1,3-Butadiene: Linking Metabolism, Dosimetry, and Mutation Induction

James A. Bond,1 Gyuri A. Csanady,2 Michael L. Gargas,3 F. Peter Guengerich,4 Teresa Leavens,5 Michele A. Medinsky,1 and Leslie Recio1

1Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina; 2GSF-Institut für Toxicologie, Oberschleissheim, Germany; 3ChemRisk, a Division of McLaren Hart, Cleveland, Ohio; 4Department of Biochemistry and Center in Molecular Toxicology, School of Medicine, Vanderbilt University, Nashville, Tennessee; 5Curriculum in Toxicology, University of North Carolina, Chapel Hill, North Carolina

There is increasing concern for the potential adverse health effects of human exposures to chemical mixtures. To better understand the complex interactions of chemicals within a mixture, it is essential to develop a research strategy which provides the basis for extrapolating data from single chemicals to their behavior within the chemical mixture. In the case of 1,3-Butadiene (BD), recent exposure data are emerging that are critical for understanding interspecies differences in carcinogenic/genotoxic response to BD. Knowledge regarding mechanisms of BD-induced carcinogenicity provides the basis for assessing the potential effects of mixtures containing BD. BD is a multisite carcinogen in B6C3F1 mice and Sprague-Dawley rats. Mice exhibit high sensitivity relative to the rat to BD-induced tumorigenesis. Since it is likely that BD requires metabolic activation to mutagenic reactive epoxides that ultimately play a role in carcinogenicity of the chemical, a quantitative understanding of the balance of activation and inactivation is essential for improving our understanding and assessment of human risk following exposure to BD and chemical mixtures containing BD. Transgenic mice exposed to 625 ppm BD for 6 hr/day for 5 days exhibited significant mutagenicity in the lung, a target organ for the carcinogenic effect of BD in mice. In vitro studies designed to assess interspecies differences in the activation of BD and inactivation of BD epoxides reveal that significant differences exist among mice, rats, and humans. In general, the overall activation/detoxication ratio for BD metabolism was approximately 10-fold higher in mice compared to rats or humans. A preliminary physiological dosimetry model was developed for BD. The model simulations for the in vitro $V_m$ and $K_m$ for BD oxidation compare favorably with the metabolic rate constants that were determined from the in vitro studies when the in vitro values were adjusted to account for microsomal content and liver weight in the intact animal. This favorable comparison suggests that in vitro biochemical parameters derived for BD could be used to predict in vivo metabolism. Using the physiological dosimetry model developed for BD, potential interactions of BD with other chemicals in the workplace (e.g., BD/styrene), the environment (e.g., BD/benzene), or through certain dietary influences (e.g., BD/ethanol) were explored. The three simulations of examples of BD chemical mixtures suggest two important general points regarding chemical interactions. The first relates to extrapolations from high to low doses. Due to the saturable enzyme systems that metabolize most toxic organic chemicals, it cannot be assumed that the inhibition effects demonstrated at high exposure concentrations will be proportional, or even significant, at low exposure concentrations. Second, patterns of enzyme induction determined in vitro may not exhibit the same magnitude of effect in the intact animal. Delivery of the chemical to the site of the enzyme chemical interaction may be, in some cases, the rate-limiting factor, rather than the quantity of the enzyme present.—Environ Health Perspect Vol 102(Suppl 9):87–94 (1994)

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Introduction

Toxicological Interactions of Chemicals from Environmental, Industrial, and Dietary Exposures

There is increasing concern for the potential adverse health effects of human exposures to chemical mixtures. As recently as the mid-1980s, a symposium was held to address health and environmental research on complex organic mixtures (1). The symposium was organized to review the existing state of knowledge regarding materials derived from energy-related and industrial activities. One of the major conclusions from this symposium was that while there is a "vast amount of information" regarding chemical mixtures, "more basic questions still need to be answered." It was concluded that additional studies were needed to determine the complex interactions among mixture components and the basic molecular mechanisms that lead to toxicologic effects in humans. Additional data must be brought to bear to reduce the inherent uncertainties in risk assessments associated with chemical mixtures.

