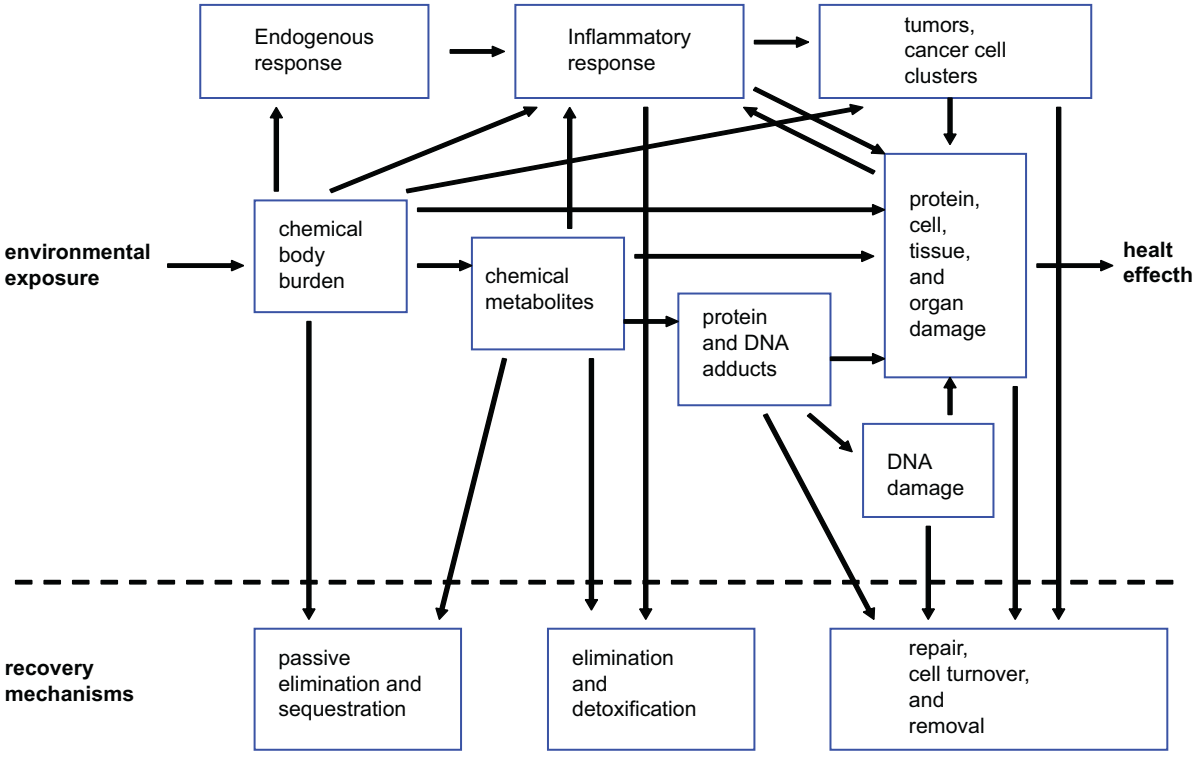

Figure 2. Conceptual model of some possible pathways for the progression from environmental exposure to eventual health outcome. The centre boxes represent, in aggregate, the concept of systems biology, and the connecting arrows represent the paths of the particular biochemical transitions. This is not intended to be complete, but rather an illustration of the complexity of susceptibility.

and analysed under Institutional Review Protocols approved by the US Air Force and Research Triangle Institute, Research Triangle Park, NC with informed consent of the subjects.

Methyl tertiary butyl ether study

As in the TCE work described above, this study also used controlled exposure scenarios. In this case, 14 subjects were exposed to MTBE in separate experiments via inhalation, ingestion and dermal exposure routes. MTBE and its primary phase 1 metabolite, tertiary butyl alcohol (TBA), were measured in venous blood (n = 14 subjects) and exhaled breath (subset of n = 7 subjects). Time series data were collected to follow the uptake and elimination for 24 h from short-term exposures: inhalation of 3.1 ppmv for 1 h, dermal exposure to one arm of 51.3 μg ml^{-1} in tap water for 1 h, and bolus ingestion by drinking 2.8 mg MTBE mixed into 250 ml Gatorade®. Breath samples were collected and analysed by the author at EPA; blood analyses were performed under the direction of David Ashley, Centers for Disease Control and Prevention, Atlanta, GA. The methods and results of these experiments have been described in detail and published (Bonin et al. 1995, Prah et al. 2004, Pleil et al. 2007). This work was performed under University of North Carolina Institutional Review Board (IRB) approval with informed consent of the subjects.

