**1,2:3,4-Diepoxybutane in blood of male B6C3-F1 mice and male Sprague-Dawley rats exposed to 1,3-butadiene**

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**Short Title:** Diepoxybutane in butadiene exposed rodents

**Abstract**

The important industrial chemical 1,3-butadiene (BD) is known as a potent carcinogen in B6C3F1 mice and a weak one in Sprague-Dawley rats. This difference is mainly related 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 blood of groups of two times 6 mice and 4 rats, each, after 6-h exposures to a series of constant concentrations of BD of between about 1 and 1200 ppm. Immediately after the end of exposure, blood samples containing perdeuterated DEB (DEB-d6) as internal standard and diethyl maleate were prepared. The hereof-obtained plasma samples were treated with sodium sodium diethyldithiocarbamate as derivatization reagent for metabolically produced DEB and DEB-d6. The resulting bis(dithiocarbamoyl) esters were 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 well be described by one-phase exponential association functions. Measured mean DEB concentrations in blood increased in mice from 11 nmol/l at 1 ppm BD to 1740 nmol/l at 1180 ppm BD and in rats from 0.4 nmol/l at 1 ppm BD to 100 nmol/l at 200 ppm BD and remaind constant up to 1220 ppm BD. Considering BD induced tumorigenicity to result mainly from the species-specific DEB burden, the present data explain why the tumorigenic potency of BD in rats is low if compared to mice.

**Keywords**

1,3-Butadiene, 1,2:3,4-Diepoxybutane analysis, Mouse, Rat, Diethyldithiocarbamate, LC/MS/MS

## 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 is 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 in 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, 2y), inhaled BD was weakly tumorigenic in Sprague-Dawley rats exposed to 0, 1000 or 8000 ppm (Owen et al., 1987) but was highly effective in B6C3F1 mice exposed to BD concentrations between 0 and 625 ppm. In female mice, lung tumors increased at a concentration as low as 6.25 ppm. In male mice, the lowest effective BD concentration was 62.5 ppm showing increased tumor incidences in several organs. 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 tumorigenic 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 mutagenic epoxide intermediates 1,2-epoxy-3-butene, 1,2:3,4-diepoxybutane (DEB), and 3,4-epoxy-1,2-butanediol (reviewed, for example, in Filser et al., 2007; Himmelstein et al., 1997; Kirman et al., 2010). These epoxides are genotoxic as was demonstrated in a large amount of studies carried out in vitro as well as in vivo. The genotoxic potency of DEB that contains two electrophilic sites and forms DNA-DNA and DNA-protein cross-link adducts is by far higher than those of 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol (reviewed in Albertini et al., 2010). In blood of BD exposed mice and

rats, all three epoxides have been found. In both species, 62.5 ppm was the lowest BD concentration at which DEB was determined (reviewed in Filser et al., 2007). Because humans are generally exposed to much lower BD concentrations (1 ppm BD is the current permissible exposure limit of the U.S. Occupational Safety and Health Administration; NTP, 2011) measured DEB concentrations are required for lower BD concentrations. Their knowledge will be highly relevant as a solid base for the development of a valid physiological toxicokinetic model that can be applied for risk assessment purposes. Therefore, the aim of the present work was to quantify DEB in blood of mice and rats exposed over 6 h to various constant BD concentrations of between 1 ppm and – for comparison with published data – 1200 ppm.

## Material and Methods

### 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, Taukirchen, 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 (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 (>99.0; DTC) was purchased from Fluka Chemie, Buchs, Switzerland. 1,2:3,4-Diepoxy-[1,1,2,3,4,4-2H6]butane (DEB-D6), 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.

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% was maintained in the animal room.

