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Metabolism of 1,3-butadiene to toxicologically relevant metabolites in single-exposed mice and rats

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Abstract

1,3-Butadiene (BD) was carcinogenic in rodents. This effect is related to reactive metabolites such as 1,2-epoxy-3-butene (EB) and especially 1,2:3,4-diepoxybutane (DEB). A third mutagenic epoxide, 3,4-epoxy-1,2-butanediol (EBD), can be formed from DEB and from 3-butene-1,2-diol (B-diol), the hydrolysis product of EB. In BD exposed rodents, only blood concentrations of EB and DEB have been published. Direct determinations of EBD and B-diol in blood are missing. In order to investigate the BD-dependent blood burden by all of these metabolites, we exposed male B6C3F1 mice and male Sprague-Dawley rats in closed chambers over 6–8 h to constant atmospheric BD concentrations. BD and exhaled EB were measured in chamber atmospheres during the BD exposures. EB blood concentrations were obtained as the product of the atmospheric EB concentration at steady state with the EB blood-to-air partition coefficient. B-diol, EBD, and DEB were determined in blood collected immediately at the end of BD exposures up to 1200 ppm (B-diol, EBD) and 1280 ppm (DEB). Analysis of BD was done by GC/FID, of EB, DEB, and B-diol by GC/MS, and of EBD by LC/MS/MS. EB blood concentrations increased with BD concentrations amounting to 2.6 μmol/l (rat) and 23.5 μmol/l (mouse) at 2000 ppm BD and to 4.6 μmol/l in rats exposed to 10000 ppm BD. DEB (detection limit 0.01 μmol/l) was found only in blood of mice rising to 3.2 μmol/l at 1280 ppm BD. B-diol and EBD were quantitatively predominant in both species. B-diol increased in both species with the BD exposure concentration reaching 60 μmol/l at 1200 ppm BD. EBD reached maximum concentrations of 9.5 μmol/l at 150 ppm BD (rat) and of 42 μmol/l at 300 ppm BD (mouse). At higher BD concentrations EBD blood concentrations decreased again. This picture probably results from a competitive inhibition of the EBD producing CYP450 by BD, which occurs in both species.

Keywords: 1,3-Butadiene; 1,2-Epoxy-3-butene; 3-Butene-1,2-diol; 3,4-Epoxy-1,2-butanediol; 1,2:3,4-Diepoxybutane; Mouse; Rat; Inhalation

1. Introduction

1,3-Butadiene (BD) is a major industrial chemical, the worldwide demand of which was almost 9 million metric tons in 2003 [1]. The gaseous olefin is primarily used in the production of synthetic rubbers and of plastics as "shock-resistant polystyrene" (a two-phase system made of polystyrene and polybutadiene), ABS or MBS, copolymers of BD with acrylonitrile and styrene and with methyl methacrylate and styrene, respectively. BD is found ubiquitously in the environmental atmosphere, mainly because it is formed in combusting processes and during burning of organic material [2]. BD concentrations in urban air generally range from less

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than 1 to 10 ppb [3]. The USEPA [4] reports for the USA in 1994 average BD concentrations of 0.18 (rural area), 0.45 (suburban area) and 0.62 ppb (urban area). In rodents, inhaled BD caused dose-dependent increases of tumors at various sites. Mice were far more sensitive than rats [5–9]. In order to understand this species difference, metabolism of BD was investigated in several laboratories. In both rodent species, BD is metabolized extensively. A considerable number of metabolic intermediates has been detected in exhaled air, blood and urine (summarized in [2,4]). These include the three mutagenic epoxides 1,2-epoxy-3-butene (EB), its oxidation product 1,2:3,4-diepoxybutane (DEB), and 3,4-epoxy-1,2-butanediol (EBD). The latter can be formed by hydrolysis of DEB or by oxidation of 3-butene-1,2-diol (B-diol), the hydrolytic product of EB (Fig. 1). All four metabolites have been found in BD perfused livers of mice and all but EBD in BD perfused livers of rats [10]. In vivo, however, only EB and DEB have been measured in blood of both species when exposed to BD ([11–14]: EB in both species and DEB in mice; [13–16]: DEB in rats). Because of the relevance of these metabolites for the species-specific toxicity and carcinogenicity of BD, it was the aim of the present study to quantify the blood burden not only by EB and DEB but especially by EBD and B-diol in male Sprague-Dawley rats and B6C3F1 mice single exposed up to 6 h to constant atmospheric BD concentrations between 0 and 1200 ppm.

2. Material and methods

2.1. Chemicals

All commercial chemicals were purchased with the highest purity available from Ridel-de-Haën, Seelze, Germany, and Merck, Darmstadt, Germany. Liquemin® N25000 (Heparin-Natrium) was obtained from Hoffmann-La Roche, Grenzach-Wyhlen, Germany, and BD 99.5% from Linde, München, Germany. EB 98%, DEB 97%, 1,2-epoxybutane 99+%, *n*-butylboronic acid 97% and 4-benzylpiperidine 99% were from Sigma–Aldrich, Steinheim, Germany. Several compounds were obtained by custom synthesis: B-diol 99% and EBD 98% from EMKA-Chemie, Neufahrn, Germany, EBD 98%, DEB-D6 98%, B-diol-D8 98%, and EBD-D6 (3,4-epoxy-[1,1,2,3,4,4-$^2H_6$]butane-1,2-diol) 98% from Synthon, Augsburg, Germany. Handling of all chemicals during different sample preparations was carried out under the hood.

