Published in final edited form as:

J Breath Res. 2008 September ; 2(3): 037018. doi:10.1088/1752-7155/2/3/037018.

Quantifying heterogeneity in exposure—risk relationships using exhaled breath biomarkers for 1,3-butadiene exposures

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Abstract

The health effects of human exposure to 1,3-butadiene (BD) have been extensively studied using both epidemiological and animal toxicology approaches. However, various data and knowledge gaps remain, one of which is an understanding of the human heterogeneity in BD dosimetry. The objective of our study was to better understand the role of individual variability in delivered tissue dose. We designed a study of laboratory exposures of a relatively large group of healthy human subjects. Subjects were then exposed to 2.0 ppm BD through a face mask for 20 min, followed by 40 min of breathing clean air. Exhaled breath concentrations of BD were measured at ten time points during and after exposure, and a three-compartment physiologically based pharmacokinetic (PBPK) model was used to quantify the kinetic behavior of BD. We implemented a Markov chain Monte Carlo procedure to fit the model to the experimental data, and used global sensitivity analysis techniques to examine the sensitivity of exhaled breath concentrations to PBPK model parameters. Uptake during exposure was strongly influenced by rebreathing of exhaled BD during exposure; inclusion of rebreathing in the model simulations resulted in a 21% increase in the amount of BD retained in the body. We found that uptake ranged from 38% to 77% across individuals. We measured considerable intra-individual variability from 11 subjects who underwent the testing twice. Most of this variation stemmed from phase I metabolism of BD, which varied by as much as a factor of 2.6 within individuals. Overall, we have sought to quantify the sources of inter- and intra-individual variabilities in the pharmacokinetic behavior of BD. The results of our research may impact the current framework for biomarker and pharmacokinetic studies by improving our understanding of the sources of heterogeneity in response to chemical exposures.

Introduction

One of the central questions for environmental health is: How much exposure to a toxic agent produces how much risk of disease? This is entirely analogous to the problem of determination of the maximally effective dose for pharmacology. In drug development and assessment, the route of administration, tissue distribution, metabolism and elimination are estimated, measured and subsequently tested *in vivo* to generate therapeutic ranges that have maximal clinical utility. Variability in individual responses, in this paradigm, is expected and has become one of the central issues in the emerging field of pharmacogenomics.

Development of quantitative exposure–risk relationships in environmental health will similarly depend strongly on the tissue dose of the agent. Two processes define the ultimate tissue dose: uptake during and after an exposure, and metabolic biotransformation that produces or removes the active agent. These processes are rate limiting for the internal dose: if there is little uptake then only a small amount of parent compound will be available for biotransformation, and if activation is slow or if deactivation is rapid then little of the active metabolite will be present, even if uptake is large. The highest risk will occur for people with high uptake, rapid activation and slow deactivation. Unlike medical pharmacology, very little human exposure–dose data have been developed to explore these relationships in environmental health or to examine gene–environment interactions in this context. While it is recognized that people with identical environmental exposures may have a wide range of internal doses, the differences have not been well explored; this is the topic of the emerging field of exposure biology.

1,3-Butadiene (BD) is an excellent example of this problem. Most people have daily exposures to low levels of BD: 0.4 ppb has been observed in city air pollution, primarily coming from vehicle exhaust [1–4]; smoky clubs and bars have higher exposure levels as a result of the approximately 2 µg BD emitted by each cigarette [5, 6]. Petrochemical and synthetic rubber worker exposures are much higher, 0.1–20 ppm [7, 8]. Human cancer risks from BD have only been studied in a few cohorts of production workers in the U.S. and around the world, and these studies have had inconsistent results [9–11]. A suggestion of elevated leukemia and lymphoma risk has been seen, but the BD exposure–risk relationship is uncertain because of very limited exposure data. Recent risk assessments by IARC, US NTP, US EPA and others have determined that BD is a suspect or probable human carcinogen based primarily on the animal evidence [12]. Although human exposure–risk relationships have been extrapolated from animal findings, because of the wide divergence in animal sensitivity and the lack of human metabolic data, such extrapolation is highly uncertain and remains controversial.

Cytochrome P-450 enzymes in mammals metabolize BD to mutagenic mono- and diepoxide compounds (possible metabolic pathways are shown in figure 1). Oxidation of BD by cytochrome P450s results in the formation of butadiene monoxide (BMO). This reaction has been demonstrated in microsomes from several rodent and human tissues; the primary P450s involved in BD oxidation in human liver are CYP2E1 and CYP2A6 [13]. BMO forms covalent adducts with DNA [14], and it is directly mutagenic in bacterial and mammalian assays [15]. BMO is further oxidized into diepoxybutane (DEB) by P450 2E1. The *in vitro*

mutagenic potency of DEB is approximately 100-fold greater than BMO [16]; thus, DEB is thought to play a critical role in the carcinogenic activity of BD. Reduction of DEB by epoxide hydrolase forms diol-epoxybutane, diol-BMO, or it can be formed by reduction of BMO to diol-butene and then oxidation of the remaining double bond. Diol-BMO also forms covalent adducts with DNA, and is mutagenic in rodent bioassays [17]. BD has been extensively studied via *in vivo* animal testing and *in vitro* experimental studies over the past 20 years [18]. It is a clear carcinogen in rats and mice, but rats are approximately 1000-fold less sensitive than mice. These large inter-species differences in carcinogen sensitivity appear to be associated with major differences in epoxide formation and detoxification rates [19]. Human formation and removal rates for BD epoxides have only been determined with *in vitro* tests on small numbers of tissue samples obtained from trauma and cancer cases, which may not be representative of the general population [20].

