ELSEVIER

Journal of Chromatography B, 730 (1999) 257–264

JOURNAL OF CHROMATOGRAPHY B

Gas chromatographic determination of 3-butene-1,2-diol in urine samples after 1,3-butadiene exposure

Tiina Anttinen-Klemetti, Raija Vaaranrinta, Kimmo Peltonen*

Finnish Institute of Occupational Health, Chemistry Laboratory, Topeliuksenkatu 41 aA, FIN-00250 Helsinki, Finland

Received 2 June 1998; received in revised form 11 May 1999; accepted 11 May 1999

Abstract

1,3-Butadiene is an important industrial chemical and a common environmental contaminant. Because of its suspected carcinogenicity butadiene-related research has gained high activity. The obvious lack of knowledge so far has been that a biomonitoring method that can detect at least one of the metabolites of butadiene from body fluids or excretas does not exist. In this communication we describe a robust and simple analytical method which can be applied for biomonitoring purposes. We have developed a method that can detect 3-butene-1,2-diol in urine samples of rats inhalation-exposed to various concentrations of 1,3-butadiene. The method is based on liquid–liquid extraction and subsequent gas chromatographic analysis. The extraction efficiency of 3-butene-1,2-diol at a concentration of 2.2 μg/ml was 95% (SD=±3%, n=3) and was achieved by using sodium chloride saturation and isopropanol as an extracting solvent. The standard deviation of the gas chromatographic analysis was ±2% (n=12), the limit of detection was 0.08 μg/ml, the limit of quantitation was 0.11 μg/ml (SD=±4.8%, n=3) and the analysis was observed to be linear from 0.11 to 486 μg/ml (R=0.9987). Animals exposed to 1,3-butadiene showed a linear excretion of 3-butene-1,2-diol into urine as a function of butadiene exposure. During the exposure saturation of metabolism or accumulation of 1,3-butadiene or 3-butene-1,2-diol into the body was not observed in any exposure levels used. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: 3-Butene-1,2-diol; 1,3-Butadiene

1. Introduction

1,3-Butadiene (BD), classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen and by the United States Environment Protection Authority (USEPA) as a known or probable carcinogen, has been produced and used as a monomer in the production of plastic and synthetic rubber since the 1930s [1,2].

BD also occurs as an environmental contaminant. It has been estimated that most BD emissions derive from mobile sources, although leaks and waste emissions from manufacturing facilities may be locally important. Burning of organic materials produces emissions containing minor amounts of BD. Low exposure to BD is thus a common characteristic of the whole human population [3–8].

BD is epoxidized by the cytochrome P-450 dependent monooxygenase CYP 2E1 to the enantiomeric monooxiranes butadiene monoepoxides (BMO). BMO can then be further epoxidized to the corresponding diastereomeric diepoxides (DEB). Alternatively, hydrolysis to a diol form can occur, fol-

*Corresponding author. Tel.: +358-90-47-471; fax: +358-90-414-634.

0378-4347/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(99)00227-3

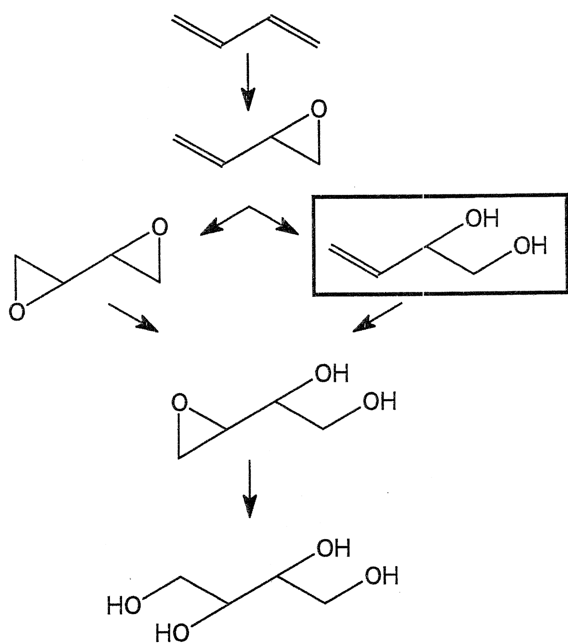

Fig. 1. The metabolism of 1,3-butadiene. The compound analyzed in this study (3-butene-1,2-diol) is framed with a rectangular box.

lowed by further oxidation to butadiene diolepoxide (BDE) Fig. 1. These electrophilic metabolites are mutagenic in different in vivo and in vitro systems and can form DNA binding products; they are, therefore, thought to be responsible for at least a proportion of the genotoxic activity seen after exposure to BD [9–16].

