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Biochemical Changes in the Central Nervous System of Rats Exposed to 1-Bromopropane for Seven Days

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1-Bromopropane is used widely as an alternative to ozone-depleting solvents. The neurotoxic effects of this agent have been described in humans and experimental animals. Here we investigated the underlying mechanisms of the neurotoxic effects of 1-bromopropane by examining the initial biochemical changes in the central nervous system. Four groups of 9 Wistar male rats each were exposed to 200, 400, or 800 ppm 1-bromopropane or only fresh air, 8 h per day for 7 days. At the end of the experiment, the cerebrum, cerebellum, brain stem and lumbar enlargement of the spinal cord were dissected out from each rat (n = 8) for biochemical analyses. Morphological examinations of the nervous system were performed in the remaining rat of each group. 1-Bromopropane dose-dependently decreased neurospecific γ-enolase, total glutathione, and nonprotein sulfhydryl groups in the cerebrum and cerebellum. Creatine kinase activity decreased dose-dependently in the brain and spinal cord. Histopathological examination showed swelling of preterminal axons in gracile nucleus and degeneration of myelin in peripheral nerves. Our results of low levels of γ-enolase suggested that 1-bromopropane might primarily cause functional or cellular loss of neurons in the cerebrum and cerebellum. Glutathione depletion or modification to functional proteins containing a sulfhydryl base as a critical site might be the underlying mechanism of 1-bromopropane neurotoxicity.

Key Words: **1-bromopropane; creatine kinase; glutathione; γ-enolase; peripheral nerve; neurotoxicity.**

1-Bromopropane is an important alternative to ozone-depleting solvents such as specific chlorofluorocarbons and 1,1,1-trichloroethane. It is used as a cleaning agent for metals, precision instruments, electronics, optical instruments, and ceramics. However, previous experimental studies from our laboratories have shown that 1-bromopropane has toxic effects on various body systems such as the central nervous system and reproductive system (Ichihara *et al.*, 2000a,b; Yu *et al.*, 1998). Specifically, our study on the neurotoxicity of 1-bromopropane showed decreased limb muscle strength, electrophysiological and morphological changes in peripheral nerves, and swelling of preterminal axons in the gracile nucleus of the medulla oblongata. No other regions of the central nervous system showed any clear morphological changes, although the weight of the cerebrum was decreased. At about the time of reporting our neurotoxicity study, a case was reported in the United States of a 19-year-old male who presented with various neurological symptoms such as weakness of the lower extremities and right hand, numbness, dysphagia, and urinary difficulties following a 2-month exposure to an industrial solvent constituted mainly of 1-bromopropane (Sclar, 1999). Several tests in that case showed specific abnormalities such as changes in periventricular white matter and in areas close to the root ganglia, as detected on magnetic resonance image (MRI), and abnormal findings on somatosensory evoked potential studies suggestive of lesions of the myelinated tracts in the central nervous system.

The cerebrum weight loss in our rats and the MRI findings in the reported patient suggested that 1-bromopropane had adverse effects on the central nervous system. Accordingly, we designed the present study to clarify the underlying mechanism of the neurotoxic effects of 1-bromopropane by examining the initial biochemical changes in the central nervous system.

For this purpose, we measured selected biochemical indices. First, we measured neuron-specific γ-enolase and glia-specific β-S100 protein in the central nervous system to elucidate the susceptible cells in each region. γ-Enolase is localized in the cytoplasm of neurons (Schmechel *et al.*, 1978), and β-S100 protein is specifically distributed in glia cells (Cicero *et al.*, 1970; Isobe *et al.*, 1990; Moore, 1975). These 2 markers are useful in estimating neurological diseases (Royds *et al.*, 1981; Vassilopoulos and Jockers-Wretou, 1987) and solvent-related neurotoxicity (Huang *et al.*, 1989, 1990, 1992). Second, we measured creatine kinase activity in the central nervous sys-

