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RESEARCH ARTICLE

Comparison and evaluation of urinary biomarkers for occupational exposure to spray adhesives containing 1-bromopropane

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Abstract

Three metabolites of 1-bromopropane (1-BP) were measured in urine samples collected from 30 workers exposed to 1-BP at two facilities making furniture seat cushions and evaluated for use as biomarkers of exposure. The mercapturic acid metabolite, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys), 3-bromopropionic acid (3-BPA), and bromide ion levels (Br^-) were quantitated for this evaluation. The high exposure group consisted of 13 workers employed as adhesive sprayers who assembled foam cushions using 1-BP containing spray adhesives and the low exposure group consisted of 17 non-sprayers, who worked in various jobs without spraying adhesives. All workers' urine voids were collected over the same 48 h period at work, and at home before bedtime, and upon awakening. Urinary AcPrCys and Br^- levels were elevated in the sprayers compared to that of non-sprayers. Following HPLC-MS/MS analysis of mercapturic acid metabolite levels, 50 urine samples having the highest levels of AcPrCys were analyzed for 3-BPA. No 3-BPA was detected in any of the samples. The data collected from this study demonstrate that AcPrCys and Br^- are effective biomarkers of 1-BP exposure, but 3-BPA is not.

Keywords: 1-Bromopropane, biomarker, exposure

Introduction

Biomarkers of exposure are important tools for use in exposure assessment and represent a key element in the methods used in toxicological research. 1-Bromopropane (1-BP, n-propyl bromide, CAS # 160-94-5) is a widely used industrial solvent used to replace many ozone-depleting chloroflurocarbons or potential carcinogens in metal and electronics degreasing, adhesive and in aerosol solvents. Although no occupational surveys of dermal exposure have been conducted, it is likely that worker exposure may occur through dermal and inhalation routes (NTP, 2004; Hanley et al., 2006; Frasch et al., 2011). Workplace area or breathing zone sampling is often not adequate for determination of the dose for an exposed worker; biomarkers of exposure are of highly significant importance in industrial health and toxicological research. Most importantly, a well-chosen biomarker of exposure should be specific for the exposure of interest and should provide good predictive value to a specific health status and dose level (B'Hymer and Cheever, 2010). 1-BP undergoes rapid metabolism after exposure and numerous metabolites are produced which are primarily excreted in urine (Jones & Walsh, 1979). Initial oxidation of 1-BP results in bromide formation and subsequent glucuronidation results in formation of several mercapturate conjugates. Previously, urinary bromide (Hanley et al. 2006) and *N*-acetyl-*S*-(*n*-propyl)-L-cysteine, the primary mercapturate metabolite in

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urine from workers exposed to 1-BP (Hanley et al. 2009), have been validated individually as biomarkers of 1-BP exposure. A third metabolite, 3-bromopropionic acid was evaluated in this study based on its reported effects on gene expression (Carstea et al., 1993, Lea and Tulsyan, 1995), *in vitro* mutagenesis (Toraason et al., 2006) and tumor formation in animals (Searle 1976, Theiss et al., 1979). The primary goal of this research was to evaluate the primary metabolites of 1-BP, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys), 3-bromoproponic acid (3-BPA) and bromide ion levels (Br^-), for use as biomarkers of occupational 1-BP exposure.

1-BP has been of toxicological interest for several years. A number of case studies report that 1-BP exposure caused central and peripheral neurological disorders in workers with some studies reporting exposures greater than 200 ppm (Sclar, 1998; Ichihara, 2005; Raymond and Ford, 2007; Majersik et al., 2007; MMWR, 2008, Li et al., 2010). Li et al. (2010) reported evidence of dose-dependent neurological and hematological changes in women employed in 1-BP production plants. Exposures during 8- and 12-h work shifts ranged from 0.07 to 106.4 ppm. A lowest adverse effect level of 1.28 ppm was identified for these women. Animal investigations of the toxicity of 1-BP studies have focused on the generation and effects of active metabolites that could account for 1-BP associated neurotoxic and reproductive effects in rodents and exposed workers (Tachizawa, 1982, Garner et al., 2006, 2007; Ghanayem and Hoffer, 2007, Lui, 2009). The NTP concluded in a summary of these animal studies that the toxicity of 1-BP likely is dependent on glutathione-dependent pathways with formation of glutathione-1-BP conjugates which deplete free glutathione (GSH). However these conjugates of oxidative metabolites do not appear to be a source of toxicity (NTP, 2004).