A number of critical questions surface regarding toxicological interactions of chemicals from environmental, industrial, and dietary exposures. For example: Do the chemical interactions influence the toxicokinetics of each chemical and, if so, how is the "dose" to target tissue influenced? What is known about the dose-dependence of the various metabolic pathways and are the chemicals metabolized via similar enzymes? Are there qualitative and/or quantitative differences in metabolism between laboratory animal species and humans? What role, if any, does enzyme induction/inhibition play in toxicity? Which laboratory animal species is/are appropriate for human risk assessment? While these questions by no means address all the important issues regarding chemical mixtures, they do raise important points regarding the influence of chemical mixtures on the dosimetry of chemicals.
1,3-Butadiene as a Case Study

1,3-Butadiene (BD) represents an interesting case study in which new data are emerging that are critical for understanding interspecies differences in responses to BD. Fundamental knowledge regarding mechanisms of BD-induced carcinogenicity provides the basis for assessing the potential effects of mixtures containing BD. BD, a widely used monomer in the production of synthetic rubber and other resins, exhibits low acute inhalation toxicity in rodents [LC50 >120,000 ppm; (2)] and causes sensory irritation in humans at concentrations of 2000 to 8000 ppm (3). Occupational exposures to BD can result during production, storage, and transport of the chemical. BD has been detected in cigarette smoke (4) and automobile exhaust (5) and is currently listed as one of the 189 hazardous air pollutants in the 1990 Clean Air Act Amendments (6).

BD induces tumors at multiple organ sites in B6C3F1 mice and Sprague-Dawley rats (7–9). In mice, several tissues are targets for BD carcinogenicity, including heart, lung, mammary gland, ovary, forestomach, bone marrow, and liver. An activated K-ras gene was observed in lung and liver tumors and lymphomas of B6C3F1 mice exposed to BD (10). The striking aspect of BD-induced carcinogenicity is the high sensitivity of mice to BD. Tumors were observed in mice at concentrations as low as 6.25 ppm and steep concentration-response curves were evident for several tumors. The tumor sites in rats are quite disparate and include the thyroid, mammary gland, Zymbal gland, uterus, testis, and pancreas. Rats exhibit a relatively low sensitivity to BD, since tumors occur at BD concentrations (1000–8000 ppm) nearly three orders of magnitude higher than in mice.

BD displays mutagenic activity in Salmonella typhimurium, but only in the presence of hepatic 9000g supernatant (11–13), indicating that BD is not mutagenic but that its metabolites, possibly 1,2-epoxybut-3-ene (butadiene monooxide; BMO) and/or butadiene diepoxide (BDE), are responsible for the observed bacterial mutagenicity. BD is also genotoxic in vivo, inducing chromosome aberrations and sister chromatid exchanges in bone marrow cells and micronucleus formation in peripheral blood of male B6C3F1 mice (14,15), but not in Sprague-Dawley rats (15).

The in vivo disposition and metabolism of BD have been reported in B6C3F1 mice (16,17), Sprague-Dawley rats (16–18), and in the nonhuman primate Macaca fascicularis (19). The data from these studies indicate that there are significant species differences in the toxicokinetics of BD and BMO. Mice appear to metabolize BD to BMO at greater rates than rats. Three enzymes appear to play major roles in the overall metabolism of BD: cytochrome P450-dependent monoxygenases, epoxide hydrolases, and glutathione (GSH) S-transferases. In addition to enzymic reactions, BD epoxides may nonenzymatically hydrolyze or conjugate with glutathione (GSH). Presently, there are insufficient data that quantitatively describe the contribution of these various pathways in Sprague-Dawley rats and B6C3F1 mice, species susceptible to BD-induced carcinogenesis, or humans, a species potentially at risk for BD. Since one or more of the BD epoxides may play a role in the carcinogenicity of BD, a quantitative understanding of the balance of activation (i.e., epoxide formation) and inactivation (i.e., epoxide hydrolysis or conjugation) is essential for improving our understanding and assessment of human health risk following exposure to BD and chemical mixtures containing BD.

The present paper reports quantitative species differences in the oxidation of BD and BMO by cytochrome P450-dependent monoxygenases and in the inactivation of BD by epoxide hydrolases and glutathione S-transferases using microsomal and cytosolic preparations of liver and lungs obtained from Sprague-Dawley rats, B6C3F1 mice, and humans. In vitro kinetic constants were used for the development of a physiologically based dosimetry model for BD. In addition, transgenic mice were used to assess the capacity of BD to induce in vivo mutations. The data from these studies provide a quantitative basis for our understanding of the relative contributions of the various pathways of BD metabolism in three animal species, including humans, and the role that metabolism plays in forming mutagenic DNA-reactive BD metabolites. These data serve as the basis for improving our understanding of the toxicological interactions of BD with chemicals resulting from environmental, industrial, and dietary exposure.

Materials and Methods

Animals

Male Sprague-Dawley (CD) rats (9–10 weeks) and male B6C3F1 mice (9–10 weeks) were obtained from Charles River Laboratories (Raleigh, NC). The Muta Mouse (MM) transgenic strain [BALB/c x DBA/2 (CD2F1)] of mice (6 weeks) was purchased from Hazleton Research Products, Inc. (Hazleton, PA). The construction of the shuttle vector in λ phage (Agt 10 lacZ), with the inserted bacterial target gene for mutagenesis (lacZ) and production of the transgenic mice, is described in detail elsewhere (20). All animals were determined to be free from viral infection and were acclimated for at least 2 weeks prior to use. Animals were fed with standard diet (NIH-07; Zeigler Brothers, Gardners, PA) and received water ad libitum. They were maintained on a 12-hr light:dark cycle beginning at 0700 and housed at 22 ± 2°C and 55 ± 5% relative humidity.