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tem, based on reduced plasma creatine kinase (CK) activity found in our previous 12-week experiment. We also measured the activities of glutamic oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) to confirm whether change in creatine kinase activity is specific to this enzyme. CK is present in neurons, astrocytes, and oligodendrocytes (Manos *et al.*, 1991) and plays a role in continuous replenishment of ATP from phosphocreatine in these cells (Wyss *et al.*, 1992). Acrylamide and ethylene oxide, which were both neurotoxic, suppressed creatine kinase activity in the brain and blood of rats (Kohriyama *et al.*, 1994; Matsuoka *et al.*, 1990, 1996). Moreover, we measured levels of creatine kinase isozymes creatine kinase-BB (CK-B) and creatine kinase-MM (CK-M) in the central nervous system and plasma using enzyme immunoassays to investigate whether decrease in CK activity is due to enzymatic inhibition or decrease in enzyme amount. Third, we measured the glutathione and sulfhydryl base of protein and nonprotein fractions in the central nervous system. We hypothesized that nucleophilic reagents such as sulfhydryl base could be a target of 1-bromopropane, because glutathione conjugates 1-bromopropane in rats (Barnsley *et al.*, 1966; Jones and Walsh, 1979).

In addition, we examined early histopathological changes in the central nervous system and peripheral nerves to clarify the relationship between these biochemical markers and biological changes.

MATERIALS AND METHODS

Animals and exposure to 1-bromopropane. Thirty-six specific pathogen-free, 10-week-old (body weight 240–260 g) male Wistar rats were purchased from Shizuoka Laboratory Animal Center, Japan. They were housed and acclimatized to the new environment for 1 week, then divided at random into 4 groups of 9 each. They were housed in a room set on 12:12 light:dark cycle (lights on at 0900 h and off at 2100 h), stable relative humidity (67–60%), and constant temperature (23–25°C). Food and water were provided *ad libitum*.

The 4 groups of rats were exposed to 200, 400, or 800 ppm 1-bromopropane or fresh air in inhalation chambers for 8 h a day, 7 days. Daily exposure commenced at 1400 and was terminated at 2200 h. The inhalation exposure system has been described previously (Ichihara *et al.*, 1997; Takeuchi *et al.*, 1989). The vapor concentration in the chamber was measured every 10 s by gas chromatography and digitally controlled to within ± 5% of the target concentration. After the 7-day exposure, the measured 1-bromopropane gas concentrations in the 3 chambers were 196 ± 11, 395 ± 8, and 798 ± 16 ppm (mean ± SD). 1-Bromopropane (99.81% purity) was supplied by Tosoh Co., Ltd., Japan. Rat body weight was measured before exposure and after 1, 3, and 7 days of exposure. Japanese law concerning protection and control of animals, the standards related to the care and management of experimental animals, and the Guide for Animal Experimentation of the Nagoya University School of Medicine were followed strictly throughout the experiment.

Organ weight and blood biochemical indices. After 7 days of exposure, 8 rats in each group were sacrificed by exsanguination through the abdominal aorta under pentobarbital anesthesia. Plasma was separated by centrifugation at room temperature and stored at −80°C until analysis. Plasma creatine kinase activity and amount of CK-M isozyme were measured. The brain and spinal cord (2 cm above the last thoracic vertebra, which corresponds with anterior lumbar enlargement) were rapidly removed. The brain was immediately dissected into the cerebral hemisphere, cerebellum, and brain stem (medulla oblongata, pons, and midbrain) on a steel plate placed on ice. Tissues of brain and spinal cord were weighed and kept frozen at −80°C until analysis.

Immunoassays of nerve-specific marker proteins. Tissue blocks of the brain and spinal cord were homogenized in 10 volumes (wt/vol) of 100 mM citrate buffer (pH 7.4) containing 20 mM EDTA at 0°C. The homogenate was centrifuged at 45,000 × *g* for 20 min at 4°C. The supernatant was used for analysis of nerve-specific proteins, activity of creatine kinase, GOT, LDH, and for estimation of soluble protein concentrations. Neuronal marker protein γ-enolase, glial cell marker protein β-S100 protein, CK-B, and CK-M were determined by the highly sensitive sandwich-type enzyme immunoassay system developed by Kato *et al.* (1981, 1982, 1986). Protein concentration of the soluble fractions of homogenates was estimated by the dye binding method of Bradford (1976) using Bio-Rad reagents (Bio-Rad, Richmond, CA).

