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DNA damage in leukocytes of workers occupationally exposed to 1-bromopropane

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Abstract

1-Bromopropane (1-BP; *n*-propyl bromide) (CAS No. 106-94-5) is an alternative to ozone-depleting chlorofluorocarbons that has a variety of potential applications as a degreasing agent for metals and electronics, and as a solvent vehicle for spray adhesives. Its isomer, 2-brompropane (2-BP; isopropyl bromide) (CAS No. 75-26-3) impairs antioxidant cellular defenses, enhances lipid peroxidation, and causes DNA damage in vitro. The present study had two aims. The first was to assess DNA damage in human leukocytes exposed in vitro to 1- or 2-BP. DNA damage was also assessed in peripheral leukocytes from workers with occupational exposure to 1-BP. In the latter assessment, start-of- and end-of-work week blood and urine samples were collected from 41 and 22 workers at two facilities where 1-BP was used as a solvent for spray adhesives in foam cushion fabrication. Exposure to 1-BP was assessed from personal-breathing zone samples collected for 1–3 days up to 8 h per day for calculation of 8 h time weighted average (TWA) 1-BP concentrations. Bromide (Br) was measured in blood and urine as a biomarker of exposure. Overall, 1-BP TWA concentrations ranged from 0.2 to 271 parts per million (ppm) at facility A, and from 4 to 27 ppm at facility B. The highest exposures were to workers classified as sprayers. 1-BP TWA concentrations were statistically significantly correlated with blood and urine Br concentrations. The comet assay was used to estimate DNA damage. In vitro, 1- or 2-BP induced a statistically significant increase in DNA damage at 1 mM. In 1-BP exposed workers, start-of- and end-of-workweek comet endpoints were stratified based on job classification. There were no significant differences in DNA damage in leukocytes between workers classified as sprayers (high 1-BP exposure) and those classified as non-sprayers (low 1-BP exposure). At the facility with the high exposures, comparison of end-of-week values with start-of-week values using paired analysis revealed non-sprayers had significantly increased comet tail moments, and sprayers had significantly increased comet tail moment dispersion coefficients. A multivariate analysis included combining the data sets from both facilities, log transformation of 1-BP exposure indices, and the use of multiple linear regression models for each combination of DNA damage and exposure indices including exposure quartiles. The covariates were gender, age, smoking status, facility, and glutathione *S*-transferase M1 and T1 (GSTM1, GSTT1) polymorphisms. In the regression models, start-of-week comet tail moment in leukocytes was significantly associated with serum Br quartiles. End-of-week comet tail moment was significantly associated with 1-BP TWA quartiles, and serum Br quartiles. Gender, facility, and GSTM1 had a significant effect in one or more models. Additional associations were not identified from assessment of dispersion coefficients. In vitro and in vivo results provide limited evidence that 1-BP exposure may pose a small risk for increasing DNA damage.
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Keywords: Bromopropane; Comet assay; DNA damage; Leukocytes; Humans; GSTM1; GSTT1

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1. Introduction

1-Bromopropane (1-BP) (CAS No. 106-94-5) is an alternative to ozone-depleting chlorofluorocarbons [1] that has a variety of potential applications as a degreasing agent for metals and electronics, and as a solvent vehicle for spray adhesives. There is concern regarding its potential toxicity because of the outbreak of poisonings associated with occupational exposure to the isomer 2-bromopropane (2-BP) (CAS No. 75-26-3) [2,3]. Toxicity of 2-BP has been assessed in animal studies and adverse effects include neurotoxicity [4,5], hematopoietic effects [6], reproductive toxicity [4,7–9], immunotoxicity [10], genotoxicity [11], mutagenicity [12], and possible DNA adduct formation [13]. Toxicological evaluation of 1-BP is less extensive, but initial studies indicate 1-BP induces the same toxicological responses as 2-BP, albeit being somewhat less potent [14–20].

Because of concern over the potential health effects of 1-BP exposure, the National Institute for Occupational Safety and Health (NIOSH) conducted two separate Health Hazard Evaluations (HHE) at facilities where 1-BP was being used as a solvent for spray adhesive in foam cushion fabrication [21,22]. The primary objective of the evaluations was to assess workers for clinical health effects that may be associated with 1-BP exposure. 1-BP exposures were assessed by measuring personal-breathing zone 1-BP concentrations and urine and serum bromide (Br) concentrations. Workers were also invited to participate in the present study to assess DNA damage in peripheral leukocytes. Both 1-BP [23] and 2-BP [12] have been reported to be mutagenic in vitro in *Salmonella typhimirium*. 1-BP also has been reported to induce mutations in cultured mouse lymphoma cells [24]. Because of the difficulty in assessing mutagenicity in humans, genotoxicity was assessed in workers exposed to 1-BP using the alkaline single-cell gel electrophoresis or comet assay. The comet assay is a sensitive method for measuring DNA damage in individual cells following in vitro or in vivo exposures. In addition to assessing DNA damage in leukocytes from workers exposed to 1-BP, DNA damage was also measured in human leukocytes exposed in vitro to 1- or 2-BP.

2. Materials and methods

2.1. In vitro assessment of DNA damage in leukocytes

All in vitro experiments were conducted on heparinized venous blood from an unexposed, non-smoking, adult male volunteer. Freshly drawn blood was diluted 1:10 by adding complete RPMI 1640 (GIBCO BRL, Rockville, MD, USA) and dispensed into 1 ml aliquots in microfuge tubes. Experiments were conducted in indirect, incandescent light.

2.1.1. Dose-response

1- or 2-BP (Sigma–Aldrich, Saint Louis, MO) was diluted in DMSO and added to cells at a final concentration of 0, 0.01, 0.1, or 1 mM. The DMSO concentration in all cultures was 1%. Leukocytes were processed for the alkaline microgel electrophoresis technique or comet assay after an 8-h incubation period at 37 °C in a 5% carbon dioxide in air atmosphere. DNA damage was not assessed in the presence of metabolic activation because 1-BP was found to be equally mutagenic in *S. typhimurium* with or without S9 activation [23].

2.1.2. Temporal-response

1- or 2-BP was diluted in DMSO and added to cells in cultures at a final concentration of 0 or 1 mM. The DMSO concentration in all cultures was 1%. Cells were processed for the comet assay after 1, 2, 4, and 8 h of incubation at 37 °C in a 5% carbon dioxide in air atmosphere.

2.2. DNA damage in workers

2.2.1. Participants

A total of 64 workers (18 males and 46 females) at two facilities participated in the present study (Table 1). In 2001, NIOSH conducted Health Hazard Evaluations at the two facilities. Complete HHE reports for facility A (Marx Industries Inc., Sawmills, NC, USA) [22] and facility B (STN Cushion Company, Thomasville, NC, USA) [21] can be viewed at http://www.cdc.gov/niosh/hhe/. A subpopulation of the HHE participants consented to participate in the present study to evaluate the potential for their exposure to 1-BP to increase DNA damage in peripheral leukocytes. All participants provided informed consent prior to participation as authorized by the NIOSH and the University of Washington Human Subjects Review Boards. Employees at facility A participated in an evaluation in January 2001. Facility B employees participated in July 2001. The HHEs [21,22] demonstrated a stratified exposure to 1-BP at the two facilities. Workers whose job classification was sprayer at the time of the HHE had the highest 1-BP exposures. In the absence of a matched control population,

Table 1
Worker demographics

Gender	Facility A		Facility B	
	Male	Female	Male	Female
N	18	24	3	19
Age	20 ± 10	31 ± 12	25 ± 5	36 ± 5
Smokers	5	7	2	9
Sprayers	3	10	0	6
GSTM1 null	12	13	2	7
GSTT1 null	5	3	1	4

Values are number of workers except for age, which is the mean ± S.D. of the participants' ages in decades.

classification of workers into sprayers and non-sprayers was a simple means of comparing workers with exposure to high concentrations of 1-BP with those exposed to relatively low concentrations of 1-BP.

