N-Acetyl-S-(n-Propyl)-L-Cysteine in Urine from Workers Exposed to 1-Bromopropane in Foam Cushion Spray Adhesives

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1-Bromopropane (1-BP) has been marketed as an alternative for ozone depleting and other solvents; it is used in aerosol products, adhesives, metal, precision, and electronics cleaning solvents. Mechanisms of toxicity of 1-BP are not fully understood, but it may be a neurological and reproductive toxicant. Sparse exposure information prompted this study using 1-BP air sampling and urinary metabolites. Mercapturic acid conjugates are excreted in urine from 1-BP metabolism involving debromination. Research objectives were to evaluate the utility of urinary N-acetyl-S-(n-propyl)-L-cysteine (AcPrCys) for assessing exposure to 1-BP and compare it to urinary bromide [Br (2)] previously reported for these workers. Forty-eight-hour urine specimens were obtained from 30 workers at two factories where 1-BP spray adhesives were used to construct polyurethane foam seat cushions. Urine specimens were also obtained from 21 unexposed control subjects. All the workers’ urine was collected into composite samples representing three time intervals: at work, after work but before bedtime, and upon awakening. Time-weighted average (TWA) geometric mean breathing zone concentrations were 92.4 and 10.5 p.p.m. for spraying and non-spraying jobs, respectively. Urinary AcPrCys showed the same trend as TWA exposures to 1-BP: higher levels were observed for sprayers. Associations of AcPrCys concentrations, adjusted for creatinine, with 1-BP TWA exposure were statistically significant for both sprayers (P < 0.05) and non-sprayers (P < 0.01). Spearman correlation coefficients for AcPrCys and Br(2−) analyses determined from the same urine specimens were highly correlated (P < 0.0001). This study confirms that urinary AcPrCys is an important 1-BP metabolite and an effective biomarker for highly exposed foam cushion workers.

Keywords: bromide; 1-bromopropane; CAS No. 106-94-5; foam cushion; N-acetyl-S-(n-propyl)-L-cysteine; spray adhesive; urine

INTRODUCTION

An international agreement restricts the use of ozone-depleting substances including some compounds which were widely used throughout the industry: 1,1,1-trichloroethane and chlorofluorocarbons (freons®). An industry consortium petitioned the Environmental Protection Agency (EPA) to accept 1-bromopropane (1-BP) as an alternative for ozone-depleting solvents for aerosols; adhesives; metals, electronics, and precision cleaning (EPA, 1999; EPA, 2000). Products containing potential carcinogens trichloroethylene, perchloroethylene, and methylene chloride are also candidates for substitute solvents. Future applications for 1-BP may include paint, printing ink, asphalt cement, and textile cleaning solvents (Dead Sea Bromine, 1999; Petroferm Inc., 2000; EnviroTech International, Inc., 2008). EPA published a final rule to accept 1-BP in solvent cleaning and a proposed rule to reject it in adhesives and aerosols (EPA, 2007). The quest for alternative solvents could expand 1-BP markets potentially exposing many workers.

Currently, occupational exposure limits for 1-BP are not available from the National Institute for
Occupational Safety and Health (NIOSH, 1992) nor the Occupational Safety and Health Administration (OSHA, 2009). Manufacturers’ exposure guidelines are inconsistent, ranging from 10 to 100 parts per million (p.p.m.) (Great Lakes Chemicals, 2005; EnviroTech International, Inc., 2006). The American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value® (TLV®) for 1-BP is a 10 p.p.m., 8-h time-weighted average (TWA) based on suspected embryonic/fetal, neurological, and hepatic toxicity (ACGIH, 2009).

Based on concern regarding toxicity, NIOSH requested that the National Toxicology Program (NTP) evaluate 1-BP (NTP, 2004). The absence of exposure information resulted in an interagency agreement between NTP and NIOSH to conduct an occupational exposure study in multiple industries. In this study, 1-BP exposures were measured for most jobs at two factories using spray adhesives to manufacture foam furniture cushions. The underlying goal of this study was to evaluate a new high performance liquid chromatography (HPLC) electrospray ionization mass spectrometry (ESI-MS) method for characterizing mercapturic acid conjugates of 1-BP in urine. Specific objectives were to measure urinary N-acetyl-S-(n-propyl)-L-cysteine (AcPrCys) levels and determine if a relationship of AcPrCys existed with workers’ 1-BP exposures and their urinary bromide [Br(\(^-\))] levels previously published for these workers (Hanley et al., 2006).