Quantitative biochemical analyses. Protein-bound and nonprotein sulfhydryl groups were quantified essentially as described by Habeeb (1972). Tissues were homogenized with 10 volumes (wt/vol) of 100 mM citrate buffer (pH 7.4) containing 20 mM EDTA. Proteins were denatured with trichloroacetic acid at a final concentration of 5% and pelleted by centrifugation at 15,000 × *g* for 20 min. Each pellet was dissolved in 40 mM NaPB buffer (pH 8.0) containing 1% SDS and 0.025% EDTA. Sulfhydryl groups in the pellet (protein-bound sulfhydryl base, PSH) and the supernatant (nonprotein sulfhydryl base, NSH) were quantified by incubation with 5,5′-dithiobis-(2-nitrobenzoic; DTMB) in 80 mM NaPB buffer (pH 8.0) containing 2% SDS and 0.05% EDTA at room temperature. For the quantification of total glutathione (total-GSH) and oxidized glutathione (GSSG), the supernatant was used and determined by the method developed by Matsumoto *et al.* (1996). For the total-GSH determination, the supernatant was diluted 100 times with 125 mM sodium phosphate (pH 7.5) containing 6.3 mM EDTA. An aliquot (50 μl) of the diluted solution was assayed at 412 nm in a 1 ml mixture consisting of 0.21 μmol NADOH, 0.6 μmol DTMB, 125 μmol sodium phosphate (pH 7.5), and 6.3 μmol EDTA. For GSSG determination, 20 μl of commercially available acrylonitrile (final concentration, 295 mM), 50–200 μl of the supernatant and 125 μmol sodium phosphate (pH 8.0) containing 6.3 μmol EDTA were mixed to give a final volume of 1 ml, and were incubated at 25°C for 10 min. An aliquot of the preincubation mixture was assayed.

Histopathological examination. The remaining rat of each group was perfused from the left ventricle with Zamboni's solution. Small tissue blocks of cerebellum (posterior vermis), the gracile nucleus of the medulla oblongata, thoracic spinal cord, dorsal root ganglion, and tibial nerve were embedded in epoxy resin, cut into semi-thin sections, and stained by toluidine blue for light microscopic examination. Segments of the posterior tibial nerve were dissected out, postfixed in 0.5% osmium tetroxide, then immersed in 75% ethanol and dehydrated in 50% glycerin. The nerve fibers were loosened and teased by needles in 50% glycerin for light microscopic examination.

Statistical analysis. Data were expressed as mean ± SD. Multiple comparisons between the exposure groups and the control were tested using Dunnett's method following one-way ANOVA. Significant statistical difference was set at $p < 0.05$.

RESULTS

Changes in Body and Brain Weights

Exposure to 800 ppm 1-bromopropane for 7 days resulted in a significant loss of body weight compared with the control. The weights of various brain regions did not change significantly following exposure to 1-bromopropane (Table 1).

Changes in Neuron-Specific Markers and Various Blood Enzymes and Isozymes

Exposure of rats to 400 and 800 ppm 1-bromopropane resulted in a significant decrease in tissue concentrations of

TABLE 1
Body and Brain Weights in Rats after Exposure to 1-Bromopropane for 7 Days

Group	Control	200 ppm	400 ppm	800 ppm
Body weight (g)	298 ± 4.9	303 ± 8.5	296 ± 7.4	281 ± 9.7*
Absolute organ weight				
Whole brain (g)	1.86 ± 0.03	1.86 ± 0.05	1.87 ± 0.04	1.82 ± 0.04
Cerebrum (g)	1.05 ± 0.02	1.07 ± 0.03	1.06 ± 0.03	1.04 ± 0.02
Cerebellum (g)	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
Brain stem (g)	0.54 ± 0.01	0.53 ± 0.02	0.54 ± 0.01	0.52 ± 0.01

Note. Values are the means ± SD for each group. For all groups, $n = 8$.
*Significantly different from the control ($p < 0.05$, Dunnett's multiple comparison).

