Human Physiologic Factors in Respiratory Uptake of 1,3-Butadiene

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1,3-Butadiene (BD), a suspected human carcinogen, is used as the raw material in industries to make synthetic butyl rubber and plastics. Simulation models using experimental animal data have shown that physiologic factors play an important role in the kinetic behavior of BD. However, human data are limited. The aim of this inhalation study was to identify influential human physiologic factors in the respiratory uptake of BD. We recruited 133 healthy volunteers in Boston, Massachusetts, into this study and tested them under an approved human subjects protocol. Each subject was exposed to 2 ppm (4.42 mg/m^3) BD for 20 min, followed by purified air for another 40 min. Five exhaled breath samples collected during exposure were used to determine the respiratory uptake of BD, which was defined as absorbed BD (micrograms) per kilogram of body weight during exposure. Although subjects were given identical administered doses (40 ppm/min), there was a wide range of uptake, 0.6–4.9 μg/kg. Of the studied physiologic factors, the blood:air partition coefficient and alveolar ventilation were most significant in determining the respiratory uptake ($p < 0.001$ for each). In addition, in the multiple regression analysis, females had significantly higher respiratory uptake of BD than males on a weight basis. For all subjects, increasing age and cigarette smoking led to significantly decreased respiratory uptake of BD. The results of this human study are consistent with previous kinetic simulations and animal studies. The findings also suggest that interindividual variation in human physiologic factors that affect the exposure–internal dose relationship should be considered while also exploring exposure–disease associations in future epidemiologic research. *Key words*: alveolar ventilation, blood:air partition coefficient, 1,3-butadiene, human, physiologic, respiratory uptake, sex. *Environ Health Perspect* 109:921–926 (2001). [Online 23 August 2001]
http://ehpnet1.niehs.nih.gov/docs/2001/109p921-926lin/abstract.html

1,3-Butadiene (BD), C_4H_6 (CAS No. 106-99-0), is a major raw material used in petrochemical manufacturing to make synthetic butyl rubber, coatings, and acrylonitrile-1,3-butadiene-styrene (ABS) plastics (*1*). Exposure to BD occurs mainly via inhalation because it is a gas at room temperature. In 1990, approximately 65,000 workers were likely exposed to BD in the United States (*2*). Additionally, BD can also be found in urban air pollution, gasoline vapor, and cigarette smoke (*3*). Animal inhalation studies have indicated that BD is a multiple-organ carcinogen in Sprague-Dawley rats and in B6C3F1 mice (*4,5*). With growing concerns about the potential risks to humans from exposure to BD, numerous epidemiologic studies of occupational workers exposed to BD have been performed. However, findings regarding BD carcinogenicity in humans have been equivocal, perhaps partly because reliable exposure assessment is lacking (*6–9*).

Understanding of the relationship between external exposure and internal dose is critical for determining a clear quantitative exposure–disease relationship (*10*); therefore, it is essential to characterize the influential factors determining the external exposure–internal dose association in humans. Physiologically based pharmacokinetic (PBPK) simulation models have suggested that physiologic parameters such as respiratory ventilation and the blood:air partition coefficient are critical in determining human internal dose to airborne pollutants (*11,12*). The blood:air partition coefficient is an indicator of blood solubility of a volatile chemical, and is one of the most important properties in the respiratory uptake of gases in humans (*13*). Relevant human data are very limited; most of the toxicokinetic information on BD has been obtained either from model simulations or extrapolation of the results of animal studies (*14,15*). However, the adequacy of such extrapolation to humans exposed to low concentrations of BD is of concern. The toxicokinetics of BD at high exposure concentration levels are different from those at low levels and there are interspecies differences in the kinetic pathways of BD (*16–18*).

The main objective of the present human inhalation study was to explore influential physiologic factors that determine the respiratory uptake of BD. Uptake is one of the rate-limiting steps for the internal dose. Individuals with low uptake cannot produce large amounts of toxic metabolites, whereas those with high uptake may or may not do so, depending upon their metabolic rates. Physiologic parameters examined in this study included the blood:air partition coefficient of BD, alveolar ventilation, sex, age, and race. Two potential additional factors that may affect metabolism, alcohol consumption and smoking, were also incorporated into the statistical analyses to estimate the associations of interest. Evaluation of these factors may lead to more reliable exposure assessment work and epidemiologic studies, and further our understanding of factors affecting the exposure–disease relationships in humans.