2.2. Animals

Male Sprague-Dawley rats (200–300 g) and male B6C3F1 mice (17–25 g) were purchased from Charles River Wiga GmbH, Sulzfeld, Germany. All experimental procedures with animals were performed in conformity with the "Guide for the care and use of

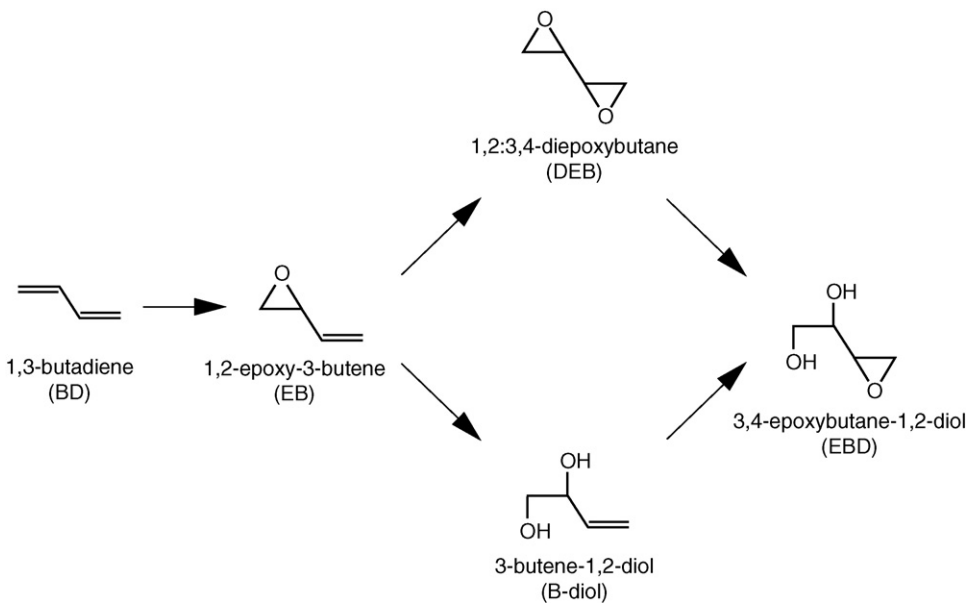

Fig. 1. Simplified scheme of the biotransformation of 1,3-butadiene (BD) showing the metabolites investigated.

laboratory animals" (7th edition, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press Washington, DC, 1996) under the surveillance of the authorized representative for animal welfare of GSF. Up to 1 week before use, two rats or five mice were housed in the GSF-Institute of Toxicology in a macrolon type III cage placed in an IVC top-flow system (Tecniplast, Buguggiate, Italy). This system provided the animals with filtered room air. A constant 12-h light/dark cycle was maintained in the chamber room. Animals had free access to standard chow (Nr. 1324 from Altromin, Lage, Germany) and tap water.

2.3. Exposure experiments

Animals were exposed to BD in closed all-glass chambers under atmospheric pressure and room temperature, similarly as described earlier [17]. Two rats or 5 mice were exposed together up to 8 h to constant BD concentrations of 1, 5, 10, 30, 100, 300, 500, 1000, 2000, 3000, 6000, 10000 ppm (rats) and of 1, 5, 10, 30, 100, 300, 1000, 2000, 4000, 6000 ppm (mice) in closed chambers of 6.5 l containing 35 g soda lime (Drägersorb 800; Drägerwerk, Lübeck, Germany) as CO_2 adsorbent. After establishing defined initial BD concentrations in the chambers, the BD concentrations were maintained in a pre-given range of ±10% by repeatedly re-injecting gaseous BD to replenish the exposure-dependent losses of BD in the gas-tight chambers. These losses resulted only from BD uptake by the exposed animals as was verified in chambers containing no animals but only BD-gas and soda lime (35 g). Measurements of BD in the chamber atmospheres were repeated in time periods between 8 and 23 min, depending on the exposure concentration.

During the exposure experiments, the atmospheric chamber concentration of exhaled EB was measured at selected time points. With increasing time of BD exposure, EB increased in the chamber atmosphere until a plateau concentration was reached. At such a steady-state condition, the air concentration of the endogenously formed EB was in thermodynamic equilibrium with its concentration in venous blood, and the rate of its endogenous formation equaled the rate of its metabolic elimination. Another possible way of EB elimination from the chamber air, the reaction with soda lime (35 g), was considered negligible. This reaction had been studied in an exposure chamber containing EB vapor and fresh or used (moistened) soda lime because soda lime could hydrolyze the epoxide with different rates dependent on its H_2O and CO_2 content. The shortest elimination half-life was obtained with moistened soda lime. Because it was longer than 2 days, the influence of soda lime on the EB concentration in the chamber atmosphere was not taken into account, and the EB concentration in venous blood [μmol/l] was calculated as the product of the EB air concentration at plateau [μmol/l] with the blood-to-air partition coefficient of EB (83.4, measured in rat blood [18]). For converting the EB air concentration from ppm to μmol/l, the air concentration in ppm was divided by 25.13, the molar volume of an ideal gas at 25 °C and 740 Torr. This procedure for determining venous blood concentrations of an internally produced, exhaled gas from its plateau concentrations in the atmosphere of a closed chamber principally yields higher blood concentrations than actually exist in open systems, where no re-inhalation occur. However, the difference between the venous blood concentrations at steady state in a closed and in an open system is less than 10% (investigated by physiological toxicokinetic modeling), provided that the blood-to-air partition coefficient is higher than 50.