2.2.2. 1-BP exposure assessment

All participants in this study worked in facilities where 1-BP was used as the solvent vehicle for spray adhesives used in the manufacture of cushions for upholstered furniture. A subgroup of workers defined as "sprayers" operated spray guns and applied adhesives dissolved in 1-BP. All other participants defined as "non-sprayers" worked elsewhere in the facilities and were considered to be at lower risk of exposure to 1-BP.

Fifty of the 64 study participants agreed to wear a personal air monitor that collected samples from their breathing zone for 1–3 days. Air sampling was conducted using the NIOSH draft sampling and analytical method for 1- and 2-BP. With this method, air is drawn through a standard charcoal tube (SKC Anasorb® CSC Lot 2000) at a nominal flow rate of 50–250 ml/min. Personal-breathing zone samples were analyzed for 1-BP using gas chromatography with flame ionization detection [25]. The 8-h time weighted average (TWA) concentration (ppm) was calculated from the entire monitoring period.

2.2.3. Biomarkers of exposure

Blood and urine samples were collected from workers at the start of the work week on Monday morning and again at the end of the same work week on Thursday afternoon. Number of days worked by individuals ranged from 3 to 4 days. Because 1-BP loses Br ion during metabolism, urine and serum were analyzed for Br ion as an internal biomarker of 1-BP exposure. Urine and serum Br concentrations represent accumulated exposure over the previous week, as Br ion is excreted slowly [26].

2.3. DNA damage

2.3.1. Comet assay

The comet assay was performed as previously described [27]. Blood from workers at the facilities was drawn into hepranized vacutainers and shipped over night on ice to the NIOSH laboratory in Cincinnati, OH. Upon arrival, 100 μl blood for comet analysis was aliquoted into iced cryovials containing 1 ml of Hank's balanced salt solution supplemented with 20 mM EDTA and 10% DMSO, pH 7. Samples were then moved to −10 °C freezer for 1 h before being transferred to a −80 °C freezer. Frozen samples were shipped on dry ice to the University of Washington laboratory in Seattle, WA, where they were stored in liquid nitrogen vapor until thawed for comet analysis. Fifty microlitres of the thawed blood sample was mixed with 50 μl of 1% agarose (high resolution agarose 3:1 from Amresco, Solon, OH) in PBS. Fifty microlitres of this mixture was quickly placed onto a clear window frosted slide (MGE slides, Erie Scientific Co, New Haven, CT) already coated with a dry and wet first layer of microgel [28]. This first layer was prepared in two steps. First, 50 μl of 0.6% high resolution agarose was pipetted on top of the frosted part of slide, and the slide was air-dried. Second, 200 μl of agarose was pipetted onto the center of the slide and a cover glass (24 mm × 50 mm, Corning Glass Works, Corning, NY) was placed over it. After adding the cell-agarose mixture, a cover glass was placed on top to make a second layer of microgel. The slide was placed on an ice-cold plate for 1 min then the cover glass was removed and a third layer of microgel was made using 200 μl of 0.5% agarose in PBS. The slides were then immersed in lysing solution (pre-warmed to 37 °C) having 1.25 M NaCl, 50 mM tetra sodium salt of EDTA, 100 mM Tris, pH 10, 0.01% sodium salt of *N*-lauroyl sarcosine with freshly added 1 mg/ml proteinase K (Amresco, Solon, OH) and 2 mg/ml of glutathione (free acid reduced form) and incubated at 37 °C for 1 h. The slides were placed in a special electrophoresis unit (Ellard Instrumentation, Monroe, WA) with 1 l of 300 mM NaOH, 1 mM EDTA, 0.2% DMSO, and 0.01% 8-hydroxyquinoline (pH > 13.5). The DNA from the lysed cells in the microgels was allowed to unwind for 20 min in this alkaline solution and then electrophoresed at 12 V (0.4 V/cm), 250 mA for 20 min at room temperature in indirect, incandescent low light. During electrophoresis, the solution was recirculated at a rate of 100 ml/min. The slides were immersed in 50% ethanol having 1 mg/ml of spermine and 20 mM of Tris–HCl (pH 7.4) for 20 min at room temperature. This neutralization and DNA precipitation step was performed two more times using fresh solution. The slides were dried in air. The slides were stained with 50 μl of 0.25 μM YOYO in 2.5% DMSO and 0.5% sucrose.

One hundred leukocytes from each blood sample were analyzed using VisComet image analysis software (Impulse Bildanalyse GmbH, Gilching, Germany) and a CCD camera CV272 (JAI Corporation, Kanagava, Japan) attached to a DMLB epifluorescent microscope (Leica, Germany) with excitation at 490, dichroic at 500, and emission at 515 nanometers. Comet tail moment was calculated by multiplying tail distance in pixels by the fraction of DNA in the tail. The mean from analysis of 100 leukocytes was the sample measure used in subsequent statistical analysis.

2.3.2. Apoptosis

Apoptosis was assessed in leukocytes exposed to 1- or 2-BP using the DNA diffusion assay [29]. Leukocytes were mixed with agarose and a microgel was formed on a microscope slide precoated with 50 μl agarose. This second layer of agarose containing cells was covered with a third layer of 200 μl of 2% SFR agarose (Amresco, Solon, OH, USA). Slides were placed in a slide-holding tray and immersed for 10 min at room temperature in a plastic container filled with a freshly-made cold lysing solution (1.25 M NaCl, 1 mM tetrasodium EDTA, 5 mM Tris, 0.01% sodium lauroyl sarcosine, 0.2% DMSO, and 300 mM NaOH). Slides were then immersed for 10 min in 50% ethanol containing 1 mg/ml spermine and 20 mM Tris, pH 7.4. This neutralization and DNA precipitation step was performed two more times using fresh solution. Slides were air-dried and stained with the fluorescent dye YOYO. Apoptotic cells have alkali labile sites spaced at approximately 180

bases or multiples of 180 bases [28,29]. Under alkali conditions, apoptotic nuclear DNA produces a gradient pattern that has a dense center consisting of the largest molecules with less dense periphery of smaller DNA molecules. This pattern is unique to apoptotic cells. Necrotic nuclei do not contain DNA with uniquely spaced alkali labile sites and do not exhibit a gradient pattern. The percentage of cells with diffuse DNA was calculated from a total of 100 cells.

2.4. Genotyping

Genomic DNA was extracted from whole blood using a PureGene DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN, USA). Glutathione *S*-transferase M1 and T1 (GSTM1, GSTT1) were genotyped on independent duplicate DNA extracts using minor modifications of the polymerase chain reaction (PCR) method as previously described [30]. All genotyping results were concordant between duplicates. PCR analyses included both positive and null controls, as well as reaction blanks which contained no DNA.

2.5. Statistical analysis

Statgraphics statistical package (STSC Inc., Rockville, MD) was used for Student's *t*-test, paired *t*-test, and ANOVA. Discrimination among means evaluated by ANOVA was made using Fisher's least significant difference procedure after determining that variances were similar. SAS® (Version 8, SAS Institute, Cary, NC, USA) was used for Pearson correlation analysis and development of multiple linear regression models that were used to determine the adjusted relationships between the 1-BP exposure indices and DNA damage. The covariates included in the models were gender, age, smoking status, facility, and GSTM1 and GSTT1 polymorphisms. The exposure measures of 1-BP TWA concentration and serum and urine Br concentrations were transformed using the base 10 logarithm.