Preparation of Liver and Lung Microsomes for Metabolism Studies

Rodents were euthanized by either sodium pentobarbital or CO2 asphyxiation and the livers and lungs were excised, frozen in liquid nitrogen, and stored at −80°C. Livers and lungs were slowly thawed while on ice, weighed, cut into pieces, and homogenized with four volumes of isotonic KCl-Tris buffer with six passes of a Teflon-glass homogenizer (1100 rpm, Braun). Microsomes and cytosol were prepared as described previously (21).

Samples of liver (n = 12) from trauma victims (Tennessee Donor Services, Nashville, TN) and samples of lung (n = 5) removed by surgery from cancer patients (Tissue Procurement Service, University of Alabama at Birmingham, Birmingham, AL) were used in these experiments. Samples were frozen (−80°C) and remained frozen during shipment. Tissues were slowly thawed on ice, homogenized, and microsomes and cytosol were isolated as described previously (21).

Protein content was determined using a modified micro-Lowry method (22). Cytochrome P450 content was estimated spectrophotometrically (23).

Enzyme-catalyzed Metabolism of BD and BMO

Experiments were conducted to assess cytochrome P450-dependent metabolism of BD, epoxide hydrolase catalyzed hydrolysis of BMO, and glutathione S-transferase catalyzed conjugation of BMO with glutathione. Details of the methods used for these experiments are reported in Csanády et al. (21). Additionally, non-enzymic rates of hydrolysis and conjugation were assessed as described by Csanády et al. (21). A two-
Physiologically Based Pharmacokinetic (PBPK) Model for BD

A PBPK model describing BD disposition was adapted from a model for styrene toxico-kinetics developed by Ramsey and Andersen (24). The BD model consisted of a description of the uptake of BD from the lungs into the blood, the distribution of BD in blood to four tissue compartments (liver, fat, a compartment representing skin and muscle, and a compartment representing richly perfused tissues such as intestines, blood, kidney and spleen). Metabolism of BD to BMO was assumed to take place in the liver compartment and to proceed by a Michaelis-Menten, or saturable, pathway. Differential equations describing mass transfer between compartments were numerically integrated using the Simusolv simulation language software (Dow Chemical, Midland, MI).

Parameters incorporated into the model were of three types: physiological, such as organ volumes, ventilation rates, and blood flow to various organ groups, chemical, such as blood/air, and tissue/blood partition coefficients, and biochemical parameters describing BD metabolism ($V_{\text{max}}$ and $K_M$). The physiological parameters were taken from the literature and partition coefficients were determined experimentally in vitro using methods of Gargas et al. (25).

The in vivo biochemical parameters were determined by exposing mice or rats to BD gas in a closed, recirculating system described previously by Gargas et al. (26). Animals were placed in a sealed chamber and different initial amounts of BD gas were introduced into the chamber. Starting BD concentrations were approximately 5000, 2000 (3000 mice), 1000, 500, and 50 ppm. In this system, uptake of BD by the animals is a function of both the solubility of BD in tissues and the rate at which the animal metabolizes the chemical. After an initial equilibration period, the decline of the concentration of BD in the chamber is a direct reflection of the rate at which its biotransformation occurs. The simulation model described above was used to model this system. With the physiological and chemical parameters set, the magnitude of the metabolic parameters ($V_{\text{max}}$ and $K_M$) was adjusted until model simulations of BD concentrations in the closed chamber reflected the experimentally collected data as determined by visual inspection.

Experimental Design for Mutagenicity Studies

The mutagenicity experiment utilized three groups of animals with five MM per group. The BD group was exposed to 625 ppm BD for 5 consecutive days for 6 hr/day. This exposure concentration was selected because previous studies indicated that exposure of B6C3F1 mice to 625 ppm BD for up to 60 weeks resulted in significant elevation of tumor incidences in a number of organs (7, 8). The two remaining groups of animals consisted of air controls that were housed in chambers similar to those of the BD-exposed group and a group of animals administered N-ethyl-N-nitrosourea (ENU) at 250 mg/kg (ip injection dissolved in DMSO) as a positive control. Animals were housed in individual wire mesh cages inside a 1-m$^3$ Inn hitter style chamber (27). During the six hours of exposure to air or BD, the animals were without food but had free access to water. Airflow in the chamber was maintained within 10% of 15 air changes per hr.