γ-enolase in the cerebrum and cerebellum. No significant changes were noted in β-S100 protein (Table 2).

The CK activity decreased dose-dependently in various regions of the brain and spinal cord in all exposed rats, but no changes were noted in GOT and LDH levels (Table 3). CK-B tissue concentrations decreased significantly in the cerebrum of rats exposed to 800 ppm 1-bromopropane, however, it increased significantly in the brain stem of the same group, and increased in the spinal cord in rats exposed to 400 and 800 ppm 1-bromopropane. No significant change was found in the CK-M of brain by region or in that of spinal cord tissue (Table 4). On the other hand, plasma CK activity decreased dose-dependently in the exposed groups, and the amount of CK-M decreased dose-dependently in parallel with the enzymatic activity (Table 5).

Changes in Total-GSH, GSSG, PSH, and NSH

Total glutathione concentrations were significantly lower in the cerebrum and cerebellum of rats exposed to 800 ppm compared to the control, but were significantly higher in the spinal cord of all exposed rats. Furthermore, GSSG was lower only in the brain stem of rats exposed to 400 ppm, and the change was not concentration-dependent. NSH was significantly lower in both the cerebrum and cerebellum of 800 ppm exposed rats, and in the brain stem of all exposed rats; however, it was higher in the spinal cord of rats exposed to 800 ppm. Exposure to 1-bromopropane did not alter the levels of PSH (Table 6).

Histopathological Changes

Examination of epoxy resin-embedded sections of the gracile nucleus of rats exposed to 800 ppm 1-bromopropane showed swelling of preterminal axon containing dark-stained material with thin myelin sheath (Fig. 1). No significant change was found in the sections of the cerebellum, dorsal root ganglion, or thoracic spinal cord.

Histopathological examination of the muscle branch of the posterior tibial nerve, both in epoxy resin-embedded section and segments that were loosened and teased by needles, showed swelling or a dense mass of myelin sheath especially near the nodes of Ranvier, hypertrophy of Schwann cell cytoplasm and less frequency of Schmidt-Lanterman's incisures in rats exposed to 800 ppm 1-bromopropane (Fig. 2).

TABLE 2
γ-Enolase and β-S100 Protein in Rat Brain and Spinal Cord Tissues following 7 Days of 1-Bromopropane Exposure

	Control	200 ppm	400 ppm	800 ppm
γ-Enolase (ng/μg soluble protein)				
Cerebrum	12.27 ± 1.02	12.86 ± 1.26	10.16 ± 2.01*	9.67 ± 1.60*
Cerebellum	20.13 ± 4.59	15.37 ± 3.48	14.43 ± 2.90*	14.85 ± 4.32*
Brain stem	9.80 ± 1.09	9.88 ± 1.51	10.04 ± 1.28	9.72 ± 1.21
Spinal cord	4.15 ± 1.15	3.82 ± 0.83	4.37 ± 0.68	4.63 ± 0.88
β-S100 (ng/μg souble protein)				
Cerebrum	2.37 ± 0.33	2.69 ± 0.28	2.50 ± 0.43	2.45 ± 0.21
Cerebellum	3.97 ± 1.08	4.20 ± 0.62	4.12 ± 0.93	3.94 ± 0.59
Brain stem	3.72 ± 0.36	3.74 ± 0.47	3.96 ± 0.49	3.98 ± 0.58
Spinal cord	4.54 ± 0.72	4.67 ± 0.61	5.11 ± 0.85	5.03 ± 1.36

Note. Values are the means ± SD for each group. For all groups, $n = 8$.
*Significantly different from the control ($p < 0.05$, Dunnett's multiple comparison).