Quartiles for Br urine and serum concentrations were obtained by adding and ranking start-of-week and end-of-week values for each individual. Individuals with missing data were assigned to quartiles based on the ranking of available data. Using this approach, 8 participants were assigned to urine Br quartiles based on a single urine Br value, 6 participants were assigned to serum Br quartiles based on a single serum Br value, and 14 participants were assigned to 1-BP TWA quartiles based on their urine and serum Br concentrations. Relationships were considered statistically significant if $p < 0.05$.

3. Results

3.1. In vitro DNA damage

1- and 2-BP induced increased DNA damage in vitro in human leukocytes as indicated by increased comet tail moment (Fig. 1) [31]. Fig. 1 illustrates that significant increases in tail moment were evident at 1 mM 1- or 2-BP,

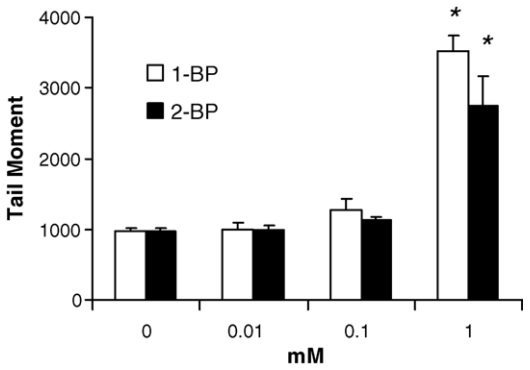

Fig. 1. DNA damage (comet tail moment) in human leukocytes induced in vitro by 8 h exposure to 1- or 2-BP. Bars are mean + standard deviation of three cultures. Comet tail moment was significantly increased by 1 mM 1- or 2-BP ($^{*}p < 0.05$, ANOVA).

which were equally effective in producing DNA damage. Fig. 2 illustrates the time dependency of the increase in DNA damage induced by 1- or 2-BP. Statistically significant increases were evident after 4 and 8 h of exposure to 1 mM 1- or 2-BP. Positive control studies (data not shown) were conducted with each electrophoresis run of in vitro and in vivo samples to ensure consistent performance of the comet assay. Results indicate that 0.5, 1, or 2 Gy of X-rays at a dose rate of 1 Gy/min consistently increased comet tail moment above controls by approximately 50, 100, and 150%, respectively.

3.2. Apoptosis

1- and 2-BP induced concentration dependent increases in the percentage of apoptotic cells on slides

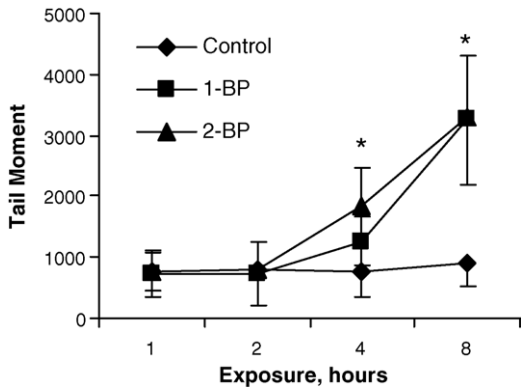

Fig. 2. DNA damage (comet tail moment) in human leukocytes induced in vitro by 1, 2, 4, or 8 h exposure to 0 or 1 mM 1- or 2-BP. Points are mean ± standard deviation of three cultures. Comet tail moments of 1- or 2-BP exposed leukocytes were statistically significantly greater than controls at 4 and 8 h ($^{*}p < 0.05$, ANOVA).

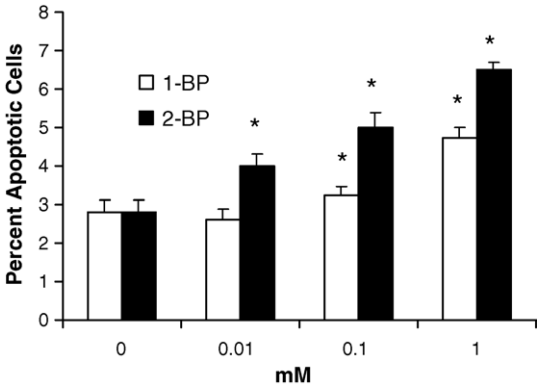

Fig. 3. Apoptosis in leukocytes exposed to 0, 0.01, 0.1, or 1 mM 1- or 2-BP. Bars are mean + standard deviation of three cultures. Compared to control cells, significant increases in the percentage of apoptotic cells were observed in cultures treated with 0.1 or 1 mM 1-BP, or with 0.01, 0.1, or 1 mM 2-BP. The percentage of apoptotic cells was significantly greater for 2-BP than for 1-PB at all concentrations examined ($p < 0.05$, ANOVA).

(Fig. 3). The lowest effective concentrations were 0.1 mM for 1-BP and 0.01 mM for 2-BP.

3.3. 1-BP exposure assessment

A summary of indices of 1-BP exposures are shown in Table 2. 1-BP TWA concentrations were highest for sprayers at facility A and were, on average, four-fold higher than sprayers at facility B. Workers classified as non-sprayers at both facilities had TWA exposures to 1- BP that were considerably lower than exposures experienced by sprayers. Similarly, urine and serum Br concentrations from all workers at facility A were an order of magnitude greater than correspondingly classified workers at facility B.

Pearson correlation analysis demonstrated a strong association between environmental concentrations of 1-BP, expressed as TWA ppm, and urine or serum Br concentration. The highest correlation was seen between 1-BP TWA and urine Br concentration (Table 3). High correlations were also evident between start-of-week and end-of-week urine and serum Br levels.

3.4. In vivo DNA damage

DNA damage in leukocytes was assessed by examining the mean tail moment of an individual sample and the corresponding dispersion coefficient. The dispersion coefficient is defined as the variance divided by the mean, and is recommended for the assessment of leukocytes from exposed humans to determine if exposures affect the distribution of comet values within individuals [32]. In the present analysis, variance for tail moment was determined from 100 leukocytes from each sample. Exposure assessment demonstrated that the workers involved in spraying adhesives had the highest 1-BP exposures in both facilities (Table 2). Furthermore, sprayer exposures at facility A were four-fold higher than exposures at facility B. Comparison of tail moments from peripheral leukocytes demonstrated that at both facilities sprayers had greater, but not significantly greater, comet tail moments. However, comet tail moment results differed dramatically at the two facilities in terms of the start-of-week, end-of-week comparison (Table 4). At facility A, comet tail moments increased during the week, while at facility B, comet tail moments decreased. The only statistically significant change was with non-sprayers at facility A where end-of-week tail moments were significantly greater than start-of-week tail moments. Tail moment dispersion coefficients increased significantly during the course of the week in sprayers at facility A (Table 4). This single significant effect is of note because it occurred in the subgroup of workers in the present study with the highest exposure to 1-BP (Table 2).

Table 5 summarizes results from linear regression models that included adjustments for gender, age, smoking status, facility, and two GST polymorphisms. Comparisons among start-of-week measurements revealed

Table 2
Environmental and internal 1-BP exposure indices

	Facility A		Facility B	
	Non-Sprayer	Sprayer	Non-Sprayer	Sprayer
N	29	13	16	6
1-BP TWA concentration (ppm)	2 ± 2	83 ± 85	5 ± 1	21 ± 5
Start-of week urine Br (mg/dl)	26 ± 14	122 ± 96	2 ± 1	6 ± 5
End-of-week urine Br, mg/dl	28 ± 9	238 ± 179	2 ± 2	10 ± 14
Start-of-week serum Br (mg/dl)	2.3 ± 0.8	14.9 ± 8.8	0.3 ± 0.1	0.8 ± 0.3
End-of-week serum Br (mg/dl)	2.6 ± 0.7	19.5 ± 11.4	0.3 ± 0.1	0.9 ± 0.3

Values are mean ± S.D.

