Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 802 (2004) 361–366
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Development of a gas chromatographic test for the quantification of the biomarker 3-bromopropionic acid in human urine

C. B'Hymer*, K.L. Cheever

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Applied Research and Technology, Taft Laboratory C-26, 4676 Columbia Parkway, Cincinnati, OH 45226, USA

Received 7 October 2003; received in revised form 2 December 2003; accepted 4 December 2003

Abstract

An accurate and precise method was developed for the detection and quantification of 3-bromopropionic acid (3-BPA), a metabolite and biomarker for exposure to 1-bromopropane (1-BP). 1-BP is used as an industrial solvent and exposure is a health concern for industrial workers due to its toxicity. It has been associated with neurological disorders in both animals and humans. Urine sample preparation for the determination of 3-BPA consisted of liquid–liquid extraction (LLE) with ethyl acetate and silylation with *N*-methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide (MTBSTFA). Quantification was by means of a gas chromatograph (GC) equipped with a mass selective detector (MSD) using a dimethylpolysiloxane (HP-1) capillary column and 3-chloropropionic acid was used as an internal standard in the procedure. Demonstrated accuracy and precision during this method's validation was good; recovery varied between 93 and 98% with relative standard deviations (R.D.S.) of 5.7% or less. The limit of detection (LOD) for the procedure was approximately 0.01 μg/ml 3-BPA in urine. These data and other factors of the development and validation of this test method will be discussed.

Keywords: 3-Bromopropionic acid; 1-Bromopropane

1. Introduction

1-Bromopropane (1-BP, CAS No. 106-95-5) is widely used as a substitute for a number of chlorofluorocarbon solvents which have been withdrawn from use because of their possible damaging effects to the ozone layer [1]. 1-BP has many industrial applications including cleaning metal, optical instruments and electronics, and its use as a component in adhesives. 1-BP has become a health concern because of recent toxicity studies; this compound has been shown to decrease body weight, decrease motor nerve conduction velocity and cause elongation in distal latency in rats exposed by inhalation at 1000 ppm for 5–7 weeks [2]. Pathology noted in these animals includes degeneration of the myelin central sheath [2]. Similar electrophysiological and nerve changes were noted in a separate rat study, when 1-BP exposure at 700 ppm for 8 weeks was investigated [3]. Neurological hyperexcitability was studied in the rat exposed to 1-BP vapor [4]. Central neurological disorders and peripheral neuropathy has been reported in workers chronically exposed to materials composed of 1-BP in the United States [5,6]. Reproductive toxicity, consisting of a dose dependent reduction in the number of antral follicles in rat ovaries, has been reported for 1-BP inhalation exposure at 400 ppm for 7 weeks [7]. Both 1- and 2-bromopropane have been shown to form DNA adducts [8], and 2-bromopropane has been associated with reproductive and hematopoietic disorders of Korean electronic workers from their occupational exposure [9,10].

The metabolism of 1-BP has been studied over several years and is complex [11,12]. Two major routes of metabolism include oxidation at C2 or C3 via the cytochrome P450 monooxygenase system [13,14] and the formation of glutathione conjugates via glutathione-*S*-alkyltransferase. Experiments with various species have shown that these conjugates are primarily excreted in the urine as mercapturic acids [8,15]. A brief summary showing the proposed and generally accepted metabolic pathways of 1-BP is shown in Fig. 1, including the formation of 3-bromopropionic acid (3-BPA) metabolite [12], which is the focus of this study. The mercapturic acids, as well as 3-BPA, have been detected in urine from the rat [12]. The

* Corresponding author.
E-mail address: cbhymer@cdc.gov (C. B'Hymer).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.12.004