BD exposure levels in the chamber were monitored by infrared spectroscopy using a Foxboro Wilkes Miran IA IR (Norwalk, CT). BD was generated directly from a liquid/gas storage tank as described by Recio et al. (28). Relative humidity in the chamber was maintained at 50 ± 10% and temperature was maintained at 22 ± 2°C. The five control mice were similarly housed in a second chamber in the same room. They were exposed to clean air of the same temperature, relative humidity, and airflow as delivered to the BD-exposed animals.

Animal Necropsy and Tissue Collection for Mutagenicity Studies

MM were euthanized by an overdose of sodium pentobarbital and exsanguinated by cardiac puncture. The liver and lungs excised from the animals were immediately frozen in liquid nitrogen and subsequently stored frozen at –80°C. Bone marrow was removed from the tibias and femurs by rinsing with Dulbecco’s phosphate-buffered saline (GIBCO, Grand Island, NY). The cell suspension was placed in a microcentrifuge tube and the bone marrow cells were pelleted in a microcentrifuge at maximum speed for 30 to 45 sec. The supernatant was discarded and the cell pellet was frozen in liquid nitrogen and stored at –80°C until the DNA was extracted.

Determination of lacZ$^+$ Mutant Frequency

To determine the lacZ$^+$ mutant frequency in bone marrow, lung, and liver tissue samples, DNA was independently extracted from each tissue for each animal as described in Recio et al. (28). The DNA samples for each animal in the same exposure group (five per each exposure group) were packaged independently into λ phage (28). After the determination of the plaque forming units (pfu) for each individual DNA sample, an equal number of pfu from each individual animal within an exposure group were combined to determine the lacZ$^+$ mutant frequency. Therefore, a single lacZ$^+$ mutant frequency was determined for each tissue for each exposure group. A total of 1 × 105 λ phage plaques were examined from the bone marrow samples, while in lung and liver samples, approximately 5 × 105 λ phage plaques were examined. The number of lacZ$^+$ mutant clear or light blue plaques were counted and "picked" for confirmation by replating on LB medium with agarose plus X-gal. The lacZ$^+$ mutant frequency was calculated by dividing the total number of confirmed mutant plaques by the total phage population analyzed, and was expressed as lacZ$^+$ mutants/10$^6$ plaques. The number of observed mutant plaques within each tissue group was used to determine statistical significance relative to the tissues from the air control group, assuming a Poisson distribution.

Results

Mutant Frequency in Different Tissues of Mice Following Exposure to BD

The lacZ$^+$ mutant frequency in three tissues of mice exposed to 619 ± 3.2 ppm BD (mean ± SD) and ENU was determined 14 days following the last exposure. The number of lacZ$^+$ mutant plaques observed and the lacZ$^+$ mutant frequency for each tissue are shown in Table 1. The background lacZ$^+$ mutant frequency in the tissues examined from control mice ranged from 2 to 4 mutants/10$^5$ plaques. The positive control, ENU, was mutagenic in all tissues examined. The lacZ$^+$ mutant frequency in the BD-exposed mice did not show a significant increase in the bone marrow or liver. However, there was a significant increase in mutant frequency in the lungs of exposed mice ($p < 0.001$).
Microsomal Metabolism of BD

For the enzyme-mediated reactions using liver and lung microsomes, both the disappearance of BD from the gas phase and the appearance of BMO in the gas phase were measured. The previously determined parameters of BMO metabolism were incorporated into equations (21) used to describe the in vitro system. The equations were then used to estimate the Michaelis-Menten constants for BD oxidation as described by Csanády et al. (21). The model was found to describe adequately both BD disappearance from the headspace and BMO appearance in the headspace of reaction vials containing liver microsomes from all species. The Michaelis-Menten parameters are listed in Table 2.

Microsomal Metabolism of BMO

Enzyme-mediated hydrolysis of BMO was not detected in cytosolic fractions of liver from any of the species. All 12 of the human liver microsomal samples were assessed for their ability to metabolize BMO using one initial BMO concentration (100 ppm). The normalized first-order rate constants for the 12 liver samples, which varied between 0.020 and 0.068 min⁻¹mg protein⁻¹, were then rank-ordered and three samples (high, median, low activity) were selected for detailed studies of BMO kinetics. In all cases, results of model simulations (21) adequately described the decline in BMO concentration in the headspace due to enzymic hydrolysis (data not shown).

The $V_{max}$ for BD oxidation to BMO for human liver microsomes was about one-half that observed with B6C3F1 mouse liver microsomes and 2-fold higher than in rat liver microsomes (Table 2). The apparent $K_m$ parameters for BD oxidation in liver microsomes from humans, Sprague-Dawley rats, and B6C3F1 mice were similar and ranged from 2 to 5 µM. There were some striking differences in the calculated $V_{max}/K_m$ (29). The calculated $V_{max}/K_m$ for BD oxidation in B6C3F1 mouse liver microsomes was 6-fold greater than for rat and human liver microsomes.