Data interpretation – fold ratio

The primary concern in this article is to assess properly the variability of the outcome of a set of biomarker measurements within the context of an exposure profile. The data from the three studies were grouped according to exposure cohorts and all statistical analyses were performed under the assumption that biomarker data were log-normally distributed within groups. This is not unreasonable as log-normality is mechanistically expected based on multiplicative influences from multiple factors (Hattis et al. 1999). There are a number of ways to assess the relative ranges of response distributions that are essentially similar; Hattis (1997) uses the $\log_{10}$ (GSD) as the dependent variable in his discussions to facilitate estimates of risk reduction through application of the traditional 10-fold uncertainty factor. Rappaport (1991) originally proposed using log-space estimates which have been implemented in metadata analyses (e.g. Lin et al. 2005) that use the 95% confidence interval (CI) fold ratio (FR_{95}) to compare results from different experiments; this approach is used in herein.

In the simplest case of a balanced dataset without repeat measures, the high and low 95% CIs for the distributions can be estimated based on the geometric mean (GM) and the geometric standard deviation (GSD): $CI_{97.5} = GM \times GSD^{1.96}$ and $CI_{2.75} = GM/GSD^{1.96}$. The extent of the variability for any given group of biomarker outcomes can then be calculated as the 'fold ratio' of the 95% CIs, that is

$$\left(FR_{95}\right) = \left[CI_{97.5}/CI_{2.75}\right]$$

which is interpreted as how many times greater the upper statistical bound is than the lower bound. In datasets with repeat measures, the fold range must be calculated using both the between- and within-subject variance components σ_b^2 and σ_w^2, respectively, and combined to find the 95% CI fold range

$$\left(FR_{95}\right) = \exp\left(3.92\sqrt{\sigma_b^2 + \sigma_w^2}\right)$$

In either case, the fold ratio allows direct comparisons among different log-normal distributions with different mean values based only on their 'spread' (Rappaport 1991, Lin et al. 2005). For example, if the mean exposure measurements have a 95% CI ranging from 0.5 to 2 pbbv, and the biomarker measurements in the population have limits of 0.02 μg l^{-1} to 12 μg l^{-1}, then the variability of the exposure (FR_{95} = 4) is less important than the biomarker response (FR_{95} = 600). If the biomarker response is interpreted as a measure of susceptibility, then one could infer that the individual's risk is primarily driven by an internal mechanism. If, however, the fold ratios were reversed, then one would conclude that reduction in exposure is of primary value for reducing risk of adverse outcome. Where appropriate, standard intraclass correlation (ICC) and ANOVA analyses were invoked to compare results. Data were post-processed using Excel 2002 sp2 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 4.03 (Graphpad Software Inc., San Diego, CA, USA).

Results and discussion

Jet fuel exposure studies

The seven bases exhibited different breath biomarker means both for baseline exposure (pre-work) and for post-work. This is expected as the bases have varying missions and a different complement of aircraft and fuel use. Data reliability was assessed based on 21 independent pairs of replicate breath samples. ICC for all compounds were >0.98, with most >0.99; this indicates that essentially all of the variance is between subjects and that sampling and analytical error is negligible.

Each of the datasets displays a log-normal character in their individual distributions. To illustrate this behaviour, Figure 3 shows the frequency distributions comparing pre-exposure and postexposure exhaled breath concentrations for fuels systems workers (only those workers who had hands-on contact with JP-8) using concentrations of decane (a major component of jet fuel) as an example. Data were adjusted for overall base mean to allow combining all bases into the histogram. The figure confirms that pre- and postexposures have log-normal distributions with similar GSDs. Table 1 shows all of the composite results across all bases for the major constituents of jet fuel, nonane, decane, undecane and dodecane, as well as a series of eight single-ring aromatic compounds ranging in volatility from benzene to 4-ethyl toluene that are associated with jet fuel and automotive fuel sources. The columns are organized as comparisons of pre- vs post-median exhaled concentrations, and as pre- vs post-comparisons of 95% confidence fold ratios as described in Eqn 1 (adjusted for base mean, as in the example for decane in Figure 3). In total, 152 individual subjects who performed hands-on fuels work were sampled post or during work shift; due to logistical constraints in the field, 23 of these subjects are not represented in the pre-work cohort.

