1,2:3,4-Diepoxybutane in blood of male B6C3F1 mice and male Sprague-Dawley rats exposed to 1,3-butadiene

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ABSTRACT

The important industrial chemical 1,3-butadiene (BD; CAS Registry Number: 106-99-0) is a potent carcinogen in B6C3F1 mice and a weak one in Sprague-Dawley rats. This difference is mainly attributed to the species-specific burden by the metabolically formed 1,2:3,4-diepoxybutane (DEB). However, only limited data exist on the DEB blood burden of rodents at BD concentrations below 100 ppm. Considering this, DEB concentrations were determined in the blood of mice and rats immediately after 6 h exposures to various constant concentrations of BD of between about 1 and 1200 ppm. Immediately after its collection, blood was injected into a vial that contained perdeuterated DEB (DEB-D_6) as internal standard. Plasma samples were prepared and treated with sodium diethyldithiocarbamate that derivatized metabolically produced DEB and DEB-D_6 to their bis(dithiocarbamoyl) esters, which were then analyzed by high performance liquid chromatography coupled with an electrospray ionization tandem mass spectrometer. DEB concentrations in blood versus BD exposure concentrations in air could be described by one-phase exponential association functions. Herewith calculated (±)-DEB concentrations in blood increased in mice from 5.4 nmol/l at 1 ppm BD to 1860 nmol/l at 1250 ppm BD and in rats from 1.2 nmol/l at 1 ppm BD to 92 nmol/l at 200 ppm BD, at which exposure concentration 91% of the calculated DEB plateau concentration in rat blood was reached. This information on the species-specific blood burden by the highly mutagenic DEB helps to explain why the carcinogenic potency of BD in rats is low compared to that in mice.

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1. Introduction

The gaseous olefin 1,3-butadiene (BD) is a major industrial chemical used primarily in the production of synthetic rubbers and plastics. In 2010, its global production and consumption were reported to have been approximately 10.5 million metric tons (IHS, 2011). Exposure to BD occurs not only at workplaces. The general population is exposed to low concentrations of this gas, which is found in indoor and outdoor air, mainly as a product from tobacco smoking and from incomplete combustion of biomass and fuel (U.S. Environmental Protection Agency, 2002). In long-term carcinogenicity studies (6 h/d, 5 d/w, 2 y), inhaled BD was weakly carcinogenic in Sprague-Dawley rats exposed to 0 ppm, 1000 ppm or 8000 ppm (Owen et al., 1987) but was highly effective by inducing tumors in B6C3F1 mice which were exposed to BD con-

centrations of up to 625 ppm. In female mice, lung tumors increased at a concentration as low as 6.25 ppm. In male mice, the lowest BD concentration showing increased tumor incidences in several organs was 62.5 ppm. In both genders, increased tumor incidences were found in every investigated tissue at 200 ppm (Melnick et al., 1990).

In order to understand the different carcinogenic potency of BD in both species, its metabolism was thoroughly investigated by several laboratories. BD is biotransformed by cytochrome P450 dependent monooxygenases (primarily CYP2E1) and the endoplasmic epoxide hydrolase to the three epoxide intermediates 1,2-epoxy-3-butene, 1,2:3,4-diepoxybutane (DEB), and 3,4-epoxy-1,2-butanediol (reviewed in Himmelstein et al., 1997; Kirman et al., 2010). In vivo metabolism of BD to 1,2-epoxy-3-butene was first shown by Bolt et al. (1983) and Filser and Bolt (1984) in BD exposed rats. The three epoxides are genotoxic as was demonstrated in numerous studies carried out in vitro as well as in vivo (reviewed in Albertini et al., 2010). DEB contains two electrophilic sites and forms DNA–DNA and DNA–protein cross-link adducts (Goggin et al., 2009; Michaelson-Richie et al., 2010). In cultured human lymphoblasts, the mutagenic activity of DEB was up to 100

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and 350 times higher than those of 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol, respectively (Cochrane and Skopek, 1994). Also, the carcinogenic potency of DEB was higher than that of 1,2-epoxy-3-butene in similarly treated Swiss mice (skin application, 3 times per week, lifelong; Van Duuren et al., 1963, 1965).

In blood of BD exposed mice and rats, all three epoxides were found. In both species, 62.5 ppm was the lowest BD concentration at which DEB was determined (reviewed in Filser et al., 2007). Humans, however, are generally exposed to lower BD concentrations: in the USA, European countries, Canada, China, Malaysia, South Africa, and New Zealand, occupational threshold limits for 8-h time-weighted average workplace concentrations of BD are between 0.5 and 21 ppm (IARC, 2008).

Knowledge of the DEB concentrations in blood will be highly relevant as a solid basis for the development of a valid physiological toxicokinetic model that can be applied for risk assessment purposes. In order to become informed about DEB in the blood of mice and rats at BD concentrations that are more relevant to human exposure concentrations as well as for comparison with published data, the aim of the present work was to quantify DEB in the blood of mice and rats exposed over 6 h to various constant BD concentrations of between 1 and 1200 ppm.

