Exposure limits of bromopropanes

Korean researchers proposed an exposure limit of 2-bromopropane of 1 ppm, based on the animal data and epidemiological data. Kim et al. (1996) and Park et al. (1997) showed convincing evidence of adverse effects of 2-bromopropane on the reproductive and hematopoietic functions of workers, but the assessment of the real exposure level was not easy. The ambient exposure level was 12.4±3.13 ppm (range 9.2–16.0 ppm), but the intermittent peak exposure up to 4,140.7 ppm, or absorption through skin, was also deemed to contribute to overall exposure and cause adverse effects. An ambient exposure level of ≥12.4 ppm might be the level at which adverse effects are likely to appear. The survey on a Chinese 2-bromopropane factory suggested possible adverse effects on hematopoiesis at exposures of less than 10 ppm, based on the association between time-weighted average concentration of 2-bromopropane and hematological indices of individual workers. However, we should be careful in interpreting this association because the exposure level was determined only once; thus, the exposure level was not necessarily representative of the long-term exposure level that would be more related to the hematological indices, if any. Thus, as for 2-bromopropane, we may be able to set the exposure limit based on the epidemiological data, although they are limited.

On the other hand, for 1-bromopropane, there are few epidemiological data that allow one to make a concrete recommendation. Severe cases showed features of the possible neurotoxicity of 1-bromopropane. However, estimation of exposure level was more problematic. Several animal studies showed a clear dose–response relationship and 200 ppm of lowest observed adverse effect level (LOAEL), but we do not have sufficient mechanism data to enable us to extrapolate from animals to humans.

Conclusions

Animal studies have shown that 2-bromopropane targets spermatogonia in the testis, oocytes in the primordial follicle, and hematopoietic cells in the bone marrow. 2-Bromopropane is more mutagenic than 1-bromopropane, but the latter is more neurotoxic. 1-Bromopropane also exhibits reproductive toxicity, but the target is different from that of 2-bromopropane. Exposure to 1-bromopropane inhibits the spermiation process, suggesting Sertoli cell dysfunction, and the development of ovarian follicles. A few suspected human cases of bromopropane intoxication have been reported. The affected individuals showed various signs or symptoms related to the nervous system, such as a decrease in the sense of vibration and perception, paresthesia in the lower extremities, decreased sensation in the ventral aspects of the thighs and the gluteal region, stumbling, and headache, as well as those related to the urinary (e.g. incontinence) and reproductive systems. The dose–response relationship of bromopropanes in humans and the mechanisms underlying the differences in the toxic effects of the two bromopropanes remain to be determined.

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Neurologic Abnormalities in Workers of a 1-Bromopropane Factory

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We reported recently that 1-bromopropane (1-BP; *n*-propylbromide, CAS Registry no. 106-94-5), an alternative to ozone-depleting solvents, is neurotoxic and exhibits reproductive toxicity in rats. The four most recent case reports suggested possible neurotoxicity of 1-BP in workers. The aim of the present study was to establish the neurologic effects of 1-BP in workers and examine the relationship with exposure levels. We surveyed 27 female workers in a 1-BP production factory and compared 23 of them with 23 age-matched workers in a beer factory as controls. The workers were interviewed and examined by neurologic, electrophysiologic, hematologic, biochemical, neurobehavioral, and postural sway tests. 1-BP exposure levels were estimated with passive samplers. Tests with a tuning fork showed diminished vibration sensation of the foot in 15 workers exposed to 1-BP but in none of the controls. 1-BP factory workers showed significantly longer distal latency in the tibial nerve than did the controls but no significant changes in motor nerve conduction velocity. Workers also displayed lower values in sensory nerve conduction velocity in the sural nerve, backward recalled digits, Benton visual memory test scores, pursuit aiming test scores, and five items of the Profile of Mood States (POMS) test (tension, depression, anxiety, fatigue, and confusion) compared with controls matched for age and education. Workers hired after May 1999, who were exposed to 1-BP only (workers hired before 1999 could have also been exposed to 2-BP), showed similar changes in vibration sense, distal latency, Benton test scores, and depression and fatigue in the POMS test. Time-weighted average exposure levels in the workers were 0.34–49.19 ppm. Exposure to 1-BP could adversely affect peripheral nerves or/and the central nervous system. *Key words:* 1-bromopropane, distal latency, nerve conduction velocity, neurobehavioral testing, neurotoxicity, ozone-depleting solvents, postural sway testing, reproductive toxicity, vibration sense. *Environ Health Perspect* 112:1319–1325 (2004). doi:10.1289/ehp.6995 available via *http://dx.doi.org/* [Online 30 June 2004]

Ozone-depleting solvents, such as specific chlorofluorocarbons and 1,1,1-trichloroethane, have been banned since 1996 in developed countries. Because they were used in large amounts in various industries, alternative compounds were introduced to the workplace. One such alternative compound is 1-bromopropane (1-BP; *n*-propylbromide, CAS Registry no. 106-94-5), which is used in the United States and Japan as a cleaning agent for metals, precision instruments, electronics, optical instruments, and ceramics (Ichihara, in press). It is also used in spray form as an adhesive in the United States (Ichihara et al. 2002). Environ Tech (2001) estimated the total amount of 1-BP commercially available for sale in the United States in the year 2000 was 1,967.9 metric tons (4,338,583 lb), which is comparable to 9.0, 31.0, and 10.6% of the amount of methylene chloride, perchloroethylene, and trichloroethylene used in adhesive/foam fabrication and metal cleaning in the same year in the United States. In Japan, the amount of 1-BP sold in 2003 was 1,125 metric tons, which is about double the 645 metric tons sold in 1998 (Association of Bromopropane Producers of Japan, unpublished data). In addition, in the workplace where cases of neurotoxicity had been reported, 1-BP was introduced as an alternative for methylene chloride (Ichihara et al. 2002). The benefits of using 1-BP instead of the chlorinated carbons are not clear. However, under pressure to regulate the use of chlorocarbons, 1-BP has been used as a surrogate, which is encouraged by the lack of measures to define the exposure limits. In this regard, previous animal studies revealed neurotoxicity and reproductive toxicity of 1-BP (Ichihara et al. 2000a, 2000b; Wang et al. 2002, 2003; Yamada et al. 2003; Yu et al. 1998, 2001). Exposure to 1-BP resulted in a dose-dependent limb muscle weakness and reduction of nerve conduction in rats (Ichihara et al. 2000a). It also resulted in myelin degeneration of peripheral nerves and swelling of preterminal axons in the medulla oblongata (Ichihara et al. 2000a). It was also revealed that 1-BP exhibits reproductive toxicity in both male and female rats (Ichihara et al. 2000b; Yamada et al. 2003). Thus, animal studies preceded human studies and warned about the potential neurotoxicity and reproductive toxicity of 1-BP in humans. The most recently reported cases also confirmed the neurotoxicity of 1-BP in humans (Ichihara et al. 2002; Sclar 1999). However, these case reports have limitations in terms of quantitative analysis. In 1999 we investigated a 1-BP factory, but this investigation was also limited because it was originally oriented to study the effects of 2-bromopropane (2-BP), which targets mainly reproductive and hematopoietic systems (Ichihara et al. 2004).

The aim of the present study was to assess the neurologic function and other health-related changes in workers exposed to 1-BP and compare the results with those of control workers in a beer factory.

Materials and Methods

Factories and workers. The subjects were female workers of a 1-BP production factory located in Yixing, Jiangsu Province, China. The survey was conducted 16–18 January 2001. The same factory mainly produced 2-BP in 1996 (Ichihara et al. 1999), but shifted the main production to 1-BP between 1996 and 1999 (Ichihara et al. 2004), and the product was only 1-BP at the time of the present survey. 1-BP was synthesized by incubating *n*-propranolol and hydrogen bromide under concentrated sulfuric acid. The product was purified by distillation and temporarily stored in ceramic containers. The crude product was then transferred to 20-L plastic vessels through hose pipe from the cock of the container and subsequently neutralized with hydrogen carbonate. The product was finally transferred to 1,000-L drums for storage and transport. The

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workers were at risk of exposure to 1-BP when *a*) placing the chemicals into the reaction pots; *b*) sitting close to the reaction pots to observe and record the temperature; *c*) taking out the crude product; *d*) adding the hydrogen carbonate and stirring; and *e*) pouring the product into the drums. In the final step, the workers added the product with hand scoops to adjust the product volume in the drum.

The surveyed factory has two similar-sized manufacturing plants, each measuring 9.7 × 24.4 × 7 m (width × depth × height). In each plant, a ventilating fan was ineffectively installed 6 m from the floor; no local ventilation fan was installed in the vicinity of the areas where workers might be exposed to 1-BP. The 27 surveyed workers who were engaged in the production of 1-BP in the factory were all female. As controls, we selected age-matched (± 2 years) females at random from 202 female workers in a beer factory in the same city. The control workers lived in the same area.

In the analysis of paired *t*-tests between 1-BP workers and controls, four 1-BP workers were excluded because no corresponding match of control workers from the beer factory could be recruited. However, the analysis by exposure level or period of exposure included those 1-BP female workers for whom no corresponding age-matched controls could be recruited. All workers who were hired after 1991 and for whom corresponding age-matched controls could be recruited were identified as 1991 workers. Among them, the workers who were hired after 1999 and were exposed only to 1-BP were defined as 1999 workers.

Medical examination. Signed informed consent was obtained from each worker for all examinations and interviews, according to the Declaration of Helsinki (World Medical Association 2002). All female workers in the 1-BP factory and the 23 age-matched beer-factory workers were clinically examined by a trained Chinese neurologist who was conducting medical research at the Department of Neurology, Nagoya University, Japan, and had a good command of both Chinese and Japanese languages.

The vibration sensation was evaluated using a vibrating tuning fork (128 Hz); the fork was placed on the dorsum of the metatarsophalangeal joint of the big toe or the dorsum of the metacarpophalangeal joint of the thumb, and the worker was asked to report when the vibration ceased. Immediately after reporting, the tuning fork was moved to the same site (big toe or thumb) of the examiner and the duration of the lasting vibration after the worker's report was recorded. It was difficult to assess the actual time when the delay time was < 2 sec, because it took some time (but < 2 sec) to move the tuning fork from the worker's body to the examiner's body. In addition, one worker reported total loss of vibration sense in the right toe. Therefore, the value could not be treated as a continuous value in the statistical analysis. The examiner was a trained female (38-year-old) neurologist who worked with every worker throughout the investigation.

Electrophysiologic studies. We conducted electrophysiologic studies in an air-conditioned room maintained at 24°C. The workers were acclimated to the room temperature for 30 min before the electrophysiologic studies. We examined distal latency (DL), motor nerve conduction velocity (MCV), F-wave conduction velocity (FWCV), and sensory nerve conduction velocity (SNCV). Electric stimulation and recordings were performed with a Neuropack evoked potential/electromyogram measurement system (model MEB5508; Nihon Kohden, Co., Tokyo, Japan). For measurement of DL and MCV, the stimulation site was just behind the medial malleolus (distal) and the center of popples (proximal), and the recording site was fixed 11 cm distal to the distal stimulation site on the abductor hallucis muscle.