For the overall study, subjects exhaled an average (median value) 16 times more hydrocarbon analytes after occupational exposure than before such work, but the average fold ratio as calculated across all alkanes and aromatics (33 vs 49) only increased by a factor of 1.5. Furthermore, if only the four primary jet fuel constituents represented by the C9 through C12 alkanes are considered, subjects exhaled on average 21 times as much of these compounds after work than before, yet the average fold ratios (42 vs. 41) remained essentially the same. These overall composite results indicate that fold ratio assessments are essentially independent of cohort exposure levels; that is, individual variance in biomarker response is probably more associated with micro-environmental contact and individual biological response rather than ecological measures of exposure. Although metabolites were not directly measured, the native compound levels reflect between-subject differences in metabolism rates through the variance in the elimination of the compounds of exposure.

In subsequent analyses, bases were treated as a fixed effect so that the within-base fold ratios could be compared without adjustment for between-base means. Table 2 shows a breakout of results for the four alkanes and eight aromatic compounds for each of the seven bases. In the table, summary statistics for median, fold ratio and activity are shown. All data subsets were assessed for log normality using

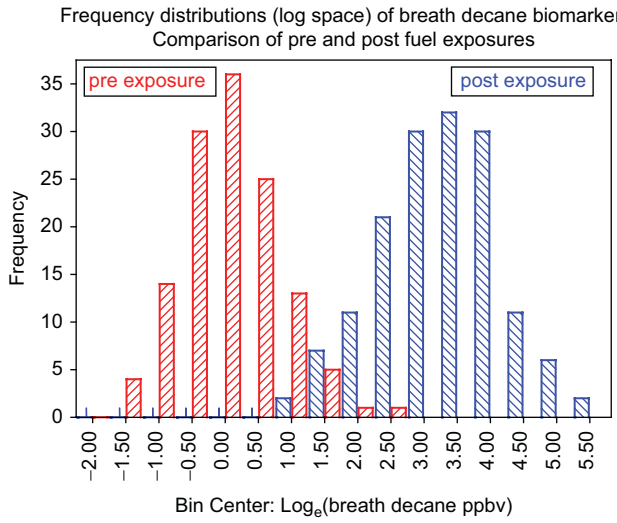

Figure 3. Illustration of log-normal character of distribution of exhaled breath biomarker decane, a primary constituent of JP-8 military jet fuel. Comparison shows that pre- and post-work exposure distributions are essentially identical, only the mean value changes. Data from seven bases are combined with adjustment for respective base means.

Table 1. Overall biomarker results for exposed fuels systems workers across seven Air Force bases.

	Pre-exp median (ppbv) (n = 129)	Post-exp median (ppbv) (n = 152)	Pre-expfold ratio (95% CI) (n = 129)	Post-expfold ratio (95% CI) (n = 152)
Nonane	0.7	28.1	79	48
Decane	1.0	26.3	20	39
Undecane	1.3	20.3	32	39
Dodecane	0.9	13.3	37	39
Mean (alkanes)	1.0	22.0	42	41
Benzene	0.6	2.2	55	62
Toluene	2.0	9.4	35	65
Ethylbenzene	0.2	3.5	25	46
m,p-Xylene	0.6	9.4	23	43
o-Xylene	0.3	5.3	23	53
4-Ethyltoluene	0.3	7.0	22	51
1,3,5-Trimethylbenzene	0.2	3.0	23	50
1,2,4-Trimethylbenzene	0.4	8.5	20	52
Mean (aromatics)	0.6	6.0	28	53

CI, confidence interval.

Shapiro–Wilk and Kolmogorov–Smirnov normality tests with most passing at p = 0.05; a combination of rare outliers and small 'n' skewed a few of the stratified results; however, there is sufficient statistical evidence that all underlying distributions have log-normal character.