For direct measurements of DEB, B-diol, and EBD in blood of BD exposed rodents, another closed exposure system was used. It consisted of a glass-sphere (63 l), equipped with an 8 cm long neck (inner diameter 15 cm) closed by a round lid with 3 ports. Two ports were closed by Teflon coated synthetic rubber septa; the third one was connected to a passive oxygen supply system described in [19]. The sphere contained a circular, foldable floor plate of solvent-inert polyvinylidene fluoride with a diameter of 40 cm. Exhaled CO_2 was trapped by 80 g soda lime that was below the floor plate. The BD concentrations in the chamber were maintained constant (maximum coefficient of variation ±9%) by injecting BD gas repeatedly through one of the septa into the chamber air in order to compensate for the loss of BD by metabolism. The target BD concentrations in the atmospheres were 0, 60, 100 (mice only), 150 (rats only), 300, 600, 900, and 1200 ppm BD for the determination of EBD and B-diol with the exception that B-diol was not quantified in rats exposed to 300 ppm BD. For the determination of DEB, exposure concentrations of BD were up to 900 ppm in rats and 0, 67, 630, and 1270 ppm in mice. For each BD concentration, two rats or 3–16 mice were exposed together for 6.0 h. Immediately thereafter, animals were sacrificed by cervical dislocation, the ribcages were opened, and blood was taken from the vena cava near to the heart using a disposable, Liquemin® moistened syringe. This procedure lasted less than 1 min.

For rats, the blood of two animals was pooled and about 1 ml was used for B-diol, 1 ml for DEB, and 6 ml for EBD analysis. For mice, blood of 12 animals (6 ml) was pooled for one EBD determination and of 2 animals (1 ml) for one B-diol determination. Between 0.3 and 0.7 ml blood was obtained from each of three mice simultaneously exposed to one BD concentration for the individual determination of DEB. Blood was collected in ice-cold 6 ml screw capped glass centrifuge tubes or 1.5 ml Eppendorf cups, each of which containing 10 μl of an ethanolic diethyl maleate solution (1 mol/l ethanol) per ml of blood to be added. After immediate addition of the internal standards (see below), centrifuge vials were closed and the exact blood volumes were determined by weighing. Thereafter, blood samples were either immediately analyzed or stored at −80 °C until use. The analytes remained stable over the required time frame as had been verified experimentally.

For safety reasons, all exposure experiments were conducted under the hood.

2.4. Analysis of 1,3-butadiene

After intensive mixing the chamber air using a disposable syringe of 100 ml, air samples of 25 μl or 10 ml were taken repeatedly from the exposure chamber via a Teflon® coated rubber septum using a gastight 25 μl syringe (RNS, series 1700, Hamilton, Bonaduz, Swizerland) or disposable syringe of 10 ml and injected on column of a GC-8A gas chromatograph (Shimadzu, Duisburg, Germany) equipped with a flame ionization detector, either directly or via a gas sample loop (5 ml). Separation was done at 155 °C on a stainless steel column (3.5 m × 1/8″ × 2 mm) packed with Tenax TA 60–80 mesh (Chrompack, Frankfurt, Germany). Detector temperature was 200 °C. Gas flows were: carrier gas (nitrogen) 13.3 ml/min, hydrogen 60 ml/min, and synthetic air 600 ml/min. Under these conditions, the retention time of BD was 2.0 min. Chromatograms were recorded and integrated by a C-R5A integrator (Shimadzu, Duisburg, Germany). Calibration curves were constructed several times by generating BD gas concentrations ranging from 1 to 10000 ppm in atmospheres of closed desiccators. Calibration curves were linear in the whole range. Analysis of linear regression through the origin revealed correlation coefficients of at least 0.997 between peak areas and atmospheric BD concentrations. Each time before starting a BD exposure, a one-point calibration was carried out in the concentration range used in the actual experiment. The limit of detection was not quantified because the smallest BD concentration of 1 ppm was more than 2 orders of magnitude above the background noise when using the gas sample loop.

2.5. Analysis of 1,2-epoxy-3-butene

EB concentrations in chamber air samples were quantified using a GC/MSD system from Agilent, Waldbronn, Germany (gas chromatograph HP 5890 Series II Plus and mass selective detector HP 5972) equipped with the Thermal Desorption Cold Trap Injector CP 4010 (Chrompack, Frankfurt, Germany). A chamber air sample of 10 ml collected by means of a gas-tight syringe (series 1010, Hamilton, Bonaduz, Switzerland) was manually injected into the injector that contained a CP Sil 5 CB capillary trap (length 30 cm, ID 0.53 mm; Chrompack, Frankfurt, Germany). During the injection period (about 3 min) and up to 2 min thereafter, the Cold Trap injector was cooled with liquid nitrogen to −100 °C. The flow of the carrier gas helium through the injector was 20 ml/min. Then the injector was heated immediately to 200 °C and analytes were transferred to the separation column (PoraPlot U, length 25 m, ID 0.32 mm, equipped with a 2.5 m long particle trap; Chrompack, Frankfurt, Germany). Helium flow was 2.4 ml/min. Column temperature was initially 50 °C for 1.0 min and then raised to 170 °C with a rate of 30 °C/min. The final temperature was held for 8 min before cooling down the column again. The temperature of the GC/MSD interface was 280 °C. The electron ionization potential of the MSD was 70 eV. Retention time of EB following transfer of the trapped material to the separation column decreased with increasing water content of the air sample from 8.2 to 7.8 min. EB was detected at BD exposure concentrations ≥100 ppm in the scan mode (identified using the Wiley 138 MS library) and at lower BD concentrations in the single ion-monitoring (SIM) mode at *m/z* 69 [$(M-1)^+$] and *m/z* 39 [$(M - H_2COH)^+$]. Generally, EB was quantified using *m/z* 69. Three calibration curves were constructed over a concentration range from 0 ppb to 10 ppm using 14 different EB concentrations. Linear regression analysis through the origin revealed correlation coefficients of at least 0.999 between peak areas and EB concentrations. Before each BD experiment for the determination of EB, a three-point calibration curve through the origin was constructed and an EB standard concentration, similar to the actual exhaled EB concentration, was prepared in a desiccator and then repeatedly measured during the exposure period. The detection limit for atmospheric EB was 3 ppb for *m/z* 69 and 1 ppb for *m/z* 39 at an injection volume of 10 ml and a signal-to-noise ratio of 3:1.