To more completely understand the role of individual variability in delivered, tissue dose and thereby to move toward mechanism-based exposure–risk estimation for BD, we undertook a study of laboratory exposures of a relatively large group of human subjects drawn from the general population. Our research questions were: given identical inhalation exposures (exposure intensity $\times$ duration = administered dose), how do BD uptake and metabolism vary in a cross-section of the general population, and what factors (e.g. age, sex, race, metabolic genetic differences, etc) are associated with significant differences in internal epoxide dose?

Methods

We used a mass balance experimental design. BD enters the body by inhalation and can only leave by either exhalation or metabolism, as shown in figure 1. The difference between inhaled and exhaled BD during exposure indicates uptake. However, during our short exposure only a portion of the material retained is metabolized. Some is also redistributed among the tissues, especially the body fat, so the uptake overestimates the biotransformation rate. This limitation was overcome by fitting the time course of exhaled breath concentrations during and after exposure with a physiologically based pharmacokinetic (PBPK) model that accounts for redistribution to the tissues and metabolic biotransformation.

Human subjects' testing

The Human Subjects Committee at the Harvard School of Public Health reviewed and approved our study protocol. The exposure was judged to be within the range of everyday risks from exposure to BD (ppm $\times$ h), which include ever smoking one or two cigarettes, or spending an evening or two in a smoky club or bar. Volunteers were told that our experimental exposure could make a small, but real increase in their lifetime risk of leukemia, which was estimated as less than 10^{-6} using the California EPA risk assessment with the female mouse as the most sensitive species [21, 22]. Only healthy adults were recruited for the tests. We studied approximately 50% males and 50% females that represented a wide range in ages (19–65 yr) and ethnicity/race (we sought subjects from the

four major ethnic groups in the US (Caucasian, African American, Hispanic American (non-black) and Asian)) (see table 1).

Test protocol and apparatus

A brief description of the test procedure is provided below; further details of the test procedure have been described elsewhere [23]. Prior to the test exposure, we obtained informed consent, had the subject complete a short questionnaire (demographics, lifestyle, diet, occupation, medical background) and took blood and baseline urine samples. Blood samples were used to determine the blood to air partition coefficient ($PC_{b/a}$) of BD and genotypes of polymorphic metabolic enzymes: CYP2E1 [24], epoxide hydrolase [25] and glutathione-S-transferases μ and θ [26, 27]. Exposure and breath sampling were performed with a simple, computer-controlled, face-mask exposure system, which collected precisely timed samples of exhaled breath at time baseline, 2, 5, 10, 15, 19, 21, 22, 28, 38 and 58 min [23]. In this system, a subject inhales the test gas or clean air through one-way valves in a breathing face mask. Solenoid valves control the type of gas inhaled, as well as the collection of timed breath samples; the operation of solenoid valves is controlled in accordance with a predetermined schedule set by a computer program. The subject's breathing activity is monitored with a real-time breathing monitor, which is calibrated by both electronically and with an external standard volume before and after an inhalation experiment. Respiratory monitoring was performed with the RespiTrace breathing monitor with the RespiEvents data processing software (Nims, Inc., Miami Beach, FL). This system estimates lung volume (total thoracic volume) of subjects by measuring the changing capacitance of wires embedded in elastic bands placed around the subject's chest and abdomen. The real-time respiratory kinetic information, calculated on the basis of lung volume, included breath-by-breath tidal volume, breathing frequency and pulmonary minute ventilation. The test exposure was 2.0 ppm (v/v) BD administered through the face mask for 20 min, followed by 40 min of breathing purified air. After the test, 250 mg chlorzoxazone was administered to phenotype CYP2E1 metabolism by measuring the quantity of 6-hydroxy-chlorzoxazone eliminated in the urine in 6 h [28]. A total of 144 subjects were tested. After removing subjects with incomplete data and those with data quality problems, a final data set of 133 remained (table 1). Eleven subjects were tested twice with the second test, 4–8 weeks after the first.

We collected mixed exhaled breaths (both airway and alveolar air) in Tedlar sampling bags; immediately after testing, the collected breath was drawn through 100/50 mg Anasorb coconut-shell charcoal sampling tubes (no. 226–73 tubes; SKC Inc., Eighty Four, PA). The charcoal was pretreated with 4-*tert*-butylcatechol (TBC) to prevent self-polymerization of the collected BD. Charcoal tubes were stored in a refrigerator at -20°C until analysis. BD was analyzed immediately using a modified analytical method (NIOSH method 1024). A Hewlett Packard gas chromatograph/flame ionization detector (HP 5890 series I) and HP model 6890 autosampler were used for the sample analysis. The analytical columns included a $50\text{ m} \times 0.32\text{ mm}$ ID fused-silica porous-layer (PLOT) column coated with aluminum oxide/potassium chloride (Cat. No. 7515, Chrompack) and a back-flushable precolumn of $10\text{ m} \times 0.5\text{ mm}$ ID, fused silica CP wax 57 CB (Cat. No. 7648, Chrompack). The initial oven temperature was held at 50°C for 2 min, and then increased at a rate of $10^{\circ}\text{C min}^{-1}$ up to

200 °C, and held at 200 °C for 1 min. The purge time to begin the back-flush process was set at 0.6 min following injection. The charcoal was desorbed with 1.6 ml of methylene chloride (99.9%, Burdick & Jackson, Inc., Muskegon, MI), and the injection volume was 2 μ l. A six-point calibration curve (range of 0.027–36.8 μ l⁻¹) was used to quantify BD concentrations. The limit of detection is 0.006 ppm of BD in a 5.0 L exhaled breath sample with a coefficient of variation of 10%.