Methods and Materials

Study subjects. Subjects were recruited from the Longwood Medical Area of Boston between 1997 and 1999. They were tested using a human subject protocol approved by the Human Subjects Committee of the Harvard School of Public Health. Before they gave consent, they were informed that BD is a suspected human carcinogen, that the experimental exposure was within the range of possible everyday exposures, and that the experimental exposure might cause a small increase in their lifetime risk of cancer, possibly leukemia. We estimated that this exposure would cause less than a one per million increase in lifetime risk of leukemia, using the California Environmental Protection Agency (EPA) Air Resources Board risk assessment, which was based on the mouse as the most sensitive species (*19*). The aim of subject recruitment was to test an approximately balanced number of males and females from four major U.S. population groups: Caucasian, African-American, Hispanic, and Asian groups. All participants were interviewed before testing to ensure that they had no metabolic or cardiovascular diseases, nor were planning to start a pregnancy in the succeeding 6 months.

Experimental procedures and conditions. Before starting the experiment, we verified each subject's health and obtained informed consent. We then administered a standardized questionnaire to collect demographic and lifestyle information such as age, sex, racial background, medical history, smoking status, and alcohol consumption. Smoking

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We are especially grateful to R.F. Herrick, H.Y. Chang, and E. Moy for helpful discussions.

This study is part of the three-year 1,3-butadiene metabolic study, funded by the National Institute of Environmental Health Sciences (NIEHS) grants 07586 and the NIEHS Center Grant ES00002.

Received 15 January 2001; accepted 8 March 2001.

status was recorded as current smoking (Yes/No). Alcohol consumption was categorized as no drinking habit, 1 to 2 drinks weekly, or more than 2 weekly drinks. The definition of a drink was defined as 12 oz of beer, 5 oz of wine, or 1.5 oz of hard liquor (*20*). Each subject chose an optimal size of breathing mask (small, medium, or large; Hans Rudolph, Inc., Kansas City, MO) to comfortably fit his or her own face. The subjects checked for mask leaks by placing the palms of hands over the mask's valves and breathing in for 10 sec to ensure the mask fit snugly without leaks. One minute of breathing or 6 breaths were collected through the breath sampling system into an 8-L spirometer (Warren E. Collins, Braintree, MA) to calibrate the RespiTrace breathing monitor (NIMS, Inc., Miami Beach, FL). As a result of operating problems with the RespiTrace, 28 subjects (20%) were not monitored; instead, data from spirometer calibrations (number/min and tidal volume) and exhaled volume in breath samples (volume in 1 min) were used. We collected a baseline breath sample before exposure to verify there were no background BD sources to interfere with the experiment. We also collected a venous blood sample before beginning the inhalation experiment to determine the blood:air partition coefficient.

Each subject was exposed to 2.0 ppm (4.42 mg/m^3) BD for 20 min, followed by charcoal purified air for another 40 min with an inhalation exposure system developed by Lin and coworkers (*21*). We collected 10 timed breath samples from each volunteer at 2, 5, 10, 15, 19, 21, 22, 28, 38, and 58 min from the start of exposure. We chose the time points on the basis of an optimized sampling time schedule developed by Bois and coworkers (*22*); each of the first seven breath samples was collected for 1 min, and the last three were collected for 2 min each to increase the sensitivity of detecting exhaled BD. We used exhaled breath samples collected during exposure (wash-in phase) to determine the respiratory uptake of BD. We used these breath samples and other measured population parameters to develop the personalized physiologically based pharmacokinetic (PBPK) model and to study the elimination of BD and excretion of urine BD metabolites (*23*).