With the exception of mice, lung microsomes displayed considerably lower capacity to metabolize BD than did liver microsomes (Table 2). For example, the $V_{max}$ for BD oxidation in lung microsomes of humans and rats was similar and about 10 and 4 times lower, respectively, than measured in liver microsomes of the same species. Apparent $K_m$ parameters for BD oxidation in lung tissue of all three species ranged from 2 to 8 µM. The calculated $V_{max}/K_m$ for lung microsomal oxidation of BD was less than liver microsomal metabolism of BD for all three species.

Table 1. Lac<sup>+</sup> mutant frequency in bone marrow, lung, and liver samples from 1,3-butadiene-exposed animals and controls.<sup>a</sup>

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|----------------------------------|
| Bone marrow | Mutant Frequency (×10⁻⁵) |
| Air control | 3 | 3.0 |
| 1,3-Butadiene | 2 | 1.8 |
| ENU-exposed | 71 | 73.1<sup>b</sup> |
| Lung | | |
| Air control | 29 | 4.4 |
| 1,3-Butadiene | 59 | 9.1<sup>b</sup> |
| ENU-exposed | 88 | 16.2<sup>b</sup> |
| Liver | | |
| Air control | 12 | 2.4 |
| 1,3-Butadiene | 18 | 3.1 |
| ENU-exposed | 71 | 13.0<sup>b</sup> |

<sup>a</sup>BD-exposed mice were exposed to 625 ppm BD (6 hr/day for five consecutive days). Controls were exposed to air only for the same duration as the BD-exposed mice. Mice dosed with ENU received a single ip injection of 250 mg ENU/kg. All mice were killed 14 days after the last inhalation exposure or injection for mutant frequency analysis (28).<sup>b</sup>Significantly greater (p<0.001) than air control for each tissue by Poisson analysis of the number of mutant plaques relative to air controls.

Table 2. Kinetic constants for the oxidation of 1,3-butadiene to butadiene monoxide.<sup>a</sup>

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|----------------------------------|
| Liver microsome | $V_{max}$ nmole/mg protein/min | $K_m$ µM | $V_{max}/K_m$<sup>b</sup> |
| Humans | 1.2 ± 0.6 | 5.1 ± 2.6 | 230 |
| B6C3F1 mice | 2.6 ± 0.06 | 2.0 ± 0.2 | 1295 |
| Sprague-Dawley rats | 0.5 ± 0.1 | 3.8 ± 0.2 | 157 |
| Lung microsomes | | | |
| Humans | 0.15 ± 0.04 | 2.00 ± 0.15 | 75 |
| B6C3F1 mice | 2.31 ± 0.26 | 5.01 ± 0.67 | 461 |
| Sprague-Dawley rats | 0.16 ± 0.01 | 7.75 ± 1.88 | 20.6 |

<sup>a</sup>Values are mean ± SD (21).<sup>b</sup>Values are in units of nmol·(min·mg protein·mmol⁻¹).

Table 3. Kinetic constants for the hydrolysis of butadiene monoxide.<sup>a</sup>

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|----------------------------------|
| Liver microsomes | $V_{max}$<sup>b</sup> | $K_m$, mM | $V_{max}/K_m$<sup>c</sup> |
| Humans<sup>a</sup> | | | |
| high | 58.1 ± 4.0 | 1.65 ± 0.12 | 35 |
| median | 18.5 ± 4.1 | 0.58 ± 0.15 | 32 |
| low | 9.2 ± 2.2 | 0.24 ± 0.10 | 38 |
| B6C3F1 mice | 5.0 ± 0.3 | 1.59 ± 0.03 | 3.6 |
| Sprague-Dawley rats | 2.5 ± 0.05 | 0.26 ± 0.01 | 9.5 |

<sup>a</sup>Values are mean ± SD (27).<sup>b</sup>Values are in units of nmole/mg protein/min. <sup>c</sup>Values are in units of nmole·L·(min·mg protein·mmol⁻¹).<sup>d</sup>Three of the 12 human liver samples were used for the kinetic analyses. Selection of the samples was as described in "Materials and Methods". Table 3 shows the Michaelis-Menten constants for BMO hydrolysis by human, rat, and mouse liver and lung microsomes. In the three human liver samples used for the detailed kinetic experiments, $V_{max}$ parameters ranged from 9 to 60 nmole/mg protein/min and apparent $K_m$ parameters ranged from 0.2 to 1.6 mM. In contrast, $V_{max}$ determined from reactions using rodent liver microsomes was one-half or less than that measured in human liver samples. Apparent $K_m$ parameters for B6C3F1 mice were approximately 7- to 8-fold higher than the $K_m$ parameters observed in rats. A comparison of the calculated $V_{max}/K_m$ reveals some striking differences across species (Table 3). For example, for the three human liver samples, calculated $V_{max}/K_m$ parameters ranged from 32 to 38, whereas in rodents calculated $V_{max}/K_m$ parameters ranged from 4 to 10.