Table 3
Association among environmental and biological exposure indices

	Start-of-week urine Br	End-of-week urine Br	Start-of-week serum Br	End-of-week serum Br
1-BP TWA	0.6188[a] <0.0001[b] 48[c]	0.9155 <0.0001 46	0.7452 <0.0001 48	0.8249 <0.0001 48
Start-of-week urine Br		0.7612 <0.0001 55	0.8383 <0.0001 57	0.9038 <0.0001 55
End-of-week urine Br			0.8026 <0.0001 57	0.8727 <0.0001 55
Start-of-week serum Br				0.9789 <0.0001 58

[a] Pearson correlation coefficient.
[b] *p*-value.
[c] Number of individuals included in analysis.

a statistically significant association between start-of-week tail moment and serum Br exposure quartiles. Despite the lack of statistical significance, all associations between exposure indices and start-of-week tail moment were positive (Table 5). End-of-week values for tail moment were significantly and positively associated 1-BP TWA quartiles and serum Br quartiles. All other associations between end-of-week comet values and exposure indices were positive with the exception of urine Br concentration. Covariates that had a significant effect in models were GSTM1, facility, and gender.

4. Discussion

4.1. Hypotheses

The present investigation extended the scope of two NIOSH HHEs [21,22] by assessing the association between 1-BP exposure indices and DNA damage in peripheral leukocytes of workers. The premise for this investigation and analysis was based upon one assumption and three hypotheses. The assumption was that worker exposure to 1-BP at the two facilities would be stratified to an extent that would allow assessment of the association between 1-BP exposure and DNA damage without relying on comparison with a matched control group not exposed to 1-BP. The hypotheses tested by the present analyses were: (1) that 1- and 2-BP would induce DNA damage in human leukocytes in vitro; (2) that increased 1-BP exposure in workers would be associated with increased levels of DNA damage in peripheral leukocytes; and (3) that DNA damage would be greater in 1-BP exposed workers at end-of-week/end-of-shift than at start-of-week/start-of-shift.

4.2. DNA damage in vitro

Wu et al. [11] reported that 2-BP induced DNA strand breaks in rat Leydig cells in vitro; effective concentrations were 0.1 and 1.0 mM. In the present study, 1 mM 1- or 2-BP was effective in inducing DNA damage. In addition to reporting 2-BP induced DNA damage, Wu et al. [11] also reported that 2-BP impaired antioxidant defenses. The delay in the appearance of 1- or 2-BP induced DNA damage until 4 h after exposure may reflect the possibility that DNA damage is secondary to depletion of antioxidants. Regardless of the mechanism, present results are consistent with previous studies demonstrating the potential for 2-BP to induced DNA damage. In the present study, 1-BP demonstrated a capacity comparable to 2-BP for inducing DNA damage in vitro.

4.3. Apoptosis

The in vitro concentrations of 1- or 2-BP that induce apoptosis are an order of magnitude lower than concentrations that induce DNA damage. Li et al. [33] also demonstrated the capacity for 2-BP to induce apoptosis in vivo. The greater sensitivity of leukocytes to apoptosis is most evident for 2-BP, which significantly increased the percentage of cells undergoing apoptosis at 0.01 mM, compared to 0.1 mM for 1-BP. The difference between 1- and 2-BP in terms of in vitro cytotoxicity in leukocytes is consistent with the increased hematopoietic toxicity of 2-

Table 4
Summary of comet analysis for 1-BP sprayers and non-sprayers

	Facility A		Facility B	
	Non-Sprayer	Sprayer	Non-Sprayer	Sprayer
N	29	13	16	6
Start-of-week tail moment	2517 ± 641	2867 ± 895	2856 ± 359	3430 ± 984
End-of-week tail moment	3080 ± 697[a]	3178 ± 762	2770 ± 504	2974 ± 280
Start-of-week tail moment dispersion coefficient	562 ± 354	496 ± 259	580 ± 243	596 ± 234
End-of-week tail moment dispersion coefficient	678 ± 422	752 ± 349[a]	653 ± 210	616 ± 165
GSTM1 null	603 ± 355	669 ± 300	633 ± 214	522 ± 98
GSTM1 positive	787 ± 504	886 ± 413	668 ± 220	709 ± 179

Values are mean ± S.D. GST1 rows are for end-of-week tail moment dispersion coefficients.
[a] End-of-week values are significantly greater than corresponding start-of-week values for same individuals (paired *t*-test, $p < 0.05$).

Table 5
Association between DNA damage and environmental and biological exposure indices

	Comet tail moment			
	Start-of-week		End-of-week	
	Mean[a]	Dispersion coefficient[b]	Mean	Dispersion coefficient
1-BP TWA log ppm	79.06[c] 0.654[d] 49[e]	−15.44 0.829 49	209.07 0.148 48 C[f]	75.62 0.386 48 A
Urine Br log mg/dl	555.86 0.075 56 B	32.31 0.815 56	393.69 0.108 53 C	−19.31 0.886 53
Serum Br log mg/dl	381.95 0.191 58	−116.06 0.365 58	370.78 0.171 57 C	80.53 0.591 57
1-BP TWA exposure quartiles	54.41 0.567 60	−46.91 0.252 60	209.59 0.016* 58 C	48.142 0.326 58
Urinary Br exposure quartiles	225.22 0.106 60 B	−33.60 0.582 60	196.33 0.141 58	61.32 0.405 58
Serum Br exposure quartiles	378.98 0.007* 60 B	7.17 0.909 60	269.37 0.049* 58 C	70.28 0.356 58

[a] Mean tail moment of 100 leukocytes from each participant.
[b] Dispersion coefficient = mean/variance of tail moment from 100 leukocytes.
[c] Slope of regression model.
[d] *p*-value for association between dependent and independent variables. *Indicates $p < 0.05$.
[e] Number of individuals included in analysis.
[f] Presence of letter indicates a significant affect ($p < 0.05$) of covariate in the regression model: A, GSTM1; B, facility; and C, gender.

BP reported in comparative studies [20]. The increased capacity for 1-or 2-BP to induce apoptosis compared to DNA damage would, in theory, limit the potential for mutation and possibly neoplasia by eliminating cells with excessive DNA damage.

4.4. Worker exposure to 1-BP

For the protection of stratospheric ozone, the US Environmental Protection Agency (EPA) [34] has published notice of approval of 1- BP for certain applications as an alternative for some ozone-depleting halogenated hydrocarbons. The US EPA recommends that users of 1-BP adhere to an exposure limit of 25 ppm over an 8-h TWA. NIOSH, the Occupational Safety and Health Administration (OSHA), and the American Conference of Governmental Industrial Hygienists (ACGIH) do have not finalized exposure evaluation criteria for 1-BP. Both facilities in the present study had workers with personal-breathing zone measurements that exceeded the EPA's recommended 25 ppm. Facility A exceeded this limit by three-fold, on average, for workers categorized as sprayers. Two workers at facility A had TWA exposures that were greater than 200 ppm, while 10 workers at this same facility had TWA exposures less than 1 ppm. Workers defined as sprayers had the highest exposures. The high exposures at facility A were attributed to the inability of the spray line exhaust fans to adequately capture the 1-BP vapors generated during spray adhesive operations [22]. Only two workers at facility B had TWA exposures greater than the recommended 25 ppm. Reduced 1-BP exposures at facility B at the time of assessment were attributed to improvements in local ventilation resulting from previous NIOSH recommendations made in 2000 [21]. The range of exposures at these two facilities provided an opportunity to assess the effects of 1-BP in the absence of matched control groups.