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 of 1.03, 6.43, 11.3, 20.7, 63.2, 108, 311, 603, or 1184 ppm (mice) and of 1.15, 2.35, 5.31, 11.5, 21.2, 32.9, 62.4, 106, 203, 624, or 1217 ppm (rats). Atmospheric BD concentrations were determined in time periods of between 5 and 35 min (depending on the BD concentration) and were maintained quasi-constant in a range of not more than ± 10% (mice) or ± 20% (rats)

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 times 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. All animals were exposed over exactly 6 h. Mice were sacrificed by cervical dislocation. Using a disposable, Liquemin-moistened syringe, up to 0.5 ml 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 DEM solution (515 µl DEM in 2760 µl $C_2H_5OH$) and 10 µl (1 and 6 ppm BD) or 30 µl (11 – 1184 ppm BD) of the internal standard DEB-D6 (14.46 µmol/l in acetone). The vial was shaken after each blood injection. The whole procedure of pooling the blood of the six mice per group lasted not more than 6 min. Rats were treated according to Lee et al. (2005). Twenty min before sacrificing a rat, it was removed from the sphere and immediately anesthetized by injecting intraperitoneally a mixture consisting of 0.88 ml ketamin/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 immediately sacrificed. 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 Liquemin-moistened syringe, and injected in an ice-cooled 5-ml-cryotube vial that contained 40 µl of the DEM solution and 10 µl (1 and 6 ppm BD) or 30 µl (11 – 1184 ppm BD) of DEB-D6

(14.46 µmol/l in acetone). The vial was closed and vigorously shaken.

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

Analytical methods

*1,3-Butadiene*

During the exposures, BD was directly determined 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 x 1/8" x 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 C-R5A 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 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 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%.

*1,2:3,4-Diepoxybutane*

*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 vigorously vortexing, 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 a sodium chloride solution (10 g/100 ml) was added and the mixture was thoroughly vortexed 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 sec 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 to 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.

*LC/MS/MS analysis.* From an autosampler vial containing the DEB- and DEB-D6-bis(dithiocarbamoyl) esters 5 µl was subjected to LC/MS/MS analysis. The LC/MS/MS system consisted of an HP1100 liquid chromatograph (from Agilent, Waldbronn, Germany) and an API 4000 triple quadrupole mass spectrometer with turbo ion spray interface (from Applied Biosystems, Darmstadt, Germany). The liquid chromatograph was equipped with a Luna C18 (2) column (150 mm x 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-D6) and 8.0 min (meso-DEB and meso-DEB-D6) 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 ratio of the solvents was A = 40% and B = 60% for the first 5 min. Up to 8 min, the percentage of A decreased linearly to 0% and that of B to 100%. From 8 to 23 min, A remained 0% and B 100%. 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-D6-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 zero to 0.08 µmol/l blood or from zero to 2.0 µmol/l blood (mice) and from zero to 0.0035 µmol/l blood or from zero 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 "Exposure experiments" 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 detection limit of DEB (3 times the background noise) was 1 nmol/l and 0.3 nmol/l (rat blood).

**Results and Discussion**

**Figure 2** shows (±)-DEB and meso-DEB in blood of BD exposed mice (**Figures 2A and 2a**) and rats (**Figures 2B and 2b**). All measured data of up to about 1200 ppm BD are given in **Figures 2A** and **2B**, excerpts that demonstrate DEB concentrations at low BD exposure concentrations of between 0 and 21 ppm are given in **Figures 2a** and **2b**. 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 a one-phase exponential association function. 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

identically exposed. In non-exposed control animals of both species, there was not any hint of a 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 exposed repeatedly over 2 weeks to BD concentrations of between 0 and 625 ppm.

In mice, calculated mean (±)-DEB blood concentrations reach 82% of the plateau concentration (1.9 µmol/l) at 600 ppm BD (**Figure 2**). In rat blood, mean concentrations of (±)-DEB amount to not more than 0.1 µmol/l. Of this concentration, 70% are reached at 100 ppm BD. 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 BD. In both species, the cross-link adducts from *meso*-DEB were much less than those from racemic DEB being in support of the present data on blood concentrations of (±)- and *meso*-DEB.

The DEB plateaus (**Figure 2**) do certainly 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 EB, DEB, EBD and B-diol at the metabolizing enzymes (Filser et al., 2010) are the main cause for these

plateaus occurring in both 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 opposed to DEB blood concentrations that were reported by three working groups for BD exposure 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, 1997). The earlier data represent of between 63% and 200%, on average 117%, of the values that were calculated for identical BD exposure concentrations by means of the exponential function fitted to the data given in **Figures 2A and 2a**. So far, only one group reported 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 either once (6 h) or repeatedly (6 h/d, 10 d) exposed to 62.5 ppm and 8000 ppm BD (Thornton-Manning et al., 1995a, 1995b, 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 and sensitive 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, 2w) 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- to 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 blood increases from 4.5 at near to zero 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. Somewhat larger differences can be calculated for the DEB exposure marker *N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine. The ratio of this DEB-hemoglobin adduct decrease from 15 to 6.2 in the range from 0.5 to 200 ppm BD and is 13 at 625 ppm BD. All three studies show that the DEB burden is substantially higher in mice than in rats and the difference increases at BD concentrations above 200 ppm. Not expectable 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 tumorigenic 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 tumorigenic 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) did not only result from DEB. It has been assumed that both 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol contribute particularly to the tumorigenicity of BD in this species (Filser et al., 2007; Fred et al., 2008). In 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).