Exposure data and uptake

Examples of typical exhaled breath data are shown in figure 2. The breath concentrations had the time course expected for an inhaled substance with moderate blood solubility, $PC_{b/a} = 1.5 \pm 0.35$ (mean $\pm$ SD). There was a rapid rise to a near plateau during exposure and an initially sharp decline post-exposure, followed by a slower decline.

The total uptake was calculated as the difference between the BD inhaled during exposure and the total BD exhaled during and after the exposure. During exposure, there was significant rebreathing of the exhaled BD because the inhaled air includes the previously exhaled air in the deadspace of the airways and the face mask. After 5 min of exposure, exhaled air has a high concentration of BD, ~80% of inhaled, because of BD's modest blood solubility. Also, the deadspace in our system was relatively large; the face mask was ~50% of the subject's tidal volume. Inhaled air is a combination of new air and pulmonary deadspace air that contains the alveolar concentration from the last breath. As a result, the total intake during exposure is

$$\text{Intake} = \text{Flow}_{\text{alv}} \times C_{\text{in}} \times 20 \text{ min} + F_{\text{ds}} \times \text{Flow}_{\text{total}} \times (C_{\text{alv}} \times \text{Duration})_{\text{interval}},$$

where C is a concentration, either inhaled or alveolar; F_{ds} is the relative fraction of each subject's tidal volume that is deadspace; Flow is a ventilation, either alveolar or total minute ventilation; and Duration is a time interval. Here, we report the total uptake, which is calculated by the total input, including the absorption of recycled exhaled breath during exposure, minus the quantity of BD exhaled during and after exposure stops.

Model fitting and data analysis

The breath data for each subject were used to estimate the rate-limiting, first step epoxidation rate of BD by a PBPK model, as shown in figure 3. Three compartments were formed from tissues with similar perfusion (blood flow per unit of tissue). The three tissue compartments are 'well perfused' (WP: liver, brain, lungs, kidneys and other organs), relatively 'poorly perfused' (PP: muscles and skin) and the 'fat' tissues (F). BD can be rapidly metabolized in liver, lung and kidney tissues, so the epoxidation of BD was placed in the WP compartment, and not just in the liver [18]. Earlier analysis confirmed that a model with metabolism only in the liver could not fit the observed data for ~30% of the subjects [28]. The tissue volumes and blood flows for each individual were constrained by basic physiologic scaling functions, which use the subject's age, sex, height and weight in the calculations, as shown in figure 3. Based on observed variances and ranges of human values, we specified *a priori* distributions for each of the parameters for each subject, which were

the starting values for the fitting process. Since the metabolic rate was *a priori* unknown, we only identified a broad range for it that we thought might include the subject's value.

A Markov chain Monte Carlo (MCMC) fitting process was used with a hierarchical set of PBPK models: one for each individual and one for the population to describe variability between subjects [29]. The calculations were performed using the MCSim software developed by Bois and Maszle [30]. The parameters for these were all fit simultaneously to develop joint posterior distributions. The fitting procedure is based on a Bayesian statistical framework that begins with the set of prior parameter distributions. The calculation is done by first drawing a random set of parameter values from the prior distributions for each subject, then these values are used in the model to calculate estimates for the measured time points, and finally the estimates are compared to the observed values for the individual. Based on the degree of agreement, the parameter estimates are adjusted using the Bayes equation and new parameter distributions are sampled and the procedure is repeated. After a large number of repetitions, e.g. 10 000 times, the revised parameter distributions for the individuals and population will converge on a set of stable estimates, unless the model cannot be fitted to the data. The final parameter distributions are the 'posterior' estimates. The advantage of this approach is that it allows the use of the extensive prior information on human physiology to constrain the models. It also fits all of the parameters simultaneously and separates uncertainty from variability.

Sensitivity analysis

The global sensitivity analysis (GSA) technique was used to evaluate the relative importance of the PBPK model parameters on the concentration of BD in exhaled air during the 20 min of exposure and the 40 min after cessation of exposure [31]. In GSA, all model parameters are varied together such that the impact of a parameter on exhaled air and its interactions with other parameters can be examined [32]. The sensitivity of exhaled air to model parameters was quantified by calculating total order sensitivity indices (TOSI). TOSI were computed with lognormal distributions representing heterogeneity in the parameters. The mean values for the physiological parameters, partition coefficients and the rate of biotransformation of BD into EB (K_{met}) were obtained from the literature [33–35]. Information on distributions was obtained from previous analyses of the pharmacokinetic behavior of BD [28].