Similar measurements made at an Air National Guard base demonstrated much lower levels than

Table 2. Comparison of two Air Force Bases (LA and PO) with the lowest and highest respective mean exposures as determined by exhaled breath measurements.

	Base LA				Base PO			
	Pre-exp (ppbv) all	Post-exp (ppbv) all	Post-exp (ppbv) fuels	Post-exp (ppbv) controls	Pre-exp (ppbv) all	Post-exp (ppbv)all	Post-exp (ppbv) fuels	Post-exp (ppbv) controls
Part A: ppbv	n=14	n=20	n=13	n=7	n=20	n=32	n=28	n=4
nonane	0.8	5.8	13.3	1.3	1.1	93.0	171.6	1.3
decane	0.9	6.0	10.9	2.0	1.1	72.1	132.9	1.0
undecane	0.8	4.9	6.8	2.7	1.8	47.5	81.8	1.1
dodecane	0.6	4.3	5.5	2.7	1.2	23.9	35.8	1.4
benzene	0.6	0.7	0.6	0.8	0.9	11.1	15.6	1.1
toluene	2.8	4.5	5.0	3.8	3.7	47.3	66.8	4.3
ethylbenzene	0.4	0.9	1.4	0.4	0.3	10.7	17.6	0.3
m,p-xylene	1.3	2.5	3.9	1.0	1.0	39.0	67.0	0.9
o-xylene	0.3	1.0	2.0	0.2	0.5	24.0	44.0	0.3
4-ethyltoluene	0.3	1.6	3.0	0.5	0.3	25.9	48.5	0.3
1,3,5-trimethylbenzene	0.1	0.6	1.2	0.2	0.3	12.4	22.4	0.2
1,2,4-trimethylbenzene	0.4	1.9	3.3	0.7	0.4	29.4	54.9	0.4

	Pre-exp (95% FR) all	Post-exp (95% FR) all	Post-exp (95% FR) fuels	Post-exp (95% FR) controls	Pre-exp (95% FR) all	Post-exp (95% FR) all	Post-exp (95% FR) fuels	Post-exp (95% FR) controls
Part B: Fold Ratios	n=14	n=20	n=13	n=7	n=20	n=32	n=28	n=4
nonane	44	452	65	60	78	3045	97	215
decane	34	107	33	23	26	2683	78	180
undecane	93	21	15	8	105	1102	45	117
dodecane	9	18	16	9	576	295	30	385
benzene	78	128	251	36	86	418	114	533
toluene	9	12	16	5	15	631	247	48
ethylbenzene	4	65	42	11	44	1462	158	86
m,p-xylene	6	55	27	15	41	2031	163	65
o-xylene	5	350	47	185	62	4006	205	80
4-ethyltoluene	18	155	43	42	41	4382	168	99
1,3,5-trimethylbenzene	22	140	40	37	91	3327	175	132
1,2,4-trimethylbenzene	20	117	46	39	32	3985	151	92

these means (Tu et al. 2004) suggesting large between-base variance. As such, the observations for the consolidated data shown in Table 1 were revisited in subsequent base-stratified analyses. Within each base, subjects were classified into fuels system maintenance (fuels) workers and controls. The control subject cohort was defined as workers who did not work directly with open aircraft fuel tanks. They comprised office workers, aircraft operations personnel, engineers, truck drivers, medical staff, etc. Table 2 presents data from two of the seven bases chosen to represent extremes in average fuels exposure level.

In Part A of Table 2, base 'LA' represents the low-exposure group with mean jet fuel compound levels in breath for fuels workers about 15 times lower than base 'PO', the high-exposure base. However, despite the obvious difference in work-related exposures, one-way ANOVA shows no statistical difference among all pre-exposure measurements and postexposure measurements for control subjects represented in data columns 1, 4, 5 and 8. This demonstrates that nominal overall exposure levels exist at these bases, but that the higher occupational exposures are eliminated overnight.