The metabolism of 1-BP is complex (Barnsley, 1966, Sklan and Barnsley, 1968, Baines et al., 1977, Jones & Walsh, 1979, Tachizawa, 1982). A simplified and condensed scheme is provided in Figure 1. In rats, some absorbed 1-BP is metabolized through direct conjugation with GSH to form AcPrCys with the eventual release of free bromide (Br^-) ions both of which are eliminated in urine (Jones & Walsh, 1979). Alternate major routes of metabolism include oxidation at C2 or C3 via the cytochrome P450 monooxygenase system (P450) to form 3-bromopropionic acid (Jones and Walsh, 1979), 1-bromo-2-propanol and bromoacetone (Barnsley et al., 1966). These products are in turn conjugated with glutathione via glutathione-*S*-alkyltransferase to form mercapturic acids *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, *N*-acetyl-*S*-(2-hydroxy-*n*-propyl)-L-cysteine and *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine, respectively.

Early human exposure studies examined urinary bromide level as a possible biomarker of 1-BP exposure using gas chromatography with electron capture detection (Kawai et al., 2001). The correlation between urinary Br^- and airborne 1-BP was significant, but background levels of urinary Br^- was substantial (~8 mg/l). Subsequent investigations found intake of fruits and sea-foods influenced urinary Br^- levels which may limit the use of urinary Br^- levels as a biomarker for estimating human occupational exposure (Kawai, 2002, Zhang et al., 2001). Bromine also is present, in some soft drinks and foods prepared with brominated vegetable oil (Horowitz, 1997). Further human exposure studies demonstrated significant correlations between airborne 1-BP exposure and urinary 1-BP levels measured by headspace analysis (Kawai et al., 2001, Ichihara, 2004). Reported oxidative P450 CYP2E1 metabolites of 1-BP in rodents, i.e. 3-bromopropionic acid, 1-bromo-2-propanol, and bromoacetone, and their mercapturic acid metabolites, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, *N*-acetyl-*S*-(2-hydroxy-*n*-propyl)-L-cysteine and *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine suggested that more specific alternative biomarkers free of dietary and other non-occupational exposures may exist (Pombrio et al., 2001, Ichihara et al., 2002). Urinary AcPrCys levels were measured by GC/MS in post-shift urine samples of 47 workers in a 1-BP production facility which demonstrated that urinary AcPrCys levels increased with increased exposure to 1-BP exposure (Valentine et al., 2007).

Currently, no occupational exposure limits for 1-BP have been recommended by the National Institute for Occupational Safety and Health (NIOSH) nor promulgated by the Occupational Safety and Health Administration (OSHA), and manufacturer recommendations are inconsistent. The American Conference of Governmental Industrial Hygienists has a recommended Threshold Limit Value® of 10 parts per million (ppm) over a full 8 h work shift (ACGIH, 2012). In December 2009, California OSHA promulgated an 8 h limit of 5 ppm (CalOSHA, 2012); this is the only enforceable regulatory standard for 1-BP in the US. In a final rule, the Environmental Protection Agency accepted 1-BP as an alternative solvent for ozone depleting solvents in vapor degreasing and cleaning applications, but did not accept it for use in spray adhesives and aerosols (EPA, 2007). No US EPA Reference Concentration (RfC) has been established. More pertinent to this study, no standardized biological monitoring techniques have been established for 1-BP. Due to concerns regarding toxicity and limited exposure data, NIOSH, with partial funding from the National Toxicology Program (NTP), conducted occupational exposure studies in multiple industries. As part of this effort, this laboratory investigated worker 1-BP exposures and urinary metabolites at foam cushion plants where 1-BP containing spray adhesives were used. Airborne 1-BP levels were monitored, and all worker urine voids for 48 hours over 2 consecutive work days were collected. Urinary bromide ion excretion (Br^-), 3-bromopropionic acid (3-BPA), and mercapturic acid metabolites *N*-acetyl-*S*-(2-hydroxy-*n*-propyl)-L-cysteine (AcPrCys), (*N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine-*S*-oxide, *N*-acetyl-*S*-(2-hydroxy-*n*-propyl)-L-cysteine, and *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine were