Blood tests. The following blood tests were performed in each worker: red blood cell (RBC) count, hemoglobin, hematocrit, white blood cell (WBC) count, and platelet count, using a hematocell counter (Coulter JT, Coulter Electronics, Hialeah, FL, USA), as well as fructosamine (colorimetric method), blood urea nitrogen [urease ultraviolet (UV) method], creatinine (enzyme method), total protein (Biuret method), total cholesterol (enzyme method), creatine kinase (UV *N*-acetylcysteine method), aspartate aminotransferase (UV method), alanine aminotransferase (UV method), γ-glutamyl transferase (L-γ-glutamyl-3-carboxy-4-nitroanilide substrate method), lactate dehydrogenase (Wroblewski-LaDue method), alkaline phosphatase (*p*-nitrophenol substrate method), serum creatinine (alkaline picric acid method), vitamin B_1 (HPLC method), iron [2-nitroso-5-(*N*-propyl-*N*-sulfopropylamino)phenol method], ferritin, thyroid-stimulating hormone [radioimmunoassay (RIA)], luteinizing hormone (LH; RIA), follicle-stimulating hormone (FSH; RIA), and estradiol (RIA).

Neurobehavioral tests and postural sway test. Neurobehavioral testing [simple reaction time, digit span, Santa Ana, digit symbol, Benton, pursuit aiming test, Profile of Mood States (POMS)] was conducted based on the Chinese edition of the World Health Organization Neurobehavioral Core Test Battery (Chen 1988; Liang 1987) by trained Chinese researchers. Because neurobehavioral tests can be influenced by education level, we also conducted analyses with controls matched for age and education level. Postural balance was measured with a Gravicorder GS-30 stabilometer (Anima Co., Tokyo, Japan). The same instrument was used in all subjects throughout the investigation. Postural sway testing was performed as described previously (Yamamoto et al. 2001; Yokoyama et al. 1997). Briefly, the subject was asked to stand with big toes touching each other on the platform of the Gravicorder. The center of gravity was recorded every 50 msec with both eyes open for 1 min and closed for 1 min. The calculated values based on the center of gravity were *a*) the total length of excursion; *b*) envelope area; *c*) length of excursion per envelope area; *d*) rectangular area, representing the product of the range of the x-component (lateral) and that of the y-component (anteroposterior); *e*) root mean square area; *f*) the mean of *x*-axis or *y*-axis component of each recorded point; *g*) the center of range of the *x*-axis or *y*-axis component of points; *h*) power spectrum of the *x*-axis or *y*-axis at 0.02–0.2 Hz, 0.2–2.0 Hz, and 2.0–10.0 Hz, obtained by frequency analysis, with both eyes open and closed; and *i*) the Romberg quotient, representing the ratio of values measured with eyes closed to the value with eyes open for items *a* through *h*.

Assessment of exposure to 1-BP. Individual exposure levels during work shifts were evaluated with passive samplers (Sibata Scientific Technology Ltd., Tokyo, Japan) using the method described previously by Ichihara et al. (2004). A passive sampler was attached to each worker during one 8-hr shift and was collected immediately after the shift and kept in separate sealed bags at 4°C until analysis. The absorbed solvent in the sampler was analyzed 2 weeks after the investigation. In our previous study (Ichihara et al. 2004), we confirmed the stability of absorbed 1-BP in charcoal at 4°C for 2 weeks. For analysis, activated charcoal particles were taken from the samplers and then immersed in 2 mL carbon disulfide (Wako Pure Chemicals, Osaka, Japan) in a glass tube with a screw cap. The tube was shaken vigorously for 5 min and left to stand

Table 1. Characteristics of workers.

Characteristic	1-BP exposed (*n* = 23)	Control (*n* = 23)
Age (years)	36.2 ± 5.7[a]	36.2 ± 5.2
Height (cm)	160.3 ± 6.6[a]	158.8 ± 5.9
Education		
Elementary school	4	4
Junior high school	19	12
High school	0	6
University	0	1
Job duration (months)	27 ± 31	168 ± 67
Past job exposure to chemicals	0	4[b]
Previous medical condition	2[c]	8[d]

Data for age, height, and job duration are mean ± SD. Other values are numbers of workers.
[a]Not significantly different from the controls (paired *t*-test). [b]Includes formalin (2), ammonia (1), alkaline (1). [c]Includes cholecystitis (1), contraceptive use (1). [d]Includes anemia (2), gastritis (2), hysteromyoma (2), oophoritic cyst (1), cholecystitis (1), taking antihypertensive medications (1).

for 1 hr; the supernatant was then injected into a gas chromatograph equipped with an electron ionization detector (GCD system G1800A, Hewlett Packard, Palo Alto, CA, USA). The concentration of 1-BP was quantified by the selected ion mode. The detection limit was 0.007 ppm by this method. The time-weighted average (TWA) was calculated based on the formula

$$\text{TWA} = \frac{\text{absorbed solvent } (\mu g)}{\text{sampling rate}\left[\frac{\mu g}{(\text{ppm} \times \text{min})}\right] \times \text{sampling time (min)}}$$

In our calculations, we used the value of 0.134 as the sampling rate of 1-BP. The value was determined by the diffusing cell method.

Statistical analysis. We used the paired *t*-test to compare continuous parameters of the exposure group and controls matched for age or age and education level. In this analysis, all indices of electrophysiologic studies, neurobehavioral tests, POMS test, stabilometer testing, and blood tests were compared with the age-matched controls, and the indices of neurobehavioral tests and POMS were also compared with controls matched for age and education level. We used the Wilcoxon test and Fisher's exact test to compare the delay time and abnormality of menstrual cycles, respectively, of the exposure group and the age-matched controls. In the analysis by exposure levels, the 27 exposed workers were classified into two groups: ≤ 2.64 ppm (n = 17) and ≥ 8.84 ppm (n = 7); data missing (n = 3). For analysis by length of exposure, the 27 exposed workers were again classified into two groups: ≤ 9.31 months (n = 10) and ≥ 16.33 months (n = 16); data missing (n = 1). We selected these cutoff values because they divided the two peak distributions when the histograms with column width of 2.5 ppm and 5 months, respectively, were drawn, whereas no values were found between 2.64 and 8.84 ppm and 9.31 and 16.33 months. In comparisons between groups stratified with fructosamine [≤ 246 μmol/L (n = 14) and 248–284 μmol/L (n = 13)] or vitamin levels [20–30 ng/mL (n = 13) and ≥ 31 ng/mL (n = 13); data missing (n = 1)], the groups were divided according to the median because there was no split in the distribution that formed two peak distributions. The *t*-test was applied when comparing continuous variables (electrophysiologic tests, neurobehavioral tests, POMS test, stabilometer tests, and blood tests) by exposure levels or length of exposure as well as the levels of fructosamine or vitamin B_1. For the analysis of delay time and frequency of menstrual cycles, we used Wilcoxon test and Fisher's exact test, respectively, for comparison according to exposure levels, length of exposure, and the level of fructosamine or vitamin B_1. We defined significance as the probability of $p < 0.05$.

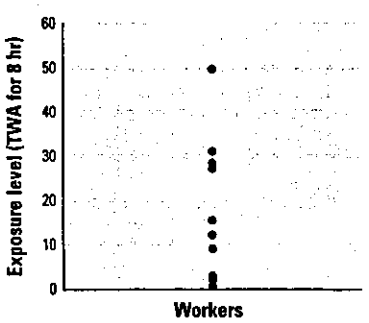

Figure 1. Exposure levels of each worker in a 1-BP factory (TWA for 8-hr shift). Values were obtained with passive samplers from workers who had age-matched controls (n = 23). Maximum = 49.19; minimum = 0.34; median = 1.61; geometric mean = 2.92 ppm.

Results

There were no differences in age and height between 1-BP workers and the age-matched controls (Table 1). The control group had a higher education level than the exposure group. Job duration of the exposure group was shorter than for the controls, probably because the area where the workers lived had been developed quite recently, so they had engaged in agriculture before employment in the factory. Four workers in the beer factory (controls) had been exposed to various chemicals (formalin, n = 2; ammonia, n = 1; alkaline reagent, n = 1) in occupational settings before their present jobs. None of the workers investigated was a smoker, and only one exposed worker and one control worker were alcohol drinkers. None of the workers investigated had a history of diabetes mellitus, which could cause polyneuropathy. Individual exposure levels ranged from 0.34 to 49.2 ppm (median, 1.61 ppm; geometric mean, 2.92; Figure 1).

Bromopropane workers, all of whom were hired after 1991 (1991 workers), had significantly longer DL and lower SNCV than did the age-matched controls (Table 2). Because the main product in the factory had shifted from 2-BP to 1-BP between 1996 and May 1999 (Ichihara et al. 2004), we also analyzed data for 1999 workers to examine the effects of exposure to 1-BP only. Examination of these workers showed the only significant change to be an increase in the DL compared with age-matched controls. However, the extent of the change in any electrophysiologic parameter in the 1999 workers tended, in general, to be similar to that of the 1991 workers. Reduced vibration sensation as tested on the right toe, left toe, right finger, and left finger was detected in 15, 13, 4, and 4 female workers, respectively (Tables 3 and 4). One worker showed complete loss of vibration sense on the right toe by tuning fork stimulation. The exposure level for this worker was 1.10 ppm, and she had a relatively high DL (8.8 msec) and low MCV (43.1 m/sec), FWCV (53.7 m/sec), and SNCV (38.8 m/sec). In contrast, none of the age-matched beer workers showed any abnormalities in vibration sensation in the toe and finger. The Wilcoxon test showed significant differences in the delay time bilaterally both in the feet and in the fingers between 1991 workers and controls. Analysis of 1999 workers also showed significant prolongation of the delay time on the toes bilaterally but not in the fingers. The percentage of 1999 workers who showed reduced vibration sensation (delay time ≥ 2 sec) on both sides of the foot and in the fingers was similar to that of 1991 workers.

Neurobehavioral tests showed lower values for the forward and backward digit span, Benton visual memory test, pursuit aiming test, POMS test (scores for tension, depression, anxiety, fatigue, and confusion) in the 1-BP workers than in the controls (Tables 5 and 6). Because the education level of 1-BP workers was different from that of the age-matched controls and because the education level could affect the results of neurobehavioral tests, these tests were analyzed after matching both education level and age (Tables 5 and 6). 1-BP workers had lower levels of backward digit span; correct scores in the Benton visual memory test; completed response in the pursuit aiming test; and tension, depression, anxiety, fatigue, and confusion in the POMS test than did controls matched for age and education level. Further analysis was conducted for these neurobehavioral tests on 1999 workers (Tables 5 and 6). Significant differences with the controls were found only in the Benton visual memory test and in POMS depression and fatigue.

The postural sway tests showed significantly lower power spectrum of the x-axis at 2.0–1.0 Hz with eyes open and y-axis at 0.02–0.2 Hz with eyes closed and significantly

Table 2. Electrophysiologic indices of workers exposed to 1-BP and of the controls.