In estimating the risk to humans from butadiene, the obvious lack of knowledge is that no robust method exists that allows biomonitoring. However, the determination of some small aliphatic diols from body fluids has been a target of several studies. Methods have been developed for example for 2,3-butanediol, a compound involved in the metabolic pathway of several industrial solvents. The methods used in these reports include packed, wide-bore or capillary column gas chromatography, gas chromatography–mass spectrometry and high performance liquid chromatography [17–22]. In many occasions the method is based on the derivatization by using boronate or acetoin derivatives [23–26].

Our report describes a simple, selective and sensitive method designed to determine 3-butene-1,2-diol from urine samples – as a marker of recent exposure to 1,3-butadiene. The method includes liquid–liquid extraction, the use of an external standard and an analysis carried out by using a capillary gas chromatograph equipped with a flame ionization detector.

2. Experimental

2-Butene-1,4-diol, sulphuric acid, mercuric sulphate and isopropanol were purchased from Aldrich (Milwaukee, WI, USA) and 1,2-butenediol was synthesized according to Rao et al. [26] The synthesis can be briefly described as: a mixture of 2-butene-1,4-diol (60 g), water (25 ml), concentrated sulphuric acid (0.35 ml) and mercuric sulphate (0.25 g) was heated under reflux. After 2 h the reaction mixture was cooled to 0°C and neutralized with 10% sodium hydroxide to pH 7.0. The content of the flask was distilled by using a Vigreux fractionating column. Three fractions were collected, the first contained mainly water, the second contained 3-butene-1,2-diol and the third mainly contained the unreacted 2-butene-1,4-diol.

The product was characterized by NMR and GC–MS. The NMR spectrum was obtained in 99.9% $CDCl_3$ at 200 mHz, using a Varian Gemini 200 spectrometer. The ^1H NMR chemical shifts (δ) are reported in ppm down field from TMS. ^1H NMR ($CDCl_3$): δ 3.5(s, 2H, OH exchanges with D_2O), δ 3.5–4.5 (m, 3H, H-1, 1′, H-2), δ 5.1–6.0 (m, 3H, CH_2=CH–). 3-Butene-1,2-diol was also analyzed with a mass spectrometer. EI spectra were recorded on a Hewlett-Packard mass spectrometer (Hewlett-Packard 5989 A mass spectrometer, Hewlett-Packard 5890 Ser II gas chromatograph with an 7673 auto-injector). EI spectra were recorded at 70 eV from 30 to 250 *m/z*, and the temperature of ionization chamber was set to 200°C.

A Hewlett-Packard (Palo Alto, CA, USA) model 5890-A gas chromatograph equipped with a flame ionization detector, a split-splitless injector and an autosampler (Hewlett Packard Model 7673 A) was used for monitoring purposes. The column was BP-20 fused-silica, 0.5 μm film thickness (20 m×0.32 mm I.D., SGE, Victoria, Australia). Helium was used as both the carrier gas at a flow-rate of 1.6 ml/min and the detector make-up gas at 45 ml/min. Hydro-

gen and air for the detector were used at flow-rates of 40 and 300 ml/min, respectively. The injector and detector temperatures were 225°C and 280°C, respectively. Sample introduction was via splitless mode using an injection volume of 1 μl and a helium purge at 20 ml/min with off times of 0.5 min. A continuous helium purge of the inlet septum was maintained at 5 ml/min. The column temperature was programmed as follows: initial temperature was 60°C for 1 min, followed by a 5°C/min increase to 170°C, which was held for 1 min, then an increase at 15°C/min to 225°C, which was held for 5 min. Data collection and management were carried out by using the Sunicom software package (Sunicom OY, Helsinki, Finland).

The identification of 3-butene-1,2-diol in urine was confirmed by mass spectral analysis. EI spectra were recorded on a Hewlett-Packard mass spectrometer (Hewlett-Packard 5989 A mass spectrometer, Hewlett-Packard 5890 Ser II gas chromatograph with an 7673 autoinjector). EI spectra were recorded at 70 eV from 15 to 250 m/z, with the temperature of ionization chamber was set to 200°C.