2. Materials and methods

2.1. Chemicals

All commercial chemicals were purchased with the highest purity available. Most of them were from Merck, Darmstadt, Germany, Riedel-deHaën, Seelze, Germany, or Sigma–Aldrich, Taufkirchen, Germany. Gases were from Linde, Unterschleissheim, Germany. Liquemin N25000 (heparin-sodium) was obtained from Hoffmann-La Roche, Grenzach-Wyhlen, Germany. Soda lime (Drägersorb 800 Plus) was from Drägerwerk, Lübeck, BD (99.5%) from Linde, racemic DEB (97%) and diethyl maleate (DEM, 97%) from Sigma–Aldrich. Ketamine 10% (aqueous solution containing 115.34 mg ketamine hydrochloride per ml) was obtained from Intervet, Unterschleissheim and Rompun 2% (aqueous solution containing 23.32 mg xylazine hydrochloride per ml) from Bayer, Leverkusen, Germany. Sodium diethyldithiocarbamate trihydrate (DTC, >99.0) was purchased from Fluka Chemie, Buchs, Switzerland. 1,2:3,4-Diepoxy-[1,1,2,3,4,4-D$_6$]butane (DEB-D$_6$), consisting of a mixture of the (±)-form (2 parts) and the meso form (1 part) as confirmed by LC/MS/MS-measurements, was custom made by Synthon, Augsburg, Germany. Handling of all chemicals during different sample preparations was carried out under the hood.

2.2. Animals

Male Sprague-Dawley rats (240–290 g) and male B6C3F1 mice (20–30 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 laboratory animals (NRC, 1996) under the surveillance of the authorized representative for animal welfare of the Helmholtz Zentrum München. Animals were acclimated for at least 3 days before exposure. They were housed in macrolon type III cages in an IVC top-flow system (Tecniplast, Buguggiate, Italy) that provided them with filtered room air. Animals were kept at room temperature of between 22 and 25 °C receiving standard diet 1324 (Altromin, Lage, Germany) and tap water ad libitum. A light and dark cycle of 12 h and a relative air humidity of between 50% and 60% were maintained in the animal room.

2.3. Exposure experiments

Closed glass-spheres (63 l) were used for exposing animals to BD. The exposure system is described in detail in Filser et al. (2007). Groups of mice or rats were exposed to mean atmospheric BD concentrations (±standard deviation) of 1.0 (±0.17), 6.4 (±0.65), 11 (±1.2), 21 (±1.8), 63 (±6.8), 108 (±8.1), 311 (±24), 603 (±35), or 1180 (±101) ppm (mice) and of 1.1 (±0.20), 2.4 (±0.67), 5.6 (±1.1), 11 (±1.1), 21 (±1.1), 33 (±2.5), 62 (±8.9), 106 (±6.0), 203 (±11), 624 (±36), or 1220 (±47) ppm (rats). During the exposure experiments, atmospheric BD concentrations were determined at varying time periods of between 6 and 14 min and were maintained quasi-constant by repeatedly injecting gaseous BD (taken directly from the gas cylinder or as a diluted gas from a storage desiccator) to replenish the losses of BD in the gas-tight spheres, which resulted from metabolic elimination and from opening the chamber for placing or removing an animal. At each exposure experiment with mice, two groups of six animals each (tail-marked by different colors) were placed with an interval of 25 min into one chamber. In experiments with rats, 4 individually tail-marked animals were successively put into one chamber at time

intervals of 20 min. Rat exposures were carried out twice at BD concentrations of 1.1, 5.6, and 11 ppm. Each animal was exposed for 6.0 h. Mice were sacrificed by cervical dislocation. Using a disposable, heparin sodium-moistened syringe, up to 0.5 ml of blood was taken from the vena cava caudalis (near to the heart) of each animal of a group and injected – one after the other – in one ice-cooled 5-ml-cryotube vial (Simport, Beloeil, Quebec, Canada) that contained 40 μl of an ethanolic solution of the glutathione depleting agent DEM (515 μl DEM in 2760 μl ethanol) and 10 μl (1.0 and 6.4 ppm BD) or 30 μl (11–1180 ppm BD) of the internal standard DEB-D$_6$ (14.5 μmol/l in acetone). The vial was shaken after each blood injection. The whole procedure of pooling the blood of the 6 mice per group lasted not more than 6 min. Rats were treated according to Lee et al. (2005). Twenty minutes before sacrificing a rat, it was removed from the sphere and immediately anesthetized by injecting intraperitoneally a mixture consisting of 0.88 ml ketamine/kg body weight and 1.1 ml Rompun/kg body weight. Directly thereafter, the anesthetized animal was returned into the exposure sphere. Within 5 min, the target concentration was readjusted by compensating for the amount of BD being lost. At the end of the exposure, the anesthetized animal was removed from the sphere and sacrificed immediately. Within 1 min after sacrifice, about 4 ml of blood was collected from the vena cava caudalis (near to the heart) by means of a heparin sodium-moistened syringe, and injected in an ice-cooled 5-ml-cryotube vial that contained 40 μl of the DEM solution and 10 μl (1.1–5.6 ppm BD) or 30 μl (11–1220 ppm BD) of DEB-D$_6$ (14.5 μmol/l in acetone). The vial was closed and shaken vigorously.