In Part B of Table 2, the assessment of fold ratios between these two extreme bases is somewhat different. Although the median values in Part A are similar, the spread of data is greater on average for the high-exposure base for pre-exposure samples and for post exposure controls. Furthermore, the stratified data confirm that the fold ratio calculation is probably not statistically useful for exposure assessment when controls and exposed subjects are naively grouped together as they are in the data columns 2 and 6. These results confirm that between-person variance (as expressed by 95% fold ratios), is much more likely to be affected by activity derived from differences in exposure than by internal biological mechanisms.

Table 3. Trichloroethylene chamber exposure results: six subjects.

Time point (min)	Blood median (μg l^{-1})	Breath median (μg l^{-1})	Blood/ breath ratio	Blood FR 95%	Breath FR 95%	Blood/ breath FR 95%
-240	0	0.0	na	na	na	na
-210	674	87.1	7.73	1.97	2.21	1.50
-180	937	95.9	9.64	1.40	2.64	2.65
-120	1167	120.6	9.68	1.59	2.16	1.60
-60	1358	90.1	13.62	2.81	3.78	6.43
-2	1321	112.0	11.79	1.60	2.41	1.62
1		114.8			2.59	
17	835	28.4	29.38	2.17	3.08	3.06
32	537	21.5	24.94	2.76	3.40	6.38
60	311	11.9	26.16	4.18	2.64	8.69
120	173	7.1	24.17	5.52	2.36	7.82
240	169	4.8	37.71	1.73	3.51	3.57
360	134	3.5	43.47	2.41	3.32	3.91
480	112	2.7	44.28	2.18	4.24	2.44
600		2.3			4.13	
720		2.5			4.08	

Biomarker	*n*	Global 95% FR
Blood	64	2.34
Breath	84	3.89
Blood/ breath	60	3.53

FR, fold ratio.

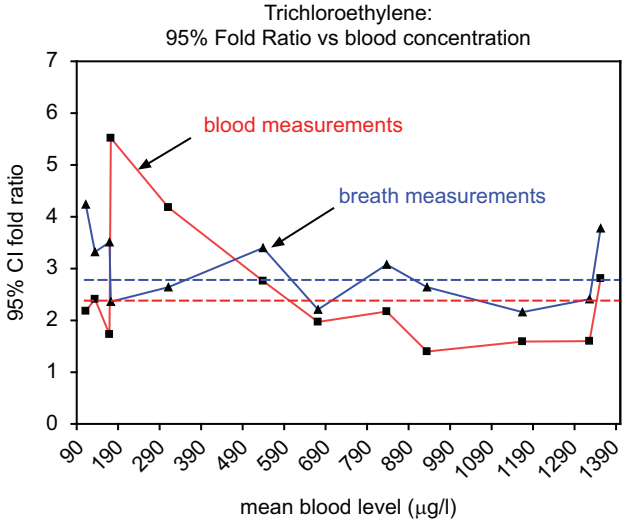

Figure 4. Illustration of variance of blood and breath biomarkers with respect to mean circulating blood level of trichloroethylene. Dashed lines indicate mean values for blood and breath biomarkers. Graph confirms that there is no consistent concentration trend, nor significant difference in variance between blood and breath media.

Trichloroethylene exposure study

This biomarker study has six subjects with multiple samples per subject ($n = 15$ for breath, $n = 12$ for blood). Because the concentration profile and the sample time within that profile are identical for all subjects, any between-subject variance is attributed solely to biological differences. Under the basic assumption that ADME is directly related to susceptibility, then these results should provide the first variance components in the progression from exposure to health effect.

This study was originally intended to assess classical pharmacokinetic properties of TCE exposure based on temporal profiles with breath sampling (Wallace et al. 1993, Wallace & Pellizzari 1995, Pleil et al. 1998). For this application (assessing variance), the sampling time points are treated as individual sample groups across all subjects. This is a legitimate use of data in that the samples within time groups are independent. Furthermore, within-subject autocorrelation is a moot point because time data are not treated as repeat measures. The basic dataset comprises 12 discrete time points for six subjects measuring blood and breath concentrations, plus an additional three time points for breath alone. Table 3 presents the general overview of the data structure, and summary statistics for mean response and fold ratio. Blood samples were not collected for $t = 1$ min, and the blood samples collected at 600 and 720 min were below analytical detection limits.