The calibration graph of 3-butene-1,2-diol was prepared in rat urine over the concentration range of 1.5–25 μg/ml. The calibration graph was obtained by using at least six different concentration of 3-butene-1,2-diol and each point in the graph was subjected to three independent analysis. To study the accuracy of the injection and the recovery of 3-butene-1,2-diol from urine samples, another calibration graph was prepared in isopropanol at a concentration range of 1.5 to 25 μg/ml. The limit of detection was measured at a level that resulted in a peak with a height three times as high as the baseline noise. Limit of quantitation was measured at a signal-to-noise ratio of 5. Day-to-day variation in sample preparation and analysis was determined by spiking rat urine and carrying out the sample preparation and analysis on 1, 3, 6, 8, 10 and 14 days after the samples were spiked. Three spiked samples at a level of 4.5 μg/ml of 3-butene-1,2-diol were analyzed in each day.

Male Wistar rats were acclimatized for five days before the start of exposure. Food and water were given ad libitum except during exposure. Animals were exposed to BD at concentrations of 250, 500 and 1000 ppm for 6 h per day for 5 days. Dynamic exposure (ventilation exchange rate was 10 times per h) of animals was performed in a 1 m^3 chamber with glass doors. All urine excreted during a 6 h exposure period and the following 18 h of recovery time was collected. The urine of five animals were pooled and samples were stored at −20°C until subjected to analysis.

Possible conjugation products of 3-butene-1,2-diol in urine samples of exposed animals were liberated by using acidic thermal hydrolysis (1 ml of urine was acidified to pH 3 by using 1 *M* HCl and heated 100°C for 1 h).

Urine samples were centrifuged at 600 *g* for 5 min and 1 ml of supernatant was transfered to a glass vial. An aliquot of sodium chloride (1.5 g) was added followed by an extraction with isopropanol. The sample was extracted two times with 1 ml of isopropanol followed by 0.5 ml extraction. Extracts were pooled and analyzed as described.

3. Results and discussion

3.1. Product identification

The synthesis of the diol was mainly performed as described in the literature and the identification of the synthesis product is based on the ^{1}H NMR spectrum and electron impact mass spectrum [27]. NMR data clearly demonstrates that a terminal olefinic functionality and exchangable protons of the hydroxy groups are present. The mass spectral data confirmed that cyclization or polymerization had not occured. Additionally, the mass spectrum was identical to the one found in mass spectral library sources [28].

The identification of 3-butene-1,2-diol from urine samples of BD exposed rats was based on the mass spectrum. The spectrum of the product detected in urine and the corresponding standard is shown in Fig. 2. The fragmentation and the relative abundance of ions are practically identical. The molecular ion is not present in the spectrum, but a fragment of M−31 can be considered as a typical loss of a primary aliphatic alcohol. Because in this instance acid hydrolysis was not performed on the urine samples, it can be concluded that the assay detects free 3-butene-1,2-diol in urine samples.