Lung microsomal preparations from all three species showed considerably less capacity to hydrolyze BMO enzymatically. This was evidenced by the more rapid decline in BMO concentration in the head space of the reaction vials when liver microsomes were incubated with BMO in the absence of NADPH compared to lung microsomes (data not shown). Over the concentration range investigated, BMO hydrolysis in lung microsomes was best described as a first-order process. First-order rate constants for enzymic hydrolysis of BMO ranged from 0.004 to 0.008 min⁻¹ mg protein⁻¹ for human liver microsomes and 0.001 to 0.002 min⁻¹ mg protein⁻¹ for rodent lung microsomes.

Enzymic oxidation of BMO was negligible for rats or humans. In contrast, mouse liver microsomes quantitatively oxidized BMO to BDE ($V_{max}$ = 0.2 nmole/mg/min; $K_m$ = 15.6 µM. This qualitative
### Table 4. Kinetic constants for conjugation of butadiene monooxide with glutathione

| Sample          | \( V_{\text{max}} \), pmole/kg/hr | \( K_m \), mM | \( V_{\text{max}}/K_m \), pmole mM \(^{-1} \) |
|-----------------|----------------------------------|---------------|---------------------------------------------|
| **Liver cytosol** |                                  |               |                                            |
| Humans          | 10.4 ± 1.04                      | 4.3           |                                             |
| B6C3F1 mice     | 35.3 ± 6.2                       | 14            |                                             |
| Sprague-Dawley  rats | 13.8 ± 0.3             | 17            |                                             |
| **Lung cytosol** |                                  |               |                                            |
| Humans          | 273 ± 31                         | 7.5           |                                             |
| B6C3F1 mice     | 44.2 ± 12.6                      | 2.5           |                                             |

\( a \) Values are mean ± standard deviation (21). \( b \) Values are in units of pmole/mg protein/min. \( c \) Values are in units of 1-nmole-(min-mg protein-mmole)\(^{-1} \). Two of the 12 human liver samples were used for kinetic analyses. Selection of the samples was as described in Materials and Methods. One of the samples displayed Michaelis-Menten kinetics and in the other sample, the reaction was best described by a second-order rate constant of \((2.56 ± 0.22) \times 10^{-4} \) liter/mmole/min/mg protein (mean ± SD). \( d \) Reactions were best described by a \( k_{\text{eq}} \) = \((2.56 ± 0.22) \times 10^{-4} \) liter/mmole/min/mg protein (mean ± SD).

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**Conjugation of BMO with GSH**

Enzyme-mediated conjugation of GSH with BMO in human and rodent liver cytosol and rodent lung cytosol could best be described by Michaelis-Menten kinetics (Table 4). The \( V_{\text{max}} \) in B6C3F1 mouse liver cytosolic fractions was about 2-fold higher than in rat liver cytosol (Table 4). Only one of the two human liver samples analyzed displayed Michaelis-Menten kinetics and had a \( V_{\text{max}} \) of 1/5 to 1/10 of that observed in rodents. Mouse liver cytosolic apparent \( K_m \) parameters were about 3-fold higher than of rats and humans. None of the human lung cytosol samples displayed Michaelis-Menten reaction kinetics. The calculated \( V_{\text{max}}/K_m \) in rodent liver cytosol was about 4-fold higher than the calculated \( V_{\text{max}}/K_m \) for the 1 human sample.

**In vivo Metabolism of BD**

Physiological model simulations for the disappearance of BD from a closed inhalation chamber compared to experimentally determined data are shown in Figure 1. Because of the generally low solubility of BD in blood and tissues (e.g., blood:air partition coefficient = 1.3 to 1.5), the early equilibration phase, in which uptake of BD into tissues dominates, is very brief as indicated by the initial portions of the model simulations for the first 20 min of the experiment. The \( V_{\text{max}} \) and \( K_m \) values used to produce these simulations were 40.7 pmole/kg/hr and 3.7 pmole/l, respectively, for rats and 163 pmole/kg/hr and 11.1 pmole/l, respectively, for mice. These values compare favorably with the metabolic rate constants that were determined from the \textit{in vitro} studies with microsomes described earlier, when these \textit{in vitro} values are adjusted to account for microsomal content and liver weight in the intact animal (21). This favorable comparison suggests that \textit{in vitro} biochemical parameters derived for BD could be used to predict \textit{in vivo} metabolism.