	1991 workers	Age-matched controls for 1991	1999 workers	Age-matched controls for 1999
No. of pairs	23		12	
DL of nervus tibialis (msec)	8.05 ± 2.17*	5.96 ± 1.38	8.36 ± 2.38*	6.06 ± 1.43
MCV of nervus tibialis (m/sec)	49.8 ± 10.3	49.9 ± 8.2	51.3 ± 12.0	51.7 ± 10.7
FWCV of nervus tibialis (m/sec)	52.8 ± 3.5	55.1 ± 3.2	51.8 ± 2.8	55.0 ± 2.9
SNCV of nervus suralis (m/sec)	39.2 ± 3.5*	46.2 ± 6.6	39.2 ± 2.6	47.5 ± 8.5

Data are mean ± SD.
*$p < 0.05$ compared with age-matched controls (paired *t*-test).

higher power spectrum of the *y*-axis at 0.2–2.0 Hz with eyes closed (Table 7) in the 1999 workers than in the age-matched controls, but other parameters were not significantly different between the two groups (Table 7; Romberg quotients for all items, which also did not show any statistical difference, are not shown). The comparison of the 1999 workers and age-matched controls did not show any significant differences in postural sway tests (Table 7; Romberg quotients not shown).

Laboratory tests did not show any significant differences between the 1991 workers and age-matched controls (data not shown) except for significantly lower levels of vitamin B_1 (31.0 ± 5.6 vs. 34.3 ± 5.4 ng/mL) and low WBC count (5.7 ± 1.7 × 10^3/μL vs. 6.7 ± 1.8 × 10^3/μL) in the 1991 workers than in age-matched controls. For 1999 workers, only the WBC count was significantly lower than in the age-matched controls. In only one worker (42 years of age) in the control group, the fructosamine level (286 μmol/L) was above the upper limit of reference value (205–285 μmol/L). This worker had rather high DL (8.24 msec) and low levels of MCV (42.5 m/sec), FWCV (49.8 m/sec), and SNCV (39.5 m/sec) but did not show abnormal vibration sensation. This worker was not included in the education-matched testing because she had no education-matched individual in the exposure group. Comparison between the two groups stratified by fructosamine levels within all exposed workers (n = 27) showed significant differences only in higher levels of total protein (8.22 ± 0.53 g/dL), total cholesterol (197.7 ± 32.1 mg/dL), choline esterase (ChE; 366.1 ± 86.7 IU/L), LH (14.3 ± 14.3 IU/L), WBC (6.62 ± 5.15 × 10^3/μL), RBC (4.19 ± 0.38 × 10^6/μL), POMS confusion (5.31 ± 4.35), and lower estradiol level (35.4 ± 25.1 pg/mL) in the high-fructosamine group compared with the low-fructosamine group (total protein, 7.64 ± 0.24 g/dL; LH, 4.2 ± 3.4 IU/L; total cholesterol, 166.5 ± 28.7 mg/dL; ChE, 288.4 ± 37.8 IU/L; WBC, 5.16 ± 0.97 × 10^3/μL; RBC, 3.84 ± 0.38 × 10^6/μL; POMS confusion, 2.36 ± 1.91; estradiol, 63.2 ± 38.3 pg/mL).

Fisher's exact test did not show any difference between the 1991 and 1999 worker groups and their corresponding age-matched control groups with regard to the frequency of menstrual abnormalities after starting working in the 1-BP factory. Two workers in the exposure group had a short menstrual cycle. Similarly, one worker in the control group had a short menstrual cycle, and another reported a prolonged period of menstrual bleeding.

On the other hand, a comparison based on the exposure levels (≤ 2.64 or ≥ 8.84 ppm) showed that workers with high exposure levels showed significantly high values of MCV (56.4 ± 12.9 m/sec), FWCV (54.7 ± 2.8 m/sec), hematocrit (0.393 ± 0.032), and POMS tension (5.14 ± 1.77) and lower values of FSH (9.0 ± 6.3 mIU/mL) and POMS vigor (18.6 ± 2.5), compared with the low-exposure group (MCV, 47.3 ± 8.3 m/sec; FWCV, 52.0 ± 1.9 m/sec; hematocrit, 0.356 ± 0.034; POMS tension, 2.73 ± 1.49; FSH, 27.7 ± 35.3 mIU/mL; POMS vigor, 24.3 ± 4.0) but did not show any significant association with other examined indices. In the comparison by the length of exposure (≤ 9.31 or ≥ 16.33 months), the longer-exposure group had high levels of LH (13.5 ± 13.7 mIU/mL) and FSH (34.9 ± 34.9 mIU/mL) and lower levels of total protein (7.77 ± 0.30 g/dL) and vitamin B_1 (29.2 ± 5.1 ng/mL) than did the shorter-exposure group (LH, 3.3 ± 1.8 mIU/mL; FSH, 5.5 ± 2.1 mIU/mL; total protein, 8.18 ± 0.66 g/dL; vitamin B_1, 33.2 ± 5.0 ng/mL) but did not show any significant association with other examined indices.

Because the mean concentration of vitamin B_1 was significantly lower in the exposure group than in the controls, the values were compared between the two groups stratified by vitamin B_1 level within all exposed workers (n = 27). The comparison did not reveal any difference in the frequency of low vibration sensation or results of electrophysiologic tests, apart from lower levels of alkaline phosphatase (ALP; 129.3 ± 30.7 IU/L) and ChE (293.8 ± 52.8 IU/L) in the low vitamin group than high vitamin group (ALP, 169.5 ± 43.1 IU/L; ChE, 361.8 ± 84.1 IU/L).

Discussion

In the tested factory, isopropanol, hydrogen bromide, and sulfuric acid were also used as materials in the process of producing 1-BP. These chemicals are not considered to have neurotoxic effects, so it is unlikely that the low vibration sensation or change in DL is due to these chemicals. In the last survey of the same factory (Ichihara et al. 2004), we found that the main product of this factory was shifted from 2-BP to 1-BP. 1991 Workers include the workers who were hired before May 1999 and might have been exposed to not only 1-BP but also 2-BP before 1999 (Ichihara et al. 1999). In contrast, 1999 workers were exposed to 1-BP only. Therefore, the observed changes in the DL, vibration sense in both feet bilaterally, Benton visual memory test score, and depression and fatigue in the POMS test that were noted in 1999 workers are considered to be due to exposure to 1-BP. However, the effects of 2-BP cannot be excluded in 1991 workers. The SNCV showed significant changes in the analysis of 1991 workers but not in 1999 workers with age-matched controls. This is most likely due to the lack of power as a result of the reduction in the number of subjects, given the fact that the extent of change in sensory nerve conduction, as well as other electrophysiologic parameters, and the percentage of workers who showed reduced vibration sense among 1999 workers was similar to that of 1991 workers. This explanation might also be valid for other parameters that showed significant change in 1991 workers but not in 1999 workers.

Table 3. Number of workers with reduced vibration sensation in the foot.

	1991 workers and age-matched controls (n = 23 pairs)				1999 workers and age-matched controls (n = 12 pairs)			
Delay time[a]	Right foot*		Left foot*		Right foot*		Left foot*	
(sec)	1-BP workers	Controls	1-BP workers	Controls	1-BP workers	Controls	1-BP workers	Controls
< 2	8	23	10	23	5	12	5	12
2	0	0	1	0	0	0	1	0
3	3	0	1	0	1	0	1	0
4	2	0	4	0	1	0	1	0
5	2	0	1	0	1	0	0	0
6	4	0	4	0	3	0	2	0
8	3	0	1	0	1	0	1	0
10	0	0	1	0	0	0	1	0
∞[b]	1	0	0	0	0	0	0	0

[a]Delay time for vibration sensation by tuning fork stimulation (see "Materials and Methods" for details); time 0 is the time when the worker reported becoming unaware of the vibration. [b]One worker felt no vibration sense in the right foot. *p < 0.05, Wilcoxon test.

Table 4. Number of workers with reduced vibration sensation in the finger.

	1991 workers and age-matched controls (n = 23 pairs)				1999 workers and age-matched controls (n = 12 pairs)			
Delay time[a]	Right finger*		Left finger*		Right finger		Left finger	
(sec)	1-BP workers	Controls	1-BP workers	Controls	1-BP workers	Controls	1-BP workers	Controls
< 2	19	23	19	23	10	12	10	12
2	3	0	2	0	2	0	2	0
3	1	0	2	0	1	0	0	0

[a]Delay time for vibration sensation by tuning fork stimulation (see "Materials and Methods" for details); time 0 is the time when the worker reported becoming unaware of the vibration. *p < 0.05, Wilcoxon test.

Our animal studies (Ichihara et al. 2000a; Yu et al. 1998) preceded human case reports in revealing the neurotoxicity of 1-BP, which is far more potent than that of 2-BP (Yu et al. 1999, 2001). However, the results of animal studies had certain limitations in predicting symptoms or signs in human cases; for example, animal studies cannot detect any subjective symptoms that might reflect abnormalities of sensation or the central nervous system. It is sometimes difficult especially for morphologic studies to substantiate the adverse effects on the central nervous system because the structure of the central nervous system is far more robust than that of peripheral nerves or other organs. It is also difficult to evaluate imbalance during walking in rodents because four-footed animals are completely different from bipedal humans regarding the clinical signs of imbalance. Thus, information from human cases should help us understand the toxicologic targets of 1-BP. The first case was reported by Sclar (1999), and three other cases were recently reported by our group (Ichihara et al. 2002). All four cases showed diminished vibration sensation in the toe. Moreover, the present study showed that more than half of the workers exposed to 1-BP suffered from reduced vibration sensation. Considered together, these results suggest that vibration sensation in the toe might be susceptible to exposure with 1-BP. The previously reported cases also complained of urinary incontinence; numbness in the perineum, low back, and front of the thighs or buttocks; or headache (Ichihara et al. 2002); however, our factory workers did not report any such symptoms. This difference might depend on the levels or period of exposure to 1-BP because it is possible that our workers adapted to low levels but longer periods of exposure, leading to unawareness of symptoms.

In comparisons with age-matched controls, both the 1991 workers and 1999 workers showed prolonged DL but no change in MCV. This prolongation of DL without decrease in MCV parallels the results of animal studies, which showed earlier changes in DL than MCV in the tail nerve (Ichihara et al. 2000a). Such a pattern of changes might indicate predominant deterioration of the distal portion of the peripheral nerve or delay in chemical transmission between nerve terminals and muscle.

Comparison of data of 1991 workers with age-matched controls showed that the exposure group had lower levels of forward and backward digit span, Benton scores, pursuit aiming test scores, and POMS tension, depression, anxiety, fatigue, and confusion than did the controls. Because education level could influence the results of neurobehavioral tests, the results of the tests were reanalyzed after matching age and education levels. This reanalysis also revealed changes in the above items excluding forward digit span. When the analysis was limited to the 1999 workers, significant differences were found only in Benton visual memory test scores, POMS depression, and POMS fatigue, which could reflect the lack of power due to the small sample number. Digit span, pursuit aiming test, and the POMS test are considered the most sensitive indicators of exposure to organic solvents or neurotoxic agents such as lead (Zhou et al. 2002). Poorer performance in the POMS test was also observed in a Venezuelan study of workers exposed to organic solvents (Escalona et al. 1995). The present results of neurobehavioral tests could also suggest that 1-BP adversely affects the central nervous system in humans. Postural sway tests showed higher power of the *y*-axis (anterior–posterior sway) at 0.2–2.0 Hz and lower at 0.02–0.2 Hz with eyes closed, although such significant differences were not observed in 1999 workers. These results might be important because the cases found in the United States also showed unstable balance in walking. Clinically, patients with cerebellar disease and anterior lobe atrophy show anteroposterior sway, often with a spontaneous high-frequency body tremor of around 3 Hz (Diener et al. 1984). This anteroposterior sway might resemble the present result of the increase in the power of the *y*-axis at 0.2–2 Hz. However, the results of the postural sway tests noted in our study await further confirmation because the presence of cerebellar disorder in the formerly reported cases or present workers is not conclusive, and it is possible to attribute the unstable balance to a disorder of the peripheral nerves or spinal cord.