Determination of alveolar BD, alveolar ventilation, and respiratory uptake of BD. Total uptake of gas/vapor traditionally has been determined as the difference between the total amount of inhaled and exhaled gas during the exposure (*24*,*25*). In this study, we reported respiratory uptake of BD as the absorbed BD micrograms per kilogram of body weight to reflect the body burden on a unit weight basis (*5*,*26*,*27*). This was the variable used for statistical analyses in the study. Total amount of inhaled BD was calculated as the inhaled concentration times the alveolar ventilation times the duration of exposure. Alveolar ventilation was used because the pulmonary minute ventilation includes mixed exhaled breath that contains both alveolar (end-tidal) gas and inhaled gas from the respiratory dead space where there is no gas exchange. Thus the BD concentration in mixed exhaled breath is a function of both the inhaled and the alveolar BD concentrations as given in Equation 1:

$$BD_{\text{mixed}} = BD_{\text{alveolar}}\left(1 - \frac{\text{total dead space}}{\text{tidal volume}}\right) + BD_{\text{dead space}}\left(\frac{\text{total dead space}}{\text{tidal volume}}\right) \quad [1]$$

where BD_{mixed} = mixed exhaled BD concentration in parts per million; BD_{alveolar} = alveolar BD concentration in parts per million; $BD_{\text{dead space}}$ = inhaled BD concentration in parts per million; tidal volume = average tidal volume in each breath (milliliters per breath); and total dead space = sum of individual physiologic dead space volume and mask dead space volume in each breath (milliliters per breath). We used Equation 1 to estimate the alveolar concentration from measured quantities. Pulmonary minute ventilation is the sum of alveolar and dead space ventilation, as shown in Equation 2.

$$\dot{V}_E = \dot{V}_A + (\text{breathing frequency}) \times (\text{total dead space}) \quad [2]$$

where $\dot{V}_E$ = pulmonary minute ventilation (liters per minute); $\dot{V}_A$ = alveolar ventilation (liters per minute); breathing frequency = breaths every minute (breaths per minute); and total dead space = sum of individual physiologic dead space and mask dead space in each breath (milliliters per breath). We used the relationship in Equation 2 to estimate the alveolar ventilation rate, $\dot{V}_A$.

Dead space in this system comes from two sources: inside the face mask and in the airways of the subject. The dead space volume of mask was 100 ± 10 mL, estimated by water displacement measured on five subjects, which was not affected by mask size used. We estimated individual physiologic dead space using Equation 3, developed by Harris and co-workers (*28*).

$$V_D = 0.834\ (\text{Age}) + 1.26\ (\text{Height}) + 0.296\ (V_T) - 879/f - 174 \quad [3]$$

where V_D = predicted physiologic dead space volume (milliliters); Age = individual subject's age in years; Height = individual subject's height in centimeters; V_T = average tidal volume in each breath (milliliters per breath); and f = breaths every minute (breaths per minute). This algorithm explained 89% of the variability for the test population (R^2 = 0.89) for predicting physiologic dead space volume.

We estimated the total uptake (absorbed BD) during exposure by the difference between the inhaled and exhaled BD during exposure. The total amount of inhaled BD was estimated by the inhaled BD concentration (BD_{inhaled}) multiplied by the alveolar ventilation rate (V_A) multiplied by the duration of exposure (T). We estimated the total amount of exhaled BD during exposure by integrating the product of exhaled alveolar BD concentration (BD_{alveolar}) by V_A across the period of exposure using the trapezoidal method (*29*). Because BD_{alveolar} during the interval of 0–2 min was not measured, we estimated its concentration as one-half the BD_{alveolar} measured in the first sample collected during the interval of 2–3 min. We also estimated the respiratory uptake fraction—the percentage of total inhaled BD retained in the body—by total uptake divided by total inhaled (*30*–*32*).

Collection of exhaled breath samples and analysis of BD. We collected mixed exhaled breaths 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 (*33*). Charcoal tubes were stored in a refrigerator at –20°C until analysis. We used methylene chloride (99.9%; Burdick & Jackson Inc., Muskegon, MI) to extract BD from the charcoal. We used a Hewlett Packard Gas Chromatograph 5890 series I (Hewlett-Packard Co., Palo Alto, CA, USA) equipped with flame ionization detector (FID) and an HP model 6890 autosampler to determine BD in the methylene chloride solution. The method was modified from the U.S. National Institute for Occupational Safety and Health analytic approach (NIOSH method 1024) (*34*). The detailed analytic conditions were described earlier in another paper (*21*). Finally, BD concentration in breath samples was determined by dividing the total amount of BD by the volume of exhaled breath. The limit of detection was 0.006 ppm of BD in a 5.0 L exhaled breath sample with a coefficient of variation of 10%.