**BACKGROUND**

A number of animal toxicity studies have implicated 1-BP as a reproductive or neurological toxicant (Ichihara et al., 2000a,b; Takeuchi et al., 2001; Yu et al., 2001; Wang et al., 2003; Yamada et al., 2003; NTP, 2004; Fueta et al., 2007; Lee et al., 2007; Ueno et al., 2007; Yoshida et al., 2007). Ichihara (2005) concluded that, in rats, 1-BP was a neurotoxicant which produced prolonged distal latencies; decreased nerve conduction velocities; cerebellum degeneration; peripheral myelin sheath deterioration; and muscle weakness.

Health surveys were conducted by Ichihara et al. (2004a,b) at a factory after production changed from 2-bromopropane to 1-BP synthesis. Significant changes in motor nerve conduction velocities were not observed but decreased vibration sensation in toes, longer distal latencies, and neurobehavioral deficits were noted for exposed workers. Ichihara proposed that 1-BP may produce adverse peripheral sensory, motor, or central nervous system effects in humans.

Majersik et al. (2007) reported on a case study of six foam cushion gluers with neurological dysfunction after using 1-BP-based adhesives. The mean TWA 1-BP exposure was determined to be 108 p.p.m. (range: 92–127 p.p.m.) after additional ventilation was used. Symptoms included lower extremity pain, numbness, weakness, labored walking, headache, and nausea. Serum Br\(^{−}\) levels were 44–170 mg dl\(^{-1}\) (reference: 0-40 mg dl\(^{-1}\)) with hyperchloremia measured at 107–139 mmol l\(^{-1}\) (reference: 98–107 mmol l\(^{-1}\)). A 2-year follow-up revealed that the two workers most severely affected had little improvement and three patients suffered chronic neuropathic pain. The authors concluded that 1-BP exposure may produce neurotoxicity in humans, principally spastic paraparesis with distal sensory loss.

Raymond and Ford (2007) described another case study involving four workers exposed to 1-BP adhesives following their hospitalization. Mean TWA 1-BP exposure, measured 9 months later, was slightly over 100 p.p.m. Primary symptoms were consistent with previous case studies: numbness, tingling, weakness, and reduced sensation in lower legs with unsteady gait. Elevated Br\(^{−}\) in serum was determined to range from 3 to 12.5 mEq l\(^{-1}\) (reference < 0.6 mEq l\(^{-1}\)). In the two workers not lost to follow-up, symptoms persisted after 8 years, albeit less severely. The authors concluded that severe neurological disease occurred in these workers but concurrent elevated arsenic levels, perhaps acting synergistically with Br\(^{−}\), prevented a definitive determination.

Metabolism of 1-BP is relatively complex and occurs by several pathways shown in Fig. 1, which includes exhalation of unaltered 1-BP; debromination; oxidation by CYP2E1; and S-conjugation with glutathione (Barnsley et al., 1966; Jones and Walsh, 1979, 1980; Garner et al., 2006). An intermediate metabolite, S-n-propyl-glutathione, is cleaved to S-n-propyl-L-cysteine which is metabolized producing several mercapturic acid conjugates excreted in urine. Bromide ions are eliminated mainly through the kidneys in urine (Ryan and Baumann, 1999). Urinary Br\(^{−}\) elevated substantially above normal (>10 mg l\(^{-1}\)) represents accumulated exposure over the previous week or two, as Br\(^{−}\) is slowly excreted (Jones and Walsh, 1979). Even though Br\(^{−}\) analysis is well established, it may not be ideal for evaluating 1-BP exposure because background levels may be influenced by diet, medications, and exposure to other brominated compounds. Hence, mercapturic acid conjugates which have been used as biomarkers of exposure to halogenated compounds (Van Welie et al., 1992; De Rooij et al., 1998) may prove more specific for occupational exposure to 1-BP than urinary Br\(^{−}\).

**METHODS**

This is a follow-up manuscript regarding two factories manufacturing furniture cushions (Hanley
An additional urinary metabolite, AcPrCys, was analyzed from aliquots of the same urine specimens previously collected from these workers. A total of 30 workers were evaluated—13 adhesive sprayers and 17 workers performing other jobs. ‘Sprayers’ used 1-BP spray adhesives to assemble furniture cushions. ‘Non-sprayers’ included material handlers and cutters, seamstresses, and supervisors who were exposed to 1-BP vapor drifting away from spraying stations.

To evaluate 1-BP metabolites, all the workers’ urine voids over a 48-h period were collected, including the amount excreted while away from work. The specimens were collected as composite samples over sequential time intervals: (i) at work, (ii) after work but before bedtime, and (iii) upon awakening. Urine collection started pre-shift before the workweek began, after nearly 3 days without exposure, and ended pre-shift 2 days later. Participants collected urine specimens in nitric acid rinsed Nalgene® bottles [high-density polyethylene (HDPE)] and immediately chilled it in small coolers with frozen gel ice. After the collection period, the total urine volume was measured with a graduated cylinder. Twenty-five milliliter aliquots were dispensed into nitric acid rinsed HDPE bottles, frozen (and shipped) on dry ice, and then stored in a laboratory freezer below −60°C.