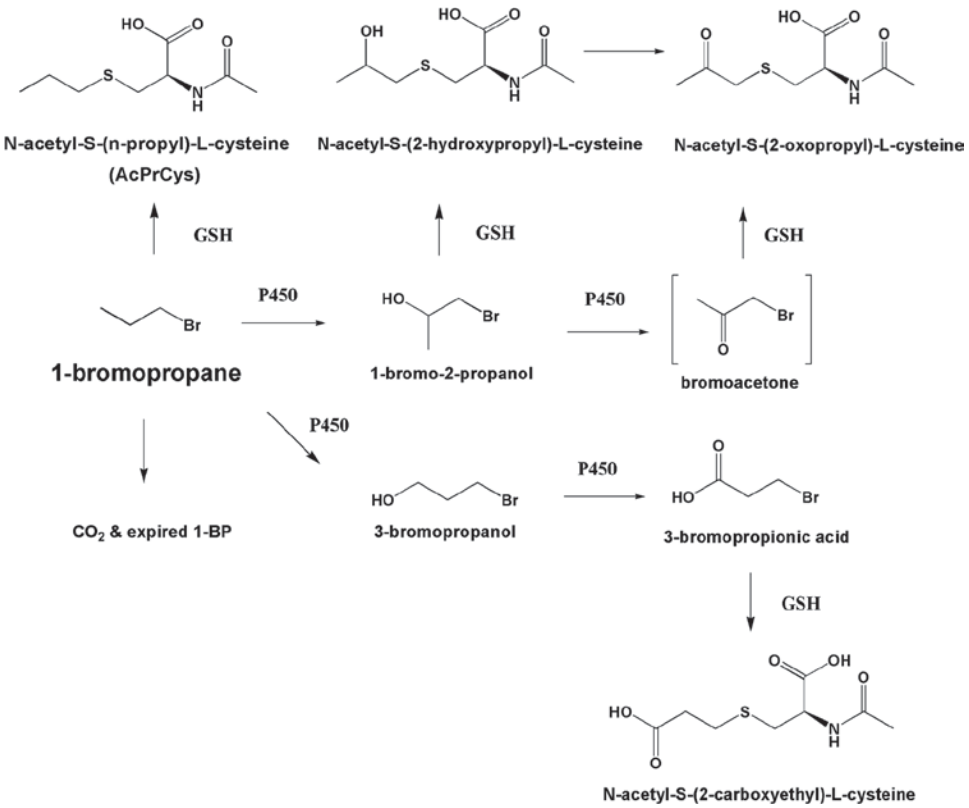

Figure 1. Metabolism of 1-bromopropane in the rat by multiple pathways. Oxidative metabolism products of cytochrome P450 2E1 or other oxidative systems are in turn conjugated with glutathione (GSH) to form mercapturic acid metabolites.

quantitated to investigate their utility as biomarkers of occupational 1-BP exposure.