**Outlook**

As a starting point for the estimation of the risk of BD to humans, who are 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 in our hands a very sensitive and highly specific method for the analysis of DEB. Considering the larger blood volumes that can be collected from humans if compared to individual rodents, the method presented here may be sensitive enough to quantify DEB in blood of BD exposed humans even if it is up to 9-fold lower than in rats as has been estimated (Kirman et al., 2010). Blood concentrations of the three epoxides, measured also in BD-exposed humans, will be highly relevant to reduce the toxicokinetic chemical-specific adjustment factors in the dose-response assessment to a minimum.

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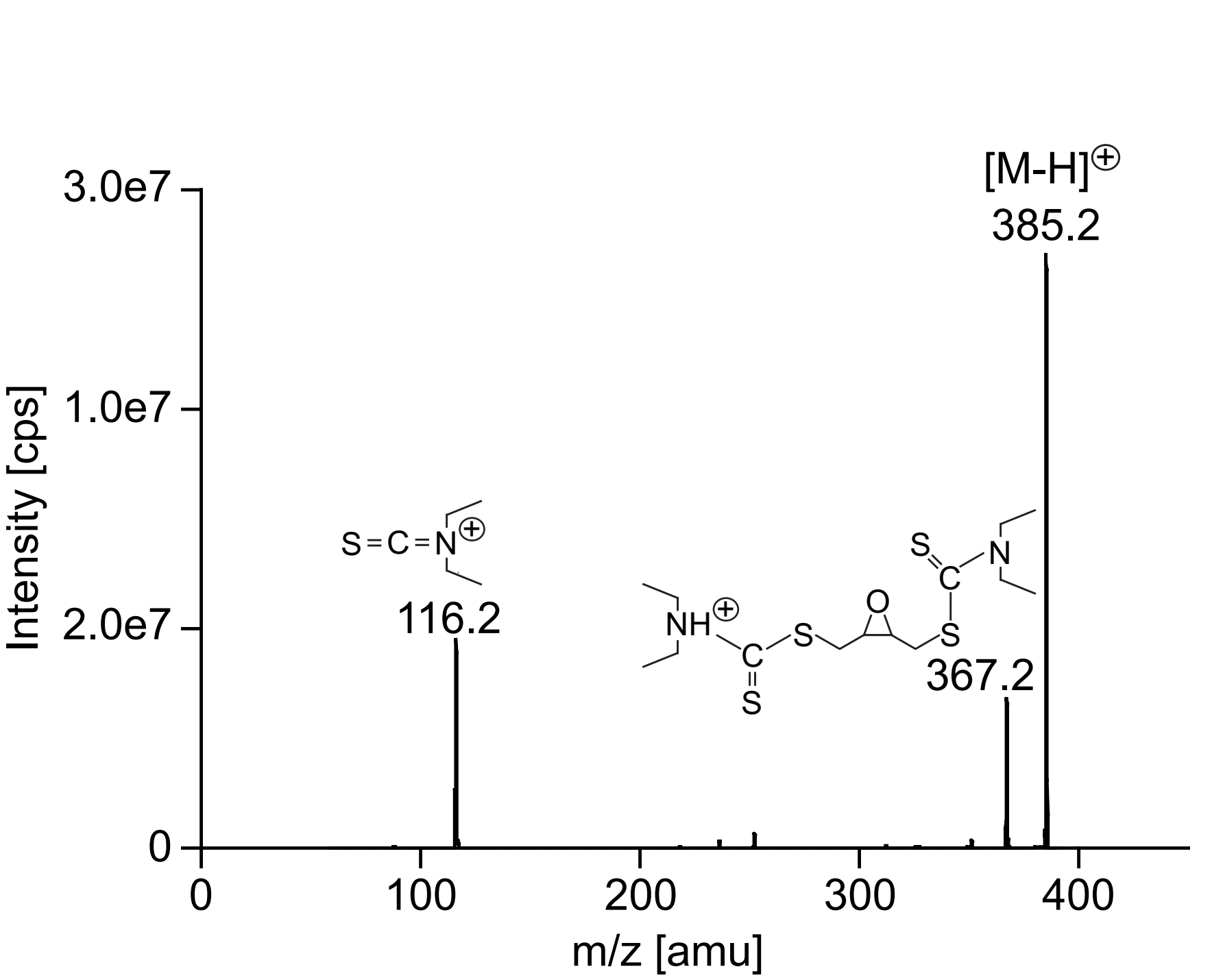
**Legends to the Figures**