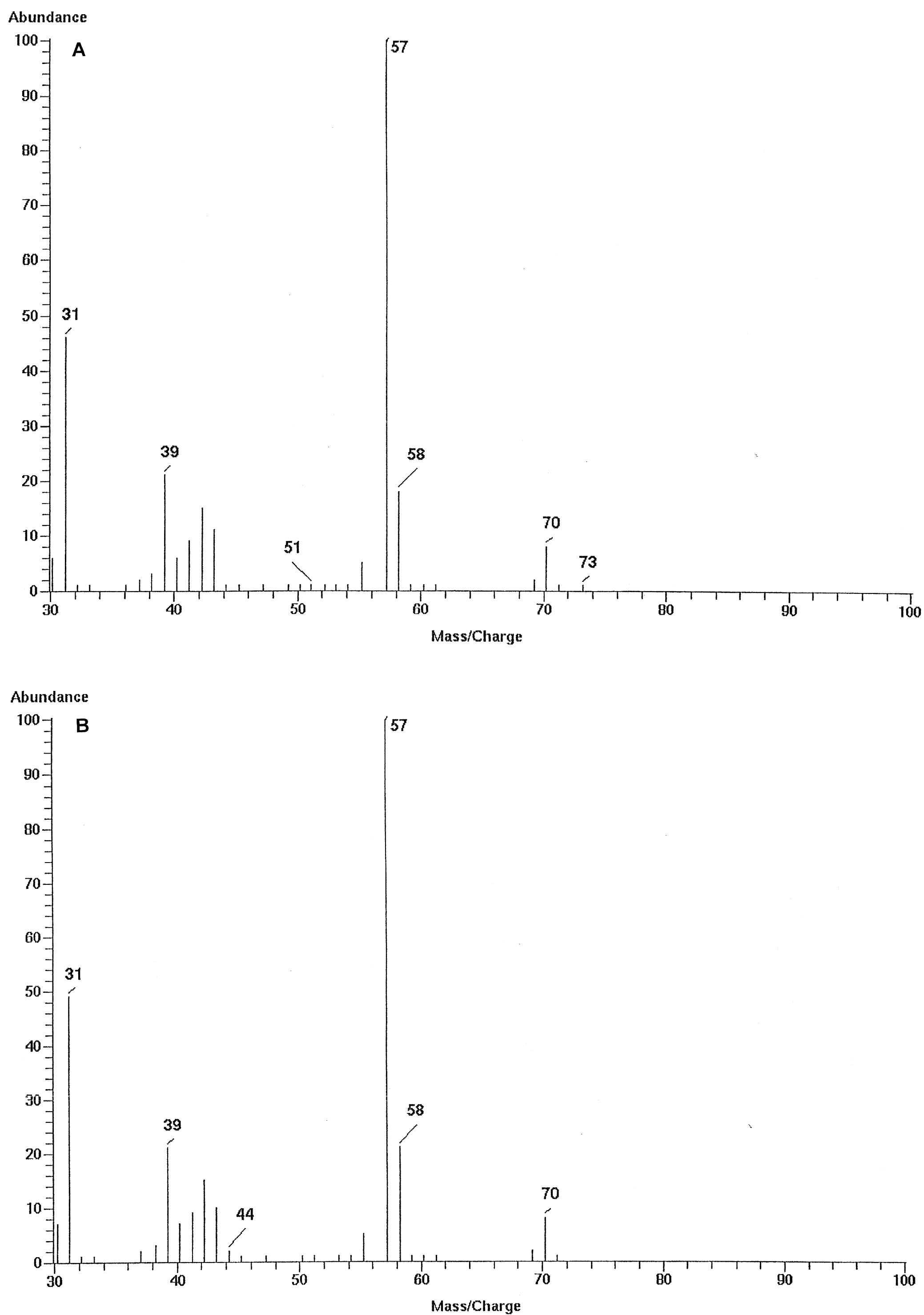

Fig. 2. The electron impact mass spectra of the synthesized 3-butene-1,2-diol (A) and the product detected in urine samples of the BD exposed rats (B).