Determination of the blood:air partition coefficient. We determined the blood:air partition coefficient using a modification of the closed-vial, headspace equilibration method (*35*). We spiked 50 µL of pure BD

vapor (> 99.99%) (Aldrich Chemical Co., Milwaukee, WI) into a closed 20-mL vial containing 6 mL of blood sample with a gas-tight syringe (Hamilton Company, Reno, NV). The vial was placed in a 37°C oven for 2 hr until reaching equilibrium (*36*). To promote gas exchange and avoid any adsorption of BD onto the walls of the vials, we agitated the vials occasionally using a shaker (Lab-Line Instruments Inc., Melrose Park, IL). We took duplicated 1-mL gas samples after 2 hr from the headspace of the closed vial using gas-tight syringes. We analyzed the gas samples using gas chromatography (GC) to determine the mass of BD in gas phase. We calculated the amount of BD in blood phase as the difference between the initially added BD and the recovered BD in gas phase, after correcting for recovery losses. Recovery loss was the average recovery, estimated from the loss of BD during the analytic procedure and from wall losses in the empty vials; we determined the latter using the same procedures for blood without adding the blood sample and shaking vials. Then we calculated the blood:air partition coefficient as the ratio of the concentration of BD between head-space air and blood.

Statistical analysis. The objective of this study was to investigate the physiologic factors that affect the respiratory uptake of BD. We performed logarithmic transformation of BD uptake to normalize the distribution before regression analyses. Initially we examined separately the relationship of BD uptake to each of the parameters of interest, including univariate regression analyses. We then investigated a stepwise regression model by including interaction terms on the basis of biologic plausibility. We examined the assumption of linearity using residual plots for testing the significance of quadratic terms. Higher-order terms are not included in the analysis. We used the *t*-test, analysis of variance (ANOVA), or nonparametric Wilcoxon rank-sum tests (if the distributions were skewed) to compare the mean BD uptake across race, sex, and age. We used the SAS standard statistical package version 7.0 for data analysis (SAS Institute Inc., Cary, NC). The level of significance was set at 0.05.

Results

We tested 144 healthy individuals. We excluded 11 subjects with incomplete data from the final analysis because of mask leaking during the experiment (n = 6), analytic instrument malfunction caused by saturation of GC guard column (n = 2), lack of blood data (n = 2), and errors in analytic preparations (n = 1). The demographic and physiologic characteristics of the 133 eligible participants are summarized in Table 1. As expected from the recruiting plan, there were nearly equal numbers of males and females. The final self-reported racial distribution was 43 Caucasians (including 6 with ancestry from the Indian subcontinent), 17 African Americans, 27 Hispanics, and 46 Asians (predominantly Chinese). Males and females were significantly different for all comparisons of physiologic parameters ($p < 0.05$ for each) such as the blood:air partition coefficient and respiratory ventilation, but we found no significant differences in age, smoking, and alcohol consumption. Figure 1 shows the time curve of alveolar BD concentration during exposure and postexposure phase, averaging the 133 subjects. Alveolar BD dropped rapidly within minutes after cessation of BD exposure and had larger interindividual variation after exposure than during exposure.

The total inhaled BD during exposure for males (301 ± 79 µg, mean ± SD) was higher than females (275 ± 77 µg) (*t*-test, p = 0.04), as shown in Table 2. The total BD uptake (absorbed BD) is also higher for males (135 ± 50 µg) versus females (121 ± 55 µg) (Wilcoxon rank-sum test, p = 0.058). However, the respiratory BD uptake (micrograms BD per kilogram of body weight) was higher for females (2.0 ± 0.9 µg/kg) than males (1.8 ± 0.7 µg/kg) but the difference was not statistically significant (Wilcoxon rank-sum test, p = 0.24). The difference between men and women in the respiratory uptake fraction of total inhaled, which ranged from 18% to 74% for all 133 participants, was also not significant.

Univariate associations between the physiologic factors and BD uptake ($\log_{10}$ transformation) found strong significant relationships for the blood:air partition coefficient and alveolar ventilation ($p < 0.001$) and nonsignificant differences between sexes (Figures 2 and 3). Current cigarette smokers had lower respiratory BD uptake than current nonsmokers (p = 0.02). Neither race nor alcohol consumption was significantly related to BD uptake (p = 0.38 and 0.31, respectively).