TABLE 3
CK, GOT, and LDH Activities in Rat Brain and Spinal Cord Tissues following 7 Days of 1-Bromopropane Exposure

	Control	200 ppm	400 ppm	800 ppm
CK (IU/mg soluble protein)				
Cerebrum	522.3 ± 34.9	494.5 ± 80.7	420.9 ± 18.5*	324.2 ± 23.9*
Cerebellum	737.0 ± 158.6	707.6 ± 65.4	680.4 ± 58.5	539.2 ± 33.7*
Brain stem	594.3 ± 53.4	531.4 ± 39.4*	471.1 ± 32.4*	354.1 ± 29.0*
Spinal cord	494.2 ± 48.6	443.0 ± 21.8*	391.0 ± 36.2*	338.6 ± 46.6*
GOT (IU/mg soluble protein)				
Cerebrum	6.8 ± 9.7	3.7 ± 0.8	3.4 ± 0.1	3.3 ± 0.2
Cerebellum	5.6 ± 1.3	5.2 ± 0.3	5.0 ± 0.2	4.8 ± 0.4
Brain stem	5.1 ± 0.5	5.1 ± 0.3	5.2 ± 0.4	5.0 ± 0.3
Spinal cord	3.3 ± 0.4	3.2 ± 0.2	3.1 ± 0.2	3.4 ± 0.6
LDH (IU/mg soluble protein)				
Cerebrum	6.8 ± 0.3	7.2 ± 1.7	6.6 ± 0.4	6.4 ± 0.4
Cerebellum	7.7 ± 1.7	7.2 ± 0.4	6.9 ± 0.3	6.6 ± 0.4
Brain stem	7.0 ± 0.6	7.1 ± 0.4	7.3 ± 0.5	7.1 ± 0.3
Spinal cord	4.9 ± 0.5	4.7 ± 0.3	4.7 ± 0.3	5.0 ± 0.8

Note. Values are the means ± SD for each group. For all groups, $n = 8$.
*Significantly different from the control ($p < 0.05$, Dunnett's multiple comparison).

DISCUSSION

Our results showed that γ-enolase and CK activity decreased after exposure to 1-bromopropane at concentrations lower than those at which morphological changes were initially detected in the central nervous system. These results indicated that biochemical indices are more vulnerable than morphological structure to 1-bromopropane exposure, and might underlie the mechanism of neurotoxic effects of 1-bromopropane. The dose-dependent decrease in γ-enolase might be specific to 1-bromoproane exposure, because exposure to toluene and n-hexane does not reduce γ-enolase in the rat brain; rather, γ-enolase and β-S100 increase following toluene exposure (Huang *et al.*, 1989, 1990, 1992). Since γ-enolase is localized specifically in neurons, a decrease in the concentration of this enzyme might suggest a decrease in the amount of the enzyme per cell or a decrease in the number of neurons. If the latter is the case, it indicates a substantial toxic effect of 1-bromopropane on neurons.

In the present study, the inhibition of CK activities in the brain and spinal cord seems to be the most sensitive indicator of 1-bromopropane exposure. Based on the results of CK activity and the amounts of CK-B and CK-M identified, we can discuss whether the decrease in CK activity was due to the decrease in CK amount. The amount of CK-M, which accounts for 0.1–5% of total CK subunits in the central nervous system, did not change significantly. The amount of CK-B, which accounts for 99.9% of total CK subunits in the brain and 95% in the spinal cord, did not parallel CK activity, and the decrease in CK activity exceeded that of CK-B concentration in the cerebrum. Furthermore, in the brainstem and spinal cord,

TABLE 4
CK-B and CK-M in Rat Brain and Spinal Cord Tissues following 7 Days of 1-Bromopropane Exposure

	Control	200 ppm	400 ppm	800 ppm
CK-B (ng/μg soluble protein)				
Cerebrum	17.75 ± 2.15	16.26 ± 1.55	16.36 ± 1.14	14.30 ± 1.76*
Cerebellum	24.59 ± 4.91	23.73 ± 1.47	22.43 ± 1.80	22.60 ± 2.49
Brain stem	16.16 ± 1.29	15.69 ± 2.55	18.37 ± 1.21	19.25 ± 2.50*
Spinal cord	12.34 ± 0.98	13.54 ± 1.11	14.78 ± 2.29*	16.33 ± 1.17*
CK-M (pg/μg soluble protein)				
Cerebrum	11.99 ± 7.26	13.51 ± 9.40	10.57 ± 4.89	9.92 ± 5.99
Cerebellum	14.20 ± 9.33	12.53 ± 9.30	9.01 ± 3.29	9.52 ± 3.82
Brain stem	24.30 ± 7.32	19.06 ± 8.48	14.32 ± 5.84	17.74 ± 4.93
Spinal cord	522.93 ± 233.63	651.65 ± 170.74	349.12 ± 98.62	430.93 ± 294.32

Note. Values are the means ± SD for each group. For all groups, $n = 8$.
*Significantly different from the control ($p < 0.05$, Dunnett's multiple comparison).