Breathing zone (BZ) air samples were collected over these two consecutive workdays with Anasorb carbon molecular sieve sorbent tubes. The sorbent was desorbed with 1 ml of carbon disulfide and analyzed for 1-BP by gas chromatography with flame ionization detection via NIOSH method 1025 (NIOSH, 2003). The limit of detection (LOD) for this method is 1 µg which equates to a minimum detectable concentration of 0.016 p.p.m. using the maximum recommended air sampling volume of 121. Dermal exposure was not quantitatively measured but was qualitatively evaluated by work method observations.

The urine specimens were analyzed for creatinine (cr), Br(−), and AcPrCys concentrations. For comparison, single ‘spot’ samples were analyzed from 21 unexposed volunteers, not employed by these facilities. Creatinine was analyzed using standard spectrophotometric procedures and a Sigma Diagnostics test kit no. 555, with a LOD of 0.1 gm l−1. Bromide ion was measured using an inductively coupled plasma mass spectrometer (Varion Ultra-mass 700) operated at radiofrequency of 1300 W yielding an LOD of 100 µg l−1; yttrium was used as an internal standard (Allain et al., 1990). Recoveries were reported to be nearly 100%, the coefficient of variation was 3%, and between-day reproducibility was ~5%. One milliliter of each sample was diluted to 10 ml with 1% nitric acid prior to analysis. Analytical standards and quality control samples were prepared using Uri-sub, a synthetic urine solution since background concentrations of Br(−) may be present in pooled urine from the general population. Calibration graphs were linear over 0−40 mg l−1; additional 2- or 5-fold dilutions were prepared for samples exceeding the standard calibration range. Five replicates, with 20 scans per replicate, were analyzed for each sample.

Urinary metabolites of 1-BP were characterized with a newly developed method using HPLC with ESI-MS (Cheever et al., 2009). Mercapturic acid standards were prepared using the general procedure of Grenby and Young (1960) as modified by van Bladeren et al. (1980). Using human specimens, Cheever et al. (2002) isolated four mercapturic acid conjugates from workers exposed to 1-BP adhesives [e.g. AcPrCys; N-acetyl-S-(n-propyl)-L-cysteine-S-oxide; N-acetyl-S-(2-carboxyethyl)-L-cysteine; and N-acetyl-S-(3-hydroxy-n-propyl)-L-cysteine]. However, only the major metabolite, AcPrCys, was chosen for quantification because it was vastly predominant in worker specimens.
Urine aliquots were spiked with 10 μg ml⁻¹ [d₇]-AcPrCys, an internal standard and prepared for analysis using BondElute C₁₈ 500 mg solid phase extraction columns conditioned sequentially with acetone; 95% methanol:5% 0.1 N HCl and 95% H₂O (pH 3.0):5% methanol. Urine specimens were adjusted to pH 3; applied to the column; rinsed with 40% methanol:60% H₂O (pH 3.0); eluted with 4 ml acetone; and reduced to dryness under nitrogen. Eluates were dissolved in methanol and injected onto a column (150 x 2 mm, Phenomenex Aqua, 3 μm, C₁₈ 300A). A 10-min linear solvent gradient was started with H₂O:methanol (85:15, 0.1% acetic acid) to 15% methanol (0.1% acetic acid) at 300 μl m⁻¹. Solvent composition was maintained with H₂O:methanol (5:95, 0.1% acetic acid). Samples were analyzed using a mass spectrometer (Agilent 1100/ MSD SL ESI), and negative ions were monitored in the selectively ion mode at m/z 204 and 211.

Standard solutions for AcPrCys are not commercially available as an in-stock reagent, but these may be purchased from a custom synthesis laboratory. Deuterated standards and non-deuterated reference compounds were prepared by NIOSH researchers which were in excess of 98% pure as determined by HPLC-ESI/MS (Cheever et al., 2009). Calibration standards were prepared at 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.625, 1.25, 5, and 10 μg ml⁻¹. Mercapturic acid conjugates are generally regarded as stable in urine (De Rooij et al., 1998). AcPrCys was demonstrated to be stable with spike samples in urine extract at room temperature and chilled at 8°C for 2 weeks and repeatedly with standard solutions refrigerated at 2–4°C after numerous months of storage (Cheever et al., 2009).