The ice-cold blood samples were immediately centrifuged at 0 °C and plasma was stored at −80 °C until sample preparation for DEB analysis.

2.4. Analytical methods

2.4.1. 1,3-Butadiene

During the exposures, BD was determined directly from 5-ml gas samples that were collected by means of disposable syringes from the chamber air and immediately injected via a 300 μl sample loop on column in a Shimadzu GC-8A gas chromatograph (GC; Shimadzu, Duisburg, Germany) equipped with a flame ionization detector using nitrogen with a pressure of 3.75 kg/cm^2 as carrier gas. Separation was done on a stainless steel column (3.5 m × 1/8 in. × 2 mm) packed with Tenax TA (60–80 mesh; Chrompack, Frankfurt, Germany). Temperatures of column oven and detector were 110 °C and 200 °C, respectively. The combustible gases were hydrogen and synthetic air, each with a pressure of 0.6 kg/cm^2. Under these conditions, the retention time of BD was 3.8 min. Chromatograms were recorded and integrated by a CR5A integrator (Shimadzu). Calibration curves were constructed several times by generating BD gas concentrations ranging from 1 to 2000 ppm in atmospheres of closed chambers. Calibration curves were linear in the whole range. Analysis of linear regression through the origin revealed correlation coefficients (r) 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 (three times the background noise) was 0.3 ppm. The coefficient of variation, as a measure for reproducibility, was determined from 6 measurements each carried out at BD concentrations that covered the whole concentration range studied. It was always below ±2.7%.

2.4.2. 1,2:3,4-Diepoxybutane

2.4.2.1. Sample preparation. The DEB determination was based on the derivatization with DTC (according to Munger et al., 1977; Dupard-Julien et al., 2007). The derivatization procedure was species-specific. Mice: To 0.5 ml of thawed plasma, 1 ml of a DTC solution (0.22 mol/l in a 50 mmol/l phosphate buffer of pH 7.4) was added. After vortexing vigorously, the mixture stood for 10 min at room temperature, then for 1 h at 50 °C. After cooling to room temperature, the obtained bis(dithiocarbamoyl) esters were extracted twice with 2 ml chloroform each. To the unified organic phase 1 ml of an aqueous sodium chloride solution (10 g/100 ml) was added and the mixture was vortexed thoroughly for 0.5 min. After centrifuging, the organic phase was carefully removed, dried in a gentle stream of nitrogen, resuspended in 50 μl methanol, and transferred in an autosampler vial for LC/MS/MS analysis. Rats: To 2 ml of thawed plasma, 1 ml of a DTC solution (1.78 mol/l in a 50 mmol/l phosphate buffer, pH 7.4) was added, followed by vortexing. After standing for 1 h at room temperature, 1 ml of acetonitrile was added. The mixture stood for further 10 min, followed by vortexing and centrifugation. The supernatant was transferred to a new vial. The pellet was vortexed for about 30 s in 1 ml of acetonitrile, centrifuged, and the supernatant was unified with the already transferred one. Thereafter, 300 mg NaCl was given to the 2–3 ml of the unified aqueous acetonitrilic solution which was then twice extracted with 3 ml chloroform each. After drying under a stream of nitrogen, the residue was solved in 40 μl methanol and transferred to an autosampler vial for LC/MS/MS analysis.

2.4.2.2. LC/MS/MS analysis. From an autosampler vial containing the DEB- and DEB-D$_6$-bis(dithiocarbamoyl) esters 5 μl was subjected to LC/MS/MS analysis. The LC/MS/MS system consisted of an HP1100 liquid chromatograph (Agilent, Waldbronn, Germany) and an API 4000 triple quadrupole mass spectrometer with turbo ion spray interface (Applied Biosystems, Darmstadt, Germany). The liquid chromatograph was equipped with a Luna C18 (2) column (150 mm × 2 mm i.d., 5 μm) obtained from Phenomenex, Aschaffenburg, Germany. Separation was carried out with retention times of around 7.1 min (racemic DEB and (±)-DEB-D$_6$) and 8.0 min

(*meso*-DEB and *meso*-DEB-D$_6$) at 30 °C (column oven) with a flow of 300 µl/min using a mobile phase consisting of aqueous ammonium acetate (5 mmol/l, pH = 7.0; solvent A) and methanol (solvent B). The composition of the solvents was A = 40% and B = 60% for the first 5 min. Up to 8 min, the percentage of B increased linearly to 100% and remained up to 23 min. Within 2 min, the composition of the buffer was then adjusted back to A = 40% and B = 60%. The column was ready for a new injection after 30 min.