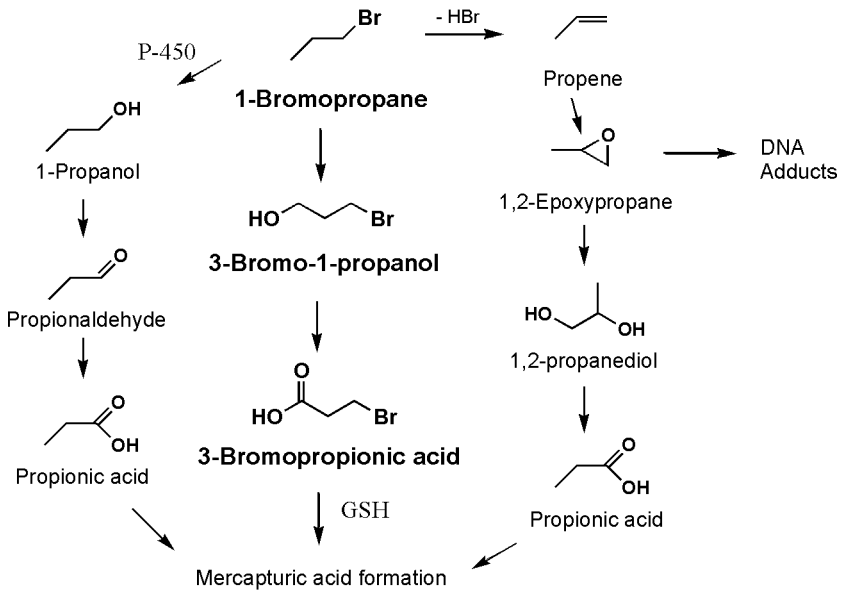

Fig. 1. The metabolic pathways for 1-bromopropane (1-BP). 3-Bromopropionic acid (3-BPA) is a metabolic product soluble in urine and is the focus of this test procedure as a measurable biomarker. 1-BP has many metabolic routes which can lead to the formation of mecapturic acid and DNA adducts.

metabolic profile has not been studied well for the human. 3-BPA has raised toxicity questions by itself; it is known to be a potent regulator of some genes [16] and is a potent inhibitor of erthroleukemia cell proliferation [17].

The current work was to devise a test method for the quantification of 3-BPA. This method could, in turn, be used in this laboratory to evaluate the use of 3-BPA as a possible biomarker for monitoring occupational exposure to 1-BP. Dermal and inhalation exposure are both possible in the work place, and although many studies have been published, much is not fully understood about 1-BP exposure in the human. Other biomarkers have been used in the literature to monitor for the exposure of 1-BP. Kawai et al. [18] noted that levels of bromide ion in urine collected from factory workers were higher after exposure to 1-BP. In an inhalation study with rats, Ishidao et al. [19] monitored levels of 1-BP in urine and blood from exposed rats by means of headspace analysis. Recently, a mercapturic acid metabolite of 1-BP, *N*-acetyl-*S*-propyl-L-cysteine, was detected in urine from workers by gas chromatograph (GC) equipped with a mass spectrometric detector (MSD) [20]. 3-BPA is more likely present in collected urine samples than 1-BP, because of 1-BP's possible volatility loss. The analysis for 3-BPA also avoids volatility sample handling problems associated with headspace analysis for the parent 1-BP. 3-BPA is more specific for 1-BP exposure and should not have either background levels or interfering sources as a total bromide ion determination would. Few frequently used brominated compounds would yield 3-BPA as a metabolite; 1,3-dibromopropane is used for intermediate chemical synthesis, not a general solvent as 1-BPA is used. Analysis for acid metabolites is generally more convenient. Extraction and derivatization for analysis by gas chromatography is usually more simple, and certainly uses less expensive analytical instrumentation such as the alternative high performance liquid chromatography–mass spectrometry (HPLC–MS). 3-BPA, therefore, was considered the most useful possible biomarker for detection in urine. The focus of this paper is the development and validation of a test method for the detection and quantification of 3-bromopropionic acid, which to our knowledge has not previously been evaluated for human urine testing.