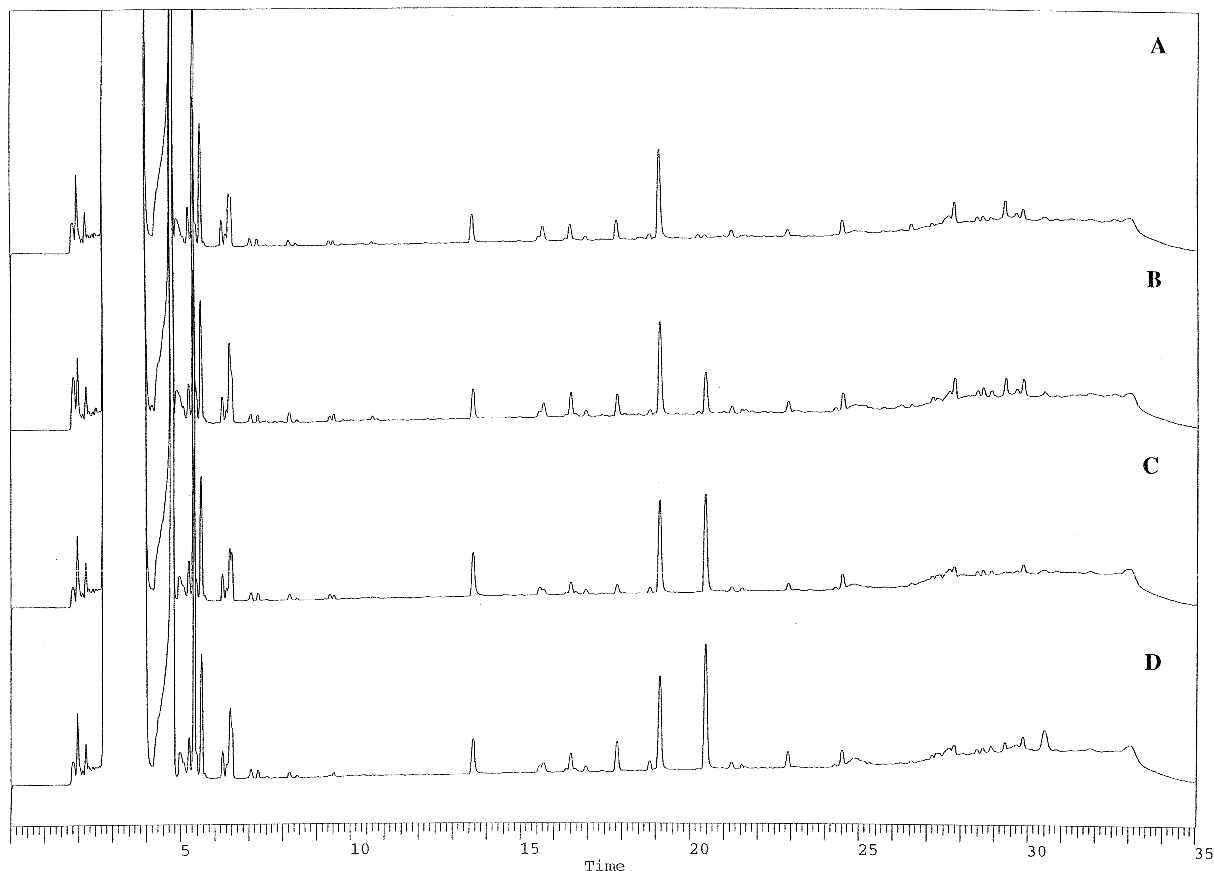

Fig. 3. Isopropanol extracts of BD exposed rat urine samples analyzed using gas chromatography: (A)=control animals, (B)=animals exposed to 250 ppm, (C)=500 ppm and (D)=1000 ppm of 1,3-butadiene for 6 h. 3-Butene-1,2-diol has a retention time 20.4 min.

3.2. The method validation

The extraction recoveries were determined by spiking nonexposed rat urine with 3-butene-1,2-diol. Naturally, urine used for spiking experiments was analyzed prior spiking to detect a possible background level of excretion of the compound under study. The 3-butene-1,2-diol concentration was found to be below the limit of detection (LOD) of the urine samples of the control animals (Fig. 3).

Using one concentration, several solvents were tested for the liquid–liquid extraction. The extraction efficiency of the solvents tested varied from 2% to 64%. The solvents and the corresponding extraction efficiency are summarized in Table 1. The water soluble organic solvents were tested only by saturat-

Table 1
The recovery of 3-butene-1,2-diol from rat urine by utilizing the salting out effect and isopropanol extraction[a]

Solvent	Recovery at 1.1 mg/l (SD, n=3)	Recovery at 2.2 mg/l (SD, n=3)	Recovery at 11 mg/l (SD, n=3)	Recovery at 16 mg/l (SD, n=3)	Recovery at 21 mg/l (SD, n=3)
Isopropanol	93% (±6%)	95% (±3%)	95% (±3%)	95% (±4%)	96% (±3%)

[a] The other solvents that were tested: acetonitrile (recovery=64%), ethyl acetate (recovery=55%), diisopropylether (recovery=34%), n-hexane (recovery=2%), n-butanole–acetonitrile (in 1:1 mixture, recovery=44%) and n-butanole–ethyl acetate (in 1:1 mixture, recovery=22%).

ing urine with sodium chloride prior to extraction. This technique allowed the separation of the organic and aqueous phases. Isopropanol showed a relatively high extraction efficiency and it proved to be a user-friendly solvent; isopropanol was, therefore, selected and the extraction efficiency was further tested at three different concentrations (Table1).

The standard deviation of injection at the concentration of 2.2 μg/ml was ±2% (n=12). Fig. 4 presents the calibration graph based on the spiked urine samples that had been saturated with sodium chloride and extracted with isopropanol. The LOD was 0.08 μg/ml and the limit of quantitation (LOQ) was determined at a signal-to-noise ratio of 5. LOQ was 0.11 μg/ml (SD=±4.8%, n=3). Linearity of the assay was tested on a range of 0.11–486 μg/ml (in addition to the calibration points shown in Fig. 4, 48.6, 121.5, 243.0 and 486 μg/ml concentrations were used to study the linearity) which gave a slope of $y=2639a-14\,241$ (R=0.9987). Day-to-day variation during a two week time period was ±4.2%.