Control urine samples fortified with AcPrCys were similarly processed to determine recovery, LOD, and limit of quantitation (LOQ). The LOD for AcPrCys is 0.01 μg ml⁻¹ and the LOQ was 0.03 μg ml⁻¹. Recovery trials were performed with blank urine fortified with 0.625, 1.25, 5, or 10 μg ml⁻¹ mixed with 1 μg ml⁻¹ of internal standard; spiked sample mean recovery was 96–103% (n = 6; SD ≤ 6.4). Calculations were based on peak area ratios of AcPrCys to [d₇]-AcPrCys. Calibration was linear from 0.01 to 10 μg ml⁻¹ with correlation coefficients of 0.98 or greater and y intercepts that approached zero before and after each set of sample analyses. Samples out of linear range were diluted 10- or 100-fold with H₂O, pH 3, and re-analyzed.

Twenty-four and forty-eight hour metabolite concentrations were calculated as volume-weighted concentrations for that period using at work, after work, and next day wake-up specimens. The mass was computed by summing the products of sample concentrations by the total urine volumes from each sample collected over the 24 (or 48)-h period. Linear regressions for the 48-h urinary AcPrCys concentrations versus the average 1-BP TWA concentrations for ‘all jobs and both factories combined’ were evaluated using both the unadjusted (AcPrCysunadj) and creatinine-adjusted concentrations (AcPrCyscr). Comparison of the associations showed that the relationships for creatinine-adjusted concentrations were stronger than the unadjusted concentrations (r² = 0.42 versus 0.36, respectively, for AcPrCyscr and AcPrCysunadj). Hence, statistical analyses were only conducted using AcPrCyscr concentration and total mass.

Simple relationships for 24-h urinary AcPrCys with 1-BP TWA for each job group were also examined to assess the proportion of metabolite variability explained by 1-BP TWA. Assumptions of normality were better met by taking logarithms of the urinary AcPrCys variables. Models of urine level logarithms were regressed on the logarithm of 1-BP TWA, day, and their interaction; these were conducted separately for sprayers and non-sprayers. Terms in the model were removed when not significant. Employee was treated as a random effect, and restricted maximum likelihood was used to estimate the covariance parameters using PROC MIXED in SAS (SAS Institute, Cary, NC, USA). In addition, Spearman correlation coefficients were computed to evaluate the relationship between AcPrCyscr or Br⁻cr concentrations with 1-BP TWA for all jobs combined and with collection intervals. Spearman coefficients were also calculated for each of the AcPrCyscr and Br⁻cr analyses measured from the same specimens.

RESULTS

The demographic data for these workers was tabulated in the initial study (Hanley et al., 2006). Most workers were female: 92.3% of the sprayers and 76.5% of the non-sprayers. Ages were also similar between job categories; average age for both groups was ~36. Nearly half (46.2%) of the sprayers were African–American while the non-sprayers were predominately Caucasian (82.4%).

Full-shift TWA BZ concentrations to 1-BP are reported in Table 1. Adhesive sprayers’ exposures were substantially greater than those for non-spraying jobs due to their immediate proximity to solvent emissions. Exposure to 1-BP among employees performing non-spraying jobs was due to ineffective general exhaust ventilation and solvent vapor drift from spraying stations. The sprayers’ GM exposure to 1-BP was 92.4 p.p.m., while the non-sprayers’ GM was 10.5 p.p.m. All BZ concentrations of 1-BP measured for adhesive sprayers exceeded 25 p.p.m., the industrial exposure guideline originally proposed by the EPA (2003). Eight out of 34 non-sprayers’ exposures exceeded 25 p.p.m.; 23 out of 34 exceeded 10 p.p.m., the TLV® recommended by ACGIH (2009).
In Table 2, descriptive statistics are tabulated for AcPrCys\textsubscript{cr} from both days combined, grouped according to collection interval and job. The sprayers’ GM concentrations were ~4 times greater than those for non-sprayers. Furthermore, the GMs of pre-week AcPrCys\textsubscript{cr} concentrations for sprayers and non-sprayers, after an extended weekend without 1-BP exposure, were over 100 and nearly 25 times higher than that for the unexposed subjects, respectively.