When all of the covariates were considered jointly in a multiple regression model on $\log_{10}$ transformed uptake, several of the variables became significant (Table 3). With the $\log_{10}$ transformation on BD uptake, the model is a multivariate model where the β values are multipliers or divisors depending on the sign of the coefficient. The blood:air partition coefficient, alveolar ventilation, smoking, age, and sex were all significant predictors of the respiratory BD uptake. Females had larger body burdens of BD per kilogram of body weight ($10^{0.094}$ or 1.24 times larger) than males. Both age (9.1% loss per decade) and smoking (smokers 18.5% less) were negatively associated with the uptake of BD after adjustment for the other explanatory variables. We compared racial groups to Caucasians as a baseline. Only Asians showed a significantly different

Table 1. Demographic and physiologic characteristics of 133 participants.[a]

Characteristics	Male (n = 71) mean ± SD	Female (n = 62) mean ± SD
Age (years)	30.3 ± 8.1	29.0 ± 8.9
Height (m)	1.74 ± 0.08	1.61 ± 0.07**
Body weight (kg)	77.7 ± 15.7	61.3 ± 15.9**
Physiologic dead space (mL)[b]	171.3 ± 38.9	127.5 ± 37.0**
Blood:air partition coefficient	1.62 ± 0.35	1.46 ± 0.34*
Minute ventilation (L/min)	7.3 ± 1.6	6.4 ± 1.5**
Alveolar ventilation (L/min)[c]	3.5 ± 0.9	3.2 ± 0.8*
Current smoking, n (%)		
Yes	14 (20)	9 (15)
No	57 (80)	53 (85)
Alcohol consumption (drinks/week in the past year),[d] n (%)		
> 2	27 (38)	16 (26)
1–2	24 (34)	26 (42)
0	20 (28)	20 (32)

[a]We made sex comparisons using *t*-tests for continuous variables and chi-square tests for categoric variables; we used Wilcoxon rank-sum tests for age, weight, physiologic dead space, and minute ventilation because of skewness in these variables. [b]Estimated from prediction formula (*28*). [c]Estimated from minute ventilation by adjusting for individual physiologic and mask dead space (100 mL). [d]A drink was defined as 12 oz of beer, 5 oz of wine, or 1.5 oz of hard liquor (*20*). *p < 0.05; **p < 0.01.

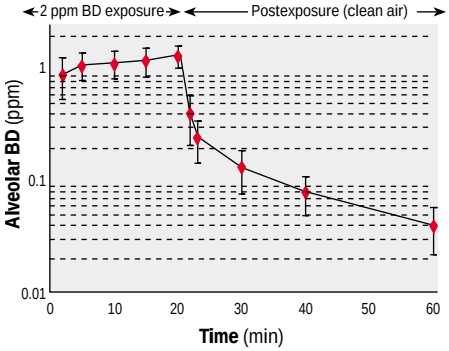

Figure 1. Time course of mean alveolar BD (logarithm scale) during exposure and postexposure phases summarized for 133 subjects exposed to 2 ppm BD for 20 min, followed by clean air for another 40 min; error bars represent 1 SD.

uptake than Caucasians (19.9% higher). Alcohol consumption did not predict the outcome variable in the multivariate model. We retained both race and alcohol consumption in the final multiple regression model to adjust for the confounding effects, and we found no significant interactions or nonlinear terms (data not shown).

Discussion

Our findings were consistent with the previous PBPK model simulations that showed that alveolar ventilation and the blood:air partition coefficient were two of the most important physiologic factors in predicting the respiratory uptake of BD (*26*). In addition, females had higher respiratory BD uptake (micrograms per kilogram) than males after adjustment for body weight and the other explanatory variables. The sex difference in respiratory BD uptake may result partly from differences in the fat composition of body compartments, indicated by the ratio of fat/lean body mass. The fatty tissue can extract and store lipophilic chemicals more effectively, which contributes to retention of absorbed lipophilic chemicals during and after exposure (*37*). Previous studies of different racial populations had consistently indicated that the fat compartment is significantly larger in females than males, but the magnitude of body fat varied across ethnic groups (*38–40*). A better method for estimating body fat is needed for pharmacokinetic studies.