The stability of 3-butene-1,2-diol in urine extracts was studied by spiking the rat urine and subsequently extract urine as described with isopropanol. After a six-month storage of the extracts in −20°C at a concentration level of 20 μg/ml, the recovery was 96% (SD=±2%, n=12). The spiked urine samples were also stored for six months in a freezer after which they were extracted. After storage in − 20 °C for six months the recovery was 95% (SD=±2%, n=12).

3.3. 3-Butene-1,2-diol in rat urine

The chromatogram A in Fig. 3 demonstrates that the background excretion of 3-butene-1,2-diol is lower than the LOD, and furthermore shows that isopropanol quite selectively extracts compounds from sodium chloride-saturated rat urine. The number of possible interfering peaks remained unexpectedly low.

Animals were exposed to 50, 250, 1000 ppm of butadiene, and excretion of the diol was linear over the exposure range (Fig. 5). The acid hydrolysis of the urine samples prior to the isopropanol extraction did not increase the amount of 3-butene-1,2-diol in any exposure groups. Metabolism of butadiene was fast – no diol was seen in urine collected the night after the exposure. The linear excretion of 3-butene-

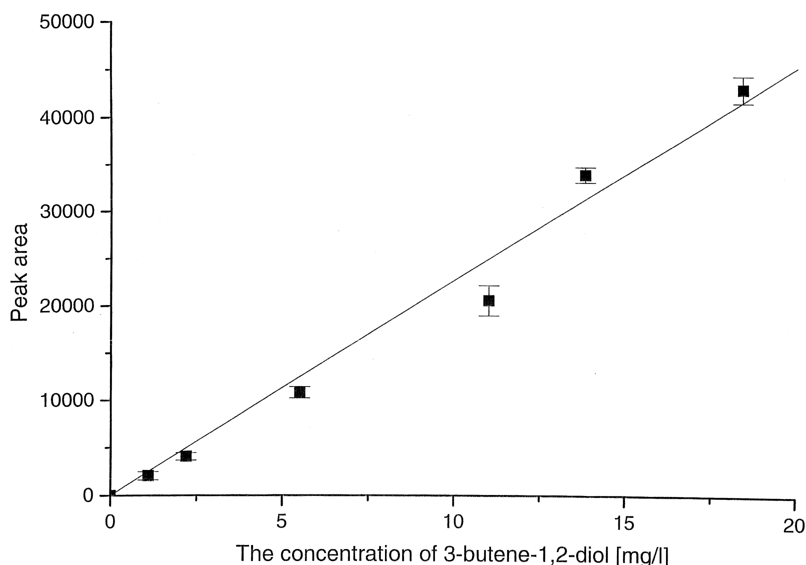

Fig. 4. The calibration graph of 3-butene-1,2-diol based on spiked and isopropanol extracted urine samples. Each point contains three separate analyses, and error bars are shown.

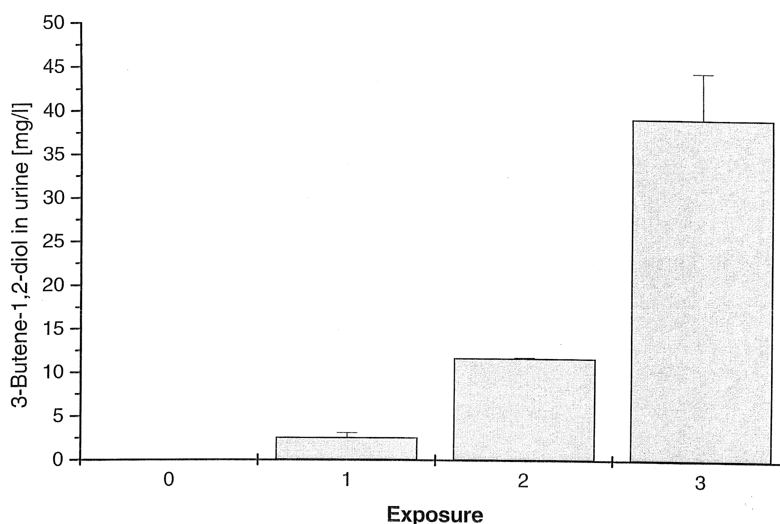

Fig. 5. The excretion of 3-butene-1,2-diol as a function of exposure. On the x-axis 0, 1, 2 and 3 corresponds to control, 250 ppm, 500 ppm and 1000 ppm exposure, respectively. Urine samples were collected during a 6 h exposure-period, and the urine of five animals were pooled.