Results

Table 2 shows that uptake during exposure was strongly influenced by rebreathing of exhaled BD during exposure. Including this effect resulted in a 21% increase in the amount of BD retained in the body, $57 \pm 7\%$ versus $36 \pm 8\%$ obtained without considering rebreathing. Although the exposures were all identical (2.0 ppm for 20 min), there was large variation in the total uptake, which ranged from 38 to 77%. Without correction for rebreathing, the observed fraction of BD retained (36%) was low relative to what was predicted based on the solubility of BD in blood (approximately 60%) [36]. In an earlier paper, Lin and coworkers found that the conventionally defined uptake during exposure

(ignoring rebreathing and post-exposure losses) varied significantly among the subjects: decreasing with age, varying among ethnic groups and reduced for cigarette smokers [37].

Examples of the PBPK model fits are shown for one individual in figure 2(B). (Note that the variability of the measurements is distorted by the log(concentration) scale for the y-axis, which overemphasizes variation for low values.) Because the timing of the breath samples was tightly controlled by the automated collection system and pulmonary ventilation was measured during each sample, the fit was very good with little variability due to sampling and measurement error. The fitted parameters are given in figure 3. The average magnitude of the posterior estimates of the metabolism rate constant was sensitive to the values chosen for the distributions of the priors. However, extensive human physiological data limited the ranges of reasonable values that could be chosen for the prior distributions. Also, the relative ordering of the subjects from low to high values in the earlier analysis was retained, even when somewhat different priors were chosen.

The average epoxidation rate constant for BD in the well-perfused tissues, K_{met} , was $0.22 \pm 0.10 \text{ min}^{-1}$ for the whole group with a ten-fold range (table 2). As reported earlier by Lin *et al*, there were no significant differences associated with age, gender, ethnic group or the genotype or phenotype characteristics of the subjects' CYP2E1 enzyme [28]. Recent, moderate consumption of alcoholic beverages (≥ 2 drinks in the past 24 h) was associated with a significantly higher metabolic rate constant, $0.33 \pm 0.021 \text{ min}^{-1}$. Less intake of alcohol and long-term alcohol intake were not associated with higher metabolism rate constants. Although *in vitro* tests with human tissue samples indicate that CYP2E1 is the primary enzyme responsible for generation of epoxides of BD, neither the variant genotypes nor the measured enzymatic phenotype were related to K_{met} , as reported earlier [28]. However, the effect of recent alcohol intake to increase K_{met} is consistent with studies of the induction of CYP2E1 [38, 39]. Thus, it is possible that CYP2E1 is one of the enzymes for human BD metabolism, but the effects of genotypic and phenotypic differences are small relative to the induction of this metabolizing enzyme by alcohol.

The results from fitting the PBPK model were used to estimate the total amount of BD metabolized (μg BD), using the area under the concentration-time curve for the well-perfused tissues. As expected from their derivation from each subject's exhaled BD time course, both K_{met} and uptake were highly associated with μg BD metabolized; they accounted for 75% of the variability. Additional significant sources of variation were identified by step-wise regression (table 3): the blood/air partition coefficient ($\text{PC}_{\text{b/a}}$), gender, weight, body mass index ($\text{BMI} = \text{mass in kg}/(\text{height in m})^2$), age and ethnic group. Both weight and BMI were importantly associated with μg BD metabolized, but epoxide production increased with weight and decreased with increasing BMI. The BMI increases with the volume of body fat, which is a non-metabolizing compartment. For a given total body weight, a high BMI will correspond to a lower relative volume of the well-perfused tissue. Small significant effects (11% of the overall variation) were also associated with age (metabolism declined with age) and gender (females had more BD metabolism, even after controlling for weight and BMI which vary significantly by gender). Overall, our measured BD dose estimates yielded a mean of 271 μg absorbed during the 20 min exposure to 2 ppm, and 98 μg was metabolized.

A set of 11 subjects was tested twice, 1–2 months apart. These data enabled us to determine if K_{met} varied significantly across time. Visual inspection of the data strongly suggested that intra-individual variability was present, as shown in figure 2(B). To test the statistical significance of this observation, we compared two multi-level population models on the basis of the Bayes factors [40]. The first model A (figure 4(A)) described explicitly inter-individual variability and lumped together the intra-individual variability, measurement error and modeling error. Model B (figure 4(B)) had one more level to account separately for intra-individual variance [41]. Given the data, posterior parameter distributions were obtained for both models via numerical Bayesian calibration [19, 42]. The logarithm of the Bayes factor for model B against model A, a measure of their relative likelihood, was 103, indicating a significantly better fit of the second model with intra-individual variability. Most of this variability stemmed from the rate of primary oxidative metabolism, K_{met} , which varied by as much as a factor 2.6 (estimated intra-individual coefficient of variation: 50%). Other parameters controlling butadiene distribution in the body also vary between occasions, but to a lesser extent.

From the global sensitivity analyses, we first observed that the relative impact of parameters on exhaled air evolves considerably with time, and that large differences in parameter importance over time exist between the inhalation and the elimination phases (see figure 5). The most influential parameters (i.e. explaining more than 10% of the total variance of exhaled air) were $PC_{b/a}$, dead space fraction (F_{ds}), ventilation perfusion ratio (R_{VP}), blood flow to poorly perfused tissue ($Flow_{pp}$), poorly perfused tissues:blood partition coefficient ($PC_{pp/b}$), minute ventilation ($Flow_{pul}$) and K_{met} . During inhalation, the influential parameters were those directly implied in the intake of BD in body ($PC_{b/a}$, F_{ds} , R_{VP} and $Flow_{pul}$). After inhalation, all the influential parameters impacted exhaled air concentrations. We observed that K_{met} had the most impact on exhaled air during the first minutes after the cessation of inhalation. With low values for TOSI, the fat compartment did not contribute much to the variation of the concentrations of BD in exhaled air.