The turbo ion spray source of the API 4000 was operated at a temperature of 470 °C in the positive ionization mode at an ion spray voltage of 4400 V. Nitrogen served as curtain (CUR = 10), nebulizing (GS1 = 35, GS2 = 45), and collision gas (CAD = 7). The mass spectrometer was used in the multiple reaction-monitoring mode. Unit resolution (at half peak height) was used for both Q1 and Q3. For identification and quantification, the peak area of the transition ion at *m/z* 385.2 → 367.2 (dwell time 150 ms, declustering potential = 50 V, collision energy = 17 V) was monitored for the DEB-derivative relative to that at *m/z* 391.1 → 373.1 (dwell time 150 ms, declustering potential = 50 V, collision energy = 19 V) monitored for the DEB-D$_6$-derivative. Additional fragmentation reactions (385.2 → 116.2 and 391.1 → 116.2) were used as qualifiers. Data processing was done by means of the software Analyst 1.4.2 from Applied Biosystems. A product ion spectrum of the DEB-diester is shown in Fig. 1.

For constructing a DEB-calibration curve consisting of 10 DEB concentrations (mice) or 9 DEB concentrations (rats) that ranged from 0 to 0.08 µmol/l blood or from 0 to 2.0 µmol/l blood (mice) and from 0 to 0.0035 µmol/l blood or from 0 to 0.4 µmol/l blood (rats), blood of naïve animals (about 30 mice or 10 rats) was pooled. Blood samples were treated as described under Section 2.3 with the difference that between 5 and 20 µl of an acetonic solution of a predefined concentration of racemic DEB was added into the samples before the preparation of plasma. In total, four calibration curves were constructed for mice and eight calibration curves for rats. Linear regression analyses revealed coefficients of determination (R^2) of between 0.992 and 0.999. The limits of detection of DEB (3 times the background noise) were 1 nmol/l (mouse blood) and 0.3 nmol/l (rat blood).

3. Results and discussion

Fig. 2 shows (±)-DEB and *meso*-DEB in the blood of BD exposed mice (Fig. 2A and a) and rats (Fig. 2B and b). All measured data are given in Fig. 2A and B, excerpts demonstrating DEB
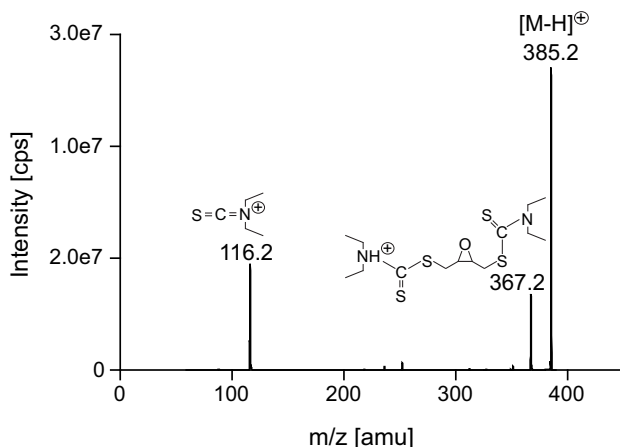
Fig. 1. Product ion spectrum of the bis(*N,N*-diethyldithiocarbamoyl) ester of DEB (*m/z* = 385.2). Presumed products are shown. The instrumental parameters are given in the text.