Materials and methods

Study participants

Thirty workers employed at two seat cushion fabrication plants were classified into two exposure groups – 13 adhesive sprayers: 1 male and 12 females as the high exposure group and 17 non-adhesive sprayers: 4 males and 13 females as the low exposure group. In the absence of a matched control population, classification of workers into sprayers and non-sprayers was a simple means of comparing workers with exposure to higher concentrations of 1-BP with those exposed to lower concentrations of 1-BP. An additional (non-statistical) comparison was made with 7 'spot' control samples collected from persons not employed at these factories. The average ages (± sd) of sprayers and non-sprayers were 35.5 (11.9) and 36.1 (8.8) years, respectively (Hanley et al., 2006). Control subjects were 4 males and 3 females with average ages (± sd) 38.8 (7.4) and 44.7 (4.0), respectively. All participants provided informed voluntary consent prior to participation as authorized by the NIOSH Human Subjects Review Board.

Collection of personal breathing zone samples

Two full-shift measurements were collected on consecutive days using Anasorb carbon molecular sieve sorbent tubes (SKC, Eighty Four, PA). Samples were desorbed using 1 ml of carbon disulfide and were analyzed for 1-BP by GC/FID as described in NIOSH method 1025 (NIOSH, 2003). The limit of detection (LOD) is 1 μg providing a minimum detectable concentration of 0.016 ppm using the recommended maximum 12 liter air sample volume.

Collection of urine samples

Urine samples were collected as previously reported (Hanley et al., 2006). All of the workers' urine voids were collected over a 48 h period including when away from work. Sampling began Monday morning pre-work week and ended Wednesday morning. Specimens were collected over sequential time: at work, after work, but before bedtime, and upon awakening. The specimens were collected in nitric acid rinsed high density polyethylene (HDPE) bottles and immediately chilled with gel ice. After each collection period, total urine volumes were measured and 25 ml aliquots were dispensed into acid rinsed HDPE bottles, and frozen with carbonic acid (dry-ice) for storage and shipment. At the laboratory the samples were stored at –80°C prior to analysis.

Mercapturic acid HPLC-MS/MS analysis

Mercapturic acid levels were measured using a recently described and validated method employing a combination of high-performance liquid chromatography (HPLC) with electrospray ionization-tandem mass spectrometry (ESI-MS/MS (Cheever et al., 2009). In brief,

3 ml of acidified urine were extracted using solid phase extraction on C18 columns (Varian Inc., Harbor City, CA). The samples on column were rinsed with acidified aqueous methanol (40% MeOH/60% H_2O, pH 3). AcPrCys was eluted with acetone, and reduced to dryness by N_2 sweep. Samples were reconstituted with 1 ml methanol and placed in screw-cap, sealed vials for injection into an Agilent 1100 LC-MSD (Agilent Technologies) using negative ion SIM mode for quantification of AcPrCys ion at $m/z = 204$. The LOD for this method was approximately 0.01 μg/ml AcPrCys in urine.

3-BPA GC/MS analysis

Urinary 3-BPA levels were measured using a previously described and validated gas chromatograpy/mass spectrometry method (B'Hymer and Cheever, 2004). Briefly, 2 ml of acidified urine were extracted four times by liquid-liquid extraction with ethyl acetate. The ethyl acetate layers were combined, dried using anhydrous magnesium sulfate, and reduced to 1 ml by nitrogen sweep at room temperature. The concentrated extract was silyated in a sealed vial for analysis using an Agilent model 6890 gas chromatograph (Avondale, PA, USA) equipped with a mass selective detector using a dimethylpolysiloxane capillary column (Agilent Technologies) with temperature programming elution. Mass selective detection in electron impact mode was used to quantitate derivatized ions of 3-BPA and 3-chloropropionic acid at m/z = 211 and 165, respectively. The limit of detection (LOD) for this method was approximately 0.01 μg/ml 3-BPA in urine.