These results demonstrate that under the conditions of controlled exposure, the response of the subjects has consistent 95% fold ratios in the 1.5–5 range, with overall composite medians for blood and breath as 2.4 and 2.8, respectively. Figure 4 shows between- subject fold range as a function of estimated blood concentration and demonstrates that there is no particular trend in variance due to compound level confirming that the variability of internal dose created by the physiological and biological differences of the subjects is independent of the exposure level. Furthermore, although the empirical blood/breath ratios are different between uptake and elimination phases of the experiment as discussed in detail in the literature (Pleil et al. 1998), the between-subject fold ranges are remarkably consistent. These results indicate that the internal (between-subject) correlation between blood and breath measurements is consistent and that a non-invasive breath measurement is a reasonable surrogate for a blood measurement as long as rapid changes in exposure are considered.

Methyl tertiary butyl ether study exposure study

Similarly to the TCE work, this study was performed with controlled chamber exposures and limited numbers of subjects. Summary data for fold ratio calculations are presented in Table 4; to avoid calculations with 'below detection entries' and to better correlate breath and blood analyses, data were truncated to begin at 15 min

Table 4. Overall between-subject fold ratios (95% confidence interval) for TBE Chamber Study.

	Dermal			Ingestion			Inhalation		
	MTBE	TBA	MTBE/TBA	MTBE	TBA	MTBE/TBA	MTBE	TBA	MTBE/TBA
Blood	4.7	5.1	4.6	4.5	4.2	3.4	2.9	2.4	5.2
Breath	5.5	6.2	11.8	3.1	3.8	2.8	2.3	2.4	4.1
Blood/breath	4.4	5.6	10.8	4.3	3.6	2.6	2.6	2.1	5.1

MTBE, methyl tertiary butyl ether; TBA, tertiary butyl alcohol.

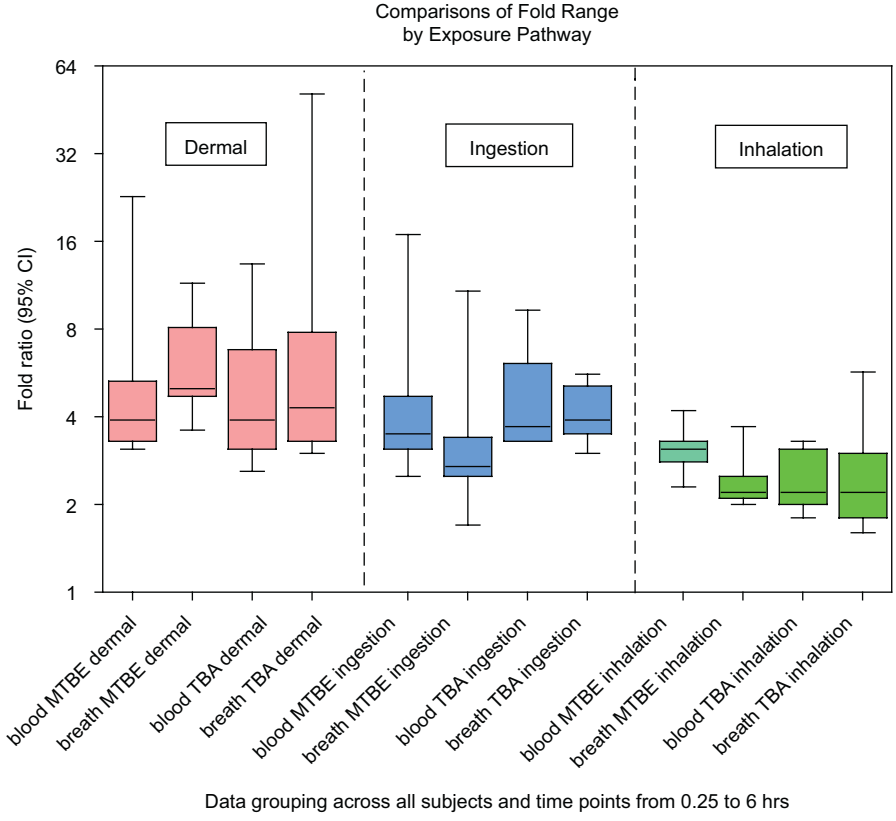

Figure 5. Fold range comparison dependence on exposure pathway, blood and breath media, and native vs metabolite biomarker. Dermal exposure has greater overall variance, blood and breath media variance are indistinguishable within pathway grouping, and metabolite conversion does not appreciably change measurement variance.