Twenty-four and forty-eight hour composite urinary AcPrCys levels were calculated to reduce the impact that individual variability of excretion rates and urination patterns may have on the time specific AcPrCys levels were calculated to reduce the impact that individual variability of excretion rates and urination patterns may have on the time specific...
Table 3. Twenty-four and forty-eight hour urinary AcPrCys concentrations, adjusted for creatinine, and total AcPrCys mass by job

<table>
<thead>
<tr>
<th>Analyte measure</th>
<th>Collection period</th>
<th>Adhesive sprayers ( (n = 13) )</th>
<th>Non-spraying jobs ( (n = 17) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcPrCys ( (\text{mg (g-cr)}^{-1}) )</td>
<td>Day 1, 24-h</td>
<td>Minimum 14.3 0.373</td>
<td>Maximum 97.9 37.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GM (GSD) 32.5 (1.78)</td>
<td>7.77 (3.50)</td>
</tr>
<tr>
<td></td>
<td>Day 2, 24-h</td>
<td>Minimum 22.1 1.23</td>
<td>Maximum 127 81.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GM (GSD) 48.5 (1.65)</td>
<td>12.1 (3.04)</td>
</tr>
<tr>
<td></td>
<td>Days 1–2, 48-h</td>
<td>GM (GSD) 41.1 (1.64)</td>
<td>10.2 (3.07)</td>
</tr>
<tr>
<td>Total AcPrCys mass ( (\text{mg}) )</td>
<td>Day 1, 24-h</td>
<td>Minimum 13.3 0.798</td>
<td>Maximum 104 47.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GM (GSD) 32.4 (1.96)</td>
<td>8.92 (3.08)</td>
</tr>
<tr>
<td></td>
<td>Day 2, 24-h</td>
<td>Minimum 15.0 1.92</td>
<td>Maximum 189 66.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GM (GSD) 43.9 (1.91)</td>
<td>13.0 (2.62)</td>
</tr>
<tr>
<td></td>
<td>Days 1–2, 48-h</td>
<td>GM (GSD) 78.3 (1.84)</td>
<td>22.8 (2.65)</td>
</tr>
</tbody>
</table>

\(^a\text{GM (GSD)} = \text{geometric mean.}\)

\(^b\text{Twenty-four and forty-eight hour concentrations were calculated from composite specimens collected at work, after work, and next day wake up as volume-weighted average concentrations.}\)

\(^c\text{Total mass was calculated by multiplying the composite concentrations by the total urine volume for each specimen followed by summation of the specimen masses for each day and 2 day period.}\)

The relation between AcPrCys\(_{cr}\) or Br\(^{-}\)\(_{cr}\) with 1-BP TWA for all jobs combined and stratified by collection interval were tested using Spearman’s correlation coefficients. The Spearman’s coefficient data from these analyses showed that both these metabolites are highly correlated with 1-BP TWA whether partial-, 1-, or 2-day concentrations were tested \([r = 0.716–0.835 \text{ for AcPrCys}_{cr} (P \leq 0.0001); r = 0.800–0.952 \text{ for Br}^{-}\)\(_{cr} (P \leq 0.0001)]\). Spearman’s correlation coefficients were also computed for each of the seven sequential Br\(^{-}\)\(_{cr}\) and AcPrCys\(_{cr}\) analyses pairs. These coefficients ranged from 0.688 for the pre-week to 0.854 for the first evening specimens; \(P < 0.0001\) for each collection interval.

**DISCUSSION**

This study used a new HPLC tandem ESI-MS analytical method to measure several mercapturic acid conjugates possible from 1-BP metabolism. These conjugates are likely to be more specific to 1-BP than Br\(^{-}\)\(_{cr}\) since there should be less non-occupational interference. Furthermore, even though the efficacy of 1-BP analysis directly measured in the headspace of urine samples has been shown (Kawai et al., 2001; Ichihara et al., 2004a; B’hymer and Cheever, 2005), it is more probable to accurately measure mercapturic acid conjugates than the parent compound due to 1-BP volatility and its rapid elimination in exhaled breath (Jones and Walsh, 1979; Garner et al., 2006). Headspace analysis requires immediate preparation upon collection and must be from a single sample or analyte loss could be expected. Hence, 1-BP in urine is not as practical of a biomarker for field studies, especially when monitoring many workers simultaneously both at and away from work.

Metabolism studies with propyl halides, including 1-BP, have shown that rats excreted \(S\)-propylcysteine, \(S\)-propylmercapturic acid, 2-hydroxypropylmercapturic acid, 3-hydroxypropylmercapturic acid, 2-carboxyethylmercapturic acid, propylmercapturic acid \(S\)-oxide, AcPrCys, and 3-bromopropionic acid in urine (Grenby and Young, 1960; Barnsley et al., 1966; Baines et al., 1977; Jones and Walsh, 1979). Initial analyses of human specimens in our study revealed that AcPrCys was the predominant mercapturic acid conjugate identified with this method (Cheever et al., 2002). When stratified by exposure group, the same relative rankings were noted for AcPrCys as with 1-BP TWA and Br\(^{-}\) (i.e. sprayers levels were much greater than those of non-sprayers). This method proved effective to evaluate highly exposed foam cushion workers and sensitive enough to measure lower exposed material cutters and unexposed controls.