2.6. Analysis of 1,2:3,4-diepoxybutane

The detailed analytical procedure is given in [20]. The internal standard DEB-D6 was immediately added to the blood sample. After extraction with dichloromethane, 4 μl were injected onto a pre-column (fused silica capillary, deactivated) protected HP-5MS capillary column (30 m × 0.25 mm I.D.; film thickness 1.0 μm; Agilent, Waldbronn, Germany) in a gas chromatograph HP 6890 equipped with the mass selective detector HP 5973 (Agilent, Waldbronn, Germany). Analytes were detected in positive chemical ionization mode using ammonia as reagent gas. In the SIM mode, ions of *m/z* 104 [DEB + NH_4^+] and 110 [DEB-D6 + NH_4^+] were monitored for quantitative analysis. Racemic DEB was separated from the meso-form. The sum of both diastereomers was used to quantify DEB. The detection limit (3 times background noise) was 10 nmol DEB/l blood.

2.7. Analysis of 3-butene-1,2-diol

The detailed analytical procedure is given in [20]. Aliquots of blood were immediately spiked with the internal standard (B-diol-D8) before erythrocytes were removed by centrifugation. The supernatant was treated with acetonic *n*-butylboronic acid (4 mg per ml acetone). The obtained butylboronate of B-diol was extracted with ethyl acetate; 4 μl thereof were injected onto a HP-5MS capillary column (30 m × 0.25 mm I.D.; film thickness 1.0 μm) in a gas chromatograph HP 6890 equipped with the mass selective detector HP 5973. Detection was in the positive chemical ionization mode with methane as reagent gas. In the SIM mode, the $(M + H)^+$ ions of the derivatized B-diol (*m/z* = 155) and B-diol-D8 (*m/z* = 161) were chosen for identification. B-diol-D8 was also quantified using the ion of *m/z* 161. Because the ion of *m/z* 155 showed a disturbing peak in contrast to the less specific ion of *m/z* 109, the latter was chosen for B-diol quantification. The detection limit (3 times background noise) for B-diol was 20 nmol/l blood.

2.8. Analysis of 3,4-epoxy-1,2-butanediol

Aliquots of blood (6 ml) were immediately spiked with the internal standard (EBD-D6) before erythrocytes were removed by centrifugation (5 min at 4000 g). Potassium carbonate (3 g) was added to the mixture of 3 ml supernatant, 27 ml isopropanol and 3 ml chloroform. After 5 min of vigorous shaking by hand, phase separation was achieved by centrifugation (5 min, 4000 g). The organic (upper) layer, warmed to 45 °C by means of a metal block thermostat, was evaporated under a stream of nitrogen. The residue containing EBD and EBD-D6 was derivatized for 1 h at room temperature with 500 μl of a methanolic 4-benzylpiperidine solution (2 mg/ml). After the addition of 250 μl acetonitrile, the samples were stored for 30 min on ice and then centrifuged (5 min, 4000 g). An aliquot (5 μl) of the supernatant containing the EBD derivative 4-(4-benzylpiperidin-1-yl)-butane-1,2,3-triol ($C_{16}H_{25}NO_3$, exact mass: 279.18 amu) and the corresponding derivative for EBD-D6 ($C_{16}H_{19}D_6NO_3$, exact mass: 285.22 amu) was injected into an LC/MS/MS system, which consisted of an HP1100 LC, an autosampler HP1100 equipped with a 100-well plate holder, and a column oven (all from Agilent, Waldbronn, Germany), an LC column Luna 150 × 2 mm I.D., 5 μm, C18(2) from Phenomenex, Aschaffenburg, Germany, and an API 3000 triple quadrupole mass spectrometer with a turbo ion spray interface from Applied Biosystems, Darmstadt, Germany. Compounds were eluted within 7.5 min at 24 °C with a flow of 250 μl/min using an isocratic mobile phase of 5 mmol/l aqueous ammonium acetate (adjusted to pH = 4 with acetic acid):methanol (60:40, v/v). The mass spectrometer was operated in the multiple reaction monitoring mode and the turbo ion spray source at a temperature of 350 °C in the positive ion mode at an ion spray voltage of 4100 V. Nitrogen was used as curtain gas (setting 9), nebulizing gas (setting 8) and collision gas (setting 4). The collision energy was set at 38. The declustering potential and focusing potential voltages were set at 40 and 150 V, respectively. Unit resolution (at half peak height) was used for both Q1 and Q3. Following HPLC separation, the peak area corresponding to the *m/z* 280 → 188 reaction ($[M + H]^+$ - $C_3H_7O_3$; dwell time 100 ms) for the EDB-derivative was measured relative to that of the *m/z* 286 → 190 reaction ($[M + H]^+$ - $C_3H_3D_4O_3$; dwell time 100 ms) of the internal standard. The peak areas of the *m/z* 280 → 202 and 280 → 91 reactions (dwell time 100 ms for both) were used as qualifiers for the EBD derivative and those of the *m/z* 286 → 205 and 286 → 91 reactions (dwell time 100 ms for both) as qualifiers for the EBD-D6 derivative. For data processing, the software Analyst 1.3 (Applied Biosystems, Darmstadt, Germany) was used. The detection limit (3 times background noise) for EBD was 9.6 nmol/l blood. Under analytical conditions, no separation of the diastereomers was achieved. For every exposure experiment, a three-point calibration covering the expected EBD concentration was performed. Analysis of linear regression through the origin revealed correlation coefficients of at least 0.995 between peak areas and EBD concentrations.