**Figure 1**

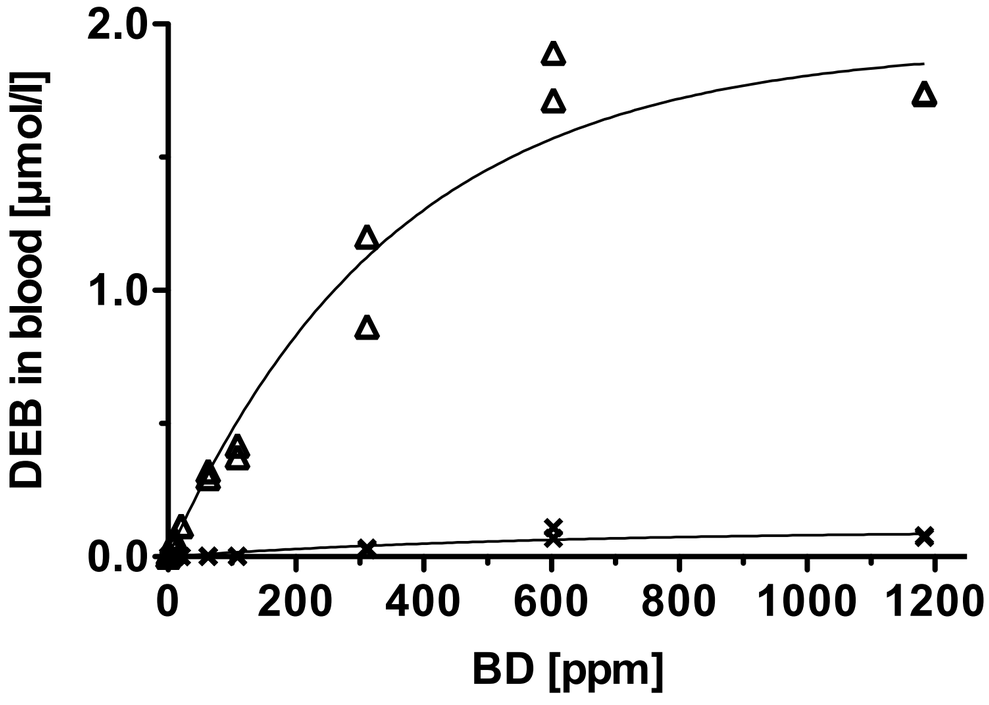
Product ion spectrum of the bis(*N,N*-diethyldithiocarbamoyl)ester of DEB (m/z = 385.2). Presumed products are shown. The instrumental parameters are given in the text.