concentrations at low BD exposure concentrations of between 0 and 21 ppm are given in Fig. 2a and b. Large standard errors are seen in rats. The individual rat data may reflect the fact that DEB is only a minor second-order BD metabolite in the rat liver (Filser et al., 2010). In mice, the figure shows only small differences in the means of two groups of 6 animals each, both of which were exposed identically. In non-exposed control animals of both species, there was no DEB background. Also no DEB-related background was found by Georgieva et al. (2010) who investigated DEB-characteristic adducts at the N-terminal valine of hemoglobin (*N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine) in mice and rats
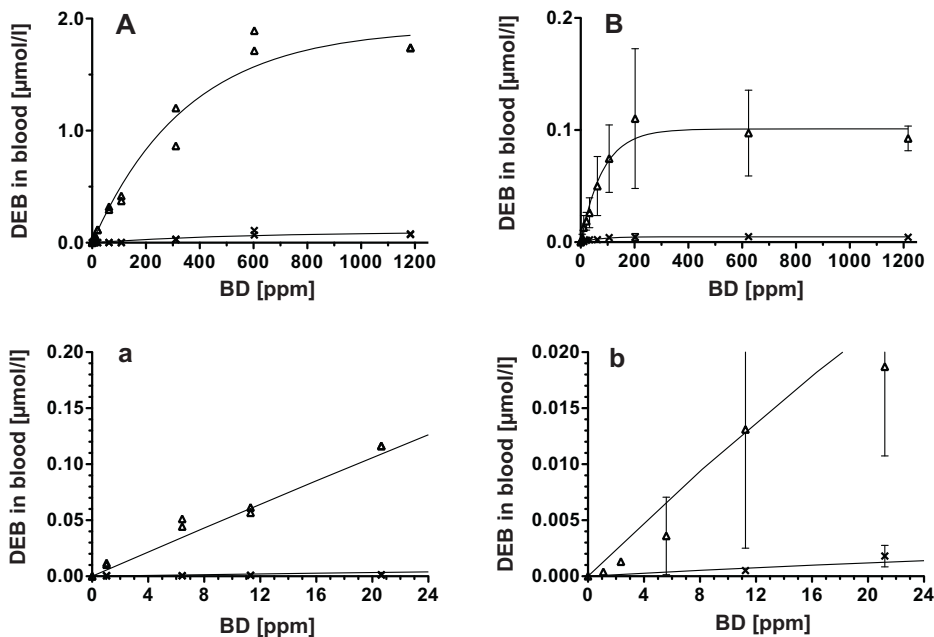
Fig. 2. DEB concentrations in blood of mice (A, a) and rats (B, b) after exposures (6 h) to constant concentrations of BD. Twelve mice, subdivided in 2 groups of 6 animals, were exposed together to each concentration. Four rats were exposed together to BD concentrations of 2.4 ppm, 21 ppm, and above and two times 4 rats were exposed together to concentrations of 1.1 ppm, 5.6 ppm, and 11 ppm. Whole exposure ranges are represented by the letters A and B, extracts by a and b. Symbols give measured data: (△) (±)-DEB; (×) *meso*-DEB; (A, a) two values per exposure concentration (each from pooled blood of 6 mice), identical values at 3 exposure concentrations; (B, b) means ± SE (deviation bars, except when smaller than the symbol size) from 4 rats or from 8 rats. Limits of detection were 0.001 µmol/l (mouse blood) and 0.0003 µmol/l (rat blood). Lines are one-phase exponential association functions ($y = y_\infty[1 - e^{-kx}]$) that were fitted to the data. The variable *x* stays for the BD exposure concentration (ppm). Maximum calculated (±)-DEB concentrations in blood (y_∞) are 1.92 µmol/l in mice (95% confidence intervals 1.62–2.22) and 0.101 µmol/l in rats (95% confidence intervals 0.091–0.11). The values of *k* for (±)-DEB were 0.00284/ppm BD in mice (95% confidence intervals 0.0017–0.0040) and 0.0122/ppm BD in rats (95% confidence intervals 0.0085–0.016). Maximum *meso*-DEB concentrations in blood (y_∞) were 0.0977 µmol/l in mice (95% confidence intervals 0.029–0.17) and 0.00478 µmol/l in rats (95% confidence intervals 0.0042–0.0054). The values of *k* for *meso*-DEB were 0.00175/ppm BD in mice (95% confidence intervals 0.000–0.0043) and 0.0146/ppm BD in rats (95% confidence intervals 0.0087–0.020). The squares of the correlation coefficients (R^2) for (±)-DEB were 0.981 in mice and 0.975 in rats. For *meso*-DEB, they were 0.881 in mice and 0.949 in rats.

repeatedly exposed over 2 weeks to BD concentrations of between 0 and 625 ppm. In mice, measured (±)-DEB blood concentrations seem to reach a plateau concentration of about 1.74 μmol/l at 600 ppm BD. In rat blood, mean concentrations of (±)-DEB amount to not more than 0.1 μmol/l. Of this concentration, 70% is reached at 100 ppm BD.

The curves, also shown in the figure, were fitted to the data by means of Prism 5 for Mac OS X (GraphPad Software, La Jolla, California) using one-phase exponential association functions. These functions were preferred to Michaelis–Menten functions because they provided higher correlation coefficients. The (±)-DEB blood concentrations in mice, calculated by means of the one-phase exponential association function, increased from 5.4 nmol/l at 1 ppm BD to 1860 nmol/l at 1250 ppm BD. In rats, they increased from 1.2 nmol/l at 1 ppm BD to 92 nmol/l at 200 ppm BD. At this exposure concentration, 91% of the calculated DEB plateau concentration in rat blood was reached.

In both species, the blood concentrations of the (±) form are much higher than those of the *meso* form. The ratio of (±)- to *meso*-DEB is similar in mice and rats and does not change very much in the whole exposure range. It is between 21 and 32 in mouse blood and between 17 and 21 in rat blood. Goggin et al. (2009) determined DNA–DNA cross-link adducts (1,4-bis-(guan-7-yl)-2,3-butanediol) of racemic and *meso*-DEB in livers of female mice and rats exposed repeatedly (6 h/d, 5 d/w, 2 w) to BD concentrations of up to 625 ppm. In both species, the cross-link adducts from *meso*-DEB were much less than those from racemic DEB, which is in agreement with the present data on blood concentrations of (±)- and *meso*-DEB.