The urinary Br\(^-\) results observed for these same worker exposure days were previously reported (Hanley et al., 2006). For sprayers, the 48-h GM urinary Br\(^{-}\) \(_{cr}\) concentration was 195 mg (g-cr)\(^{-1}\) [geometric standard deviation (GSD) = 1.23]; the non-sprayers’ 48-h GM Br\(^{-}\) \(_{cr}\) was 42.9 mg (g-cr)\(^{-1}\) (GSD = 2.19). These levels were 40 and 10 times greater than for unexposed subjects, respectively. Strong relations of urinary Br\(^-\) excretion with 1-BP exposure were found which demonstrated that urinary Br\(^-\) is an important biomarker for higher exposure. Moreover, the total mass of Br\(^-\) from 1-BP metabolism over these 2 days were 378 mg (GSD = 1.58) and 98 mg (GSD = 1.98) for sprayers and non-sprayers, respectively. These levels in urine may implicate bromism as a plausible etiologic explanation, at least in part, to some of the observed neurologic symptoms from 1-BP exposure in reported case studies (Ichihara et al., 2002; Majersik et al., 2007; Raymond and Ford, 2007).

Although AcPrCys\(_{cr}\) and Br\(^{-}\)\(_{cr}\) results analyzed from the same specimens were highly correlated \((P < 0.0001)\), some differences are apparent. First, the peak AcPrCys\(_{cr}\) GM concentrations were observed...
in post-shift samples each day, whereas higher $\text{Br}^{(-)}_{\text{cr}}$ GMs were found mostly in work-shift samples (Figs 2 and 3), which suggests different elimination rates of these metabolites. In human trials, the half-life ($T_{1/2}$) of $\text{Br}^{(-)}$ in serum was reported to be 7–13 days (Vaiseman et al., 1986). From Figs 2 and 3, $\text{Br}^{(-)}_{\text{cr}}$ in urine appears to have a longer ($T_{1/2}$) than AcPrCys$_{\text{cr}}$ based on the higher $\text{Br}^{(-)}_{\text{cr}}$ levels observed in pre-week specimens relative to concentrations throughout working days.

In Fig. 3, the peak AcPrCys$_{\text{cr}}$ concentrations measured post-shift decreased in the next day wake-up sample. This pattern is similar on the second exposure day except that it is slightly elevated from the day before. This figure implies that a more defined saturation plateau near the end of the workweek should occur, consistent with the rat data described by Valentine et al. (2007). Although pre- and post-shift specimens were not collected, all individual time interval samples were analyzed separately. Hence, the GM wake-up concentration may serve as an approximation of the next day, pre-shift concentration when adjusted for creatinine. The end-of-shift concentration most likely lies between

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![Fig. 2. Geometric mean concentrations of urinary bromide over time, adjusted for creatinine.](image)

![Fig. 3. Geometric mean concentrations of urinary AcPrCys over time, adjusted for creatinine.](image)
the work-shift and post-shift GM concentrations since the peak GM AcPrCys concentrations were measured in the post-shift samples on each day.

The urine collection protocol of this field study does not allow for a definitive determination of AcPrCys biological $T_{1/2}$ in urine. However, these data, when considered with the literature, may allow inferences to be drawn. Valentine et al. (2007) evaluated the rate of elimination of AcPrCys in rats and concluded that excretion of this metabolite showed a linear dose response which appeared biphasic. Based on the data provided in that paper, the biological $T_{1/2}$ of the initial rapid, primary elimination phase was $\sim$18-h; the $T_{1/2}$ of the second slower phase excretion was $\sim$8 days. It is recognized that glutathione-S conjugation with halogenated hydrocarbons produces mercapturic acids that typically have relatively short biological $T_{1/2}$ (Van Welie et al., 1992; De Rooij et al., 1998). Two reports addressed the rates of excretion of radiola-

Table 4. Equations and $P$-values for relationships between 24-h urinary AcPrCys or bromide adjusted concentrations* and mass with TWA BZ concentrations of 1-BP by job*