TABLE 5
Plasma CK Activity and CK-M Content in Rats Exposed to 1-Bromopropane for 7 Days

Group	Control	200 ppm	400 ppm	800 ppm
CK activity (IU/l)	464.7 ± 113.4	438.0 ± 147.1	345.1 ± 124.4	173.7 ± 49.3*
CK-M (ng/mg soluble protein)	3.38 ± 2.08	3.15 ± 1.87	1.94 ± 1.00	1.23 ± 1.17*

Note. Values are the means ± SD for each group. For all groups, $n = 8$.
*Significantly different from the control ($p < 0.05$, Dunnett's multiple comparison).

CK-B concentration did not decrease but rather increased, although CK activity decreased after exposure to 1-bromopropane. Thus, the decrease in CK-B amount cannot fully explain the fall in CK activity. It is possible that a considerable amount of CK-B lost its enzymatic activity, suggesting enzymatic inhibition probably through chemical modification of the enzyme. On the other hand, the plasma CK-M level almost paralleled CK activity. It is also possible that only a small proportion of CK-M that lost its activity was remaining in the blood plasma, although it is also possible that the CK-M antibody used in our study could not recognize CK-M denatured by 1-bromopropane exposure.

Our results also showed that exposure to 1-bromopropane resulted in a significant decrease in tissue concentrations of glutathione in the cerebrum and cerebellum, the site that also showed reduced levels of γ-enolase and CK activity. Glutathione depletion is thought to be associated with increased vulnerability of the brain to certain neurotoxic agents and contributes to oxidative damage of neurons and glial cells (Hu *et al.*, 1999; Trenga *et al.*, 1991). Nonprotein SH levels almost correspond with those of GSH, and this was also valid under exposure to 1-bromopropane. Glutathione has a SH-base, which plays a role in reduction or conjugation of oxidative agent or other toxic substances. CK also has a SH-base functional site, and the behavior of its enzymatic activity might represent other functional proteins with SH-base (Zhou and Tsou, 1987). It is possible that the neurotoxic effects of 1-bromopropane might include possible modification of functional proteins containing a SH-base, represented by CK, in addition to glutathione depletion. There was no increase in GSSG and hence no evidence of oxidative stress. Our results showed increased levels of total-GSH only in the spinal cord, contrary to that in the cerebrum or cerebellum. It is possible that this increase in total-GSH in the spinal cord represented a compensatory effect.

The exposure levels in the present study followed those in

TABLE 6
Total-GSH, GSSG, NSH, and PSH Levels in Rat Brain and Spinal Cord Tissues following 7 Days of 1-Bromopropane Exposure