Diabetes mellitus could be a common confounding factor related to neurologic disorders by solvent intoxication. HbA1C and fructosamine are used as long-term (Bunn et al. 1976) and intermediate-term (1–3 weeks) (Baker et al. 1983) indicators of glucose levels in clinical settings. In the present study, we measured serum fructosamine levels. For the measurement of HbA1C, the blood samples had to be kept at 4°C but not frozen. However, the long transportation from the factory site to the laboratory could have potentially caused

Table 5. Results of neurobehavioral tests in the 1-BP group and controls matched for age or for age and education (mean ± SD).

Test	1991 workers (age-matched controls)	1991 workers (age/education-matched controls)	1999 workers (age/education-matched controls)
No. (pairs)	22	12	6
Simple reaction time (sec)	0.38 ± 0.12 (0.36 ± 0.12)	0.38 ± 0.12 (0.36 ± 0.12)	0.40 ± 0.14 (0.39 ± 0.12)
Digit span (digits recalled) forward	10.6 ± 2.3* (11.7 ± 1.4)	10.8 ± 2.5 (11.8 ± 1.3)	10.2 ± 3.1 (12.0 ± 1.1)
Digit span (digits recalled) backward	4.5 ± 2.2* (5.8 ± 1.8)	5.0 ± 2.6* (5.6 ± 1.4)	4.2 ± 2.3 (6.2 ± 1.6)
Santa Ana preferred hand	35.2 ± 3.6 (36.6 ± 4.8)	35.3 ± 4.0 (36.1 ± 4.3)	36.0 ± 2.4 (35.8 ± 5.2)
Santa Ana nonpreferred hand	33.5 ± 4.6 (32.8 ± 5.1)	33.8 ± 5.2 (33.7 ± 5.6)	32.8 ± 4.4 (35.5 ± 5.9)
Digit symbol (no. completed)	47.0 ± 17.5 (54.0 ± 10.2)	48.6 ± 19.8 (55.5 ± 5.6)	45.3 ± 21.9 (56.7 ± 6.7)
Benton (no. correct)	7.2 ± 1.7* (8.3 ± 1.4)	7.8 ± 1.5* (8.2 ± 1.3)	7.3 ± 1.8* (8.3 ± 1.0)
Pursuit aiming test (no. completed)	103.1 ± 16.9* (119.9 ± 19.1)	101.6 ± 17.9* (119.3 ± 20.4)	98.0 ± 11.4 (125.7 ± 17.0)

*$p < 0.05$, paired *t*-test.

Table 6. Results of POMS tests in the 1-BP group and controls matched for age or for age and education (mean ± SD).

Test	1991 workers (age-matched controls)	1991 workers (age/education-matched controls)	1999 workers (age/education-matched controls)
No. (pairs)	20	12	6
Profile of mood state			
Tension	4.4 ± 3.9* (7.7 ± 7.1)	4.1 ± 5.2* (10.2 ± 8.5)	6.8 ± 7.0 (9.6 ± 7.2)
Depression	4.8 ± 7.5* (10.5 ± 13.0)	5.6 ± 10.1* (13.3 ± 17.1)	10.0 ± 14.4* (12.8 ± 16.0)
Anxiety	4.1 ± 5.0* (10.2 ± 10.2)	4.7 ± 6.3* (12.6 ± 13.2)	7.0 ± 9.0 (13.4 ± 12.7)
Vigor	22.2 ± 4.3 (20.7 ± 6.7)	23.7 ± 3.9 (20.9 ± 6.9)	23.4 ± 4.5 (21.4 ± 9.2)
Fatigue	3.1 ± 2.6* (6.4 ± 4.0)	3.0 ± 3.4* (6.7 ± 5.2)	4.4 ± 4.8* (7.2 ± 3.8)
Confusion	3.7 ± 3.7* (7.1 ± 4.3)	3.3 ± 4.4* (7.7 ± 5.6)	5.0 ± 6.2 (5.6 ± 5.0)

*$p < 0.05$, paired *t*-test.

hemolysis of the collected blood and thus may have resulted in marked variability and errors in estimations. For this reason, HbA1C was not measured in the present study. The comparison between the exposed group and the controls did not show any difference in the level of fructosamine, and the comparison between the high-fructosamine group and low-fructosamine group within the exposed group also did not show any difference in indices related to the nervous system.

The levels of vitamin B_1 were lower in the entire exposure group than in the controls and in the longer-exposure group compared with the shorter-exposure group. Lack of vitamin B_1 is known to cause polyneuropathy, but the relatively low level of vitamin B_1 in the 1-BP factory workers could not fully explain the neurologic abnormalities. First, the level of vitamin B_1 in the exposed workers ranged from 20 to 43 ng/mL, which was within the normal range (20–50 ng/mL). Second, the low-level vitamin B_1 group showed no neurologic deficit such as vibration sensation or electrophysiologic indices, apart from a low score of POMS confusion, which would be weak evidence in substantiating the adverse effects on the nervous system.

Letz and Gerr (1994a, 1994b) investigated the confounding factors that could affect nerve conduction velocity and amplitude as well as vibrotactile and thermal thresholds, based on data from 4,464 subjects. Their studies revealed that the major covariates were height, examiner, skin temperature, and body mass index for sural sensory nerve and height, examiner, age, and body mass index for peroneal motor nerve conduction velocities. For vibrotactile threshold in toe, the major covariates were height, examiner, age, and body mass index. Our study design could control for the effect of examiner-, sex-, and age-matching pairs but not skin temperature-, body height-, or body mass index-matching pairs. Although body height was comparable on average between the exposure group and the controls and workers were acclimated to the room temperature before the electrophysiologic studies, the lack of pair matching for height, skin temperature, and body mass index should be carefully noted as a limitation of this study. Previous animal experiments demonstrated that exposure to 1-BP disrupted the estrous cycle and inhibited follicular development (Yamada et al. 2003). Two patients who worked in a cushion company in the United States also reported temporary irregularities of menstrual cycle (Ichihara et al. 2002). Although the exposure level for the two patients was not evaluated directly, such levels would be higher than 60–261 ppm, which were determined with the third case from the same factory after the former two cases were identified and ventilation was improved in the workplace. On the other hand, our study did not demonstrate significant differences in the prevalence of menstrual cycle abnormalities between the two groups. This might be due to the difference in exposure levels between U.S. cases (≥ 60–261 ppm) and our Chinese 1-BP factory workers (0.34–49.19 ppm).

Comparisons based on the exposure period showed higher levels of FSH and LH in the longer-exposure group than in shorter-exposure group. One explanation for this difference is that our group included four elderly women, who were excluded from the paired *t*-test analysis because of the lack of matched controls and who had high levels of FSH (42–100 mIU/mL) and LH (16–42 mIU/mL). Analysis based on exposure level did not show any relationship between exposure levels and these parameters, which were different between the exposure group and age-matched controls (paired *t*-test). The present analysis by exposure period and level has certain limitations. First, the number of subjects was too small and did not control for age. Second, the experimental design allowed only a single measurement of the exposure level, although the task of workers was not fixed and thus the exposure levels could vary. The exposure levels in 1999 in the same factory ranged from 0.9 to 170.5 ppm (geometric mean = 52.5 ppm) (Ichihara et al.

Table 7. Stabilometer test results of 1-BP exposure group and controls.

	1991 Workers	Age-matched controls	1999 Workers	Age-matched controls
No. (pairs)	23		12	
LNG (cm)				
Eyes open	71.7 ± 15.5	69.9 ± 20.8	71.5 ± 19.3	74.0 ± 19.4
Eyes closed	100.4 ± 25.1	91.1 ± 27.3	106.3 ± 29.7	95.0 ± 26.0
E AREA (cm^2)				
Eyes open	3.38 ± 1.26	3.69 ± 2.86	3.60 ± 1.52	3.88 ± 2.64
Eyes closed	4.94 ± 2.27	4.56 ± 3.62	5.65 ± 2.67	4.80 ± 4.15
LNG E AREA (per cm)				
Eyes open	22.9 ± 6.3	24.9 ± 10.9	21.8 ± 6.6	26.3 ± 13.7
Eyes closed	23.1 ± 7.9	26.3 ± 10.4	22.0 ± 8.8	28.8 ± 12.2
REC AREA (cm^2)				
Eyes open	7.53 ± 2.76	8.26 ± 6.39	7.87 ± 3.42	8.51 ± 6.08
Eyes closed	10.5 ± 5.6	10.3 ± 8.6	12.8 ± 6.1	10.5 ± 9.6
RMS (cm^2)				
Eyes open	1.62 ± 0.70	1.98 ± 1.67	1.82 ± 0.91	2.09 ± 1.46
Eyes closed	2.05 ± 1.01	2.05 ± 1.65	2.30 ± 1.25	2.22 ± 2.01
Mx (cm)				
Eyes open	0.019 ± 0.581	−0.123 ± 1.162	−0.166 ± 0.485	−0.008 ± 0.724
Eyes closed	0.010 ± 0.573	0.053 ± 1.228	−0.044 ± 0.557	0.217 ± 0.679
My (cm)				
Eyes open	−2.43 ± 1.15	−2.09 ± 1.37	−2.70 ± 1.05	−2.20 ± 1.41
Eyes closed	−2.29 ± 1.06	−2.06 ± 1.28	−2.50 ± 0.95	−2.41 ± 1.07
XO (cm)				
Eyes open	−0.004 ± 0.621	−0.142 ± 1.170	−0.186 ± 0.627	−0.009 ± 0.756
Eyes closed	0.119 ± 0.657	−0.003 ± 1.347	0.106 ± 0.771	0.231 ± 0.788
YO (cm)				
Eyes open	−2.50 ± 1.15	−2.07 ± 1.39	−2.79 ± 1.14	−2.22 ± 1.41
Eyes closed	−2.29 ± 1.01	−2.30 ± 1.42	−2.48 ± 0.86	−2.48 ± 1.09
Power spectrum of *x*-axis (lateral)				
Eyes open (%)				
0.02–0.2 Hz	61.1 ± 12.4	54.7 ± 17.0	62.5 ± 12.1	53.7 ± 14.5
0.2–2.0 Hz	38.5 ± 12.3	42.1 ± 13.6	37.1 ± 11.9	45.8 ± 14.4
2.0–10 Hz	0.36 ± 0.21*	0.46 ± 0.21	0.38 ± 0.24	0.46 ± 0.21
Eyes closed (%)				
0.02–0.2 Hz	45.7 ± 17.3	47.9 ± 12.2	48.5 ± 17.8	46.1 ± 12.7
0.2–2.0 Hz	52.5 ± 19.7	49.2 ± 16.3	48.4 ± 21.8	49.2 ± 19.7
2.0–10 Hz	0.53 ± 0.37	0.59 ± 0.33	0.60 ± 0.44	0.58 ± 0.34
Power spectrum of *y*-axis (anteroposterior)				
Eyes open (%)				
0.02–0.2 Hz	66.6 ± 14.0	70.7 ± 11.4	73.5 ± 11.1	70.9 ± 10.1
0.2–2.0 Hz	32.4 ± 12.8	28.9 ± 11.4	26.1 ± 11.1	28.6 ± 10.1
2.0–10 Hz	0.97 ± 2.62	0.42 ± 0.28	0.35 ± 0.16	0.45 ± 0.35
Eyes closed (%)				
0.02–0.2 Hz	51.6 ± 14.0*	61.2 ± 13.7	54.0 ± 11.6	55.0 ± 11.2
0.2–2.0 Hz	47.3 ± 13.0*	38.3 ± 13.7	45.4 ± 11.6	44.4 ± 11.2
2.0–10 Hz	1.03 ± 2.38	0.50 ± 0.28	0.56 ± 0.26	0.57 ± 0.25