**Figure 2**

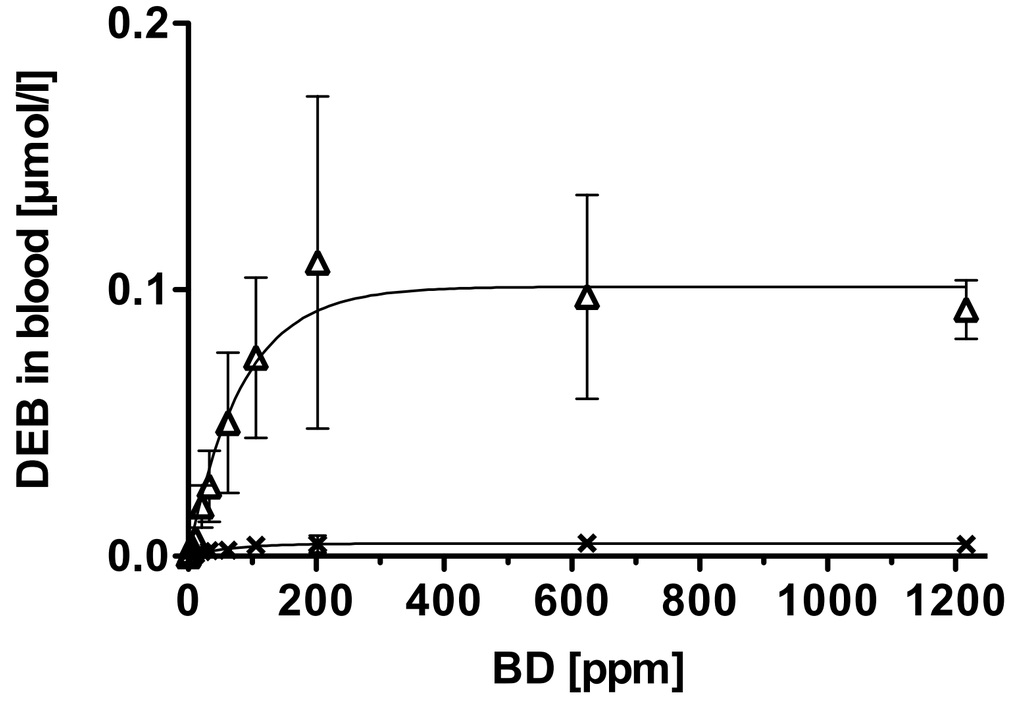
DEB concentrations in blood of mice (A, a) and rats (B, b) after exposures (6 h) to constant concentrations of BD. Whole exposure ranges are represented by the letters A and B, extracts by a and b. Symbols give measured data, $\Delta$ stays for (±)-DEB, x for *meso*-DEB and bars for standard errors. Lines are one-phase exponential association functions ($y = y_\infty \cdot (1 - e^{-k \cdot x})$ that were fitted to the data. Maximum (±)-DEB concentrations in blood ($y_\infty$) are in mice 1.918 µmol/l (95% confidence intervals 1.62 to 2.22) and in rats 0.1012 µmol/l (95% confidence intervals 0.090 to 0.11). The values of k are for (±)-DEB in mice 0.002835 (95% confidence intervals 0.0017 to 0.0040) and in rats 0.01195 (95% confidence intervals 0.0082 to 0.016). Maximum *meso*-DEB concentrations in blood ($y_\infty$) are in mice 0.09769 µmol/l (95% confidence intervals 0.029 to 0.17) and in rats 0.004778 µmol/l (95% confidence intervals 0.0042 to 0.0054). The values of k are for *meso*-DEB in mice 0.001754 (95% confidence intervals 0.0 to 0.0043) and in rats 0.01452 (95% confidence intervals 0.0086 to 0.020). The coefficients of determination ($R^2$) were for (±)-DEB in mice 0.981 and in rats 0.973. For *meso*-DEB, they were in mice 0.881 and in rats 0.948.


[M-H]⊕
385.2
116.2
367.2
Intensity [cps]
m/z [amu]
3.0e7
1.0e7
2.0e7
0
0
100
200
300
400