The objective of this reported work was to develop a simple and effective test method to measure the levels of 3-BPA in human urine samples for evaluation as an exposure biomarker. It was also an objective to have a validated [21,22] test in place for use in monitoring dermally exposed individuals in future field studies by this laboratory. The analysis procedure was designed to be simple and straightforward. Liquid–liquid extraction (LLE) has been noted in the literature for its inherent simplicity and ease of use over other sample extraction and enrichment techniques [23] and was chosen for the extraction of 3-BPA from urine in this study. The use of *N*-methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide (MTBSTFA) as a derivatizing reagent has been reported often in the literature [24–26]. *Tert*-butyl-dimethylsilane (TBDMS) derivatives have been reported to have high intensity ions for mass spectrometric detection [24] and have reasonable stability; they are more stable to hydrolysis than trimethylsilane (TMS) derivatives. Therefore, MTBSTFA was used ultimately in this study. Capillary gas chromatography using a dimethylpolysiloxane (HP-1) column and detection by means of a mass selective detector proved to be selective for the test method and eliminated interferences for the analysis of the urine sample matrix.

3-Chloropropionic acid was chosen as an internal standard for this chromatographic method.

2. Experimental

2.1. Instrumental and chromatographic conditions

The chromatographic analysis was carried out using an Agilent Technologies model 6890 gas chromatograph (Avondale, Pennsylvania, USA) equipped with a model 5973 mass selective detector and an autosampler. The detector output was connected to a Chemstation (Agilent Technologies) where all raw data were evaluated and integrated. The column was a capillary HP-1 (Agilent Technologies) with a length of 50 m, internal diameter of 0.20 mm, and film thickness of 0.33 μm. The instrumental conditions for analysis were as follows: helium carrier flow was 0.8 ml/min constant, injector port temperature was 200 °C, and the detector source temperature was 230 °C with the quadrupole set at 150 °C. The column program was as follows: the initial temperature was 60 °C (at time 0), then increased to 180 °C at a rate of 4 °C/min (30 min ramp), and finally increased to 255 °C at a rate of 15 °C/min (5 min ramp). A post run of 270 °C for 5 min was included with each run, making a total run cycle time of 40 min. The mass selective detector was operated in electron impact mode with an electron energy of 70 eV and selected ions were monitored at ions of $m/z = 209$ and 211 for the derivative of 3-BPA and ions of $m/z = 165$ and 167 for the 3-chloropropionic acid derivative. Ions of $m/z = 211$ and 165 were used for quantification. Also, the mass selective detector was used in the scanning mode for verification of the identity of peaks during the initial development phase of this analytical test method. The injection size of the final solution was 0.5 μl using splitless mode injection.

2.2. Chemicals and reagents

Commercial sources of the standard compounds, 3-bromopropionic acid (CAS No. 590-92-1, Sigma–Aldrich Chemical Company, St. Louis, Missouri, USA), and 3-chloropropionic acid (CAS No. 107-94-1, Sigma) were used. Purity of these to reagents were reported as 99% (GC analysis) or greater by the manufacturer. A deuterated analog of 3-BPA was also used (Cambridge Isotopes Laboratories, Inc., Andover, Massachusetts, USA). Ethyl acetate used in the extractions was commonly available spectral grade (CAS No. 0141-78-6, Burdick and Jackson, Muskegon, Michigan, USA). The anhydrous magnesium sulfate (Mallinkrodt Specialty Chemical, Paris, Kentucky, USA) was ACS reagent grade. The hydrochloric acid (37%, Fisher Scientific) was ACS reagent grade. All stock standard solutions were prepared in deionized water (Barnstead NANOpure, Dubuque, Iowa, USA). The derivatizing reagent, *N*-methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide plus 1% *tert*-butyldimethylchlorosilane (TBDMCS) was also commercially available (Aldrich).

2.3. General urine sample preparation

Actual, non-spiked, urine samples or 3-BPA-spiked blank urine samples were treated identically. A 2.0 ml portion of the urine was placed in a screw capped tube and acidified with 40 μl of concentrated (12 M) hydrochloric acid. A 0.5 ml aliquot of a 20 μg/ml of 3-chloropropionic acid was added as the internal standard. A 0.5 ml portion of deionized water for test samples or standard 3-BPA spiking solution was added. The urine sample was extracted four times with 4.0 ml of ethyl acetate using a vortex mixer for 1 min for each extraction. The ethyl acetate layers were combined and dried by the addition of 100–200 mg of anhydrous magnesium sulfate. The extract solution was reduced in volume to 1 ml by evaporation by nitrogen sweep at room temperature. The concentrated extract solution was then placed in an crimp sealed vial with 50 μl of the MTBSTFA with 1% TBDMCS reagent and sealed. The solution was heated for 90 min at 70 °C. The sample was allowed to cool to room temperature and then ready for injection into the gas chromatograph.