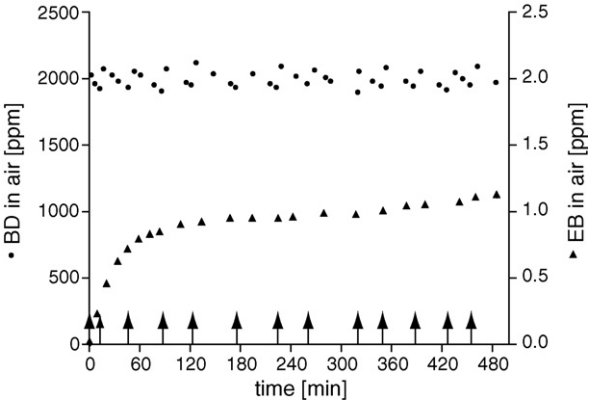

Fig. 2. Representative concentration-time courses of 1,3-butadiene (BD) and 1,2-epoxy-3-butene (EB) measured in a closed chamber containing two rats exposed to an average BD concentration of 2000 ppm. Arrows indicate the time points at which BD was administered into the chamber air.

3. Results and discussion

A representative figure of the methodological procedure for maintaining BD exposure concentrations constant in the exposure chamber is given in Fig. 2. The declines of the BD concentrations due to uptake by the two exposed rats become obvious from the repeated measurements. The arrows on the timeline represent the time points at which defined amounts of BD gas were injected into the chamber atmosphere in order to maintain the BD concentration in the designed range. The figure shows also the appearance of the exhaled metabolite EB. Its concentration increased with time and reached after about 2 h a plateau representing the equilibrium between formation and metabolic elimination of EB.

Fig. 3 shows the EB concentration-time courses in the air of the exposure chambers obtained with rats and mice exposed to the different BD concentrations. In rats, EB plateau concentrations were reached at all BD exposure concentrations. Up to 2000 ppm BD, the plateaus increased with the BD concentrations, but at 6000 and 10000 ppm BD, almost the same EB plateau concentration of 1.3 ppm was obtained. This behavior results from saturation of BD metabolism at BD concentrations above 2000 ppm [21]. In mice, the EB plateau concentrations were much higher than in rats at comparable BD concentrations. Furthermore, at 2000 ppm BD no real EB plateau was seen because after about 4 h of exposure, the atmospheric EB concentration started to increase again. This effect, which was even more pronounced at the higher concentrations, had also been observed earlier [22]. It was related to the breakdown of the glutathione S-transferase mediated BD conjugation with cytosolic