**Simulations of Interactions of BD with Styrene, Benzene, and Ethanol**

Using the physiological model developed for BD one can explore the potential for interactions of BD with other chemicals in the workplace, the environment, or through certain dietary influences. Model predictions of the results of such interactions then form the basis for hypothesis that can be tested experimentally. Three types of situations were simulated representing potential industrial, environmental, and lifestyle exposure scenarios.

Styrene-BD mixtures are often encountered in the workplace where both of these monomers are used in the production of synthetic rubber. Styrene, unlike BD, is highly soluble in tissues, especially lipid rich tissues. Oxidation of styrene to styrene oxide occurs by cytochrome P4502E1 (30), the same P450 isozyme responsible for oxidation of BD to BMO (21). Thus, we hypothesized that the interaction between these two chemicals could be described within a physiological model as a strictly competitive inhibition of each chemical on the metabolism of the other. Using published physiological models for both styrene (24) and competitive inhibition \([K_i = K_m; (3)]\), as well as the dosimetry model for BD described above, we simulated the amount of BD or styrene metabolized both during and after inhalation of mixtures of BD and styrene (Figure 2A, B). Thus, model predictions for the total amount of BD or styrene metabolized was used as a predictor of the extent of inhibition. Under the conditions of these simulations (i.e., strictly competitive

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**Figure 1.** Disappearance of butadiene from closed, recirculating chambers containing mice (top panel) or rats (bottom panel) at various starting concentrations of butadiene. Data points (+) represent samples of the chamber atmosphere analyzed by gas chromatography. Lines represent the results of physiological model simulations of the uptake of butadiene from the chamber by the animals.

**Figure 2.** Physiological model predictions for the uomles BD (A) or uomles styrene (B) metabolized by rats exposed to various combinations of BD and styrene assuming a simple competitive inhibition. Bars represent model predictions of the amount of each chemical metabolized both during and after 8-hr simulated inhalation exposures to each of the combinations of BD and styrene.

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**MICE**

**RATS**
known that ethanol is an inducer of P4502E1 (33). We simulated the inductive effect of ethanol as a 2-fold increase in the $V_{\text{max}}$ for BD oxidation. The simulations assumed an 8-hr exposure to 10, 100, or 1000 ppm BD, and the total amount of BD metabolized during and after exposure was predicted. The results of the simulations indicated that for 10, 100, or 1000 ppm BD, metabolism increased 113, 115, and 158% of control, suggesting that ethanol induction would not have a substantial effect on BD metabolism at concentrations relevant to human exposure. This marginal effect of a 2-fold increase in the maximal rate of BD metabolism predicted by the model is due to the fact that at low inhaled concentrations of BD, BD metabolism is probably blood flow-limited and not enzyme capacity-limited. Thus, model simulations predicted sufficient enzyme at low BD concentrations to metabolize all the BD presented to the liver by the blood; increasing the capacity for BD oxidation does not increase the total BD metabolized. However, at higher BD concentrations, when metabolism begins to become saturated, increasing enzyme capacity does increase the total amount of BD metabolized.

**Discussion**

An understanding of the mechanisms by which BD induces tumors in rats and mice is essential for extrapolating to humans, a species for which BD carcinogenic potency is at present unknown. This becomes particularly important for assessing the effects of chemical interactions with BD resulting from environmental, industrial, and dietary exposures. It is likely that one of the critical biochemical determinants of BD-induced carcinogenicity is the extent to which BD is activated to epoxide metabolites that can react with DNA to ultimately induce mutations. The induction of mutations in tissues of a transgenic mouse is a novel approach to determine *in vivo* mutation induction following exposure to carcinogens. The induction of mutations can be studied within the context of the endogenous pharmacokinetic and biotransformation processes that determine the tissue levels of reactive metabolites that can interact with and alter the cellular DNA in various tissues.

Exposure of the MM strain of transgenic mouse to 625 ppm of BD for five consecutive days (6 hr/day) followed by a 14-day expression period did not result in significant mutagenicity in liver and bone marrow. However, significant mutagenicity was observed in the lung, a target organ for the carcinogenic effect of BD in B6C3F1 mice at 6.25 ppm. Specific mutation of the K-ras oncogene has also been detected in lung adenocarcinomas in BD-exposed mice (10). Since there are no carcinogenicity data in the MM strain of mouse exist for the B6C3F1 mouse, it is difficult at present to relate the relatively small increases in mutagenicity observed in the present studies to another biological endpoint (e.g., tumor formation). Studies in this laboratory are in progress to evaluate the mutagenicity of BD using a B6C3F1 mouse mutation system (34). Studies on the biotransformation of BD by MM liver microsomes indicate that MM enzymes catalyze the conversion of BD to BMO and hydrolysis of BMO (28). A comparison of the initial rates ($V_{\text{max}}/K_{\text{m}}$) of BD metabolism to BMO and BMO hydrolysis between MM and B6C3F1 mouse liver microsomes indicates that there are also mouse strain differences in BD metabolism. The ratio of $V_{\text{max}}/K_{\text{m}}$ for BD to BMO and BMO hydrolysis in MM liver microsomes was 225:1, while the ratio in B6C3F1 mouse liver microsomes is 360:1 (28). The lack of mutagenicity of BD in the liver of the MM may be due to an ineffective concentration of BMO in the liver. Detailed parallel studies on the biotransformation of BD, *in vitro* and *in vivo*, in B6C3F1 and MM are required to establish the role of BD bioactivation/detoxification in mediating the genotoxic effects of BD.