2.4. Standard sample preparation and recovery studies

3-BPA standards for calibration were prepared at the 0.1, 0.25, 0.5, 1, 2, 5, 20, 50, 100, 150, and 200 μg/ml eq. levels in urine plus a blank 0 μg/ml level sample. Blank urine was spiked at the 2, 10, and 50 μg/ml eq. 3-BPA level for each experimental day of the primary recovery study. A secondary recovery study consisted of collecting urine from 20 non-exposed volunteers (NIOSH employees who were not exposed to any known source of 1-BP). Urine samples containing no 3-BPA and no 3-chloropropionic acid internal standard, urine samples with 3-chloropropionic acid only, and urine samples spiked with 20 μg/ml 3-BPA level and 3-chloropropionic acid internal standard were prepared. All these standard samples and the spiked samples used in the recovery studies were prepared as described previously using an ethyl acetate extraction and derivatization with MTBSTFA.

2.5. Calculations

Calculations were based on peak area ratios of derivatives of 3-BPA and 3-chloropropionic acid. Standard calibration curves were linear within the 0.1–200 μg/ml 3-BPA range used; correlation coefficients were 0.98 or greater and *y*-intercepts approached zero for all curves generated with this method. Two calibration curves, at the beginning and end of the batch run, using all the standards were collected for the first recovery study. This generated six calibration curves for the data presented in the first recovery study. Three calibration curves, at the beginning, middle, and end

of a sample run, were collected during the second recovery study. Since two batch runs were made during the second recovery study of spiked and blank urine from unexposed volunteers, this created an additional six calibration curves. Calibration curve slope drift was minimal during a sample run; less than 2% was observed within any sample batch run.

The limit of detection (LOD) was calculated in the traditional way, three times noise level divided by the slope of the calibration curve [21]. The average baseline level was determined for each batch run in chromatograms at the retention time window for 3-BPA from the 0 μg/ml blank standard. The standard deviation from the baseline as height was determined for the noise level using 100 data points. The slope from the calibration curve using peak height ratios of all the standard solutions was used for this calculation.

3. Results and discussion

3.1. Chromatographic separation and detection

The optimized chromatographic conditions developed for this test method proved to be selective and have no interferences. The unspiked urine samples chromatographed showed no interfering peaks for the derivative of 3-BPA; the blank samples from the 20 non-exposed volunteers showed no interfering peaks for either the derivatives of 3-chloropropionic acid internal standard or 3-BPA analyte. Therefore, this chromatographic procedure appears to be specific for testing for the presence of 3-BPA. Typical total ion chromatograms from urine samples of a non-exposed volunteer are shown in Fig. 2. A blank from an unspiked urine sample is shown on the lower chromatogram (Fig. 2A) and a spiked sample showing the peaks for the derivatives of 3-chloropropionic acid and 3-BPA is displayed in the upper chromatogram (Fig. 2B). Peak shape was good and there was no evidence of carry over between injections; blank urine samples injected after the 5 and 200 μg/ml standard displayed no peak for the 3-BPA derivative.