Bromide ion analysis

Urinary bromide ion levels were measured using the method described by Allain et al., 1990 and adjusted for creatinine to determine bromide ion excretion (Br^-_{cr}). In brief, 1 ml of urine was diluted to 10 ml with 1% nitric acid. Analytical standards and quality assurance samples were prepared using Uri-Sub (CST Technologies, Great Neck, NY) a bromide-free synthetic urine substrate. Samples were analyzed by Inductively Coupled Plasma-Mass Spectrometry (Varian Ultra-Mass 700) operated at radiofrequency 1300 W yielding a LOD of 0.1 μg/ml; yttrium internal standard.

Creatinine analysis

Creatinine levels were determined using standard spectrophotometry and Sigma-Aldrich (St. Louis, MO) diagnostic kit no. 555 with a LOD of 100 μg/ml. Creatinine values were used to calculate normalized excretion of bromide ion.

Results and discussion

The results of the 1-BP exposure and bromide ion levels from this study have been reported previously (Hanley et al, 2006). As anticipated from workplace observations, sprayers were exposed to greater quantities of 1-BP than non-sprayers. Full-shift time-weighted average Geometric Mean ± Geometric Standard Deviation (GM (GSD), and maximum breathing zone concentrations for 1-BP were 92 ppm (1.43), 200 ppm for sprayers and 11 ppm (3.01), 60 ppm for non-sprayers (Hanley et al., 2006). To assemble seat cushions, sprayers used compressed air-spray guns to apply adhesive to seat-cushion components. Some sprayers used bare hands to smooth edges and pinch corners of foam components. Non-sprayers were employed as sewing machine operators, cloth and foam cutters, pillow stuffers, product wrappers, and line supervisors. Exposure to 1-BP among employees performing non-spraying jobs was due to ineffective general exhaust ventilation and solvent drift from spraying stations where 1-BP vapors and mists were created. Higher exposure of non-spraying workers occurred at work positions located closest to the adhesive spraying stations.

Higher exposure for sprayers relative to non-sprayer is reflected also in the GM (GSD) for 48-h urinary Br^-_{cr} excretion concentrations (mg/gm-cr) of 195 (1.23) and 42.9 (2.19), respectively. Workers employed as sprayers excreted over four times the amount of urinary bromide as workers employed as non-sprayers. These results indicate that urinary Br^- excretion is a reasonable index of 1-BP workplace exposure, but care must be taken to identify non-occupational exposure to brominated compounds which may act as an interference. For these highly exposed workers, however, the GM Br^-_{cr} concentrations (mg/gm-cr) for sprayers and non-sprayers were over 50 and 10 times greater than that for control subjects 3.8 (1.32), respectively (Hanley et al., 2006). Thus, non-occupational Br^- interference is more likely for workers with much lower 1-BP exposures (>1 ppm) than those described herein (Hanley et al., 2010).

Selected urine samples were analyzed for identification of mercapturic acid conjugates AcPrCys, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine and *N*-acetyl-*S*-(2-hydroxy-*n*-propyl)-L-cysteine. However, only the major metabolite AcPrCys, was chosen for quantification as it was vastly predominant in these workers' urine specimens. To reduce the effect of individual variability of excretion rates and urination patterns, 24 and 48 h volume-weighted excretion were calculated from workers' specimens collected on the first, second, and for both days. Statistical testing was conducted on 24-h and 48-h urinary metabolite concentrations as well as for collection intervals with 1-BP TWA. It was determined the data were appropriately described by a log-normal distribution (Hanley et al., 2009). The 48-h geometric mean concentration for total AcPrCys excretion was over 4 times higher in workers employed as sprayers (43.9 mg/l) than workers in non-spraying activities (9.68 mg/l) which are shown in Table 1. The data in Table 1 clearly demonstrates differences in AcPrCys excretion between the exposure groups throughout the collection period. In addition, the GM concentration calculated from seven

Table 1. AcPrCys excretion (mg/l) by exposure group and specimen collection period.