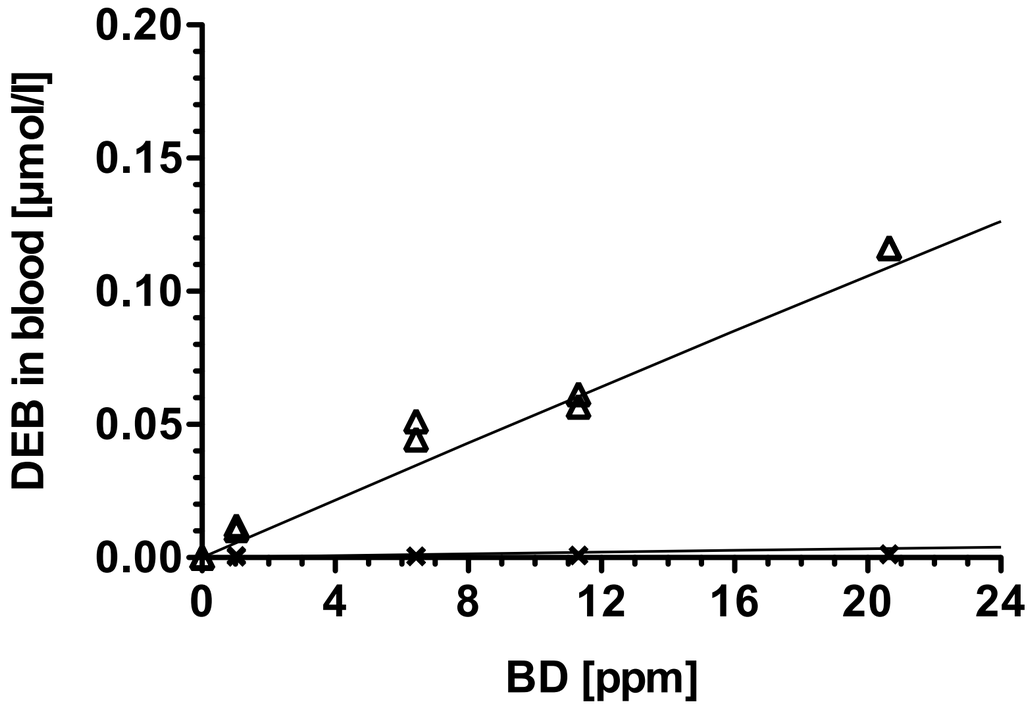
A


2.0
1.0
0.0
DEB in blood [µmol/l]
0
200
400
600
800
1000
1200
BD [ppm]

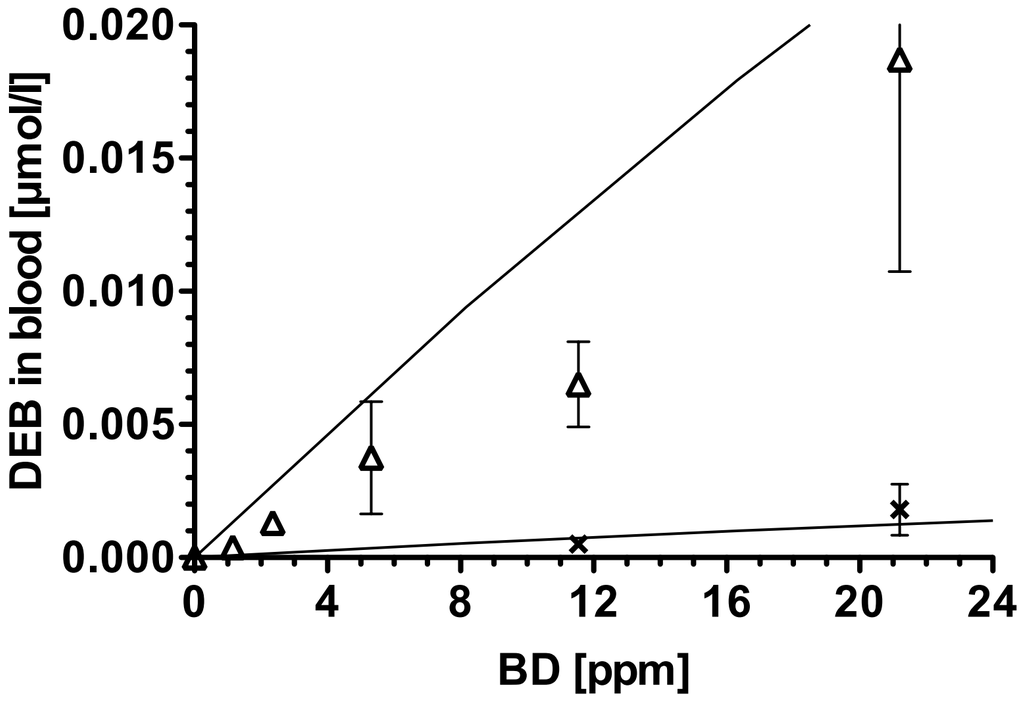
B


0.2
0.1
0.0
DEB in blood [µmol/l]
0
200
400
600
800
1000
1200
BD [ppm]

a


0.20
0.15
0.10
0.05
0.00
DEB in blood [µmol/l]
0
4
8
12
16
20
24
BD [ppm]

b


0.020
0.015
0.010
0.005
0.000
DEB in blood [µmol/l]
0
4
8
12
16
20
24
BD [ppm]