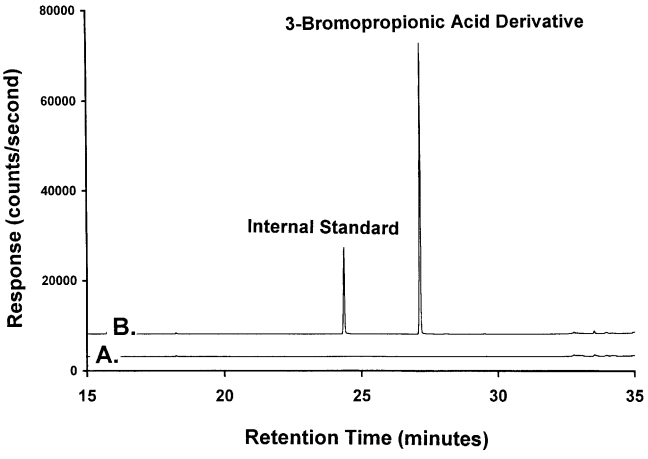

Fig. 2. A total ion chromatogram of a (A) blank non-exposed volunteer urine sample analyzed by the described procedure on using the HP-1 column; (B) 20 μg/ml spiked 3-bromopropionic acid (3-BPA) urine solution with 5 μg/ml eq. 3-Chloropriopionic acid used as the internal standard. No interfering peaks were evident in any of the group of 20 non-exposed volunteer samples.

The mass selective detector was useful in adding additional selectivity of the test method. The ion of m/z = 211 was chosen for monitoring the calibration used in the calculation because of its high abundance and because it was a characteristic fragment for the derivative of 3-BPA. It represents the molecular ion minus 57, the mass of the *tert*-butyl group from the *tert*-butyl-dimethylsilane derivative of 3-BPA (see Fig. 3). Ion of m/z = 165 was monitored for the derivative of 3-chloropropionic acid for the same reason. Both chlorine and bromine have two major isotopes, ^{35}Cl(75.5%), ^{37}Cl(24.5%), and ^{79}Br(50.5%), ^{81}Br(49.5%). The 3:1 ratio of chlorine 35–37, and the 1:1 ratio of bromine 79–81 permits qualifying ions of m/z = 165 and 167 for the 3-chloropropionic acid derivative and m/z = 209 and 211 for the 3-BPA derivative. No chromatographic interferences or co-eluting analytes were observed during this method's development; therefore, the need to use qualifying ions was not necessary.

3.2. Liquid–liquid extraction and the selection of the internal standard

The liquid–liquid extraction (LLE) followed by silylation using MTBSTFA proved to be effective for this test method. Early in this method's development, acid catalyzed esterification using ethanol was attempted; however, yields of the esters were generally low by a second liquid–liquid extraction. The initial extraction efficiency of the ethyl acetate was determined to be greater than 80% for 3-BPA. An ethyl acetate solution containing a known concentration of 3-BPA was derivatized with MTBSTFA beside a spike urine sample extracted following the sample preparation procedure. Responses were compared chromatographically and the extraction yield estimated.

3-Chloropropionic acid, was chosen as the internal standard for a number of reasons. Generally, a worker would not be expected to be exposed to 1-chloropropane solvent,

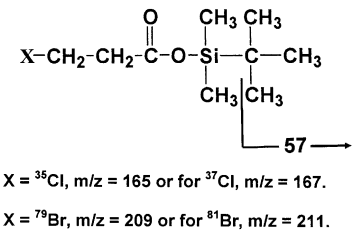

Fig. 3. The major ions monitored for the *tert*-butyl-dimethylsilane derivatives were the molecular ions less the *tert*-butyl group (m/z = 57). Ion of m/z = 165 for the internal standard and ion of m/z = 211 for the 3-bromopropionic acid (3-BPA) derivative were used for quantitation.

Table 1
Multilevel recovery study of 3-bromopropionic acid

Spike level (μg/ml)	Mean 3-bromo-propionic Acid recovered ($n = 9$) (μg/ml)	Average percent recovery	S.D. (μg/ml)	%R.S.D.
2	1.91	96	0.11	5.7
10	9.32	93	0.13	1.4
50	48.9	98	0.36	0.7

Notes: Three different spiked samples were prepared at each level and chromatographed on three separate experimental trial runs (a total of nine samples at each spike level were analyzed). The same HP-1 GC column was used for experimental batch trials 1 and 2; a second HP-1 column was used on trial run 3. %R.S.D.: percent relative standard deviation.

and the acid metabolite should not be present. An internal standard compensates for changes in solvent volume, however, the use of 3-chloropropionic acid as a procedural internal standard reduces analysis variation to acceptable levels. Variability caused by differences in the extraction and derivatization procedure can be compensated by the use of 3-chloropropionic acid added initially to the urine sample. 3-Chloropropionic acid is chemically similar to 3-BPA and has similar solubility and extraction properties. Other internal standards were evaluated during the development stage of this method. 4-Bromobutyric acid did not extract at a high yield using ethyl acetate and was dropped from consideration. The deuterated analog of 3-BPA was tried with greater success. The recovery and early calibration curves generated using the D-3-bromopropionic acid as an internal standard were as good as those using 3-chloropropionic acid. However, due to the high cost of using a deuterated analog, 3-chloropropionic acid was chosen as the internal standard for the final test method. Also, the chromatographic conditions used in this procedure will resolve the deuterated analog from the 3-BPA derivative should an alternative internal standard be needed in the future.