Discussion

The measured values for metabolism were compared to estimates in the literature. Filser and coworkers [43] used a PBPK model to extrapolate human BD metabolism from rat data. They estimated that a 70 kg male would metabolize 0.095 mmole of BD, given an applied dose of a 5 ppm exposure for 8 h (240 ppm $\times$ min). Assuming linearity, the rate is 0.04 $\mu\text{mole ppm}^{-1} \times \text{min}$. Using their model, our 2 ppm for 20 min (40 ppm $\times$ min) applied dose would result in 1.6 μmoles or 82 μg BD metabolized, which is quite comparable to the average 98 μg we observed. Kohn and Melnick [44] conducted a similar PBPK model projection of human absorption and metabolism of BD, but their model consisted of parameters that were substantially different from those found by us. They projected that a human would absorb 16.6 $\mu\text{mole kg}^{-1}$ and metabolize 0.303 $\mu\text{mole kg}^{-1}$ during a 6 h, 100 ppm (36 000 ppm $\times$ min) exposure. For a 70 kg male, that corresponds to 1.74 $\mu\text{g ppm}^{-1} \times \text{min}$ absorbed and 0.032 $\mu\text{g ppm}^{-1} \times \text{min}$ metabolized. Assuming linearity, for our 40 ppm $\times$ min experimental exposure, there should be 70 μg BD absorbed and 1.28 μg BD metabolized. These amounts are much lower than our observations of 271 μg absorbed and 98 μg metabolized. Both of the published models had metabolism only in the liver, which

we found was inadequate to account for the metabolism in some individuals. The advantage of our study is that it also examined the distribution of metabolic values in a population, which will be more useful for population risk assessment.

Most long-term dose assignments for epidemiology and risk assessments are based on an underlying assumption that individuals with the same exposure intensity and duration will receive approximately the same dose or dose per kg of body weight respectively, and therefore will have approximately the same risk. This implicitly assumes that uptake and metabolic activation are approximately the same across all members of the exposed population. Even intuitively, this seems naïve and efforts to improve upon this are drastically needed. While these assumptions are understandable in the face of ignorance, they have had a great impact upon our framework for acceptance of data as representative of real public health risk from chemical exposure.

A common finding of epidemiologic studies of risks for chemicals is an overall increased risk in the exposed group compared to unexposed, but no evidence of a dose-response using various crude markers of applied dose [45, 46]. Evidence of a dose-response (or lack thereof) is widely regarded as one cornerstone of the evaluation of causality in population-based research. We hypothesized that, in the case of low-dose exposure, the absence of appreciable dose-response might be due to the poor correlation between external exposure and internal dose of active metabolites. This possibility is nicely highlighted by our BD studies. Epidemiologic investigations of populations that have a wide range of exposure, including some that are quite high, may still show a significant exposure-response relationship. However, studies of populations with modest exposures and a limited exposure range may not show a significant exposure-response relationship, even though a very real one exists because of the wide variation in uptake and metabolic activation for people with the same external exposures; we observed an overall coefficient of variation of 24% for uptake and 45% for metabolic activation. Hence, application of the criteria for causality that demand evidence of dose-response must be carefully applied in many instances; we propose that considerably more human research is needed to better define the dose to target tissues from common environmental contaminants and thereby to truly understand many exposure-risk relationships.

The results of our research have important implication for better understanding BD dosimetry in humans from general population exposure levels. First, the use of humans, rather than rodents or other typical animal models, has allowed us to make direct assessments of human response to BD exposures. The use of exhaled breath biomarkers has allowed us to make rapid measurements that are critical to making time-dependent assessments of uptake and elimination of BD. Second, we have examined the impact of heterogeneity in oxidative metabolism on potential health risks associated with BD exposures. By use of a PBPK model that identifies the two major clearance pathways (i.e. exhalation and metabolism) for BD, we were able to quantify the impact of inter-individual differences in the rate-limiting first step in metabolism of BD. Thus, our research can be used to quantify the sources of heterogeneity in the exposure-dose-response relationship for BD. Third, the simplicity of our exposure system has allowed us to study a fairly large number of human volunteers from both genders and a mix of ethnic backgrounds; thus, we

have more confidence to extrapolate our findings to the general population than studies based on a few human volunteers (e.g. $N = 10$). Further, our results allow for better quantification of heterogeneity, which can be used to inform Monte Carlo simulation exercises. Finally, our research can be used with improved inputs of realistic population exposure scenarios to quantify the differences in internal dosimetry of BD, allowing for stronger linkages between exposure and response to be made. Few studies of human exposure to environmental toxicants have attempted to understand and quantify the exposure-dose relationship, and to better understand how perturbations to human biology are made from exposures. Our studies on BD exposures have used novel tools (i.e. exhaled breath biomarker) to study a larger human population, thus giving us greater confidence in understanding the human response to BD exposures.