after exposure start and end at 6 h postexposure for dermal and inhalation (equivalent to 7 h after bolus ingestion). These results demonstrate that there are essentially no differences between paired blood and breath biomarker fold ratios, nor is there appreciable additional variance created via the metabolic conversion from MTBE to TBA. The ratio of exhaled MTBE to TBA is somewhat higher across the board probably due to the additive effect of differences in blood flow and tissue distribution. Furthermore, the exposure pathway influences biomarker variance the most for dermal, and the least for inhalation exposures with the greatest perturbation observed in breath levels of the MTBE to TBA ratio resulting from dermal exposures. Ingestion exposure demonstrates the least variance in metabolic conversion presumably due to the 'first pass' liver metabolism effect; that is, all of the blood from the stomach first travels to the liver before distribution to rest of the body and so the circulatory differences between subjects are minimized.

Figure 5 illustrates the influence of exposure pathway; box and whisker plots are shown for MTBE and TBA biomarker results from breath and blood, grouped by dermal, ingestion and inhalation exposures. Each box plot represents a collection of between-person fold ratios that treat time points as independent measures as in the previous section regarding TCE exposures. Not only are the overall mean values (as indicated in Table 4) decreasing from dermal to ingestion to inhalation, the scatter making up the global numbers is also reduced depending on the progression of exposure pathway.

Comparison of study results

The exhaled breath study of jet fuel exposure shows that small cohorts of subjects with nominally similar (but uncontrolled) exposures have 95% fold ratios ranging from 20 to 80 (median 50) regardless of the specific compound or of mean exposure level. In contrast, the chamber studies of subjects with identical controlled exposures for TCE, MTBE and the metabolite TBA have 95% fold ratios ranging from 2.3 to 6.2 (median 3.8) regardless of mean exposure level, biological medium (blood and breath) or compound. Furthermore, when subjects are grouped by location only (disregarding activity codes), the fold ranges can increase to the multi-thousands, as indicated in Table 2 for the high-exposure location, base PO. This demonstrates empirically that the subtle effects of external exposure parameters (activity patterns, micro-environmental concentrations, timing, etc.) are of critical importance in contrast to the internal biological parameters of uptake and distribution of internal dose.

Implications for exposure reconstruction

As an example for the influence of variance on exposure reconstruction, previous work has estimated that exhaled TBA concentration can be multiplied by a correction factor of 25.8 to estimate the mean population value of previous mean inhalation exposure to MTBE (Pleil et al. 2007). So, if 1 ppbv TBA is observed in an individual's breath, then a previous mean exposure of 25.8 ppbv to MTBE is assigned. From the above discussions, this value is bounded by the individual's internal systems biology variance by about a fold ratio of 4.1 (based on MTBE/TBA ratio, Table 4) implying a 95% CI of 12.5–52.6 ppbv MTBE previous mean exposure. Similarly, if another subject breathes out 10 ppbv TBA, this implies a previous MTBE exposure to 258 ppbv with 95% CI from 125 to 526 ppbv. Based on these hypothetical biomarker measurements, one could safely assign statistically different exposures to these two subjects. However, suppose similar subjects were active in an environment with an expected integrated environmental level of 100 ppbv MTBE. Their expected mean exhaled TBA level would be about 3.9 ppbv. However, depending on their activity patterns, different subjects would have actual internal doses resulting in TBA values with a 40-fold ratio of 95% confidence (Table 1). As such, in the absence of a biomarker measurement, one would assign the same exposure to all subjects that would actually have a 95% CI exposure range from 0.25 to 644 ppbv MTBE. The results from this work show that a biomarker measurement is not perfect; however, it can reduce the exposure level assignment error appreciably. Using personal dosimetry monitors (e.g. breathing zone) rather than meso-scale environmental monitors may improve the links between external level and internal dose but still does not address multipathway exposures.

Comparisons to other studies

The particular observations and fold ratio interpretations developed here are new and thus direct comparisons to other studies are not easily achieved. There are, however, two recent articles that explore variance concepts that support the conclusions here.