Collection period	Adhesive sprayers $n = 12^{\dagger}$ or $13^{\ddagger}$	Non-adhesive sprayers $n = 17$
Pre-week[‡]		
GM (GSD)	5.95 (1.76)	0.814 (5.46)
Mean (standard deviation)	6.83 (3.56)	1.79 (1.62)
Minimum–Maximum	1.77–12.6	0.019–5.14
1st day work shift[‡]		
GM (GSD)	34.0 (2.03)	7.26 (4.03)
Mean (standard deviation)	44.2 (34.0)	14.9 (16.4)
Minimum–Maximum	14.4–127	0.542–56.1
1st day post shift[†]		
GM (GSD)	40.0 (2.44)	10.8 (4.13)
Mean (standard deviation)	56.6 (51.3)	22.5 (25.5)
Minimum–Maximum	6.97–166	0.760–94.4
2nd day pre-shift[‡]		
GM (GSD)	33.6 (1.97)	6.87 (3.53)
Mean (standard deviation)	40.9 (25.0)	12.0 (12.0)
Minimum–Maximum	13.0–77.7	0.365–41.9
2nd day work shift[‡]		
GM (GSD)	47.0 (2.15)	10.8 (3.32)
Mean (standard deviation)	62.7 (54.2)	20.1 (22.8)
Minimum–Maximum	17.6–181	1.76–77.6
2nd day post shift[†]		
M (GSD)	51.1 (2.08)	12.4 (3.91)
Mean (standard deviation)	65.6 (58.7)	29.4 (42.9)
Minimum–Maximum	10.0–242	1.69–161
3rd day wake up[‡]		
GM (GSD)	51.6 (2.11)	8.59 (2.58)
Mean (standard deviation)	67.3 (57.6)	12.5 (11.5)
Minimum–Maximum	12.6–221	1.09–48.4
Total 24 h excretion, day 1[†]		
GM (GSD)	36.8 (1.92)	7.97 (3.48)
Mean (standard deviation)	45.7 (35.7)	13.6 (12.7)
Minimum–Maximum	15.5–138	0.609–50.6
Total 24 h excretion, day 2[†]		
GM (GSD)	52.8 (1.81)	10.8 (3.14)
Mean (standard deviation)	63.1 (46.3)	19.2 (22.0)
Minimum–Maximum	23.4–165	1.78–78.5
Total 48 h excretion[†,*]		
GM (GSD)	43.9 (1.81)	9.68 (3.09)
Mean (standard deviation)	40.6 (43.9)	16.4 (17.0)
Minimum–Maximum	21.3–170	1.31–55.6

*Pre-week values excluded. [§]Control specimens: mean (SD) = 0.049 (0.043); maximum = 0.140; minimum = 0.007.

control specimens was 0.035 mg/l which is three and two orders of magnitude less than that of the 24-h and 48-h GM concentrations for the sprayers and non-sprayers, respectively. It should be noted excretion (volume-weighted) data are not corrected for creatinine. Since the renal proximal tubule is a major site of mercapturic acid elimination (Pombrio et al., 2001), these results do not necessarily need to be normalized via creatinine adjustments. Further, associations of AcPrCys concentrations with 1-BP exposures were statistically significant for both sprayers ($p < 0.05$) and non-sprayers ($p < 0.01$, Hanley et al., 2009). These results suggest AcPrCys is an important 1-BP urinary metabolite in humans, and is an effective biomarker for highly exposed workers. Moreover, the LOD for AcPrCys detection is ten times more sensitive than Br^- ion detection (Cheever et al., 2009; Allain et al., 1990) which is shown by the control subject levels that ranged from 0.007 to 0.14 mg/l. Other studies by this laboratory with workers exposed to vapor degreasing operations also showed statistically significant associations of much lower urinary AcPrCys levels in conditions of lower 1-BP exposure (Hanley et al., 2010).