The data presented in this paper reveal that there are significant species differences in the $V_{\text{max}}$ observed for BD oxidation to BMO. For example, B6C3F1 mouse liver microsomes displayed a capacity for BD oxidation exceeding that seen in either human or rat liver. This was evidenced by a comparison of both the maximum velocity for the reaction, $V_{\text{max}}$, and $V_{\text{max}}/K_{\text{m}}$. Two putative detoxication enzymic reactions can occur with BMO: hydrolysis by epoxide hydrolase and conjugation with GSH by glutathione transferase. The results from our studies reveal that liver tissues from all species can detoxify BMO by both pathways. In general, human liver microsomes hydrolyzed BMO at greater rates than either rats or mice, as evidenced by the higher $V_{\text{max}}$ and $V_{\text{max}}/K_{\text{m}}$. While there was a considerable range of epoxide hydrolase activities in liver microsomes of the three human samples investigated ($V_{\text{max}}$ = 9 to 58 nmol/mg protein/min), values for the human samples were at least 2-fold greater than the $V_{\text{max}}$ for rats and mice. The value reported by Kreuzer et al. (35) for epoxide hydrolase-catalyzed
hydrolysis of BMO by microsomes from a single human liver sample ($V_{\text{max}} = 14$ nmol/mg protein/min) falls within the range of values for $V_{\text{max}}$ in human liver microsomes reported in this paper. Values for $V_{\text{max}}/K_m$ reported by Kreuzer et al. (35) for rodents and the one human liver sample were similar to the values reported in this paper. For all species, apparent $K_m$ parameters for hydrolysis and GSH conjugation were significantly greater than for BD oxidation and, in the case of mice, BMO oxidation.

The three examples of simulations with BD (i.e., BD-styrene, BD-benzeno, BD–ethanol) suggest two general important points regarding chemical interactions. The first relates to extrapolations from high to low doses. Due to the saturable enzyme systems that biotransform most toxic organic chemicals, one cannot assume that the inhibition effects demonstrated at high exposure concentrations will be proportional, or even significant, at low exposure concentrations. Second, patterns of enzyme induction determined in vitro may not exhibit the same magnitude of effect in the intact animal. Delivery of the chemical to the site of enzyme–chemical interaction may be, in some cases, the rate-limiting factor, rather than the quantity of the enzyme present.

The simulations regarding the potential interactions of styrene exposures on BD metabolism and vice versa used a strictly competitive inhibition model and suggested that styrene would be an effective inhibitor of BD metabolism but that BD would not be an effective inhibitor of styrene metabolism. A recent publication supports these predictions. For example, Laib et al. (36) reported that for Sprague-Dawley rats coexposed to styrene and BD, styrene decreased the rate of metabolism of BD significantly, but BD had no effect on the metabolism of styrene. Additionally, these authors determined that the $K_i$ for styrene in their system was much lower than its apparent Michaelis-Menten constant, suggesting that the interaction between BD and styrene might be more complex than the simple competitive inhibition description used in our model simulations.

The development of quantitative linkages between exposure and response, which are based on biologically plausible mechanisms of action at exposure levels that are likely to be encountered by people, will significantly improve the risk assessments for human exposures to DNA-reactive chemicals. The present study was initiated to begin to establish in vitro linkages between exposure to BD, internal dose, and a biological response (mutation). The induction of mutations is a determinant of BD-induced carcinogenicity. Although the present studies did not investigate the carcinogenicity of BD in MM, the induction of mutations in a transgene integrated into the genome of the MM represents an initial step towards establishing dose-response relationships for molecular events (mutation) that are part of the carcinogenic process. These relationships need to be established with endogenous genes that are known to be involved in the carcinogenic process (e.g., oncogenes and tumor suppressor genes). The in vitro metabolic constants from these studies can be incorporated into physiological models that can simulate in vivo behavior and the models can be used to predict blood and tissue concentrations of BD and BMO. Experiments using whole animals can then be used to verify model predictions based on in vitro-derived rates. These models are critical for predicting epoxide metabolite concentrations in tissues from exposures to chemical mixtures containing BD.

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