	Control	200 ppm	400 ppm	800 ppm
Total-GSH (nmol/g brain tissue)				
Cerebrum	1031.9 ± 156.9	1038.7 ± 109.7	968.9 ± 47.9	813.9 ± 80.7*
Cerebellum	755.3 ± 65.6	743.5 ± 61.4	691.8 ± 69.9	596.4 ± 39.7*
Brain stem	613.8 ± 34.0	682.4 ± 65.0	640.8 ± 92.6	602.9 ± 100.8
Spinal cord	181.2 ± 34.9	279.6 ± 31.0*	333.9 ± 38.5*	371.0 ± 69.5*
GSSG (nmol/g brain tissue)				
Cerebrum	18.7 ± 1.1	19.8 ± 3.0	20.3 ± 2.3	19.7 ± 2.3
Cerebellum	11.8 ± 2.6	15.1 ± 5.6	10.7 ± 3.7	12.6 ± 3.2
Brain stem	18.5 ± 3.4	17.1 ± 3.6	12.8 ± 2.98	14.5 ± 3.4
Spinal cord	17.1 ± 3.4	18.7 ± 2.9	19.9 ± 3.8	20.4 ± 2.1
NSH (umol/g brain tissue)				
Cerebrum	1.567 ± 0.094	1.520 ± 0.105	1.472 ± 0.056	1.276 ± 0.067*
Cerebellum	1.191 ± 0.082	1.133 ± 0.081	1.171 ± 0.146	0.946 ± 0.093*
Brain stem	1.403 ± 0.149	1.149 ± 0.083	1.154 ± 0.178*	1.082 ± 0.020*
Spinal cord	0.606 ± 0.080	0.653 ± 0.052	0.707 ± 0.105	0.741 ± 0.105*
PSH (umol/g soluble protein)				
Cerebrum	105.49 ± 24.06	99.47 ± 17.59	88.24 ± 7.17	104.43 ± 22.00
Cerebellum	172.17 ± 39.29	164.56 ± 16.04	149.48 ± 20.02	146.14 ± 20.66
Brain stem	136.43 ± 16.12	130.87 ± 3.17	136.01 ± 11.34	130.69 ± 4.61
Spinal cord	98.43 ± 9.39	97.56 ± 9.99	102.83 ± 3.69	99.91 ± 9.77

Note. Values are the means ± SD for each group. For all groups, $n = 8$.
*Significantly different from the control ($p < 0.05$, Dunnett's multiple comparison).

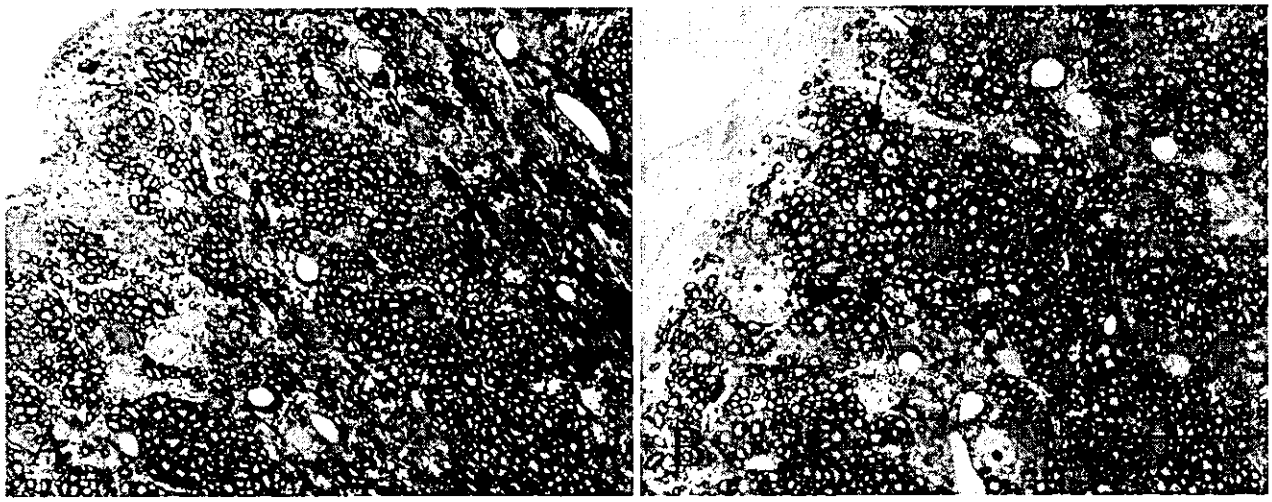

FIG. 1. Photomicrographs of preterminal axons in gracile nucleus of rats. (A) Control, no abnormal changes. (B) Rat of exposed to 1-bromopropane 800 ppm. Note the swelling of the preterminal axon containing dark-stained material (arrowhead) and the thin myelin sheath (arrows). Toluidine blue. Magnification of (A) and (B) is the same.

our previous 12-week study (Ichihara *et al.*, 2000a,b). It was reported that some workers were exposed to 1-bromopropane at 18–381 ppm (mean = 142) in the plants where 1-bromopropane was used as a solvent of spray adhesive without sufficient ventilation or containment (NIOSH, 1999, 2000). One case report with neurological disorders showed exposure levels ranging from 60–261 ppm (mean = 133 ppm; Ichihara *et al.*, 2002). The exposure levels in the present study covered the range of the real exposure levels of workers using 1-bromopropane under worse conditions as seen in the above cases. However, it should be also noted that it might be difficult to extrapolate from the present data to humans at this moment, because the possibility of metabolic enhancement or species difference of susceptibility has not been clarified yet.