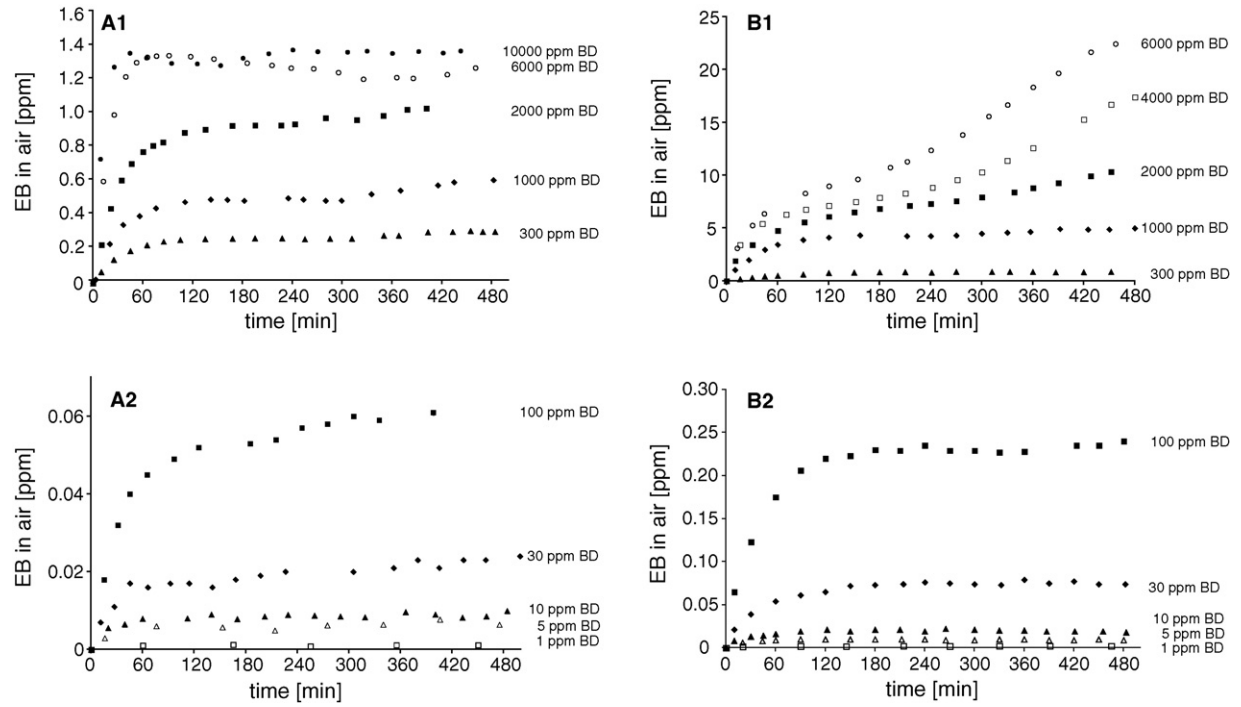

Fig. 3. Concentration-time courses of 1,2-epoxy-3-butene (EB) measured in the air of closed chambers each containing either two rats (A1 and A2) or five mice (B1 and B2) exposed to various 1,3-butadiene (BD) concentrations.

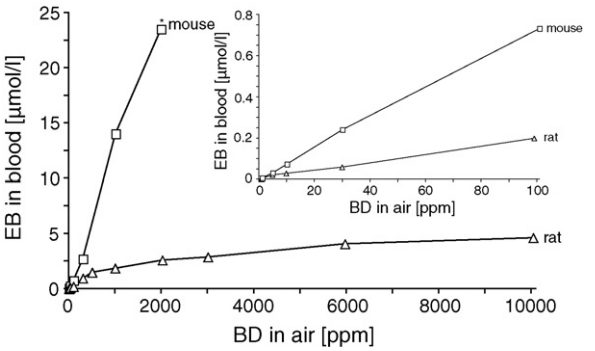

Fig. 4. Calculated steady-state concentrations of 1,2-epoxy-3-butene (EB) in venous blood of rats (△) and mice (□) versus the exposure concentrations of 1,3-butadiene (BD). All EB concentrations reflect single BD exposures over 2–8 h with the exception marked by the asterisk. *: The EB concentration in blood of mice exposed to 2000 ppm is reached after 3 h of BD exposure and will increase, if BD exposure continues due to depletion of the EB conjugating glutathione.

glutathione (GSH) in livers of mice [18]. In this species, continuous exposure to 2000 ppm BD for 7 and 15 h led to a reduction of the non-protein thiol (NPSH) content in the liver to 20% and less [23] and to about 4% [22], respectively. At 1000 ppm BD (7 h of exposure [24]) and 1250 ppm BD (6 h of exposure [25]), the GSH decline was only about 50%. In livers of equally BD exposed rats, GSH depletion was much less expressed [22,23]. Even at a BD concentration of 8000 ppm, hepatic GSH did not fall below 50% of the control level [25].

Fig. 4 shows the EB concentrations in venous blood of rats and mice at steady-state conditions of single 8 h exposures to BD between 1 and 10000 ppm in rats and 1 and 1000 ppm in mice, calculated from the plateau concentrations in Fig. 3. Also plotted is the EB concentration in venous mouse blood that was reached after 3 h of exposure to 2000 ppm BD. Because EB in blood of mice did not reach constant concentrations at higher BD exposure concentrations, they were not included in the figure. In mice, EB blood concentrations increased almost linearly with the BD exposure concentration up to 1000 ppm BD. In blood of rats, the increase of EB deviates from linearity at much lower BD concentrations. This flattening of the EB curve is due to the saturability of CYP450 mediated BD metabolism (see above). In mice, a corresponding effect is counteracted at high BD concentrations by the loss of glutathione S-transferase mediated EB elimination due to depletion of glutathione. Because of the species-specific saturation kinetics of BD metabolism, the ratio of the EB blood concentrations mouse-to-rat was not constant, even at BD concentrations far below 2000 ppm. It was between 2.0 and 2.6 at BD concentration below 10 ppm, 3.8 at 100 ppm, 4.9 at 625, and 8.0 at 1250 ppm BD (Table 1). Similar ratios were observed by comparing levels of the EB adduct with the N-terminal valine in hemoglobin of mice and rats following one- and four-week exposures (6 h/d, 5 d/w) to 10 and 100 ppm BD [26]: At 10 ppm BD the adduct ratio mouse-to-rat was about 2 and at 100 ppm BD about 4 [26].

Table 1 compares the EB blood concentrations obtained in the present work with data published by other authors at BD concentrations ≥62.5 ppm. Although different experimental methods were used in the three laboratories, there is pretty good agreement between the old and new blood concentration data for EB.

Fig. 5 demonstrates the concentrations of DEB (mice only), EBD, and B-diol in venous blood of mice and rats, measured after 6 h of exposures to constant atmospheric BD concentrations and, for comparison, the EB concentrations that are given in more detail in Fig. 4.