Three alternative approaches were taken to determine if there was an association between DNA damage in workers and their exposure to 1-BP at facilities where 1-BP was used in foam cushion fabrication. The first was to categorize workers by job title at each of the two facilities (Table 1). The second was to create regression models to determine if exposure indices were associated with DNA damage as assessed in peripheral leukocytes with the comet assay (Table 5). The third was to place the workers into quartiles based on the different exposure indices (TWA, serum Br, urine Br). Quartile analysis placed all workers into four exposure levels (low, medium low, medium high, high) with equal number of workers in each level.

4.5. Biomarkers of exposure

1-BP has been reported to result in adverse neurological health effects in workers exposed to TWA concentrations ranging from 60 to 261 ppm [35] and to 0.34–49.19 ppm [36]. These concentrations are comparable to those observed at the two facilities in the present study where the 8-h TWA concentrations of 1-BP ranged from 0.2 to 271 ppm at facility A, and from 4 to 27 ppm at facility B. At each facility, the highest exposures were experienced by sprayers. Sprayers at facility A had exposures that were, on average, four-fold higher than facility B. In the NIOSH HHE's [21,22], workers reported symptoms of headache, dizziness, and blurred vision that could have been related to 1-BP exposure, but findings were not definitive. Furthermore, there was no evidence that 1-BP exposure caused measurable nervous system problems, or alterations in blood counts.

In the absence of a control group for comparison, the range of 1-BP exposures provided an opportunity to determine if an association existed between 1-BP exposure and DNA damage. The evaluation was enhanced by the use of blood and urine Br concentrations as biomarkers of exposure to 1-BP because only 78% of the workers who provided blood and urine samples also wore personal-breathing zone monitors.

Regardless of whether personal environmental exposure levels were compared to start-of- or end-of-week urine or serum Br levels, correlation coefficients were statistically significant. However, the highest correlation was between 1-BP TWA concentration and end-of-week urine Br. Ichihara et al. [37] assessed the relationship between 1-BP TWA concentration and urinary 1-BP concentration. The correlation coefficient, while statistically significant, was only 0.4. In contrast, Kawai et al. [38] reported a correlation coefficient greater than 0.9 for the association between environmental 1-BP and urinary 1-BP. The correlation coefficient for environmental 1-BP and urinary Br was 0.5. Because Br can arise from both the diet [39] and exposure to 1-BP, urinary Br measurement lacks the specificity of urinary 1-BP. Nonetheless, in the present study, both serum and urine Br concentrations were highly correlated with environmental 1-BP levels. This demonstrates the utility of measuring biological Br levels to assess worker exposure to 1-BP.

4.6. Biomarkers of biologically effective dose—DNA damage

Initial assessment of the in vivo data indicated that there was not a consistent association between worker 1-BP exposure based on job category and DNA damage as

assessed by the comet assay. A clear association between DNA damage and 1-BP exposure would have included workers at facility A having greater DNA damage than workers at facility B, sprayers having greater DNA damage than non-sprayers, and end-of-week DNA damage being greater than start-of-week values. At both facilities, sprayers tended to have greater damage than non-sprayers. At facility A, end-of-week DNA damage levels were greater that start-of-week values and in some cases, the increases were significant. These findings have to be considered in light of the higher, though not statistically significantly higher, levels of DNA damage at facility B, which had lower 1-BP exposures, and the decrease in DNA damage between start-of-week and end-of-week assessments at facility B. The absence of consistent statistical significance and partially contrasting results between facilities A and B suggested that confounders may have been impacting the outcome. One such confounder is temporal variation in the comet assay. Positive control assays run with each lot of samples provide some assurance that the assay was performing consistently. However, unrecognized variation in sampling handling may contribute to variation in comet outcome. To address this and other possible confounders, multivariate analysis included the covariates of gender, age, smoking status, GST polymorphisms, and facility, which would encompass comet temporal variability as each facility was analyzed as a separate batch. For the 24 models listed in Table 5, only three exhibited statistically significant associations. All three included grouping workers into the exposure quartiles of 1-BP TWA, urinary Br, or serum Br. One possible reason for the increased statistical sensitivity of the quartiles was the inclusion of participants in an exposure quartile for which some exposure data was missing. For example 14 of the 64 participants did not wear environmental sampling pumps. Nonetheless, these workers could be ranked based on serum and urine Br levels, placed in an equivalent 1-BP TWA quartile, and then included in the statistical analysis. Using this approach, all participants could be included in the quartile regression models, which improved the power of the assessments over models that required discrete exposure data for each participant. Even under this scenario, there were only a limited number of significant associations between exposures indices and DNA damage. None of the models that examined associations between DNA damage and dispersion coefficients were statistically significant. This contrasts with the increased dispersion coefficient in sprayers from facility A at the end-of-week relative to start-of-week. Elevated dispersion coefficients would suggest comets in a sub-population of cells were affected by 1-BP. It may be that this sub-population is only detectable under a repeated measures assessment. This conclusion is supported by the observation that tail moment dispersion coefficients were increased at the end-of-week for all 1-BP exposed subgroups in Table 4. However, validation of this conclusion would require a demonstration that dispersion coefficients were not increased during the week in an unexposed control group which this study lacks. These results, thus, provide only limited evidence that 1-BP exposure may be inducing DNA damage.

Both gender and facility had a significant effect in several of the regression models when included as covariates. This is not surprising as there were marked differences between the two facilities in the exposure levels, and the highest exposures were experienced by sprayers who were predominately women. GSTM1 had a significant effect ($p < 0.05$) in one model and may have impacted several other associations between 1-BP exposure and DNA damage where p was >0.05 but <0.1 (data not shown). These results are not conclusive, but indicate that worker susceptibility to the effects of 1-BP could be influenced by the balance between activation and detoxification of 1-BP. In vitro work has demonstrated that 1-BP is a substrate of GST and depletes glutathione in rat hepatocytes [40] presumably through conjugation with a reactive intermediate of 1-BP [41]. The GSTM1 results indicate the presence of the gene is associated with a trend toward increased dispersion coefficients of tail moment (Table 4). A possible explanation for this is that depletion of glutathione would result from increased conjugation and could contribute to DNA damage via increased susceptibility to other sources of oxidative stress.

Leukocytes are frequently employed as surrogates of target organs. Under such circumstances, the absence of an apparent effect in a toxicological evaluation can be attributed to the surrogate status of the assessed organ. While the central nervous system is a primary target of 1- and 2-BP [42,43], blood cells have repeatedly been demonstrated to be sensitive as well [36]. Therefore, leukocytes may be a target organ for 1-BP. The capacity for 1-or 2-BP to induce DNA damage in leukocytes in vitro supports this contention. However, present results indicate that 1-BP exposure concentrations well above the recommended exposure limit of 25 ppm had limited affect on DNA in leukocytes in vivo. The maximum mean serum Br level observed in sprayers in facility A was 19.5 mg/dl, and was nearly two orders of magnitude greater than the 0.3 mg/dl observed in non-sprayers in facility B. These concentrations are approximately 2.4 and 0.04 mM, respectively. The 0.04 mM is comparable to that reported in controls where the only source of Br

is the diet [37,44]. Serum Br levels observed in sprayers are achievable by administering daily doses of 4 mg/kg Br [44]. In the present study, the assumption is made that the difference between non-sprayers and sprayers in serum Br concentration is attributable to 1-BP exposure. If each Br ion in serum of sprayers that is above that observed in non-sprayers is assumed to arise from 1-BP, then blood concentrations 1-BP attained in sprayers is comparable to the concentration (1 mM) of 1-BP that induced a marked increase in DNA damage in leukocytes in vitro. However, DNA damage in worker's leukocytes was not strongly associated with their exposure to 1-BP. One possible explanation is that 1-BP blood levels never achieved the 2.4 mM level suggested by the serum Br concentration. As a consequence, blood 1-BP levels may have only been high enough to induce marginal DNA damage. The comet assay is most effective in detecting short-lived damage from acute exposures. Worker exposures in the present study were chronic. While 1-BP may be inducing DNA damage in workers, it may also be inducing DNA repair; the result being a net effect in workers that is small or inconsistent. It is also possible that the study population size and variability in DNA damage levels among individuals may have partially masked an association between 1-BP exposure and DNA damage.