Abbreviations: E, envelope; LNG, length of excursion; Mx, mean of *x*-axis (lateral) component of each recorded points; My, mean of *y*-axis (anteroposterior) component of each recorded points; REC AREA, rectangular area; RMS, root mean square area; XO, center of range of *x*-axis component of points; YO, center of range of *y*-axis component of points. Data are mean ± SD.

*$p < 0.05$, paired *t*-test. No significant difference was found between the exposed group and the controls in the Romberg quotient for all items (the ratio of values measured with eyes closed to the values with eyes open; data not shown).

2004), which was far higher than in the present study. It is possible that the workers were exposed to 1-BP at higher levels than those measured in our study. Further assessment of long-term exposure levels is required to determine the relationship between 1-BP and exposure levels.

In summary, the present study suggested that exposure to 1-BP produces adverse effects on peripheral sensory and motor nerves and/or the central nervous system in humans. Estimation of long-term exposure levels is required to confirm the precise association between the health effects of 1-BP and exposure levels.

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Correction

In the manuscript published online, the numbers of workers listed in Table 1, especially in the footnotes, were incorrect; also, the statistical significance of values for the right and left fingers for 1999 workers and age-matched controls was incorrect. These errors have been corrected here.

Subcutaneous Injection of Mercury: "Warding Off Evil"

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Deliberate injection of mercury, especially subcutaneous injection, is rare but is seen in psychiatric patients, individuals who attempt suicide, those who are accidentally injected, and boxers who wish to build muscle bulk. Metallic mercury plays a major role in ethnic folk medicine. Neurologic and renal complications can result from high systemic levels of mercury, and subcutaneous injection usually results in sterile abscesses. Urgent surgical evacuation and close monitoring for neurologic and renal functions as well as chelation (if toxicity is indicated) are key aspects of treatment. Education of the adverse effects and dangers of mercury is important, especially in pregnant women and children. As increased immigration changes demographic patterns, proper disposal of mercury and preventing its sale and use should become urgent societal priorities. Psychiatric consultation should be obtained whenever appropriate. *Key words:* case report, local abscesses, mercury injection, subcutaneous. *Environ Health Perspect* 112:1326–1328 (2004). doi:10.1289/ehp.6891 available via *http://dx.doi.org/* [Online 22 July 2004]

Case Presentation

Injection of elemental mercury is uncommon, and only 72 cases have been reported in the literature over the past 75 years. Of these 72 cases 46 were deliberate; most involved direct intravenous administration, usually with suicidal intent (Kayias 2003), or they were a complication of drug abuse. Bradberry et al. (1996) reported an attempted homicide by this means. Self-injection has also been reported in psychiatric patients (Soo et al. 2003), and accidental injections have been reported (Ellabban et al. 2003). Subcutaneous injection of mercury by accident (including injuries from broken thermometers), self-injection, and suicide attempts has been reported (Chodorowski et al. 1997; Ellabban et al. 2003; Smith et al. 1997; Soo et al. 2003).

A search in MEDLINE and PubMed (National Library of Medicine, Bethesda, MD) did not reveal any study or report on injection of mercury in the subcutaneous space of the hands for the sole purpose of preventing infections and "evil" during foreign travel. This practice is apparently common in several Central and South American countries. In this case report, I present such an injection received by a couple in Honduras before they traveled to the United States.

G.B., a 41-year-old Hispanic woman, and her partner, V.V., a 35-year-old Hispanic male, came to the clinic together. Both had wet towels wrapped around both their forearms and hands. They reported having pain for 5 days as well as swelling in the hands and low-grade subjective fever. The pain was localized to the dorsum of the hand and forearm, with no radiation, and was moderate in intensity and continuous, with no specific aggravating or relieving factors. The swelling and redness was localized to the same areas on the dorsum of the hand. They reported no history of bites or stings, and they had no swollen glands or joint pain. A review of systems was otherwise negative.

Both patients gave a history of having received multiple injections of mercury at a roadside nonmedical facility in Honduras about 1 week before their clinic visit. They did not know about the sterility of the procedure or if needles/syringes used were disposable. On further questioning, they indicated that the injection of mercury is a common practice among people who wish to travel abroad. The reason for their injections was to ward off "evil" and also to protect against exposure to any unknown diseases while traveling in a foreign country. The patients estimated that the injections for both hands in both patients was < US$1.00.

Both G.B. and V.V. denied any significant allergies or past medical history. They were both nonsmokers and denied alcohol or drug abuse.

A physical exam revealed G.B. to be an obese Hispanic woman in obvious distress due to pain in both hands and forearms. The general exam was unremarkable, and a local exam revealed a diffuse soft tissue swelling on the dorsum of both hands, with fluctuation, redness, and pointing (most prominent part of swelling in an abscess that marks the area of imminent rupture) in the first web space of both hands. Redness and swelling was also noted all along both forearms, with significant tenderness. No lymphadenopathy was noted. Lungs and heart were normal, and there was no renal angle tenderness and no hepatosplenomegaly. The neurologic exam was normal.

V.V. was a tall, medium-built Hispanic male in distress from pain. The general exam was unremarkable, and the local exam revealed findings similar to those for his partner, with fluctuation, redness, and tenderness in the dorsum of the hand and first web space and in the forearms. Otherwise, the exam was unremarkable.

Laboratory values for G.B. were as follows: glucose, 101 mg/dL; blood urea nitrogen (BUN), 14 mg/dL; creatinine, 0.8 mg/dL; sodium, 138 mmol/L; potassium, 4.1 mmol/L; chloride, 105 mmol/L; carbon dioxide, 22 mmol/L; calcium, 9.5 mmol/L; liver function tests, normal; white blood cell (WBC) count, 8,700/μL; hemoglobin, 12.6 g/dL; hematocrit, 37.6%; urine mercury, 11.3 μg/L; and serum mercury, < 5.0 μg/L.

Laboratory values for V.V. were as follows: glucose, 108 mg/dL; BUN, 26 mg/dL, creatinine, 1.1 mg/dL; sodium, 138 mmol/L; potassium, 4.2 mmol/L; chloride, 97 mmol/L; carbon dioxide, 26 mmol/L; calcium, 10.2 mg/dL; liver function tests, normal except for alanine aminotransferase, 64 U/L (normal, 4–60 U/L); WBC count, 8,700/μL; hemoglobin, 16.0 g/dL; hematocrit, 48.3%; and blood mercury, 100 μg/L (normal < 10 μg/L). Urine mercury analysis was not performed because V.V.'s urine samples were lost by the laboratory.

A diagnosis of abscess was made, and both patients underwent incision drainage of both hands. Thick pus was evacuated along with beads of metallic mercury (Figures 1–3). Complete evacuation of all visible mercury, about 0.5 mL, was performed and wounds were thoroughly washed with copious amounts of saline. The fluid removed was sterile pus (result of milder inflammation caused by irritants, foreign bodies, etc., but not due to infection). The soaked gauze and dirty sheets were disposed in regular waste.

Postoperatively, the wounds granulated and healed well by secondary intention (left open to heal by epithelization). Since that time, the patients have been lost to follow-up.

Discussion

Mercury is sold as "azogue" in religious stores, or botanicas, for use in Esperitismo (spiritual belief in Puerto Rico), Santeria (Cuban practices), and voodoo. The mercury is often carried personally in a pouch or spread around the house or bed, mixed in the bath, or burned in devotional candles. Mexican-Americans take it orally to relieve *empacho*

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A Survey on Exposure Level, Health Status, and Biomarkers in Workers Exposed to 1-Bromopropane

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Background *The aim of this study was to evaluate the health effects of exposure mainly to 1-bromopropane, which is an alternative to ozone-depleting solvents, and to establish biomarkers for assessing 1-bromopropane exposure.*

Methods *Twenty-four female and 13 male workers of a 1-bromopropane-factory were interviewed, and their urine and blood samples were collected. Measured parameters included 1-bromopropane levels in the factory, as well as individual exposure levels, urinary 1-bromopropane levels, enzymatic activity and M subunit's concentration of serum creatine kinase (CK).*

Results *Frequent symptoms reported by workers exposed to 1-bromopropane were nose, throat, and eyes irritation or malaise and/or headache. Urinary 1-bromopropane levels correlated significantly with individual exposure levels, but enzymatic activity or CK–M subunit did not.*

Conclusions *The symptoms suggested irritation of the mucous membrane and possible adverse effects on the central nervous system. There were no severe chronic symptoms suggestive of neurological damage in workers exposed to less than 170 ppm. Urinary 1-bromopropane level may be a good indicator of exposure.* Am. J. Ind. Med. 45:63–75, 2004. © *2003 Wiley-Liss, Inc.*

KEY WORDS: 1-bromopropane; ambient concentration; individual exposure level; subjective symptoms; biomarkers; creatine kinase

INTRODUCTION

In the developed industrial countries, production of specific chlorofluorocarbons and 1,1,1-trichloroethane has been prohibited due to their ozone-depleting potential. As alternatives to these compounds, 2-bromopropane and 1-bromopropane were recently introduced into the workplace. However, the use of 2-bromopropane was minimal in Japan and Korea due to its reproductive and hematopoietic toxicities in humans and rats reported soon after its introduction [Ichihara et al., 1996, 1997; Kim et al., 1996; Kamijima et al., 1997; Lim et al., 1997; Nakajima et al., 1997; Park et al., 1997; Yu et al., 1997, 1999]. However, the other isomer, 1-bromopropane, has become an important alternative and is currently used as a cleaning agent for

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metals, precision instruments, electronics, optical instruments, and ceramics. Two and half years after our survey of a 2-bromopropane factory [Ichihara et al., 1999], we investigated the same factory with respect to the health status of workers exposed to 2-bromopropane. However, we found that the main product was shifted from 2-bromopropane to 1-bromopropane in that factory. Our recent animal studies [Yu et al., 1998, 2001; Ichihara et al., 2000a,b; Wang et al., 2002, 2003; Yamada et al., 2003] showed that 1-bromopropane is more neurotoxic than 2-bromopropane. Rats exposed to 1-bromopropane at 800 ppm showed a region-specific decrease in cerebral weight, swelling of the preterminal axons in the gracile nucleus of the medulla oblongata, degeneration of peripheral nerves, low nerve conduction velocity, and delayed the distal latency in the tail [Ichihara et al., 2000b]. Furthermore, both the 7-day and 12-week inhalation studies showed reduced amount of neurospecific γ-enolase in the brain [Wang et al., 2002, 2003]. Apart from its neurotoxic effects, 1-bromopropane was found to inhibit spermiation from the testis, although it was less toxic to spermatogonia than 2-bromopropane [Ichihara et al., 2000a]. Moreover, recent case studies suggested the presence of severe neurotoxicity in workers exposed to 1-bromopropane [Sclar, 1999; Ichihara et al., 2002].