The DEB concentrations in blood of mice (means ± S.D., $n = 3$), representing the sums of racemic and

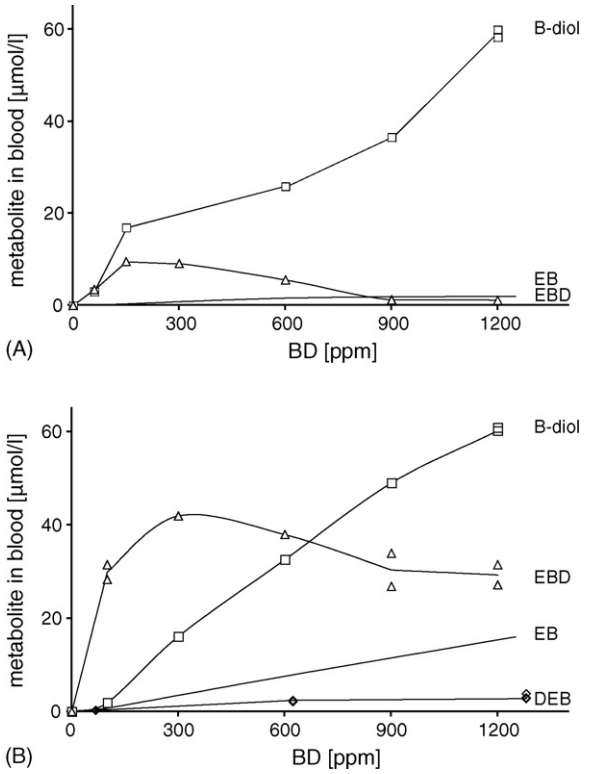

Fig. 5. Metabolite concentrations in venous blood of rats (A) and mice (B) at the end of 6 h exposures to various concentrations of 1,3-butadiene (BD). Symbols, each representing one measurement: □ 3-butene-1,2-diol (B-diol) pooled from 2 rats or 2 mice ($n = 1$–2); △ 3,4-epoxy-1,2-butanediol (EBD) pooled from 2 rats or 12 mice ($n = 1$–2); ◊ 1,2:3,4-diepoxybutane (DEB) in 1 mouse ($n = 3$). Lines: 1,2-epoxy-3-butene (EB) in blood (from Fig. 4).

Table 1
Concentrations of 1,2-epoxy-3-butene (EB) in venous blood of single 1,3-butadiene (BD) exposed male Sprague-Dawley rats and B6C3F1 mice

BD in air (ppm)	EB in venous blood (μmol/l)		EB ratio mouse to rat	Reference
	Rat	Mouse		
1	0.003	0.006	2.0	This study[a]
10	0.028	0.073	2.6	This study[a]
62.5	0.12	0.46	3.8	This study[a]
62.5	0.07	0.6	8.6	[11][b]
62.5	0.036	0.295	8.2	[13][c]
100	0.19	0.73	3.8	This study[a]
100	0.1	0.38	3.8	[12][c]
625	1.6	7.9	4.9	This study[a]
625	0.94	3.7	3.9	[11][b]
1250	2.0	16	8.0	This study[a]
1250	1.3	8.6	6.6	[11][b]
8000	4.3	Not done		This study[a]
8000	4.03	Not done		[16][c]

[a] Measured or calculated based on EB plateau concentrations in chamber air using the EB blood-to-air partition coefficient.
[b] Mean values of the concentrations measured between 2 and 6 h of exposure.
[c] After 4 h of exposure.

meso DEB (about 10% of the racemate), were 0.0, 0.30 ± 0.07, 2.2 ± 0.2, and 3.2 ± 0.6 μmol/l at 0, 67, 630, and 1270 ppm BD, respectively. They compare well with values obtained under similar exposure conditions in the same strain. Himmelstein et al. [11] determined DEB concentrations of 0.65, 1.9, and 2.5 μmol/l following 6 h exposures to 62.5, 625, and 1250 ppm BD. Thornton-Manning et al. [13] and Bechtold et al. [12] exposing male mice for 4 h to 62.5 and 100 ppm BD, respectively, reported DEB blood concentrations of 0.204 μmol/l [13] and of 0.33 μmol/l [12]. Following single (6 h) and repeated (6 h/d, 10 d) exposures of female B6C3F1 mice to 62.5 ppm BD, DEB blood concentrations of 0.345 and 0.247 μmol/l, respectively, were published [14].

In blood of male rats, no DEB could be detected at all in the present study (detection limit 10 nmol/l), even at the highest tested exposure concentration of 900 ppm BD. Other authors, too, were unable to determine DEB in rats exposed either to 100 ppm BD (4 h, DEB detection limit 10 nmol/l, [12]) or up to 1200 ppm BD (6 h, DEB detection limit 130 nmol/l, [11]). After altering the method described in [12] to reach a DEB detection limit of 1.6 nmol/l blood, DEB concentrations of 5 nmol/l [13] and 2.4 nmol/l [15] were measured following single exposures of male rats to 62.5 ppm BD. Higher concentrations of DEB in blood were reported for female Sprague-Dawley rats either once exposed over 6 h to 62.5 ppm BD (14 nmol DEB/l [14]) and 8000 ppm BD (11 nmol DEB/l [16]) or repeatedly exposed over 10 d (6 h/d) to 62.5 or 8000 ppm BD (17 nmol/l at both concentrations [14,16]). In summary, the data from different laboratories demonstrate for mice and rats a species difference in the DEB blood concentrations of more than one order of magnitude, when exposed to around 65 ppm BD. Also from the results of a hemoglobin binding study in which the levels by *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)valine, the DEB-specific ring adduct to the N-terminal valine, were compared in both species following a 2 week (6 h/d, 10 d) exposure to 3 and 62.5 ppm BD, the authors concluded "that mice are much more efficient at forming 1,2:3,4-diepoxybutane than rats, particularly at low exposures" [27].