The DEB plateaus (Fig. 2) do not result from saturation of CYP2E1 mediated oxidation of its metabolic precursor EB, considering that the Michaelis constant of this metabolic step is around 140 μmol/l in liver microsomes of both rodent species (Seaton et al., 1995). Most probably, the experimentally demonstrated concurrent metabolic interactions of BD and its metabolites 1,2-epoxy-3-butene and 3-butene-1,2-diol at the metabolizing cytochrome P450 species (Filser et al., 2010) are the main cause for these plateaus occurring in both animal species at very low DEB concentrations of less than 2 μmol/l. For a more detailed discussion see Filser et al. (2007).

The present DEB mouse data can be compared to DEB blood concentrations that had been published for the same strain. After single exposures (between 4 and 6 h) of male mice to BD concentrations of between 62.5 and 1270 ppm (Bechtold et al., 1995; Filser et al., 2007; Himmelstein et al., 1994; Thornton-Manning et al., 1995a), DEB concentrations were reported that are between 77% and 209%, on average 121%, of the values calculated for identical BD exposure concentrations by means of the exponential function fitted to the data given in Fig. 2A and a. So far, only one group reported measured DEB concentrations in BD exposed rats. After a vacuum line-cryogenic distillation of the blood of male Sprague-Dawley rats exposed to a BD concentration of 62.5 ppm, DEB concentrations of 5 nmol/l (Thornton-Manning et al., 1995a) and of 2.4 nmol/l (Thornton-Manning et al., 1995b) had been determined by gas chromatography using a mass selective detector operating in selected ion monitoring mode. By means of the same method, DEB blood concentrations of up to 17 nmol/l had been found in female Sprague-Dawley rats exposed either once (6 h) or repeatedly (6 h/d, 10 d) to 62.5 ppm or 8000 ppm BD (Thornton-Manning et al., 1995a,b, 1997, 1998). These DEB concentrations are drastically lower than those of about 50 nmol/l at 62.5 ppm and 100 nmol/l at ≥200 ppm detected in the present work by means of the distinctly more selective LC/MS/MS method compared to that of Thornton-Manning and co-workers.

Although the present data were obtained after single 6-h BD exposures of male animals and those of Goggin et al. (2009) and Georgieva et al. (2010) after repeated (6 h/d, 5 d/w, 2 w) BD exposures of female animals, it may be meaningful to compare the ratios mouse-to-rat, calculated from the present (±)-DEB blood concentrations to the calculated ratios mouse-to-rat of racemic 1,4-bis-(guan-7-yl)-2,3-butanediol in livers (Goggin et al., 2009) and of N,N-(2,3-dihydroxy-1,4-butadiyl)-valine in blood (Georgieva et al., 2010). The inter-gender comparison is justified because the amounts of cross-link adducts were 2–2.5-fold higher in females of both species compared to males when subjected to the same exposure conditions (Goggin et al., 2009). The ratio of (±)-DEB in mouse blood compared to rat blood increases from 4.5 at near to 0 ppm BD up to 16 at 625 ppm BD (calculated using the one-phase exponential association functions). The ratio of 1,4-bis-(guan-7-yl)-2,3-butanediol increases from 4.2 at 62.5 ppm BD up to 11 at 625 ppm BD. In the exposure range between 0.5 and 625 ppm BD, ratios of between 6 and 15 can be calculated for the DEB exposure marker N,N-(2,3-dihydroxy-1,4-butadiyl)-valine. All three studies show that the DEB burden is substantially higher in mice than in rats and that the difference increases at BD concentrations above 200 ppm. Not expected from the present DEB data are the drastically larger mouse-to-rat ratios in the N,N-(2,3-dihydroxy-1,4-butadiyl)-valine levels which were reported for longer BD exposures (6 h/d, 5 d/w, 4 w) (Georgieva et al., 2010; Swenberg et al., 2007). It has been speculated that the exposure of the erythrocytes to DEB decreased the lifespan of the rat erythrocytes and diluted the adduct levels in rat erythrocytes by increased hematopoiesis (Georgieva et al., 2010).

The present data help to explain the findings on the species-specific carcinogenic potency of BD in mice and rats. In blood of male rats, mean concentrations of DEB do not surpass 0.1 μmol/l, a concentration reached at an exposure concentration of 19 ppm in blood of male mice. In male mice, the lowest statistically significant carcinogenic BD exposure concentration was 62.5 ppm in a two-year inhalation study (Melnick et al., 1990), which corresponds to a DEB concentration of 0.3 μmol/l in blood. Considering that male rats never reach this blood concentration, it seems probable that BD induced gland tumors in rats exposed to 1000 and 8000 ppm BD (Owen et al., 1987) resulted not so much from the DEB burden but primarily from the burdens of both 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol as has already been suggested earlier (Filser et al., 2007; Fred et al., 2008). In the blood of rats, concentrations of 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol of about 1 μmol/l and 2 μmol/l, respectively, are found at BD concentrations of 1000 ppm (Filser et al., 2007).