Increased age and current cigarette smoking were associated with decreasing respiratory uptake of BD after adjustment for the other factors. Age and smoking effects might both decrease pulmonary gas exchange efficiency, which might also reduce the respiratory uptake of BD (*41,42*). Although reasonable, this must be demonstrated with further testing and concurrent measurement of gas exchange efficiency.

The observed uptake of BD is caused by solubility in the blood, retention in fat and other tissues, and metabolism. BD is rapidly taken up and released by vessel-rich tissues, and more slowly by poorly perfused tissues. Within the time scale of the experimental exposure, all of the BD in blood passing through the body fat will be retained and only very slowly be released during or after exposure. However, the blood flow through the fat is only a small fraction of the total, 5%–9% (*43*). A small amount of the BD inhaled is exhaled after exposure stops, as BD is rapidly cleared from most of the tissues.

Because of fat retention and slow distribution to and from the poorly perfused tissues on the short time scale of the experimental exposure, if there was no metabolism, approximately 10%–20% of the inhaled BD would not be exhaled during the exposure period. The average percent retained by the subjects ranged from 18% to 74%, which strongly implies that some individuals metabolized a significant quantity of BD. Mezzetti and coworkers (*23*) did pharmacokinetic modeling with the exhaled breath data to estimate BD metabolism. This modeling found large population variability for the metabolism rate, which was much larger than the uncertainty in model fitting. Estimated clearance values for some individuals (approximately 20% of the total) exceeded the likely total blood flow of the liver.

In the current study, all of the subjects received the same administered dose, 2.0 ppm for 20 min. The wide range of BD uptake clearly shows that administered dose is a poor estimator of the absorbed dose, even absorbed dose per kilogram of body weight. Because significant effects on uptake were observed for age, sex, and smoking, these factors are likely to be important modifiers of risk from exposure to BD. The BD

Table 2. Respiratory uptake of BD in humans exposed to 2 ppm BD for 20 min.[a]

Uptake	Male (n = 71) Mean ± SD	Female (n = 62) Mean ± SD
Total inhaled BD (µg)[b]	301 ± 79	275 ± 77*
Total uptake (absorbed BD) (µg)[c]	135 ± 50	121 ± 55
Respiratory uptake of BD (µg BD absorbed/kg body weight)[d]	1.8 ± 0.7	2.0 ± 0.9
Respiratory uptake fraction (%)[e]	45.6 ± 13.9	43.4 ± 2.9

[a]We made sex comparisons using t-tests; we used Wilcoxon rank-sum tests for total uptake and respiratory uptake of BD because of skewness in these variables. [b]We based the calculation on alveolar ventilation; 1 ppm BD = 2.21 µg BD/L at room temperature and atmosphere pressure. [c]The difference between total inhaled BD and total exhaled BD during exposure. [d]Respiratory uptake of BD was derived from absorbed BD divided by body weight. [e]Percentage of total inhaled BD absorbed, equal to absorbed BD*100% /total inhaled BD. *p < 0.05

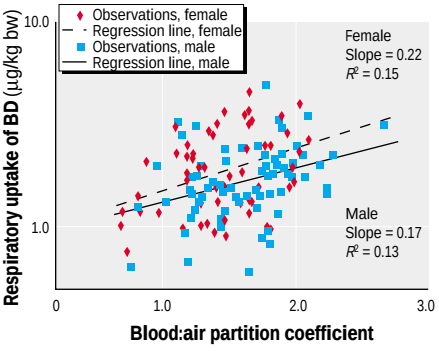

Figure 2. The relationship of respiratory uptake of BD to blood:air partition coefficient by sex. bw, body weight. The difference in slopes between sexes is not statistically significant (p = 0.61).

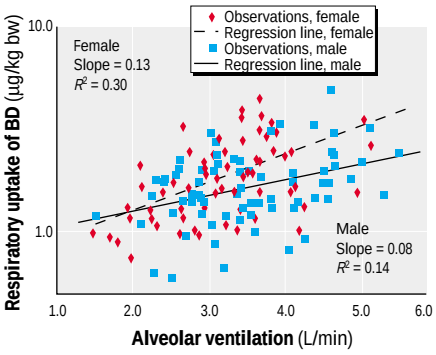

Figure 3. The relationship of respiratory uptake of BD to alveolar ventilation by sex. bw, body weight. The difference in slopes is not statistically significant (p = 0.11).