4.7. Summary

The participants in the present study were clearly stratified in terms of exposure to 1-BP. Exposure to 1-BP ranged from TWA concentrations of 0.2 ppm up to 270 ppm. Internal biomarkers of exposure were highly correlated with environmental exposure indices. Furthermore, biomarkers of exposure indicated that 1-BP exposures in the week(s) prior to the study were probably comparable to those during the study. This is demonstrated by the high correlation between start-of-week and end-of-week urine and serum Br concentrations. While the 1-BP was capable of inducing DNA damage in vitro, results of analysis of the in vivo data were inconsistent and provided only limited evidence that 1-BP increased DNA damage in workers in the two facilities. The most likely explanation for the disparity between in vitro and in vivo results is the high concentration (1 mM) of 1-BP required to produce unequivocal DNA damage in vitro. The marginal affect of GSTM1 on the relationship between 1-BP exposure and DNA damage is not definitive, but suggests that a better understanding of individual susceptibility to 1-BP would aid characterization of the association between 1-BP and DNA damage.

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Appendix A

See Tables A.1 and A.2.

Table A.1
Independent variables

Worker	Facility	Age decades	Gender	GSTM1	GSTT1	Smoker	Sprayer	1-BP TWA (ppm)
1	A	2	F	+	+	+	–	0.7
2	A	2	M	–	+	–	–	3.9
3	A	5	F	+	+	–	–	0.3
4	A	4	F	+	+	–	–	4.7
5	A	3	M	–	+	+	+	79.5
6	A	2	M	–	+	–	–	3.4
7	A	3	M	–	+	–	–	ND
8	A	4	M	–	–	–	–	3.0
9	A	4	F	+	+	–	–	1.1
10	A	2	M	–	+	–	–	10.4
11	A	2	M	–	–	–	–	1.0
12	A	2	F	+	+	–	+	77.0
13	A	2	F	–	+	–	+	207.6
14	A	3	F	–	+	–	–	0.5
15	A	5	F	+	+	+	–	0.2
16	A	3	M	–	+	+	–	1.3
17	A	2	F	–	+	–	+	19.4
18	A	2	M	+	+	–	–	0.5

Table A.1 (*Continued*)

Worker	Facility	Age decades	Gender	GSTM1	GSTT1	Smoker	Sprayer	1-BP TWA (ppm)
19	A	5	F	+	+	–	–	3.1
20	A	3	F	–	+	+	+	20.4
21	A	5	M	+	+	–	–	0.5
22	A	2	M	–	–	+	–	0.9
23	A	2	F	+	+	–	–	3.4
24	A	3	F	–	+	–	+	7.1
25	A	2	F	–	+	–	+	166.3
26	A	4	M	+	–	–	–	1.1
27	A	2	F	+	–	+	+	37.9
28	A	ND	F	–	+	ND	–	0.2
29	A	5	F	–	+	–	–	4.8
30	A	3	M	–	+	+	–	1.2
31	A	3	M	+	+	–	+	18.8
32	A	2	F	+	+	–	+	51.9
33	A	3	M	–	+	–	–	1.3
34	A	5	M	–	–	–	–	2.0
35	A	2	F	–	–	–	+	271.4
36	A	4	F	+	+	–	–	0.5
37	A	2	M	+	+	+	+	4.3
38	A	2	M	+	+	–	–	3.0
39	A	4	F	–	+	–	–	0.4
40	A	3	F	–	–	+	–	ND
41	A	2	F	–	+	+	+	116.0
42	A	4	F	–	+	+	–	1.1
43	B	2	F	–	+	+	–	ND
44	B	3	F	–	+	–	–	6.1
45	B	3	F	+	+	+	–	ND
46	B	2	M	+	+	+	–	ND
47	B	2	M	–	+	+	–	ND
48	B	2	F	+	+	+	–	4.8
49	B	3	F	+	+	+	–	4.4
50	B	2	F	+	+	–	–	ND
51	B	5	F	+	+	+	–	ND
52	B	4	F	+	+	+	–	ND
53	B	3	F	+	+	ND	–	ND
54	B	3	F	+	+	–	+	20.7
55	B	3	F	–	+	–	+	12.2
56	B	5	F	–	–	–	+	21.2
57	B	5	F	+	–	+	+	26.9
58	B	5	F	+	+	–	–	5.5
59	B	3	F	–	+	–	–	ND
60	B	3	F	–	+	–	+	23.3
61	B	2	M	–	–	–	–	ND
62	B	2	F	+	–	+	+	23.7
63	B	4	F	+	–	–	–	ND
64	B	2	F	–	+	+	–	ND

ND = no data.

Table A.2
Dependent variables

Worker	Start-of-week				End-of-week			
	Urine Br (mg/dl)	Serum Br (mg/dl)	Comet tail moment	Moment dispersion coefficient	Urine Br (mg/dl)	Serum Br (mg/dl)	Comet tail moment	Moment dispersion coefficient
1	9.1	1.4	3539	1186	34.6	3.3	1739	318
2	43.4	3.3	2056	161	40.8	3	2619	525

Table A.2 (*Continued*)

Worker	Start-of-week				End-of-week			
	Urine Br (mg/dl)	Serum Br (mg/dl)	Comet tail moment	Moment dispersion coefficient	Urine Br (mg/dl)	Serum Br (mg/dl)	Comet tail moment	Moment dispersion coefficient
3	43.7	2.3	2204	213	33.3	ND	ND	ND
4	25.5	2.3	1891	183	37.9	2.9	2408	373
5	19.2	10.1	1904	326	53.1	14.7	2835	630
6	5.3	1.7	2037	463	18.9	ND	2913	516
7	26.7	3.7	1922	210	15.4	2.8	4892	546
8	27	2.2	1694	472	33.9	2.7	3156	264
9	26	1.8	2033	424	32.9	2.3	2009	276
10	31.7	2.3	1804	639	27.1	2.7	2675	211
11	27.3	2.6	1831	203	28.6	2.2	3802	534
12	55.3	14.9	2176	305	204.3	16.9	4023	360
13	159.9	22.1	2253	181	507.9	26.6	2760	392
14	64	2.4	2118	291	34.5	2	4068	318
15	13.3	1.5	2603	262	35.4	2.1	2218	552
16	17.1	1.6	1972	253	28.7	2.3	3928	614
17	66.1	9	2190	170	46	9.3	2603	453
18	17	ND	2425	564	15.4	2	2408	566
19	28.6	2.2	3392	523	29.3	3.1	3773	670
20	39	7.3	2609	387	58.4	9.4	2598	569
21	22.9	1.4	2015	433	35.3	2	3190	849
22	13.6	2.9	2295	439	17.5	2.3	3341	1068
23	30.5	1.7	2337	619	18.6	2	2911	1971
24	59.7	5.7	4388	874	ND	5.6	3162	981
25	371.8	ND	3783	859	364.2	43.5	2570	518
26	19.4	1.9	3371	1638	35.8	3	2956	1124
27	174	13.3	3203	619	238.9	17.3	3794	1112
28	14	1.4	3509	760	21.4	1.7	2758	826
29	51.8	3.6	2981	564	34.4	4.6	3348	1630
30	31.1	2.1	3824	1310	18.4	2.4	3557	819
31	90.4	8.3	1734	239	109.8	10	5007	1398
32	225.5	20.7	2761	449	332	28	2129	592
33	25.9	3.6	3524	881	34.7	3.6	3693	623
34	8.4	4.4	3056	575	ND	3.9	3051	375
35	98.8	20.3	3730	610	595.4	27.8	3453	541
36	51.3	2.5	2538	430	42.2	2.4	2821	673
37	73.7	10.8	4181	882	150.9	11.1	3093	970
38	19.4	2.7	2173	779	16.2	2.6	3615	1280
39	17.2	2	2024	380	17.4	2	2536	394
40	24	2	2690	942	17.7	ND	ND	ND
41	149.4	36.7	2353	551	197.9	33.5	3281	1268
42	27	1.8	3122	493	33.6	2.5	2766	379
43	1.6	0.32	2514	1027	ND	0.4	2367	526
44	2.33	0.25	2682	589	2.65	0.28	3113	540
45	ND	0.44	ND	ND	4.77	0.39	ND	ND
46	2.64	0.33	2627	490	2.17	0.36	2825	642
47	ND	0.26	2581	440	2.24	0.25	2562	870
48	1.09	0.14	2893	321	1.56	0.2	2500	643
49	ND	0.33	2680	514	ND	0.37	3085	1020
50	1.82	0.26	3574	472	1.95	ND	ND	ND
51	1.69	0.28	2907	611	5.9	0.31	2557	499
52	0.9	0.15	2936	378	1.47	0.2	2182	325
53	0.65	0.14	2982	512	0.27	0.22	2389	553
54	2.76	0.76	2481	270	3.12	0.73	2671	913
55	5	0.57	5141	669	2.53	0.62	2816	435
56	14.47	0.94	3811	961	ND	1.1	3054	629
57	ND	1.3	3566	676	34.55	1.4	3486	638