The present study was designed to evaluate the health effects and establish biomarkers for biological monitoring of workers exposed to 1-bromopropane.

SUBJECTS AND METHODS

Plant and Worker Tasks

The survey was conducted on May 10–12, 1999. The factory was located in Yixing City, Jiangsu Province, China, and employed 37 workers (24 females, 13 males) at the time of the investigation. The plant consisted of two equal-size one-story buildings, which measured $9.7 \times 24.4 \times 7$ m ($W \times D \times H$). A ventilator was installed at 6 m from the floor in each plant. The main product was 1-bromopropane when the survey was conducted. Between the present survey in 1999 and the previous survey in 1996 [Ichihara et al., 1999], the plant produced 1-bromopropane as well as 2-bromopropane according to clients' orders. The main product had recently become 1-bromopropane, but the exact date of making 1- instead of 2-bromopropane the main line of production was not clear. The synthesizing process of 1-bromopropane was basically the same as that of 2-bromopropane described previously [Ichihara et al., 1999], except that 1-propanol was used instead of 2-propanol as the raw material. 1-Bromopropane was synthesized by heating 1-propanol and hydrogen bromide in the presence of concentrated sulfuric acid. It was then distilled for refinement and kept in ceramic containers. The distilled solution was placed in 20-L plastic vessels and then neutralized with sodium hydrogen carbonate. The processed 1-bromopropane was poured into 1,000-L drums for storage. Assigned jobs included operators, repairmen (who repaired the distillation apparatus or boiler), boiler operators, gas chromatograph (GC) analyzers (who analyzed the product using a GC), electrician (electric wiring or repair of electronic instruments), laborers (who carried materials and products), and accountants. The operators poured materials into the reaction pots, checked the temperature in the pots during reaction, and poured the distilled solution into 20-L plastic containers. They also were responsible for mixing the produced solvent with sodium hydrogen carbonate. Thus, the operators were at high risk of exposure to the produced solvents. Repairmen were not frequently exposed to the solvents, but could be exposed to high concentration solvents when they repaired the reaction system. Since boiler operators ran the water heater outside the plant to heat the reaction pots, they were at a relatively low risk of exposure to 1-bromopropane. A GC analyzer collected the produced solvents and measured their purity with GC in a facility separate from the plant. Two assistant managers entered the plants zero to several times a day according to work schedule, but the accountants worked in a separate office and did not enter the plant. GC analyzers and accountants worked in daytime for 8 hr, but all other workers worked for 12 hr per day in two shifts.

Ambient Concentration Level and Product Analysis

The ambient concentrations of bromopropanes were measured with a detection tube (Kitagawa type, Komyo Rikagaku Kogyo KK), 8–10 times in the breathing zone of the workers between 7:00 and 8:00 on May 14, 1999. Although this detection tube could not differentiate 1-bromopropane from 2-bromopropane, the same concentrations of bromopropanes indicate the same values, no matter which is being measured. Therefore, the value indicates the total amounts of 1-bromopropane and 2-bromopropane as long as other brominated hydrocarbons are negligible. The latter assumption is supported by the GC–MS results showing 96.74% purity (see "Results") of products, if the environmental gas simply reflects the constituents of product solvents. The product of the plant was analyzed by a GCD (gas chromatograph electron ionization detector) system G1800A (Hewlett Packard Palo Alto, CA) following the previous method [Ichihara et al., 1999].

Exposure Levels

Individual exposure levels were estimated with passive samplers (Sibata Scientific Technology, Tokyo, Japan) according to the method used in the previous study in 1996 [Ichihara et al., 1999]. A passive sampler was attached to each worker during one 12-hr shift (7:00–19:00 and

19:00–7:00). The tubes were collected immediately after the shift and kept in separate sealed bags at 4°C until analysis. The absorbed solvent in the sampler was analyzed 2 weeks after collection. For analysis, activated charcoal particles were extracted with 2 ml carbon disulfide and the solution was then injected into a GC equipped with an electron ionization detector (GCD system G1800A, Hewlett Packard). 1-Bromopropane and 2-bromopropane were quantified by the selected ion-mode. The TWA concentration of each solvent was calculated based on the formula: TWA = absorbed solvent (μg)/sampling rate (μg/ppm × min) × sampling time (min). In our calculations, the values of 0.134 and 0.117 were used as the sampling rates of 1-bromopropane and 2-bromopropane, respectively. Those values were determined by the diffusing cell method.

Interview and Blood Analyses

Workers were interviewed by Chinese medical physicians who were occupational health specialists, following completion of a check-up form similar to that used in the previous investigation in 1996. Both male and female workers were asked about subjective symptoms, past or present illness, work history, history of exposure to hemotoxic and neurotoxic substances, history of medications, marital status, and if they had children. Menstrual status was recorded for female workers. The following items were measured in a clinical laboratory: red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), white blood cells (WBC), and platelet (Plt) using a hematocell counter (Coulter JT, Miami, FL), as well as serum iron (Fe, Nitroso–PSAP method), total iron-binding capacity (TIBC, Nitroso–PSAP method), ferritin (Ft, EIA), creatinine kinase (CK, UV [NAC] method), glutamic–oxaloacetic transaminase (GOT, UV method), glutamic–pyruvic transaminase (GPT, UV method), γ-glutamyl transferase (γ-GTP, L-glutamyl-3-carboxy-4-nitroanilide substrate method), lactate dehydrogenase (LDH, Wroblewski–LaDue method), alkaline phosphatase (ALP, PNP substrate method), total protein (Biuret method), total bilirubin (alkaline azobililrubin method), creatinine (alkaline picric acid method), luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex steroid hormone (estradiol for females and testosterone for males) (radioimmunoassay [RIA] method).

Interviews and health examinations were performed with the subjects' informed consent as outlined in the Helsinki Declaration.

Subunit Creatine Kinase (CK)–M Assay

Serum CK–M (muscle), which is a mainly found subunit of CK in blood, was determined by means of the sandwich-type enzyme immunoassay system developed by Kato and Shimizu [1986]. The system consisted of a solid phase (polystyrene balls) with immobilized rabbit antibodies monospecific to the subunit, CK–M, and the same antibodies labeled with β-D-galactosidase from *Escherichia coli*. Ten microliters of plasma was subjected to the immunoassay.

Measurement of Urinary 1-Bromopropane Concentrations

At the time of collection of the passive samplers, urine samples were collected into a paper cup. The urine was immediately transferred to 20 ml vials so that it filled the vials (Chromacol, Trumbell, CT) which were then sealed with aluminum caps. The samples were then stored at 4°C for 2 weeks until the analysis. The concentration of urinary 1-bromopropane was measured with a GC (HP 5890A, Hewlett Packard) connected to a purging and trapping system (HP 7675A, Hewlett Packard). The column was a Chrompak PoraPlotQ (ID = 0.52 mm) and the trap was a TenaxGC (Enka Research Institute, Arnhem, the Netherlands). Then, 2 ml of the urine sample was diluted with MiliQ water to 5 ml. 1-Bromopropane in urine was purged with helium gas at a flow rate of 20 ml/min at room temperature. The concentrations of 1-bromopropane purged for 20 min in urine samples containing up to 1,300 ng/ml of the same compound showed linearity with the gas chromatographic peak area. More than 98% of solvents were trapped in the TenaxGC after 5 min. The trap was heated at 180°C for 5 min to purge 1-bromopropane, which was then introduced into the GC. It took 1 min to transfer all 1-bromopropane to the column. The program for controlling column temperature was initially 60°C for 1 min and increased by 6°C/min to 180°C, and then kept for 3 min. This program for column temperature commenced just before beginning to heat the trap. The detectable limit was 0.5 ng/ml in urine.

Stability of 1-Bromoproane

Two sets of 16 sampler tubes were placed in the inhalation chambers for 8 hr at 50 and 200 ppm of 1-bromopropane. Details of the inhalation exposure system were described previously [Ichihara et al., 2000a]. After exposure, the samplers were kept in sealed bags at 4°C until analysis. The analysis was conducted 1, 7, 14 days (n = 6, each) following the method mentioned above. To test the stability of urinary 1-bromopropane in sealed vials, a urine sample from a non-exposed human was spiked with 1-bromopropane to a concentration of 10 ng/ml and placed into 20 ml vials (n = 18) full to the brim. The vials were sealed with aluminum caps and silicon septum and kept at 4°C until analysis. Urinary 1-bromopropane in vials was measured with the headspace method according to Kawai et al. [2001] with slight modifications, after keeping the sealed vials at 4°C for 1, 7, and 14 days (n = 6, each). The analysis method was modified as follow: GC carrier flow: 5 ml/min, split ratio: 3:1, column

temperature: initially 40°C for 3 min and elevated at 5°C/min to 110°C and finally 230°C for 10 min, used urine: 1 ml, used vials: 7 ml vials (clear vials with screw top, Supelco, Bellefonte, PA), vial incubation time: 15 min, and temperature: 42°C.

The reducing rate of 1-bromopropane trapped in passive samplers and urinary 1-bromopropane after 7 and 14 days were calculated based on the value obtained 1 day after the exposure.