The observed variance behaviour of external exposures under nominally similar conditions is discussed by Vermeulen et al. (2004) who measured personal exposures to benzene and toluene of 2783 Chinese shoe workers in 14 different work categories and presented 90% fold ratios; when recalculated for 95% confidence, benzene had a median fold range of 70 (min. 17, max. 221), and toluene had a median fold range of 32 (min. 21, max, 187). Because this external exposure measure only represents the inhalation pathway, one would expect that the fold ranges represented by biomarker measurements would be greater due to dermal exposure influence. Such fold ranges for cohorts with observed similar exposures are of the same magnitude as observed in the JP-8 study presented above.

A metadata study of 127 datasets representing in total 12 077 observations by Lin et al. (2005) compared the within- and between-person variance for both air and biological marker measurements. The purpose was to provide guidance if and when a biomarker design is a better indicator of personal exposure than ambient air measurements. The authors presented a variety of statistical parameters of which their estimates of between-person 95% fold range for biomarker measurements and for air measurements are all in the range of from 4 to 9. The within-person fold range estimates, however, are consistently higher for air measurements than their biomarker counterparts ranging from 5 to 104. From these summary results and additional detailed analyses, they conclude that '…biomarkers appear to provide less biased estimates of true exposure levels for epidemiological studies'. Although direct numerical comparisons to the present work are not possible, Lin et al. (2005) have come to the same interpretation that the variance in the biological measurements is less likely to skew results than variance in external individual exposures, at least in the case of the inhalation exposure pathway.

In conclusion, interpretation of empirically measured biomarkers (as presented above) provides three observations regarding the variance components of the initial steps of exposure uptake, internal dose and phase 1 metabolism: (1) there is no apparent effect on between-person biomarker variance due to biological sample medium (breath or blood), nor due to the

particular type of compound of exposure (hydrocarbon, chlorinated hydrocarbon, ether or alcohol), nor due to the measurement of a native compound (MTBE) vs a phase 1 metabolite biomarker (TBA); (2) there is a measurable effect on between-person biomarker variance due to exposure pathway as shown in Figure 4; dermal exposures result in greater biomarker variance than ingestion and inhalation pathways; (3) there is a pronounced effect on between-person biomarker variance due to exposure group assignment; when subjects are grouped into nominally identical exposure cohorts based on activity and location, they exhibit 10 times greater biomarker variance than when subjects experience identical controlled chamber exposures.

From the perspective of using biomarkers to reconstruct previous exposure of a specific individual to an exogenous compound, the best one could expect to achieve is about a factor of 4 within 95% confidence; this variance is internal to the person, and thus independent of the reconstructed exposure level. However, because even nominally identical conditions of exposure to a cohort of subjects results in 10 times higher biomarker variance, a biomarker measurement from an individual within that cohort would still have great value in that it would discriminate among specific exposures beyond the factor 4 limit. Furthermore, exposure assignments to larger cohorts of individuals (e.g. on a regional level) that are based on ecological data can represent biomarker variance for individuals on the order of 2000 or more at 95% confidence. For such scenarios, the factor of 4 variance from an individual's systems biology represents a very small fraction of the total range of potential individual exposure. As such the biomarker measurement greatly reduces uncertainty in exposure assessment in contrast to assigning exposure with ecological environmental measurements.

Some caution must be observed for generalizing these results. First, the results are based on relatively volatile and thus relatively short-lived compounds measured in breath and blood; it is as yet unclear how the statistics would turn out for species with longer half-lives. Furthermore, this assessment is based on three relatively small studies with nominally healthy non-smoking adults, for a limited number of compounds of exposure, and with overlapping inferences regarding scenarios and metabolite availability. Nonetheless, every analysis and comparison is consistent across the disparate studies suggesting that there is a commonality in reducing error in exposure assignment when using a biomarker approach for assessing exposures in the general public.

The philosophical impact of this work is to demonstrate with empirical biomarker measurements that external environmental cumulative exposure variability dominates the internal biological mechanisms with regards to overall biomarker response. This confirms the value and effect of mitigation strategies that reduce environmental toxicants and that quantitative absorption/distribution of environmental chemicals is only marginally affected by interindividual systems biology.

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