Table A.2 (*Continued*)

Worker	Start-of-week				End-of-week			
	Urine Br (mg/dl)	Serum Br (mg/dl)	Comet tail moment	Moment dispersion coefficient	Urine Br (mg/dl)	Serum Br (mg/dl)	Comet tail moment	Moment dispersion coefficient
58	3.67	0.35	3503	515	3.33	0.31	4175	869
59	2	0.24	3090	1118	1.45	0.38	2987	669
60	3.68	0.49	2906	551	3.17	0.7	2909	504
61	1.04	0.2	2269	323	0.33	0.25	2374	324
62	3.72	0.7	2672	451	5.55	0.83	2909	577
63	ND	0.15	2741	810	3.52	0.21	2631	795
64	2.92	0.22	ND	ND	0.88	0.29	3031	870

ND = no data.

References

[1] T. Mirza, M. Gerin, D. Begin, D. Drolet, A study on the substitution of trichloroethylene as a spot remover in the textile industry, AIHAJ 61 (2000) 431–438.

[2] Y. Kim, K. Jung, T. Hwang, H. Kim, J. Park, J. Kim, J. Park, D. Park, S. Park, K. Choi, Y. Moon, Hematopoietic and reproductive hazards of Korean electronic workers exposed to solvents containing 2-bromopropane, Scand. J. Work Environ. Health 22 (1996) 387–391.

[3] J.-S. Park, Y. Kim, D.W. Park, S.K. Choi, S.-H. Park, Y.-H. Moon, An outbreak of hematopoietic and reproductive disorders due to solvents containing 2-bromopropane in an electronic factory South Korea: epidemiological survey, J. Occup. Health 39 (1997) 138–143.

[4] I.J. Yu, Y.H. Chung, C.H. Lim, S.H. Maeng, J.Y. Lee, H.Y. Kim, S.J. Lee, C.H. Kim, T.G. Kim, C.H. Lim, J.S. Park, Y.H. Moon, Reproductive toxicity of 2-bromopropane in Sprague–Dawley rats, Scand. J. Work Environ. Health 23 (1997) 281–288.

[5] X. Yu, G. Ichihara, J. Kitoh, Z. Xie, E. Shibata, M. Kamijima, N. Asaeda, N. Hisanaga, Y. Takeuchi, Effect of inhalation exposure to 2-bromopropane on the nervous system in rats, Toxicology 135 (1999) 87–93.

[6] T. Nakajima, S. Shimodaira, G. Ichihara, N. Asaeda, T. Kumazawa, H. Iwai, I. Ichikawa, M. Kamijma, X. Yu, Z. Xie, H. Kondo, Y. Takeuchi, 2-Bromopropane-induced hypoplasia of bone marrow in male rats, J. Occup. Health 39 (1997) 228–233.

[7] M. Omura, Y. Romero, M. Zhao, N. Inoue, Histopathological changes of the testis in rats caused by subcutaneous injection of 2-bromopropane, J. Occup. Health 39 (1997) 234–239.

[8] X.-D. Wu, J.-M. Yang, X.-Y. Wu, X.-C. Ding, B. Pang, X.-Z. Jiang, Z.-S. Ji, K. Shin, The effects of 2-bromopropane on viability and testosterone production ability of rat Leydig cells in primary culture, Biomed. Environ. Sci. 12 (1999) 43–49.

[9] X. Yu, M. Kamijima, G. Ichihara, W. Li, J. Kitoh, Z. Xie, E. Shibata, N. Hisanaga, Y. Takeuchi, 2-Bromopropane causes ovarian dysfunction by damaging primordial follicles and their oocytes in female rats, Toxicol. Appl. Pharm. 159 (1999) 185–193.

[10] T.C. Jeong, E.-S. Lee, W. Chae, W.S. Koh, B.-H. Kang, S.S. Han, Immunotoxic effects of 2-bromopropane in male Sprague–Dawley rats: a 28-day exposure study, J. Toxicol. Envrion. Health, Part A 65 (2002) 383–394.

[11] X. Wu, A.S. Faqi, J. Yang, B.P. Pang, X. Ding, X. Jiang, I. Chahoud, 2-Bromopropane induces DNA damage, impairs functional antioxidant cellular defenses, and enhances the lipid peroxidation process in primary cultures of rat Leydig cells, Reprod. Toxicol. 16 (2002) 379–384.

[12] S.H. Maeng, I.J. Yu, Mutagenicity of 2-bromopropane, Ind. Health 35 (1997) 87–95.

[13] L.-X. Zhao, E.K. Kim, H.-T. Lim, Y.-S. Moon, N.H. Kim, T.-H. Kim, H. Choi, W. Chae, T.C. Jeong, E.-S. Lee, Synthesis, characterization and in vitro identification of N^7-guanine adduct of 2-brompropane, Arch. Pharm. Res. 25 (2002) 39–44.

[14] G. Ichihara, J. Kitoh, Y. Xiaozhong, A. Nobuyuki, H. Iwai, T. Kumazawa, E. Shibata, T. Yamada, H. Wang, Z. Xie, Y. Takeuchi, 1-Bromopropane, an alternative to ozone layer depleting solvents, is dose-dependently neurotoxic to rats in long-term inhalation exposure, Toxicol. Sci. 55 (2000) 116–123.

[15] G. Ichihara, X. Yu, J. Kitoh, N. Asaeda, T. Kumazama, H. Iwai, E. Shibata, T. YamadaT, H. Wang, Z. Xie, K. Maeda, H. Tsukamura, Y. Takeuchi, Reproductive toxicity of 1-bromopropane, a newly introduced alternative to ozone layer depleting solvents, in male rats, Toxicol. Sci. 54 (2000) 416–423.

[16] H.-Y. Kim, Y.-H. Chung, J.-W. Jeong, Y.-M. Lee, G.-S. Sur, J.-K. Kang, Acute and repeated inhalation toxicity of 1-bromopropane in SD rats, J. Occup. Health 41 (1999) 121–128.