Another benefit of using an internal standard, is in the increased precision of chromatographic injections. Five replicate injections of the 5 μg/ml standard sample gave relative standard deviations (R.D.S.) of peak area ratios ranging form 0.6 to 3.5% during the collection of data for the recovery studies.

3.3. Analyte recovery studies

A primary recovery study of blank urine spiked with 3-BPA was performed over three separate experimental batch runs to demonstrate accuracy and precision of the procedure. These data are presented in Table 1; average recovery was between 93 and 98% for the three 3-BPA sample levels investigated. For each run, the experimental trial consisted of three samples at three concentration levels making nine samples for each trial. The 10 μg/ml level had the lowest average recovery at 93%, and the 50 μg/ml had the highest average recovery at 98%. The percent relative standard deviation (%R.D.S.) ran as high as 5.7%, and although the recovery was consistently below 100%, any low bias would be not significant. The second recovery study used urine samples from 20 non-exposed volunteers and demonstrated that the procedure was relatively accurate and precise (Table 2). The 20 μg/ml spiked samples showed an average recovery of 95% and a percent relative standard deviation of 2.5%. The lowest individual sample recovery was 18.3 μg/ml and the highest recovery was 19.7 μg/ml. The second recovery study also would indicate that differences in individual urine samples do not cause matrix variation which would affect recovery significantly. Again, no interferences were noticed in any of the samples analyzed.

3.4. Method reproducibility, figures of merit and future work

Two different HP-1 capillary columns of different manufacturing lots were used during the recovery studies of this method's development; the results, therefore, are expected to be consistent and reproducible with different HP-1 GC columns. The limit of detection was found to be approximately 0.01 μg/ml 3-BPA in urine. Aqueous stock 3-BPA solutions were verified to be stable for 2 weeks while stored in a refrigerator at 4 °C. Aqueous stock standard solutions of 3-BPA gave full recovery values when compared to freshly prepared standards. No significant instability of the derivative samples was noticed within a week period during this method's development phase. This test method should be considered accurate for the quantification of free 3-BPA in human urine within the 0.1–200 μg/ml standard range. Although high levels, such as 200 μg/ml, are not expected from field samples, the detector/chromatographic linearity does allow for this level.

Finally, a comment should be made about the possibility of conjugates of 3-BPA. The described method was designed for the analysis of the free acid. For routine study of field samples, additional sample preparation or pretreatment to

Table 2
Recovery of 20 μg/ml 3-bromopropionic acid spikes from urine samples of 20 non-exposed volunteers

Mean recovery (μg/ml)	Average percent recovery	Lowest value (μg/ml)	Highest value (μg/ml)	%R.S.D.
19.0	95	18.3	19.7	2.5

Notes: All non-spike samples showed no 3-bromopropionic acid derivative peak in their chromatograms. %R.S.D.: percent relative standard deviation.

include enzymatic incubation may be necessary to account for any conjugates of 3-BPA. This step was not explored for the recovery of spiked sample described in this manuscript which was part of the validation procedure for the free acid. Again, the metabolism of 1-bromopropane has not been well studied in the human. This and the testing of field samples are currently under study within this laboratory, and is part of planned future work. This larger comprehensive study is beyond the scope of this short manuscript and will be reported in detail elsewhere.