As a starting point for the estimation of the risk of BD to humans who may be exposed to low BD concentrations, knowledge of the internal burden by the epoxy-metabolites of BD is required. In addition to the earlier sensitive methods for the determination of 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol in blood (Filser et al., 2007, 2010), we have now a very sensitive and highly specific method for the analysis of DEB in our hands. Considering the larger blood volumes that can be collected from humans compared to individual rodents, the novel method demonstrated here may be sensitive enough to quantify DEB in blood of BD exposed humans even if this substance is present at up to 9-fold lower concentrations than in rats as had been estimated (Kirman et al., 2010). Data on blood concentrations of the three epoxides in BD-exposed humans will also support the use of a chemical specific toxicokinetic adjustment factor over the default factor in the dose–response assessments reducing the uncertainty in BD risk assessments.

Conflict of interest statement

No conflict of interest.

Acknowledgements

References

Albertini, R.J., Carson, M.L., Kirman, C.R., Gargas, M.L., 2010. 1,3-Butadiene: II. Genotoxicity profile. Crit. Rev. Toxicol. 40, 12–73.

Bechtold, W.E., Strunk, M.R., Thornton-Manning, J.R., Henderson, R.F., 1995. Analysis of butadiene, butadiene monoxide, and butadiene dioxide in blood by gas chromatography/gas chromatography/mass spectroscopy. Chem. Res. Toxicol. 8, 182–187.

Bolt, H.M., Schmiedel, G., Filser, J.G., Rolzhäuser, H.P., Lieser, K., Wistuba, D., Schurig, V., 1983. Biological activation of 1,3-butadiene to vinyl oxirane by rat liver microsomes and expiration of the reactive metabolite by exposed rats. J. Cancer Res. Clin. Oncol. 106, 112–116.

Cochrane, J.E., Skopek, T.R., 1994. Mutagenicity of butadiene and its epoxide metabolites: I. Mutagenic potential of 1,2-epoxybutene, 1,2,3,4-diepoxybutane and 3,4-epoxy-1,2-butanediol in cultured human lymphoblasts. Carcinogenesis 15, 713–717.

Dupard-Julien, C.L., Kandlakunta, B., Uppu, R.M., 2007. Determination of epoxides by high-performance liquid chromatography following derivatization with N,N-diethyldithiocarbamate. Anal. Bioanal. Chem. 387, 1027–1032.

Filser, J.G., Bolt, H.M., 1984. Inhalation pharmacokinetics based on gas uptake studies. VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. Arch. Toxicol. 55, 219–223.

Filser, J.G., Hutzler, C., Meischner, V., Veereshwarayya, V., Csanády, G.A., 2007. Metabolism of 1,3-butadiene to toxicologically relevant metabolites in single-exposed mice and rats. Chem. Biol. Interact. 166, 93–103.

Filser, J.G., Bhowmik, S., Faller, T.H., Hutzler, C., Kessler, W., Midpanon, S., Pütz, C., Schuster, A., Semder, B., Veereshwarayya, V., Csanády, G.A., 2010. Quantitative investigation on the metabolism of 1,3-butadiene and of its oxidized metabolites in once-through perfused livers of mice and rats. Toxicol. Sci. 114, 25–37.

Fred, C., Törnqvist, M., Granath, F., 2008. Evaluation of cancer tests of 1,3-butadiene using internal dose, genotoxic potency, and a multiplicative risk model. Cancer Res. 68, 8014–8021.

Georgieva, N.I., Boysen, G., Bordeerat, N., Walker, V.E., Swenberg, J.A., 2010. Exposure-response of 1,2:3,4-diepoxybutane-specific N-terminal valine adducts in mice and rats after inhalation exposure to 1,3-butadiene. Toxicol. Sci. 115, 322–329.

Goggin, M., Swenberg, J.A., Walker, V.E., Tretyakova, N., 2009. Molecular dosimetry of 1,2,3,4-diepoxybutane-induced DNA–DNA cross-links in B6C3F1 mice and F344 rats exposed to 1,3-butadiene by inhalation. Cancer Res. 69, 2479–2486.

Himmelstein, M.W., Acquavella, J.F., Recio, L., Medinsky, M.A., Bond, J.A., 1997. Toxicology and epidemiology of 1,3-butadiene. Crit. Rev. Toxicol. 27, 1–108.

Himmelstein, M.W., Turner, M.J., Asgharian, B., Bond, J.A., 1994. Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3-butadiene by inhalation. Carcinogenesis 15, 1479–1486.