Acknowledgments

The authors wish to acknowledge the contributions of Dr Chin-Hsiang Tsai and Chu-Yun Huang in the subject testing and data development. We also gratefully acknowledge the support of NIEHS grant nos. ES07586, 'Exposure-Dose Relationships: 1,3-Butadiene', and ES000002, 'Harvard Environmental Health Center Grant'.

References

- [1]. Dollard GJ, Dore CJ, Jenkin ME. Ambient concentrations of 1,3-butadiene in the UK. *Chem. Biol. Interact.* 2001; 135–136:177–206.
- [2]. Liu Y, et al. Distributions and source apportionment of ambient volatile organic compounds in Beijing city China. *J. Environ. Sci. Health. A.* 2005; 40:1843–60.
- [3]. Serrano-Trespalacios PI, Ryan L, Spengler JD. Ambient, indoor and personal exposure relationships of volatile organic compounds in Mexico City Metropolitan Area. *J. Expo Anal. Environ. Epidemiol.* 2004; 14(Suppl 1):S118–32. [PubMed: 15118753]
- [4]. Grant RL, et al. Spatial and temporal trend evaluation of ambient concentrations of 1,3-butadiene and chloroprene in Texas. *Chem. Biol. Interact.* 2007; 166:44–51. [PubMed: 17011534]
- [5]. Kim YM, Harrad S, Harrison RM. Concentrations and sources of VOCs in urban domestic and public microenvironments. *Environ. Sci. Technol.* 2001; 35:997–1004. [PubMed: 11347947]
- [6]. Brunneemann KD, et al. Determination of benzene, toluene and 1,3-butadiene in cigarette smoke by GC-MDS. *Exp. Pathol.* 1989; 37:108–13. [PubMed: 2637141]
- [7]. Macaluso M, et al. Historical estimation of exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate among synthetic rubber workers. *J. Occup. Environ. Hyg.* 2004; 1:371–90. [PubMed: 15238328]
- [8]. Fajen JM, et al. Occupational exposure of workers to 1,3-butadiene. *Environ. Health Perspect.* 1990; 86:11–8. [PubMed: 2401251]
- [9]. Cole P, Delzell E, Acquavella J. Exposure to butadiene and lymphatic and hematopoietic cancer. *Epidemiology.* 1993; 4:96–103. [PubMed: 8452913]
- [10]. Higashino H, et al. Exposure and risk assessment of 1,3-butadiene in Japan. *Chem. Biol. Interact.* 2007; 166:52–62. [PubMed: 17092494]
- [11]. Sielken RL Jr, et al. Cancer risk assessment for 1,3-butadiene: Dose-response modeling from an epidemiological perspective. *Chem. Biol. Interact.* 2007; 166:140–9. [PubMed: 16876150]
- [12]. Koppikar AM. Future research needs for the health assessment of 1,3-butadiene. *Chem. Biol. Interact.* 2001; 135–136:629–36.
- [13]. Csanady GA, Guengerich FP, Bond JA. Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from humans, rats and mice. *Carcinogenesis.* 1992; 13:1143–53. [PubMed: 1638680]
- [14]. Selzer RR, Elfarra AA. *In vitro* reactions of butadiene monoxide with single- and double-stranded DNA: characterization and quantitation of several purine and pyrimidine adducts. *Carcinogenesis.* 1999; 20:285–92. [PubMed: 10069466]