4. Conclusions

An accurate and precise procedure to monitor the level of the biomarker 3-bromopropionic acid in human urine has been developed and validated. Extraction and silylation with MTBSTFA was followed by analysis with capillary gas chromatography using a mass selective detector. Average recovery of known 3-BPA fortified blank urine samples was between 93 and 98% with relative standard deviations as high as 5.7% using samples at 3-BPA concentrations of 2, 10, 20, and 50 μg/ml. Standard curves generated linear responses in the range of 0.1–200 μg/ml with correlation coefficients of 0.98 and greater. The limit of detection was found to be approximately 0.01 μg/ml eq. 3-BPA levels in urine. The procedure has been demonstrated to be useable for the quantification of 3-BPA in urine.

References

[1] 1-Bromopropane, Hazard Evaluation System and Information Service, California Department of Health Services, Oakland, California, July 2003.
[2] X. Yu, G. Ichihara, J. Kitoh, Z.L. Xie, E. Shibata, M. Kamijima, N. Asaeda, Y. Takeuchi, J. Occup. Health 40 (1998) 234.
[3] W. Zhao, K. Aoki, T. Xie, J. Misumi, J. Occup. Health 41 (1999) 1.
[4] Y. Fueta, K. Fukunaga, T. Ishidao, H. Hori, Life Sci. 72 (2002) 521.
[5] G. Scalar, Clin. Neurol. Neurosur. 101 (1999) 199.
[6] G. Ichihara, J.K. Miller, A. Ziolkokwska, S. Itohara, Y. Takeuchi, J. Occup. Health 44 (2002) 1.
[7] T. Yamada, G. Ichihara, H. Wang, X. Yu, K. Maeda, H. Tsukamura, M. Kamijima, T. Nakajima, Y. Takeuchi, Toxicol. Sci. 71 (2003) 96.
[8] E. Lee, Y. Moon, L. Zahao, E. Kim, H. Lim, A. Basnet, T. Jeong, W. Chae, Toxicol. Sci. 72 (2003) S–1.
[9] Y. Kim, K. Jung, T. Hwang, G. Jung, H. Kim, J. Park, J. Park, D. Park, S. Park, K. Choi, Y. Moon, Scand. J. Work Environ. Health 22 (1996) 387.
[10] J. Park, Y. Kim, D. Park, K. Choi, S. Park, Y. Moon, J. Occup. Health 39 (1997) 138.
[11] A.R. Jones, Xenobiotica 5 (1975) 155.
[12] A.R. Jones, D.A. Walsh, Xenobiotica 9 (1979) 763.
[13] H.G. Bray, S.P. James, Biochem. J. 69 (1958) 24P.
[14] A.E.R. Thomson, G.A. Maw, L. Young, Biochem. J. 69 (1958) 23P.
[15] H. Tachizawa, T.L. MacDonald, R. Neal, Mol. Pharmacol. 22 (1982) 745.
[16] E.D. Carstea, S.P.F. Miller, H. Christakis, R.R. O'Neill, Biochem. Biophys. Res. Commun. 192 (1993) 649.
[17] M.A. Lea, N. Tulsyan, Anticancer Res. 15 (1995) 879.
[18] T. Kawai, A. Takeuchi, Y. Miyama, K. Sakamto, Z.W. Zhang, K. Higashikawa, M. Ikeda, Biomarkers 6 (2001) 303.
[19] T. Ishidao, N. Kunugita, Y. Fueta, K. Arashidani, H. Hori, Toxicol. Lett. 134 (2002) 237.
[20] G. Ichiara, K. Amarth, V. Amarnath, H. Valentine, W. Li, X. Ding, S. Peng, T. Yamada, H.Wang.X. Yu, E. Shibata, Y. Takeuch, W. Valentine, Toxicol. Sci. 60 (2001) S–1.
[21] J.M. Green, Anal. Chem. 68 (1996) A305.
[22] The Federal Register 60 (1995) 11260.
[23] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, J. Sep. Sci. 24 (2001) 331.
[24] R.F. Arrendale, J.T. Stewart, R.L. Tackett, J. Chromatogr. 432 (1988) 165.
[25] M.R. Harkey, G.L. Henderson, C.H. Zhou, J. Anal. Toxicol. 15 (1991) 260.
[26] R.E. West, D.P. Ritz, J. Anal. Toxicol. 17 (1993) 114.