Statistical Analysis

All data were expressed as mean ± SD unless otherwise indicated. Fisher's test was applied for comparison of frequencies of symptoms at work between high- and low-exposure female groups and comparisons between 1996 and 1997. Fisher's test was used for this analysis because the expected frequencies were less than five in all comparisons of symptoms (apart from strange smell). Student's *t*-test was used for comparisons of biochemical and hematological indices between groups divided according to exposure levels ($\leq$56.9 and $\geq$76.02 ppm for females and $\leq$3.8 and 40 ppm for males) or length of service ($\leq$24 and $\geq$36 months for females and $\leq$19 and $\geq$23 months for males). The values of γ-GTP, ALP, CK, CK–M, creatinine, Ft, LH, FSH, estradiol, WBC, MCV, MCH, and Plt were log transformed before *t*-test, because their distributions were not normal by Shapiro–Wilk W test. In the comparison by exposure levels, accountants were classified into the low-exposure group based on their job content and work place, even if the exposure levels were not evaluated. Other workers whose exposure levels were not measured were excluded from this analysis. In the comparison by length of service, accountants were excluded because they were not exposed to 1-bromopropane throughout their employment. In the comparison by exposure levels in males, the Fisher's exact test was used to confirm the results of *t*-tests. This is because presupposition of normal distribution required for the *t*-test could not be evaluated because of the small population sample (n = 2) of the high-exposure group. This analysis compared the proportion of individuals with less than or above the reference values. Since the above analyzes could be affected by grouping, Spearman's nonparametric correlation analysis was also performed to evaluate the correlation between exposure levels or length of service and biochemical or hematological indices. To determine the association between possible biomarkers and exposure levels, regression analysis of urinary 1-bromopropane was conducted against exposure levels with or without logarithmic transformation of values. A paired *t*-test was applied to the analysis of changes in the hematological indices; RBC, Hb, Ht, and WBC over the last 2.5 years. To examine the healthy worker effect, the *t*-test and Fisher's exact test were applied to compare hematological indices and frequency of symptoms between workers who continued to work and those who left the job after 1996. Analysis of covariance (ANOVA) followed by Dunnett's multiple comparison was applied to compare the values after 7 or 14 days with those after 1 day in passive samplers or urinary 1-bromopropane. Probability of $P < 0.05$ was accepted as significant.

RESULTS

The investigators themselves complained of nasal and conjunctival irritation during the study following visits to the two plants. The ambient concentrations of bromopropanes (sum of 1- and 2-bromopropane) at different location inside the plant are listed in Table I. The concentration was highest during the transfer of produced solvents into the containers. The purity of 1-bromopropane produced in the plants was 96.74%. The impurities were di-*n*-propyl ether 1.02%, 2-bromopropane 0.83%, 1,2-dibromopropane 0.4%, 1,2-dibromoethane 0.26%, and an unknown peak 0.75%.

Table II lists the subjective symptoms reported by both males and females. Symptoms of nose, eyes and throat

TABLE I. Ambient Concentrations of Bromopropanes (Sum of 1-Bromopropane and 2-Bromopropane) Measured With Detection Tube (ppm); Jiangsu Province, China

Site	Max	Median	Min	Geometric mean
In front of reaction pot (plant 1)	7.7	5.5	3.3	5.3
In front of stock vessel (near window, plant 1)	14.3	11.6	9.9	11.9
Above bottle when pouring the solution into bottles (plant 2, point A)	90.2	85.8	45.1	79.7
Above bottle when pouring the solution into bottles (plant 2, point B)	90.2	86.9	42.9	65.6
In front of the reaction pot (plant 2)	8.8	6.6	5.5	6.8
In front of stock vessel (plant 2)	33.0	28.1	23.1	28.2
Passage (plant 1)	4.4	3.3	2.2	2.8
Room for product analysis	3.3	2.2	1.1	2.1
Site for washing vessel outside the plants	14.3	11.0	8.8	11.4

TABLE II. Subjective Symptoms at Work in Male and Female Workers by Exposure Level; Jiangsu Province, China

	Females				
		Exposure level (ppm)			
	Total	≥56.9[b]	≥76.02	Unknown	Males[a]
n	24	10	10	4	13
Age	38.0 ± 7.8	34.6 ± 8.8	40.6 ± 6.7	40.3 ± 6.2	42.7 ± 12.5
Strange smell	14	8*	3	3	6
Irritated nose	10	4	3	3	6
Sore throat	9	4	3	2	3
Painful eyes	9	3	3	3	2
Dizziness or vertigo	7	3	1	3	0
Strange taste	5	3	2	0	2
Light headed	4	2	0	2	0
Heavy headed	3	1	0	2	1
Headache	3	1	1	1	0
Dim eyesight	2	1	1	1	3
Flushed face	1	1	0	1	0
Feeling intoxicated	1	1	0	1	0

Chi-square test was conducted to compare high- and low-exposure group in female workers.
[a]The same test was not performed on male workers, because distribution of exposure levels was deviated and it was difficult to divide into two groups with sufficient number.
[b]The low-exposure group includes two accountants, although their exposure levels were not evaluated.
*$P < 0.05$.

irritation were common, in addition to headache, dizziness or feeling heavy headedness, which was probably related to the central nervous system. The frequency of subjects who complained of a strange smell correlated significantly with exposure levels in females. We also investigated the most recent subjective symptoms. One female (no. 3, Tables IIIa,b) complained of headache while another woman (no. 11, Tables IIIa,b) complained of dizziness and constipation. One male (no. 36, Table IV) complained of throat discomfort. Tables IIIa,b, and IV show the time-weighted average of bromopropanes, including the data obtained in 1996 survey, hematological indices, iron-related biochemical data, hormonal data, and history of employment with exposure to reproductive or neurotoxic substance (hormonal data of males are not shown in Table IV). The concentration of 1-bromopropane in the environment was higher than that of 2-bromopropane. Ten of 24 female and 4 of 13 male workers had low values of RBC, Hb, or Ht but normal values of MCV, MCH, and MCHC. Among these workers, six females and one male had lower Fe value than the reference. Four female workers complained of disruption or failure of menstruation. High gonadotropin levels were observed in cases with amenorrhea, and moderate estradiol levels were detected in females with irregular menses. All workers were married except for one female (no. 24). One married male (no. 29) did not have children, but all other married workers had at least one child and none of the workers complained of infertility.

The present plant was built in a rural area and almost all workers were formerly engaged in agriculture. However, some workers had been previously exposed to other chemicals in other factories. One female worker (no. 3) had worked previously in a lead oxide (PbO_2)-production factory, while another female (no. 19) and two male workers (no. 30 and 32) had worked previously in other chemical plants, although the exact chemicals were not identified. Two workers were taking medications for more than 3 months, contraceptives by no. 18 and antihypertensive drugs by no. 36, but other workers did not. None of the females were smokers or on regular alcohol intake, but nine male workers other than no. 25, 27, and 35 smoked 2–30 cigarettes per day, respectively, and three males (no. 37, 26, 28) drank 100, 150, and 200 ml of Bai jiu (local spirit, >38% alcohol) per day, respectively.

The results of biochemical and hematological tests are summarized in Tables V and VI. Low Hb levels were detected in 29.2% of females and 30.7% of males, relative to the reference values. Low Fe and Ft levels were noted in 26.1 and 4.3% of females and 46.2 and 15.4% of males, respectively. After log-transformation, the *t*-test indicated that female workers with longer period of service had significantly higher creatine (0.63 ± 0.11 mg/dl) and higher Plt count ($1.67 \pm 0.44 \times 10^5/\mu l$) than those with shorter period of service (0.44 ± 0.21 mg/dl; $1.29 \pm 0.27 \times 10^5/\mu l$, respectively). The high-exposure male group had significantly lower WBC, RBC, Hb, Hct values than their low-exposure

TABLE IIIa. Exposure Concentration and Hematological Indices With Iron-Related Laboratory Data in Female Workers, Yixing City, Jiangsu Province, China, 1999

	Job title	Age (years)	Dn (months)	Previous work or involved product (years)	1-BP conc (ppm, TWA)	2-BP conc (ppm, TWA)	2-BP conc in 1998 (ppm, TWA)	RBC ($\times 10^6/\mu l$)	Hb (g/dl)	Ht (%)	Plt ($\times 10^3/\mu l$)	WBC ($\times 10^3/\mu l$)	Iron ($\mu g/dl$)	Ferritin (Ft) (mg/dl)	TIBC (mg/dl)
1	Operator	49	115		106.8	0.2	—	4.71	13	41.1	171	5.8	70	120↑	296
2	Operator	50	113		24.8	1.5	—	4.66	13.8	41.1	154	6.4	112	110↑	394
3	Operator	42	102	Chemical (lead, PbO_2) 2–3	48.8	0.2	4.14	4.13	13	40.5	113↓	8.2	103	32	334
4	Operator	38	76		90.6	0.3	7.25	3.89↓	11.3↓	32.3↓	240	5.3	40↓	15	395
5	Operator	28	48		40.9	3.2	4.09	3.59↓	10.9↓	31.7↓	173	5	45↓	16	375
6	Operator	31	48		76.0	1.7	—	4.03	12	34.9	133	4.7	108	12	343
7	Operator	43	36	Brick 4–5	56.9	1.8	5.80	3.67↓	10.6↓	32↓	219	4.5	23↓	4.3	398
8	Operator	43	24	Brick 2.5, furnace 1.5	—	—	—	3.8↓	11↓	33.6↓	91↓	3.5↓	96	3.8	431↑
9	Operator	38	19		47.0	0.2	—	4.28	13.5	38.8	198	6.1	69	16	437↑
10	Operator	44	19	Furnace 1	—	—	—	3.76↓	11.6	33.7↓	118↓	3.4↓	179	8.1	381
11	Operator	30	18	Stockings 3	41.3	3.3	—	4.17	12.1	36.9	116↓	4.3	47↓	23	300
12	Operator	44	17		164.4	0.6	—	3.86↓	11.4	35.1	112↓	4	119	10	390
13	Operator	48	16		80.7	1.3	—	2.99↓	10.3↓	30↓	134	4.3	49	71	265
14	Operator	45	16		95.6	0.5	—	4.65	13.9	42.4	127↓	7.7	49	29	336
15	Operator	38	13		100.3	0.3	—	3.73↓	10.2↓	30.4↓	119↓	3.9↓	21↓	2.6↓	397
16	Operator	38	13	Heat-resistant materials 7	77.2	0.2	—	4.76	13.8	40.1	123	7.9	123	14	302
17	Operator	31	13		0.9	0.1	—	4.95	11.2↓	34.2↓	135	4.5	24↓	6.6	466↑
18	Operator	43	12		—	—	—	3.87↓	12.4	35.3	177	3.9↓	156	39	420↑
19	Operator	31	12	Chemical 2, restaurant 3	—	—	—	4.02	13.1	39.5	124↓	3.7↓	86	34	329
20	Operator	30	2		169.1	0.5	—	4.12	12.4	37	113↓	6.7	77	23	319
21	Operator	45	2	Glass and brick 20	170.5	0.4	—	3.17↓	10.1↓	29.3↓	114↓	3.6↓	68	130↑	264
22	GC analyzer	27	43		9.0	0.5	2.87	4.08	12.3	36.1	129	6.1	95	35	277
23	Accountant	37	35	Management 4	—	—	N.D.	4.16	13	38.1	136	8.5	108	45	340
24	Accountant	21	11		—	—	—	4.56	13.1	39.3	109	5.7	—	—	—
	Reference value							3.9–4.8	11.5–13.6	35–45	130–369	4–10	48–154	3.4–89	246–410

TWA, time weighted average; N.D., not detectable; detection limits of 1-bromopropane and 2-bromopropane 0.13 ppm; ↑, above the reference value; ↓, below the reference value. RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; WBC, white blood cell; TIBC, total iron-binding capacity; conc, concentration; Dn, duration of employment.