- [15]. de Meester C, et al. Mutagenicity of butadiene and butadiene monoxide. *Biochem. Biophys. Res. Commun.* 1978; 80:298–305. [PubMed: 341894]
- [16]. Cochrane JE, Skopek TR. Mutagenicity of butadiene and its epoxide metabolites: II. Mutational spectra of butadiene, 1,2-epoxybutene and diepoxybutane at the *hprt* locus in splenic T cells from exposed B6C3F1 mice. *Carcinogenesis*. 1994; 15:719–23. [PubMed: 8149486]
- [17]. Powley MW, et al. Quantification of DNA and hemoglobin adducts of 3,4-epoxy-1,2-butanediol in rodents exposed to 3-butene-1,2-diol. *Carcinogenesis*. 2005; 26:1573–80. [PubMed: 15888494]
- [18]. Himmelstein MW, et al. Toxicology and epidemiology of 1,3-butadiene. *Crit. Rev. Toxicol.* 1997; 27:1–108. [PubMed: 9115622]
- [19]. van Sittert NJ, et al. Biomarkers of exposure to 1,3-butadiene as a basis for cancer risk assessment. *Toxicol. Sci.* 2000; 56:189–202. [PubMed: 10869468]
- [20]. Csanady GA, Guengerich FP, Bond JA. Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from humans, rats and mice. *Carcinogenesis*. 1992; 13:1143–53. Erratum 1993 14 784. [PubMed: 1638680]
- [21]. Cagen SZ, MacDonald RL, Van Gelder G. Review of risk assessments on 1,3-butadiene (1985–1991). *Toxicology*. 1996; 113:215–20. [PubMed: 8901900]
- [22]. Henderson RF, et al. Metabolism of 1,3-butadiene: species differences. *Toxicology*. 1996; 113:17–22. [PubMed: 8901878]
- [23]. Lin YS, Smith TJ, Wang PY. An automated exposure system for human inhalation study. *Arch. Environ. Health*. 2002; 57:215–23. [PubMed: 12507174]
- [24]. Kim RB, O'Shea D, Wilkinson GR. Relationship in healthy subjects between CYP2E1 genetic polymorphisms and the 6-hydroxylation of chlorzoxazone: a putative measure of CYP2E1 activity. *Pharmacogenetics*. 1994; 4:162–5. [PubMed: 7920697]
- [25]. McGlynn KA, et al. Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. *Proc. Natl Acad. Sci.* 1995; 92:2384–7. [PubMed: 7892276]
- [26]. Comstock KE, et al. GST1 gene deletion determined by polymerase chain reaction. *Nucleic Acids Res.* 1990; 18:3670. [PubMed: 2362832]
- [27]. Pemble S, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem. J.* 1994; 300:271–6. [PubMed: 8198545]
- [28]. Smith TJ, et al. Genetic and dietary factors affecting human metabolism of 1,3-butadiene. *Chem. Biol. Interact.* 2001; 135–136:407–28.
- [29]. Bernillon P, Bois FY. Statistical issues in toxicokinetic modeling: a Bayesian perspective. *Environ. Health Perspect.* 2000; 108(Suppl 5):883–93. [PubMed: 11035998]
- [30]. Bois FY, Maszle D. MCSim: a simulation program. *J. Stat. Softw.* 1997; 2 <http://www.stat.ucla.edu/journals/jss/v02/i09> (also available at <ftp://sparky.berkeley.edu/users/fbois>).
- [31]. Brochot C, Smith TJ, Bois FY. Development of a physiologically based toxicokinetic model for butadiene and four major metabolites in humans: global sensitivity analysis for experimental design issues. *Chem. Biol. Interact.* 2007; 167:168–83. [PubMed: 17397815]
- [32]. Saltelli A, Tarantola S, Chan KPS. A quantitative model-independent method for global sensitivity analysis of model output. *Technometrics*. 1999; 41:39–56.
- [33]. Brown RP, et al. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health*. 1997; 13:407–84. [PubMed: 9249929]
- [34]. Sweeney LM, Himmelstein MW, Gargas ML. Development of a preliminary physiologically based toxicokinetic (PBTK) model for 1,3-butadiene risk assessment. *Chem. Biol. Interact.* 2001; 135–136:303–22.
- [35]. Mezzetti M, et al. A Bayesian compartmental model for the evaluation of 1,3-butadiene metabolism. *J. R. Stat. Soc.* 2003; 52:291–305.
- [36]. Astrand, I. Effect of physical exercise on uptake, distribution, and elimination of vapors in man. In: Fiserova-Bergerova, V., editor. *Modeling of Inhalation Exposure to Vapors: Uptake Distribution, and Elimination*. CRC Press; Boca Raton, FL: 1983. p. 108–30.

- [37]. Lin YS, et al. Human physiologic factors in respiratory uptake of 1,3-butadiene. *Environ. Health Perspect.* 2001; 109:921–6. [PubMed: 11673121]
- [38]. Cederbaum AI. CYP2E1—biochemical and toxicological aspects and role in alcohol-induced liver injury. *Mt Sinai J. Med.* 2006; 73:657–72. [PubMed: 16878272]
- [39]. Liangpunsakul S, et al. Activity of CYP2E1 and CYP3A enzymes in adults with moderate alcohol consumption: a comparison with nonalcoholics. *Hepatology.* 2005; 41:1144–50. [PubMed: 15841467]
- [40]. Gilks, WR.; Richardson, S.; Spiegelhalter, DJ. Markov Chain Monte Carlo in Practice. Vol. 17. Chapman and Hall; Boca Raton, FL: 1998. p. 486
- [41]. Gelman A, Rubin DB. Markov chain Monte Carlo methods in biostatistics. *Stat. Methods Med. Res.* 1996; 5:339–55. [PubMed: 9004377]
- [42]. Huelsenbeck JP, et al. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science.* 2001; 294:2310–4. [PubMed: 11743192]
- [43]. Filser JG, et al. A pharmacokinetic model to describe toxicokinetic interactions between 1,3-butadiene and styrene in rats: predictions for human exposure. *IARC Sci. Publ.* 1993; 127:65–78. [PubMed: 8070888]
- [44]. Kohn MC, Melnick RL. Species differences in the production and clearance of 1,3-butadiene metabolites: a mechanistic model indicates predominantly physiological, not biochemical, control. *Carcinogenesis.* 1993; 14:619–28. [PubMed: 8472325]
- [45]. Divine BJ, Hartman CM. A cohort mortality study among workers at a 1,3 butadiene facility. *Chem. Biol. Interact.* 2001; 135–136:535–53.
- [46]. Ott MG. Assessment of 1,3-butadiene epidemiology studies. *Environ. Health Perspect.* 1990; 86:135–41. [PubMed: 2205483]
- [47]. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br. J. Nutr.* 1991; 65:105–14. [PubMed: 2043597]
- [48]. Harris EA, Seelye ER, Whitlock RM. Revised standards for normal resting dead-space volume and venous admixture in men and women. *Clin. Sci. Mol. Med.* 1978; 55:125–8. [PubMed: 668265]

Figure 1.

Metabolic pathway for 1,3-butadiene (BD) for the formation and removal of three epoxide compounds by cytochrome P-450 oxidative enzymes (CYP), and their removal by epoxide hydrolase (EH). The large arrows show the predominant pathway. The dashed box identifies the components we are studying. There is also a small amount of removal of epoxides by glutathione-S-transferase.