<table>
<thead>
<tr>
<th>Job</th>
<th>Parameter</th>
<th>24-h Equation</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive sprayers</td>
<td>Br concentration</td>
<td>$= -138.81 + 73.7782\ln(\text{TWA})$</td>
<td>0.0536</td>
</tr>
<tr>
<td></td>
<td>Br mass</td>
<td>$= 42.7951 + 34.4720\ln(\text{TWA})$</td>
<td>0.3889</td>
</tr>
<tr>
<td></td>
<td>$\ln(\text{AcPrCys concentration})$</td>
<td>$= 1.1499 + 0.5553\ln(\text{TWA})$</td>
<td>0.0283</td>
</tr>
<tr>
<td></td>
<td>$\ln(\text{AcPrCys mass})$</td>
<td>$= 1.4589 + 0.4761\ln(\text{TWA})$</td>
<td>0.0747</td>
</tr>
<tr>
<td>Non-spraying jobs</td>
<td>$\ln(\text{Br concentration})$</td>
<td>$= 3.1309 + 0.2756\ln(\text{TWA})$</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>$\ln(\text{Br mass})$</td>
<td>$= 3.3553 + 0.2237\ln(\text{TWA})$</td>
<td>0.0033</td>
</tr>
<tr>
<td></td>
<td>$\ln(\text{AcPrCys concentration})$</td>
<td>$= 0.4538 + 0.7731\ln(\text{TWA})$</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>$\ln(\text{AcPrCys mass})$</td>
<td>$= 0.6363 + 0.7391\ln(\text{TWA})$</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Concentrations were adjusted for creatinine.

*Statistical testing was performed over both days to compute $P$-values using PROC MIXED (SAS Institute, Cary, NC, USA).

*For adhesive sprayers, bromide data were normally distributed and AcPrCys were log-normally distributed; both bromide and AcPrCys were log-normal for non-sprayers. Appropriate models were applied to each group.

Fig. 4. Twenty-four hour urinary AcPrCys versus 1-BP TWA concentrations for workers exposed to spray adhesives, adjusted for creatinine.

beled 1-BP metabolites in urine of rats. Baines et al. (1977) found that 40–52% of the total dose was collected during the first 24-h post exposure, whereas Jones and Walsh (1979) measured 40% within 48-h. Moreover, the reduction of the GM AcPrCys concentration after work to the next day wake-up concentrations in our study (e.g. $\sim$24% for sprayers and 32% for non-sprayers per Fig. 3) suggests that the initial, primary $T_{1/2}$ measured in these workers is likely in the order of 1–3 days.

The GM concentration of AcPrCys from the pre-week specimens were over 100 and 20 times higher for sprayers and non-sprayers, respectively, than the GM concentration observed in unexposed subjects. These very high levels were measured following nearly 3 days without exposure. This may suggest that adipose tissue may serve as a storage depot for 1-BP similar to other low molecular weight, halogenated hydrocarbons such as vinyl chloride and
halothane (Wolff, 1976; Holaday, 1977; Gargus, 1989; Clewell et al., 1995; Reitz et al., 1996).

There are multiple pathways proposed for 1-BP metabolism primarily derived from animal studies; however, consensus regarding selection of a biomarker and analytical method for evaluating human exposure has not been established (Valentine et al., 2007). Garner et al. (2006) observed that 1-BP metabolic pathways differed between species; in rats, but not mice, the predominant pathway was shown to vary with dose. The authors reported that oxidation via cytochrome P-450 produced the principal metabolite, 1-bromo-2-hydroxypropanol, which is conjugated or metabolized further. When P-450 oxidation was inhibited (or saturated at high doses), AcPrCys became predominant from S-glutathione conjugation. In the Valentine et al. (2007) study, a dose response of 1-BP exposure with urinary AcPrCys in rats was established and post-shift urinary AcPrCys increased with increased worker exposure to 1-BP. The authors concluded that AcPrCys could be a biomarker of human exposure to 1-BP. Our study provides additional evidence that AcPrCys, in addition to Br\(^{-}\), can be an effective biomarker of workers’ exposures to 1-BP.

Statistical comparisons of AcPrCys with the Br\(^{-}\) data are presented in this manuscript. When jobs were stratified by sprayers and non-sprayers, statistical significance of urine metabolites with 1-BP TWA exposure, using PROC MIXED for repeated measures, was stronger for non-sprayers. The statistical significance of AcPrCys with 1-BP exposure was also greater than for Br\(^{-}\) with 1-BP TWA. For sprayers, only AcPrCys\(_{ct}\) concentrations were significant, although Br\(^{-}\)\(_{ct}\) concentrations were nearly significant (P = 0.0536). The lower statistical relationships for sprayers may be due to frequent but variable dermal contact with wet adhesives and the slower systemic distribution and metabolism via skin absorption.

Statistical testing was conducted on 24-h metabolite concentrations as well as for collection intervals with 1-BP TWA. This required grouping normally distributed data (i.e. Br\(^{-}\)\(_{ct}\) for sprayers) with log-normal data (i.e. all other data). Hence, Spearman’s correlation coefficients were used since these are not affected by log transformations. In this analysis, the coefficients were slightly stronger for Br\(^{-}\)\(_{ct}\) on 1-BP TWA than those for AcPrCys\(_{ct}\) on 1-BP TWA and all these coefficients were highly significant. The data were also grouped according to daily collection interval to determine if a particular collection time yielded the highest correlations with 1-BP TWA exposure. However, the time interval most predictive of 1-BP TWA was not apparent, presumably due to large collection intervals (e.g. 8–10-h), variable metabolic rates and excretion patterns, and mixed exposures between consecutive days.