TABLE IIIb. Hormonal Data and Menstruation Cycle in Female Workers, Yixing City, Jiangsu Province, China, 1999

	Type of job	Age (years)	LH (IU/L)	FSH (IU/L)	Estradiol (ng/dl)	Menstruation	
						Irregularity	Amenorrhea
1	Operator	49	48↑	100	10		+ (since Feb 95)
2	Operator	50	26	58	10		+ (since 94)
3	Operator	42	59↑	30	239	+ (Feb 97)	
4	Operator	38	5.4	6.5	53.5	Regular	
5	Operator	28	4.5	9	22.9	Regular	
6	Operator	31	2.4	4.6	63.2	Regular	
7	Operator	43	3.1	5.7	64.7	Regular	
8	Operator	43	3.2	2.6↓	120	Regular	
9	Operator	38	6.6	4.2	78.2	Regular	
10	Operator	44	4.8	4.6	558↑	Regular	
11	Operator	30	3.2	3.1	51.9	Regular	
12	Operator	44	2.5	5	91.6	Regular	
13	Operator	48	13	40	38	Regular	
14	Operator	45	4.4	3.3	124	Regular	
15	Operator	38	7.3	8.2	95.2	Regular	
16	Operator	38	3.2	3.1	61.3	Regular	
17	Operator	31	18	6	66.2	Regular	
18	Operator	43	2.3	1.9↓	10	Regular	
19	Operator	31	6	3.6	124	Regular	
20	Operator	30	15	8.1	32.8	Regular	
21	Operator	45	42	74	10	+ (97)	+ (since 99)
22	GC analyzer	27	6.8	6.5	50.7	Regular	
23	Accountant	37	17	3.6	108	Regular	
24	Accountant	21	—	—	—	Regular	
	Reference value		1–38	2.9–113.3	9–390		

LH, luteinizing hormone; FSH, follicle stimulating hormone.

counterparts (*t*-test), although Fisher's test did not show any difference in the frequencies of subjects with values above and below the reference values by exposure levels. Spearman's correlation analysis showed a significant correlation between the duration of employment and creatine level ($r = 0.5121$) and Plt count ($r = 0.4246$). The same analysis showed significant correlation between FSH and exposure level in males ($r = 0.7866$).

There was a significant correlation between urinary 1-bromopropane and levels of exposure to 1-bromopropane (Fig. 1), and the correlation coefficient increased when the log values were used. Normalization to urinary specific gravity did not increase the value of the correlation coefficient.

Comparison of symptoms between the two surveys in 1996 and 1999 showed a decrease in the prevalence of headache over the period, but the frequencies of other symptoms were not significantly different (Table VII). This comparison was made only in female operators, and the data of males were not analyzed due to the small sample size. Six females and three males who participated in the study in 1996, were still working in the same factory. It was unknown whether the other workers examined in 1996 had retired or changed their jobs. The six females did not complain of any change in menstrual cycle, and comparison of hematological indices showed no significant change over the period, both when the analysis was applied to all 1996–1999 workers and when accountants, who were not exposed to chemicals, were excluded (Table VIII). Furthermore, there were no differences in hematological indices or frequencies of subjective symptoms between 1996 and 1999 workers and those who had left the job after 1996 (data not shown).

Stability testing showed that 1-bromopropane trapped in passive samplers were stable at $100 \pm 3.0\%$, $99.8 \pm 3.2\%$, and $99.6 \pm 3.2\%$ at 50 ppm and $100 \pm 6.0\%$, $98.7 \pm 3.3\%$, and $98.7 \pm 1.2\%$ at 200 ppm, 1, 7, and 14 days (not significant by ANOVA) after exposure, respectively, when the average value obtained on the first day after exposure was defined as 100%. Similarly, urinary 1-bromopropane levels were $100 \pm 3.6\%$, 94.9 ± 2.3*% after 7 days and 91.2 ± 1.8*%, 1, 7, and 14 days at 10 ng/ml after sealing vials (*significantly different from the value after 1 day by Dunnette's method following ANOVA).

TABLE IV. Exposure Concentration and Hematological Indices With Iron-Related Laboratory Data in Male Workers, Yixing City, Jiangsu Province, China, 1999

	Job title	Age (years)	Dn (months)	Previous work or involved product (years)	1-BP conc (ppm, TWA)	2-BP conc (ppm, TWA)	2-BP conc 1996 (ppm, TWA)	RBC ($\times 10^6/\mu l$)	Hb (g/dl)	Ht (%)	Plt ($\times 10^3/\mu l$)	WBC ($\times 10^3/\mu l$)	Iron ($\mu g/dl$)	Ft (mg/dl)	TIBC (mg/dl)
25	Management and testing of machines	38	76	Teacher 8, instructor 2	1.7	0.2	N.D.	5.75	163	48.2	186	8.8	48↓	100	331
26	Repairmen	49	66		2.9	0.2	1.2	4.95	153	44.2	148	6.2	125	150	278
27	Repairmen	48	44	Concrete 3, macadam 2	40.0	0.2	0.95	4.11↓	128↓	37↓	144	4.3	138	110	293
28	Management	51	35	Heat-resistant materials 13	—	—	—	5.12	144	43.3	119↓	4.5	165	99	319
29	Electronics	27	27	Repair of agricultural machine 3, maintenance of standard scale 1.5	0.6	0.1		4.91	157	45.7	199	10.2	156	110	320
30	Repairmen	48	18	Chemical 1	—	—	—	3.67↓	121↓	36.1↓	96↓	3.1↓	102	150	265
31	Boiler operator	26	12	Water service and electrical work	—	—	—	4.82	145	43.2	133	4.1	172	140	302
32	Boiler operator	58	8	Repairman in chemical plant 20	3.8	0.0	—	4.8	159	45.7	199	8.4	76	80	340
33	Operator	52	7		43.3	0.3	—	3.67↓	114↓	33.7↓	140	4.2	40↓	24↓	308
34	Repairmen	25	5	Electrician 2, manufacture of drying machine 3	1.3	0.1	—	4.82	142	42	212	6.1	56	170	355
35	Laborer	28	2		2.4	0.1	—	5.03	149	43.4	178	7.7	74	42	320
36	Operator	45	1		—	—	—	4.88	140	41.9	117↓	5.3	107	120	355
37	Accountant	60	11		N.D.	N.D.	—	4.15↓	134	38.9↓	140	5.9	76	210	247↓
	Reference value							4.3–5.4	13.0–15.0	0.41–0.51	130–369	4–10	54–200	27–320	253–365

TWA, time weighted average; N.D., not detectable; detection limit of 1-bromopropane and 2-bromopropane is 0.13 ppm; ↑, over the reference value; ↓, below the reference value; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; WBC, white blood cell; TIBC, total iron-binding capacity; Dn, duration of employment; conc, concentration.

TABLE V. Summary of Biochemical and Hematological Indices in Female Workers (n = 24), Yixing City, Jiangsu Province, China, 1999

	Unit	Mean ± SD	Minimum value	Maximum value	Percentage below reference values	Percentage above reference values	Reference values
ALT	IU/L	22 ± 4.7	14	35	0	0	10–40
γ-GTP	IU/L	16.2 ± 8.6	9	40		8.7	≤30
ALP	IU/L	157.6 ± 46.8	103	267	0	4.3	80–260
LDH	IU/L	310.2 ± 45.9	233	407	0	0	230–460
CK activity	IU/L	88 ± 41.8	39	227	0	4.2	32–180
CK–M subunit	ng/ml	73.4 ± 44.9	22.7	226			
Total protein	g/dl	7.9 ± 0.3	7.2	8.5	0	8.3	6.7–8.3
Albumin	g/dl	4.8 ± 0.2	4.3	5.2	0	4.3	3.7–5.5
Total bilirubin	mg/dl	0.62 ± 0.22	0.3	1.1	0	0	0.2–1.0
BUN	mg/dl	11.8 ± 2.8	7	17.5	0	0	6–20
Creatinine	mg/dl	0.7 ± 0.07	0.6	0.8	0	0	0.6–1.3
Creatine	mg/dl	0.5 ± 0.19	0.1	0.92	26.1	8.7	0.31–1.10
Iron	μg/dl	81.2 ± 41.9	21	179	4.3	13	48–154
Ft	mg/dl	34.7 ± 37.4	2.6	130	0	17.4	3.4–89
TIBC	mg/dl	356 ± 58	264	466	0	13	246–410
LH	mIU/ml	13.2 ± 15.9	2.3	59	4.3	0	1.0–38.0
FSH	mIU/ml	17 ± 26.3	1.9	100	0	4.3	2.0–113.7
Estradiol	pg/ml	90.6 ± 114.4	10	558	13.0	4.3	11–390
WBC	10^3/μl	5.32 ± 1.58	3.4	8.5	4.2	0	3.5–9.1
RBC	10^4/μl	4.07 ± 0.49	359	495	8.3	0	376–500
Hemoglobin	g/L	120.8 ± 12.1	101	139	29.2	0	11.3–15.2
Ht		0.36 ± 0.039	0.293	0.424	25	0	0.334–0.449
MCV	Fl	88.8 ± 6.1	69.1	100.3	4.2	4.2	79.0–100
MCH	Pg	29.8 ± 2.2	22.6	34.4	4.2	0	26.3–34.3
MCHC	%	33.5 ± 1	31.3	35.1	0	0	30.7–36.6
Plt	10^3/μl	140.8 ± 37.3	91	240	54.2	0	130–369

ALT, L-alanine 2-oxoglutarate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactose dehydrogenase; CK, creatine kinase; BUN, blood urea nitrogen; TIBC, total iron-binding capacity; LH, luteinizing hormone; FSH, follicle stimulating hormone; WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

DISCUSSION

The change in the main product of the tested factory from 2-bromopropane to 1-bromopropane could be related with the fact that 1-bromopropane became the new alternative for ozone-depleting solvents instead of 2-bromopropane. The ambient concentration of 1-bromopropane in the factory (3.3–90.2 ppm) was similar to that of 2-bromopropane in our previous study (2.5–110.8 ppm) [Ichihara et al., 1999]. However, the time-weighted average of individual exposure concentrations in 1999 (0–170.5 ppm) was far higher than in 1996 (0–16.18 ppm). This change might be due to the increase in the amount of labor and chance of exposure per individual. The highest level of bromopropanes was found near the site of pouring the processed product into bottles (Table I). In our study, the frequency of this task seemed to have increased compared to the 1996 survey.

Neurotoxicity of 1-bromopropane was first described in animal studies, antedating human studies [Yu et al., 1998, 2001; Ichihara et al., 2000a]. The symptoms of dizziness or vertigo, light headedness, heavy headedness, headache or feeling intoxicated may suggest adverse effects on the central nervous system in humans. It is interesting that irritation of the mucous membrane is rather dominant to the above symptoms, because sore throat was one of the initial symptoms in a case reported earlier by our group [Ichihara et al., 2002]. However, the present neurological or mucous-irritating symptoms may not be specific to 1-bromopropane exposure but could be also caused by 2-bromopropane. This argument is based on the fact that the frequencies of almost all symptoms did not differ from those of the 1996 investigation, when 2-bromopropane was the main product, although the frequency of headache rather decreased significantly. NIOSH reported that workers complained of headache, nausea and vomiting, near-syncope, and mucous membrane irritation soon after the introduction of 1-bromopropane as a degreaser cleaning solvent in a factory [Reh, 2001]. In another factory using 1-bromopropane as an