Figure 2. Time course of exhaled butadiene. (A) Average time course for 133 subjects; error bars for one SD. (B) Time courses for two separate tests of subject 5; the top line is the first test and bottom line is the second test, the middle line fits all data for the subject.

Figure 3.

Physiologically based pharmacokinetic model and the derivation of its parameters. Notes (1) The fraction of lean body mass in the poorly perfused compartment, which is the largest, was calculated as the difference of 0.9 minus the other compartments. The skeleton was not included (0.1 of body mass). $F_{\text{BDW}_{\text{pp}}} = 0.9 - F_{\text{BDW}_{\text{wp}}} - F_{\text{BDW}_{\text{fat}}}$. (2) The fraction of body mass in the fat compartment was calculated using the algorithm of Deurenberg *et al* [47], which has been calibrated against a variety of physiologic measurements of body fat. $F_{\text{BDW}_{\text{fat}}} = (1.2 \cdot (\text{BDW}/\text{Ht}^2) - 10.8 \cdot \text{Sex} + 0.23 \cdot \text{Age} - 5.4)$, where Ht is in meters, BDW is in kilograms and Sex is 1 if male and 0 if female. (3) The alveolar ventilation is the total pulmonary ventilation (minute volume) minus the dead space ventilation. Dead space ventilation (Flow_{DS}) is the volume of dead space times the number of breaths per minute, where the dead space (DS) is the sum of the anatomical airway dead space plus the dead space in the face mask. The anatomical dead space was estimated by the equation developed by Harris and coworkers [48], and the mask dead space was measured for several subjects by water displacement. (4) The fraction of blood flow in the well-perfused compartment, which is the largest, was calculated as the difference of 1.0 minus the other compartments: $F_{\text{Flow}_{\text{wp}}} = 1.0 - F_{\text{Flow}_{\text{pp}}} - F_{\text{Flow}_{\text{fat}}}$. (5) The partition coefficient for blood to well-perfused tissues was taken from *in vitro* tissue measurements [43].

Figure 4. Repeated observations for 11 subjects were fitted with hierarchical PBPK models. (A) Subjects were fitted with a two-level population and an individual model and (B) subjects' data were fitted with a three-level population, individual and intra-individual models.

Figure 5.

Time evolution of the relative impact of the model parameters on the concentration of 1,3-butadiene (BD) in exhaled air [31]. Abbreviations: K_{met} , metabolic rate constant; $PC_{\text{b/a}}$, blood:air partition coefficient; $PC_{\text{wp/b}}$, well-perfused tissue:blood partition coefficient; $PC_{\text{pp/b}}$, poorly perfused tissue:blood partition coefficient; Flow_{pp} , fractional blood flow to poorly perfused tissue; Flow_{pul} , pulmonary ventilation rate; R_{vp} , ventilation:perfusion ratio; V_{wp} , volume of well-perfused tissues; F_{ds} , fraction dead space.

Table 1

Test subject characteristics.

	Male	Female
Number (133 total)	71	62
Age (years)	30.3 ± 8.1	29.0 ± 8.9
Height (m)	1.74 ± 0.08	1.61 ± 0.07
Weight (kg)	77.7 ± 16	61.3 ± 16
Partition coefficient for blood to air ($PC_{b/a}$)	1.62 ± 0.35	1.46 ± 0.34
Alveolar air flow ($L \min^{-1}$)	3.5 ± 0.9	3.2 ± 0.8

Notes. Values shown are mean ± SD.

Table 2

Uptake including rebreathing during exposure and post-exposure washout, and fitted metabolic rate.

	Mean	SD	CV (%) ^a	Minimum	Maximum
Uptake (μg)	271.0	65.3	24.1	109.1	519.8
Uptake per body weight (μg kg ⁻¹)	4.03	1.07	26.5	1.64	8.36
Percentage uptake	57%	7%	12.6	38%	77%
K_{met} (per min)	0.22	0.10	45.0	0.046	0.477
Butadiene metabolized (μg)	98.6	30.8	31.2	31.7	199
Per cent of uptake metabolized	36%	8%	22.3	16.9%	57.2%

^aCV (%) is the coefficient of variation, SD/mean, expressed as a percentage.

Table 3

Step history for step wise regression of variables on μg butadiene metabolized.

Step entered ^a	Parameter	Significance probability	Sequential sum of squares ^b	Cumulative R^2 ^c	C_p ^d
1	Uptake (μg)	<0.000 01	66 182.68	0.5295	308.47
2	K_{met} (min^{-1})	<0.000 01	27 711.14	0.7512	104.31
3	Weight (kg)	<0.000 01	11 274.42	0.8414	22.43
4	Sex (F–M)	<0.000 01	2 735.017	0.8633	4.09
5	Age (yr)	<0.05	501.5262	0.8673	2.36

^aParameters enter the model based on the amount they reduce the overall sum of squares.

^bThe sequential sum of squares is the quantity removed from the total by the parameter's entrance into the regression.

^cCumulative R^2 is the proportion of the variation that is attributable to that parameter and the ones above.

^dMallow's C_p criterion is calculated by $(n) \ln(\text{SSE}/n) + 2p$, where n is the number of observations, SSE is the sum-of-squares error and p is the number of model parameters. Smaller values of C_p indicate better model fit.