Worker AcPrCys excretion in Table 1 indicates exposed workers began the study week with significantly elevated levels of this metcapturate in their urine. Previous studies have demonstrated that urinary excretion of bromide ions following 1-BP exposure was does-dependent in workers (Kawai et al. 2001) and in animals (Ishidao et al. 2002). In rats exposed to 1-BP vapor, bromide ion decreased with dose and weeks of exposure (Ishidao et al. 2002). Generally metabolism of inhaled organic compounds increases due to induction of metabolic enzymes due to exposure. However, the high levels of exposure observed in the current study combined with chronic occupational exposure to 1-BP vapor, a decrease in the urinary metabolite excretion in these workers would not be unexpected.

Following HPLC-MS/MS analysis to determine urinary AcPrCys levels, 50 urine samples having the highest levels of AcPrCys were analyzed for 3-BPA using GC/MS (B'Hymer and Cheever 2004). No 3-BPA was detected in any of the 50 samples examined (<LOD 0.01 μg/ml). This unexpected result suggests that in humans 1-BP is directly conjugated with GSH, and that P450 oxidation at C2 or C3 seen in rodents is not a major route of 1-BP metabolism in humans. Human 1-BP metabolism has been investigated only through examination of urinary metabolites bromide (Kawai et al., 2001a) and AcPrCys (Ichihara, 2001, Valentine et al., 2007); and these studies offer no information to explain the current result.

Garner et al. (2006) investigated 1-BP metabolism and disposition in rodents characterizing urinary metabolites following inhalation or intravenous administration. Exposure of rodents by inhalation or by tail vein injection do not involve hepatic first pass metabolism that follows oral or i.p. routes of administration used in earlier studies, and may better represent metabolism following inhalation exposure in human workplace or environmental exposure (Garner et al. 2006). Garner observed dose-dependent P450 metabolism of 1-BP in rats. In rats, but not in mice, the proportion of 1-BP metabolized via P450 oxidation pathways decreased with increasing dose. As dose increased to 100 mg/kg, the production of AcPrCys increased as the production of mercapturic acids derived from conjugation products of P450 oxidation decreased, indicating saturation of oxidation pathways. Use of 1-aminobenzotriazole, an inhibitor of P450 eliminated oxidative urinary metabolites, reducing the number of urinary metabolites from 10 to 1 with AcPrCys as the predominant metabolite (Garner et al. 2006). In

the current study, 3-BPA, a product of P450 oxidative metabolism, was absent in urine samples from workers highly exposed to 1-BP; samples which contained predominately AcPrCys. In light of Garner's findings, this results suggests that metabolism of 1-BP in highly exposed workers may be dose dependent. The abundance of AcPrCys found in the current study in urine from exposed workers, and the limited quantities of N-acetyl-S-(2-carboxyethyl)-L-cysteine and N-acetyl-S-(2-hydroxy-n-propyl)-L-cysteine, underscore an apparent difference between human and rat and mouse 1-BP metabolism. Future studies of workers having lower 1-BP exposure and analysis for 3-BPA could possibly answer this dose-dependency question in human metabolism, but that is beyond the scope of the current study. Results from the current study demonstrate that urinary AcPrCys and Br^- concentrations are reliable biomarkers to assess human exposure to 1-BP, however, urinary 3-BPA is not.

Conclusion

Although urinary bromide analysis offers rapid high-throughput of samples, it lacks specificity and should be used only when dietary, medicinal or other background sources are considered. The results of recent and current studies at this laboratory indicate determination of AcPrCys levels in urine is both practical and accurate, as the analytical method has been validated and is highly sensitive by HPLC-MS/MS. In general, mercapturic acid metabolites are specific for exposure, and as in the case of 1-BP, allows for deduction of up-stream metabolites produced by multiple pathways from the mercapturic acids present in a urine sample.

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Declaration of interest

The authors hereby report that we have no conflict of interest with the material reported in this paper. The authors alone are responsible for the content and writing of this paper.

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