[17] A. Ohnishi, T. Ishidao, T. Kasai, Arashidani, H. Hori, Neurotoxicity of 1-bromopropane in rats, J. UOEH 21 (1999) 23–28.

[18] W. Zhao, K. Aoki, T. Xie, J. Misumi, Electrophysiological changes induced by different doses of 1-bromopropane and 2-bromopropane, J. Occup. Health 41 (1999) 1–7.

[19] X. Yu, G. Ichihara, J. Kitoh, Z. Xie, E. Shibata, M. Kamijima, N. Asaeda, Y. Takeuchi, Preliminary report on the neurotoxicity of 1-bromopropane, an alternative solvent for chlorofluorocarbons, J. Occup. Health 40 (1998) 234–235.

[20] X. Yu, G. Ichihara, J. Kitoh, Z. Xie, E. Shibata, M. Kamijima, Y. Takeuchi, Neurotoxicity of 2-bromopropane and 1-bromopropane, alternative solvents for chlorofluorocarbons, Environ. Res. 85 (2001) 48–52.

[21] National Institute for Occupational Safety and Health, Health Hazard Evaluation Report No 2000-0410-2891: STN Cushion Company, Thomasville, NC. Cincinnati OH: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, 2002. http://www.cdc.gov/niosh/hhe/reports/pdfs/2000-0410-2891.pdf.

[22] National Institute for Occupational Safety and Health, Health Hazard Evaluation Report No 99-0260-2906: Marx Industries Inc., Sawmills, NC. Cincinnati OH: United States Department of Health and Human Services, Public Health Service, Centers for

Disease Control and Prevention, 2003. http://www.cdc.gov/niosh/hhe/reports/pdfs/1999-0260-2906.pdf.

[23] E.D. Barber, W.H. Donish, K.R. Mueller, A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames Salmonella/microsome assay, Mutat. Res. 90 (1981) 31–48.

[24] National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, Monograph on the Potential Reproductive and Developmental Effects of 1-Bromopropane. United States Department of Health and Human Services, National Institute of Health Publication No. 04-4479, 2003.

[25] National Institute for Occupational Safety and Health, Halogenated Hydrocarbons; Method #1003, in: NIOSH Manual of Analytical Methods, fourth ed., P.M. Eller, M.E. Cassinelli (Eds.), United States Department of Health and Human Services (NIOSH) Publication No. 94-113, 2003. http://www.cdc.gov/niosh/nmam/pdfs/1003.pdf.

[26] N. Vaiseman, G. Koren, P. Pencharz, Pharmacokinetics of oral and intravenous bromide in normal volunteers, J. Toxicol. Clin. Toxicol. 24 (1986) 403–413.

[27] N.P. Singh, P.E. Penn, W.R. Pendergrass, N.S. Wolf, White light-mediated DNA strand breaks in lens epithelial cells, Exp. Eye Res. 75 (2002) 555–560.

[28] N.P. Singh, Microgels for estimation of DNA strand breaks DNA protein crosslinks and apoptosis, Mutat. Res. 455 (2000) 119–135.

[29] N.P. Singh, A simple method for accurate estimation of apoptotic cells, Exp. Cell Res. 256 (2000) 328–337.

[30] A. Hirvonen, S.T. Saarikoski, K. Linnainmaa, K. Koskinen, K. Husgafvel-Pursiainen, K. Mattson, H. Vainio, Glutathione S-transferase and *N*-acetyltransferase genotypes and asbestos-associated pulmonary disorders, J. Natl. Cancer Inst. 88 (1996) 1853–1856.

[31] N.P. Singh, C.H. Muller, R.E. Berger, Effects of age on DNA double-strand breaks and apoptosis in human sperm, Fertility Sterility 80 (2003) 1420–1430.

[32] R.J. Albertini, D. Anderson, G.R. Douglas, L. Hagmar, K. Hemminki, F. Merlo, A.T. Natarajan, H. Norppa, D.E. Shuker, R. Tice, M.D. Waters, A. Aitio, IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans International Programme on Chemical Safety, Mutat. Res. 463 (2000) 111–172.

[33] G.X. Li, K.S. Kang, Y.S. Lee, 2-Bromopropane induced germ cell apoptosis during spermatogenesis in male rat, J. Vet. Med. Sci. 63 (2001) 373–382.

[34] Environmental Protection Agency, Protection of Stratospheric Ozone: Listing of Substitutes for Ozone-Depleting Substances—*n*-propyl Bromide: Proposed Rule, United States Federal Register 2003, June 3, 40 CFR Part 82.

[35] G. Ichihara, J.K. Miller, A. Ziolkokwska, S. Itohara, Y. Takeuchi, Neurological disorders in three workers exposed to 1-bromopropane, J. Occup. Health 44 (2002) 1–7.

[36] G. Ichihara, W. Li, E. Shibata, X. Ding, H. Wang, Y. Liang, S. Peng, S. Itohara, M. Kamijima, Q. Fan, Y. Zhang, E. Zhong, X. Wu, W.M. Valentine, Y. Takeuchi, Neurologic abnormalities in workers of a 1-bromopropane factory, Environ. Health Perspect. 112 (2004) 1319–1325.

[37] G. Ichihara, W. Li, X. Ding, S. Peng, X. Yu, E. Shibata, T. Yamada, H. Wang, S. Itohara, S. Kanno, K. Sakai, H. Ito, K. Kanefusa, Y. Takeuchi, A survey on exposure level, health status, and biomarkers in workers exposed to 1-bromopropane, Am. J. Ind. Med. 45 (2004) 63–75.

[38] T. Kawai, A. Takeuchi, Y. Miyama, K. Sakamto, Z.-W. Zhang, K. Higashikawa, M. Ikeda, Biological monitoring of occupational exposure to 1-bromopropane by means of urinalysis for 1-brompropane and bromide ion, Biomarkers 6 (2001) 303–312.

[39] Z.W. Zhang, T. Kawai, A. Takeuchi, Y. Miyama, K. Sakamoto, T. Watanabe, N. Matsuda-Inoguchi, S. Shimbo, K. Higashikawa, M. Ikeda, Urinary bromide levels probably dependent to intake of foods such as sea algae, Arch. Environ. Contam. Toxicol. 40 (2001) 579–584.

[40] S. Khan, P.J. O'Brien, 1-Bromoalkanes as new potent nontoxic glutathione depletors in isolated rat hepatocytes, Biochem. Biophys. Res. Commun. 30 (1991) 436–441.

[41] B.M. De Rooij, J.N.M. Commandeur, N.P.E. Vermeulen, Mercapturic acids as biomarkers of exposure to electrophilic chemicals: applications to environmental and industrial chemicals, Biomarkers 3 (1998) 239–303.

[42] H. Wang, G. Ichihara, H. Ito, K. Kato, J. Kitoh, T. Yamada, X.X. Yu, S. Tsuboi, Y. Moriyama, R. Sakatani, E. Shibata, M. Kamijima, S. Itohara, Y. Takeuchi, Biochemical changes in the central nervous system of rats exposed to 1-bromopropane for seven days, Toxicol. Sci. 67 (2002) 114–120.

[43] H. Wang, G. Ichihara, H. Ito, K. Kato, J. Kitoh, T. Yamada, X. Yu, S. Tsuboi, Y. Moriyama, Y. Takeuchi, Dose-dependent biochemical changes in rat central nervous system after 12-week exposure to 1-bromopropane, Neurotoxicology 24 (2003) 199–206.

[44] F.X. Rolaf van Leeuwen, B. Sangster, The toxicology of bromide ion, CRC Crit. Rev. Toxicol. 18 (1987) 189–213.