The present work is the first demonstrating EBD and B-diol concentrations in blood of BD exposed rodents (Fig. 5). EBD showed a maximum of 9.5 μmol/l at 150 ppm BD in rats and of 42 μmol/l at 300 ppm BD in mice. With further increasing BD concentrations, the EBD concentrations decreased in the rat to become even smaller than the EB concentrations at 900 ppm BD (EBD: 1.2 μmol/l) and 1200 ppm BD (EBD: 1.1 μmol/l). In mice, the decrease of the EBD concentration was much smaller. At BD concentrations of 900 and 1200 ppm mean blood concentrations of EBD were about 30 μmol/l. Up to now, the formation of EBD in BD exposed rodents was concluded indirectly from the determination of *N*-(2,3,4-trihydroxybutyl)valine in hemoglobin [27,28] and of N-7-(2,3,4-trihydroxybutyl)guanine in DNA [29,30]. Although these adducts are formed from EBD [31,32], they might also result from DEB [33–35]). However, the conclusion that *N*-(2,3,4-trihydroxybutyl)valine results primarily from EBD is supported by comparing our

data with the findings of Perez et al. [27] who measured the *N*-(2,3,4-trihydroxybutyl)valine levels in male Wistar rats, repeatedly exposed to BD concentrations of 50, 200, and 500 ppm (6 h/d, 5 d). The authors described a similar dependency of the adduct levels from the BD exposure concentration as obtained in the present study for EBD concentrations in blood. The adduct levels following exposures to 50 and 500 ppm BD were almost identical but the level in the 200 ppm exposed rats was twice as high. Also, the findings of Koc et al. [29], who determined N-7 guanine adducts of EB, DEB, and EBD in mice and rats exposed to BD concentrations of 0, 20, 62.5, and 625 ppm BD, do not disagree with our EBD data. The N7-(2,3,4-trihydroxybutyl)guanine levels were the most abundant exhibiting a nonlinear dose–response curve. The findings that N7-(2,3,4-trihydroxybutyl)guanine reached "a plateau after 62.5 ppm" in rats but "continued to increase in mice between 62.5 and 625 ppm BD" appear consistent with our results considering that the authors had no information on the adduct levels at BD concentrations between 62.5 and 625 ppm and above 625 ppm.

B-diol was first detected as a minor urinary BD metabolite in mice and rats exposed up to 5 h to 800 ppm ^{13}C-BD [36]. Fig. 5 shows that B-diol concentrations in blood of rats were higher than those of the epoxides at all BD concentrations above 60 ppm. In blood of mice, B-diol concentrations surpassed those of EBD only at 600 ppm. In both species, the B-diol concentrations increased continuously with the BD concentrations amounting to 60 μmol/l at 1200 ppm. Below 60 ppm BD, the slopes of the curves yielding B-diol in blood versus BD in air were less steep than at higher concentrations.

The complex interaction pattern of the BD metabolites can be understood considering that they are not only formed in a chain after re-uptake of their actual parent compounds into the liver from its blood flow, as Fig. 1 might suggest, but almost simultaneously in the endoplasmic reticulum as immediate metabolites of BD. This concurrent metabolite production was hypothesized [10,37] and strongly supported from experiments with isolated, BD-perfused livers [10]. The immediate formation of EB, B-diol, EBD, and DEB results from the close spatial vicinity of CYP450 and microsomal epoxide hydrolase both situated in the endoplasmic reticulum. In the mouse liver with the higher CYP450 activity towards BD [38,39], the rates of EB and DEB formation are higher than in that of the rat. However, the immediate hydrolysis rates of EB to B-diol are probably higher in the rat than in the mouse as evidenced from the ratios B-diol to EB (compare Fig. 5). As a consequence, less DEB is formed in the rat. Its release into the blood leaving the liver is so small that it could not be detected in vivo by the present analytical method.

In both species, most of the produced DEB is assumed to be immediately hydrolyzed to EBD before becoming systemically available. The EBD concentrations in mouse blood are higher than in rat blood because mice produce more DEB (due to their higher CYP450 activity to EB [40] and because more EB is available for oxidation), which is immediately hydrolyzed. Additionally, B-diol is in part directly epoxidized to EBD as shown in B-diol exposed rodents by EBD adducts to hemoglobin and DNA of liver and lung [30]. However, only about 18% of the circulating B-diol was detected in blood as EBD after intraperitoneal administration of B-diol (12 mg B-diol/kg body weight) to male Sprague-Dawley rats (unpublished results). The group of Elfarra investigating intensively the metabolism of B-diol detected the oxidation product hydroxymethylvinyl ketone [41]. From the urinary excretion of mercapturic acids derived from B-diol, the formation of hydroxymethylvinyl ketone was suggested to be a prominent route for B-diol metabolism in rats and mice [42].

Considering the continuous increase of the direct BD oxidation product EB in blood of both species at BD concentrations up to 1200 ppm, the EBD maxima reached at much lower BD concentrations (150–300 ppm rat and about 300 ppm mouse), the steeper B-diol slopes at BD concentrations above 60 ppm (both species), and the nonlinear increase of DEB, it seems probable that BD inhibits in both species the oxidation of EB to DEB and of B-diol to EBD in a competitive manner by displacing EB, B-diol and DEB from CYP450.

Mutagenic potencies of EB and EBD are comparable [43,44]. In rats, EB blood concentrations amount to 4 μmol/l at 8000 ppm BD (Table 1; Fig. 4), the highest BD concentration used in the carcinogenicity long-term study with rats (0, 1000, 8000 ppm, 6 h/d, 5d/w, 2y; [9]). Considering that EBD concentrations reach a maximum of about 10 μmol/l at a BD concentration of 150 ppm (Fig. 5), it can be suggested that rats are at a similar mutagenic risk at BD concentrations of about two hundred ppm BD as they had been in the long-term study at 8000 ppm BD.

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