Table 3. Multiple regression of $\log_{10}$ transformed respiratory uptake of BD[a,b] (n = 133).

Explanatory variables	β (SE)	p-Value
Blood:air partition coefficient	0.172 (0.039)	< 0.001
Alveolar ventilation (L/min)	0.102 (0.017)	< 0.001
Age (years)	–0.004 (0.002)	0.03
Sex (female = 1, male = 0)	0.094 (0.027)	< 0.001
Current smoking (yes = 1, no = 0)	–0.089 (0.036)	0.01
Race[c]		
African American	–0.004 (0.043)	0.93
Hispanic	0.046 (0.039)	0.24
Asian	0.079 (0.036)	0.03
Caucasian	Reference	
Alcohol consumption[d] (drinks/week during the past year)		
> 2	0.028 (0.036)	0.45
1–2	0.019 (0.032)	0.55
0	Reference	

[a]Respiratory uptake of BD: micrograms BD absorbed per kilogram body weight during exposure. [b]R^2 and adjusted R^2 for the multivariate regression model were 0.41 and 0.36, respectively. [c]Overall race is not significant in the multivariate model ($F_{3,122}$ = 1.96, p = 0.12). [d]Overall alcohol consumption is not significant in the multivariate model ($F_{2,122}$ = 0.31, p = 0.73).

uptake is an absorbed dose, but it is not a measure of biologically effective dose because BD must be metabolized to active epoxides and perhaps other materials to cause adverse effects. However, the absorbed dose is a major limit to the quantity of active agents that may be formed. Estimation of physiologic factors as rate limiting steps for internal dose has received little attention by risk assessors. Our study clearly shows the importance of including consideration of variation in uptake in risk assessment.

There were two major limitations in the current study. First, it was not practical to collect data on cardiac output. Cardiac output is an influential factor in the inhalation kinetics of gases and vapors in humans, especially for less soluble chemicals (*44,45*). In contrast, alveolar ventilation is more important for highly soluble gases. The blood:air partition coefficient was critical for both highly and less lipid soluble chemicals (*46*). BD has an intermediate lipid solubility, compared to the commonly used anesthetics (*47*), so both cardiac output and alveolar ventilation are important. This is reflected in the positive relationship of BD uptake with the blood:air partition coefficient and alveolar ventilation, but the strength of the relationships might be improved with data on cardiac output. Further investigations that include cardiac output measurement are needed to clarify this assumption.

The second limitation was lack of direct measurement of alveolar BD. In this inhalation study, the alveolar BD was estimated from mixed exhaled breath by adjusting for the total dead space. Physiologic dead space was estimated using a highly predictive model ($R^2 = 0.89$) and the mask dead space was measured. With the extra dead space from the mask, the total was nearly as large as the alveolar volume. As a result, errors in the estimation of dead space and variation in the relative amount of dead space with depth of inhalation will add error to the estimates of both alveolar ventilation rate and alveolar BD concentration. Absorption and release of BD in the upper airways was assumed to be negligible because BD has a low water solubility (*48,49*). Thus, an important source of measurement error in estimating alveolar BD was the uncertainty in estimating physiologic dead space. An improvement in the estimation of the alveolar BD concentration could be obtained with real-time measurement of exhaled BD in breath; then the concentration in end-exhalation alveolar gases could be measured directly.

In summary, this study found that physiologic parameters play an important role in determining BD uptake, which will subsequently affect the risk from exposure to BD. Thus, it is necessary to consider variability in physiologic factors across populations to project risk from these exposures in the workplace and urban air pollution. In addition, it is also likely that the physiologic factors may also change within the same individual across time, such as increased respiratory ventilation caused by physical workload and a decline with age. Thus, we recommend that future epidemiologic work account for physiologic, environmental, and metabolic variability whenever possible to detect and quantify differences in the factors that affect the relationships between exposure and internal dose. These factors may also identify previously unrecognized sensitive subpopulations with potentially increased risk. There may be policy limitations that prevent development of separate exposure standards for more sensitive subgroups, but these populations should be considered when assessing the adequacy of proposed standards for exposure.

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