1,2-diol indicates nonsaturated metabolic activation and/or detoxification of 1,3-butadiene.

Acknowledgements

Mr. Yrjö Peltonen is acknowledged for his very skilfull assistance to maintain a stable BD atmosphere during animal exposure and Ms. Marja Pihlaja is thanked for the animal care.

References

[1] IARC, Monograph on 1,3-butadiene, in: Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 54, 1992, pp. 237–285.
[2] Environmental Protection Agency (1991) Preliminary draft list of categories and subcategories under Section 112 of the Clean Air Act. Fed. Reg., 56 28548-28557
[3] K. Brunnemann, R. Kagan, E. Cox, D. Hoffmann, Carcinogenesis 11 (1990) 1826.
[4] B. Kruschel, R. Bell, R. Chapman, M. Spencer, K. Smith, J. High Res. Chromatogr. 17 (1994) 187.
[5] G. Barrefors, G. Petersson, Environ. Technology 17 (1996) 643.
[6] L. Duffy, F. Nelson, Atmospheric Environ. 30 (1996) 2759.
[7] K. Peltonen, R. Vaaranrinta, J. Chromatogr. A 710 (1995) 237.
[8] J. Fajen, R. Lundsford, D. Roberts, in: M. Sorsa, K. Peltonen, H. Vainio, K. Hemminki (Eds.), Butadiene and Styrene: Assessment of Health Hazards, IARC Scientific Publications No. 127, Lyon, 1993, p. 3.
[9] L. Van Duuren, N. Nelson, L. Orris, D. Palmes, L. Schmitt, J. Natl. Cancer Inst. 31 (1963) 41.
[10] A. Elfarra, J. Duescher, M. Pasch, Arch. Biochem. Biophys. 286 (1991) 244.
[11] C. de Mester, F. Poncelet, M. Roberfroid, M. Mercier, Biochem. Biophys. Res. Commun. 80 (1978) 298.
[12] I. Neagu, P. Koivisto, C. Neagu, S. Kaltia, R. Kostiainen, K. Peltonen, in: K. Hemminki, A. Dipple, D.G.E. Shuger, F.F. Kadlubar, D. Segerbäck, H. Bartsch (Eds.), DNA Adducts: Identification and Biological Significance, IARC Scientific Publications No. 125, Lyon, 1994, p. 419.
[13] P. Koivisto, R. Kostiainen, I. Kilpeläinen, K. Steinby, K. Peltonen, Carcinogenesis 16 (1995) 2999.
[14] C. Leuratti, N. Jones, E. Marafante, R. Kostiainen, K. Peltonen, R. Waters, Carcinogenesis 175 (1994) 1903.
[15] P. Koivisto, M. Sorsa, F. Pacchierotti, K. Peltonen, Carcinogenesis 18 (1997) 439.
[16] I.-D. Adler, U. Kliesch, L. Nylund, K. Peltonen, Mutagenesis 12 (1997) 339.
[17] E. Moffat, A. Hagardorn, K. Ferslew, J. Anal. Toxicology 10 (1986) 35.
[18] N. Smith, Clinica Chimica Acta 144 (1984) 269.
[19] J. Johnsson, A. Eklund, L. Molin, J. Anal. Toxicology 13 (1989) 25.
[20] N. Wu, T. Malinin, J. Anal. Toxicology 11 (1987) 63.
[21] H. Maurer, C. Kessler, Arch. Toxicol. 62 (1988) 66.
[22] N. Ferrala, B. Ghanayem, A. Nomeir, J. Chromatogr. B 660 (1994) 291.

[23] A. Kezic, S. Kuiper, A. Monster, J. Chromatogr. 496 (1989) 209.
[24] M. Balikova, J. Kohlicek, J. Chromatogr. 434 (1988) 469.
[25] M. Otsuka, S. Ohmori, J. Chromatogr. 577 (1992) 215.
[26] M. Otsuka, S. Ohmori, J. Chromatogr. B 654 (1994) 1.
[27] R. Rao, S. Bose, K. Gurjar, M. Raviddranathan, Tetrahedron 45 (1989) 7031.
[28] F.W. McLafferty, D.B. Stauffer (Eds.), The Registry of Mass Spectral Data, Vol. 1, J. Wileys & Sons, New York, USA, 1989.