EPA recently published a proposed rulemaking not to accept 1-BP in aerosols and spray adhesives as an alternative to ozone-depleting solvents based on the expectation that workers would have excessive exposure to 1-BP (EPA, 2007). Indeed, the study presented here has identified many workers’ exposures to grossly exceed exposure guidelines of 25 p.p.m., recommended by some solvent manufacturers and the ACGIH TLV\(^{\circ}\) of 10 p.p.m. Most of the sprayers’ exposures approached or were greater than an order of magnitude higher than the TLV\(^{\circ}\) and many non-sprayers’ exposures exceeded 10 p.p.m. This was due to spraying substantial quantities of adhesive, absence of local exhaust ventilation, and ineffective wall-mounted vane axial fans used for general ventilation. Although half-mask organic vapor respirators were available, the workers were not required to nor did they use these respirators. In addition, sprayers frequently used bare hands to pinch cushion corners and smooth edges causing substantial skin contact with adhesive. Protective gloves were not viewed as a viable solution since the glove would become glued into the cushion. Moreover, common industrial glove materials do not provide adequate protection against 1-BP permeation for extended periods. Viton\(^{	ext{TM}}\) and Silvershield/4H\(^{	ext{TM}}\) are reported to provide good skin protection (EnviroTech International, Inc., 2008) but these materials have not been tested with 1-BP, whereas polyvinyl alcohol and Barrier\(^{	ext{TM}}\) (PE/PA/PE) materials have been shown to effectively resist 1-BP permeation for >8-h (Forsberg and Mansdorf, 2007).

The purpose of the biological monitoring conducted in this study was to evaluate exposure to 1-BP and excretion of metabolites. It was not intended to determine health and toxicological outcomes. Although, the health significance of these elevated metabolite levels observed at these foam cushion factories is unclear, the recently reported case studies (Majersik et al., 2007; Raymond and Ford, 2007) regarding neurological toxicity in highly exposed foam cushion workers are cause for concern.

CONCLUSIONS

At these furniture cushion plants, extremely high exposures to 1-BP were measured without the benefit of effective control measures or personal protective equipment, particularly for workers using spray adhesives. Several non-sprayers’ and all the sprayers’ TWA BZ concentrations of 1-BP exceeded a proposed occupational exposure limit of 25 p.p.m.; most of the workers’ exposures exceeded the recommended ACGIH TLV\(^{\circ}\) of 10 p.p.m.

This study demonstrates that urinary elimination of AcPrCys, in addition to Br\(^{-}\), can be an effective biomarker of 1-BP metabolism. First, when several mercapturic acid conjugates were identified from human specimens, AcPrCys was detected in the highest
quantities. Second, the effectiveness of the metabolite was demonstrated by the consistent relative ranking for all measures (e.g. 1-BP in air, urinary Br\(^{-}\) and AcPrCys) when stratified by job exposure group. In accordance with air concentrations, urinary AcPrCys concentrations were substantially higher for sprayers than for non-sprayers. Moreover, the GM AcPrCys\(_{cr}\) were two orders of magnitude greater and nearly 25 times greater for the sprayers and non-sprayers, respectively, when compared to non-exposed controls. Third, Spearman’s coefficients of daily AcPrCys\(_{cr}\) concentration on 1-BP TWA were highly significant. Spearman’s coefficients showed urinary Br\(^{-}\) and AcPrCys\(_{cr}\) were also highly correlated. More importantly, using PROC MIXED (SAS Institute, Inc.), there were strong positive, statistically significant relationships for 1- and 2-day AcPrCys\(_{cr}\) and mass with BZ exposure to 1-BP in non-sprayers. For sprayers, AcPrCys\(_{cr}\) concentration was associated with 1-BP, but total AcPrCys mass was not significant. Consistent with the Br\(^{-}\)\(_{cr}\) findings reported by Hanley et al. (2006), the lower statistical association of AcPrCys and 1-BP for sprayers may be due to frequent, but variable skin contact and absorption of 1-BP.

Overall, Br\(^{-}\)\(_{cr}\) concentrations and mass we previously published were substantially greater than AcPrCys levels presented here. Although urinary Br\(^{-}\) is a useful index measured with a validated, commercially available method, AcPrCys has the advantage that it is a more sensitive analytical method with less potential interferences than Br\(^{-}\)\(_{cr}\). However, the HPLC ESI-MS methodology is more expensive requiring special equipment and knowledge.

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