IARC, 2008. 1,3-Butadiene. In: 1,3-Butadiene, Ethylene Oxide and Vinyl Halides (Vinyl Fluoride, Vinyl Chloride and Vinyl Bromide). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 97. WHO, IARC Press, Lyon, France, pp. 45–184.

IHS, 2011. http://www.sriconsulting.com/WP/Public/Reports/butadiene/ (downloaded 25.06.11).

Kirman, C.R., Albertini, R.J., Sweeney, L.M., Gargas, M.L., 2010. 1,3-Butadiene: I. Review of metabolism and the implications to human health risk assessment. Crit. Rev. Toxicol. 40, 1–11.

Lee, M.S., Faller, T.H., Kreuzer, P.E., Kessler, W., Csanády, G.A., Pütz, C., Ríos-Blanco, M.N., Pottenger, L.H., Segerbäck, D., Osterman-Golkar, S., Swenberg, J.A., Filser, J.G., 2005. Propylene oxide in blood and soluble nonprotein thiols in nasal mucosa and other tissues of male Fischer 344/N rats exposed to propylene oxide vapors – relevance of glutathione depletion for propylene oxide-induced rat nasal tumors. Toxicol. Sci. 83, 177–189.

Melnick, R.L., Huff, J., Chou, B.J., Miller, R.A., 1990. Carcinogenicity of 1,3-butadiene in C57BL/6 × C3HF$_1$ mice at low exposure concentrations. Cancer Res. 50, 6592–6599.

Michaelson-Richie, E.D., Loeber, R.L., Codreanu, S.G., Ming, X., Liebler, D.C., Campbell, C., Tretyakova, N.Y., 2010. DNA–protein cross-linking by 1,2,3,4-diepoxybutane. J. Proteome Res. 9, 4356–4367.

Munger, D., Sternson, L.A., Repta, A.J., Higuchi, T., 1977. High-performance liquid chromatographic analysis of dianhydrogalactitol in plasma by derivatization with sodium diethyldithiocarbamate. J. Chromatogr. 143, 375–382.

NRC, 1996. Guide for the Care and Use of Laboratory Animals, 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, DC.

Owen, P.E., Glaister, J.R., Gaunt, I.F., Pullinger, D.H., 1987. Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. Am. Ind. Hyg. Assoc. J. 48, 407–413.

Seaton, M.J., Follansbee, M.H., Bond, J.A., 1995. Oxidation of 1,2-epoxy-3-butene to 1,2:3,4-diepoxybutane by cDNA-expressed human cytochromes P450 2E1 and 3A4 and human, mouse and rat liver microsomes. Carcinogenesis 16, 2287–2293.

Swenberg, J.A., Boysen, G., Georgieva, N., Bird, M.G., Lewis, R.J., 2007. Future directions in butadiene risk assessment and the role of cross-species internal dosimetry. Chem. Biol. Interact. 166, 78–83.

Thornton-Manning, J.R., Dahl, A.R., Bechtold, W.E., Griffith Jr., W.C., Henderson, R.F., 1997. Comparison of the disposition of butadiene epoxides in Sprague-Dawley rats and B6C3F1 mice following a single and repeated exposures to 1,3-butadiene via inhalation. Toxicology 123, 125–134.

Thornton-Manning, J.R., Dahl, A.R., Allen, M.L., Bechtold, W.E., Griffith Jr., W.C., Henderson, R.F., 1998. Disposition of butadiene epoxides in Sprague-Dawley rats following exposures to 8000 ppm 1,3-butadiene: comparisons with tissue epoxide concentrations following low-level exposures. Toxicol. Sci. 41, 167–173.

Thornton-Manning, J.R., Dahl, A.R., Bechtold, W.E., Griffith Jr., W.C., Henderson, R.F., 1995a. Disposition of butadiene monoepoxide and butadiene diepoxide in various tissues of rats and mice following a low-level inhalation exposure to 1,3-butadiene. Carcinogenesis 16, 1723–1731.

Thornton-Manning, J.R., Dahl, A.R., Bechtold, W.E., Griffith Jr., W.C., Pei, L., Henderson, R.F., 1995b. Gender differences in the metabolism of 1,3-butadiene in Sprague-Dawley rats following a low level inhalation exposure. Carcinogenesis 16, 2875–2878.

U.S. Environmental Protection Agency (EPA), 2002. Health Assessment of 1,3-Butadiene. National Center for Environmental Assessment, Washington, DC, EPA/600/P-98/001F. Available from: National Technical Information Service, Springfield, VA, http://www.epa.gov/ncea/.

Van Duuren, B.L., Nelson, N., Orris, L., Palmes, E.D., Schmitt, F.L., 1963. Carcinogenicity of epoxides, lactones, and peroxy compounds. J. Natl. Cancer Inst. 31, 41–55.

Van Duuren, B.L., Orris, L., Nelson, N., 1965. Carcinogenicity of epoxides, lactones, and peroxy compounds. II. J. Natl. Cancer Inst. 35, 707–717.