In conclusion, we demonstrated in the present study that 1-bromopropane induced dose-dependently a decrease in neurospecific γ-enolase in the cerebrum and cerebellum that suggested functional or cellular loss of neurons. This was accompanied by decreases in sulfhydryl base, total glutathione, and creatine kinase activity. Glutathione depletion or modification of functional proteins containing a sulfhydryl base might be the underlying mechanism of 1-bromopropane-induced neurotox-

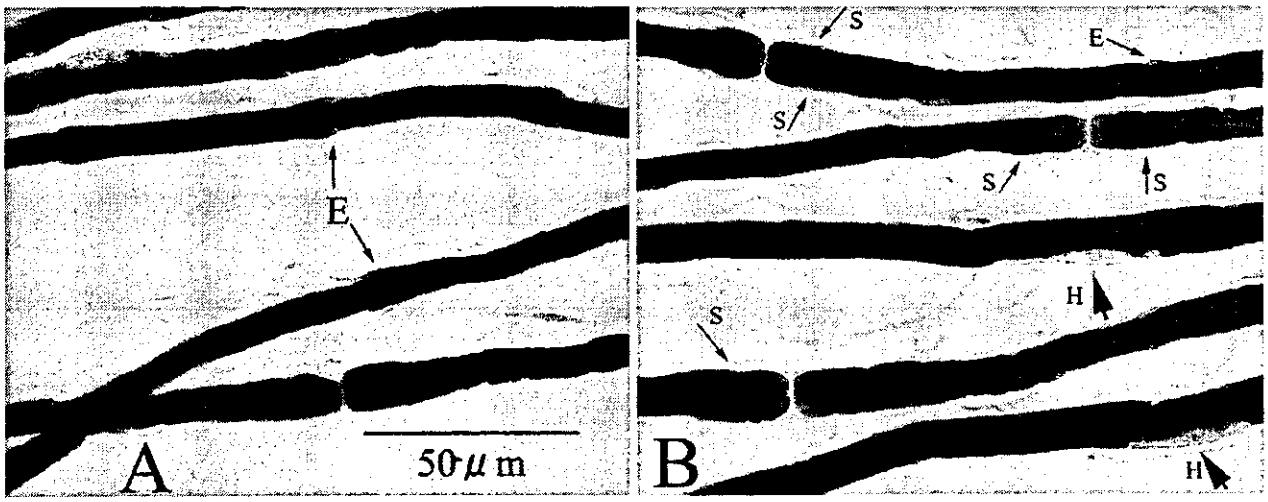

FIG. 2. Photomicrographs of the muscular branch of the posterior tibial nerve. (A) Control, no abnormal changes, Schmidt-Lanterman's incisures (E) can be seen clearly. (B) A representative rat exposed to 800 ppm 1-bromopropane. Note swelling or dense mass of myelin sheath (S) especially near the nodes of Ranvier, and hypertrophy of Schwann cell cytoplasm (H). Magnification of (A) and (B) is the same.

icity. Our results also showed that the medulla oblongata and peripheral nerves start to show morphological changes within 7 days of exposure.

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Rapid Communication

Neurological Disorders in Three Workers Exposed to 1-Bromopropane

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Abstract: Neurological Disorders in Three Workers Exposed to 1-Bromopropane: Gaku ICHIHARA, *et al.* Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine—A 35-yr-old female worker developed sore throat, stumbling, dysphagia, incontinence of urination and numbness with a burning sensation in the legs, thighs, hips and lower back as well as numbness in the perineum. She was spraying a glue to compose a cushion with polyurethane foam parts. One year after beginning to use a glue containing mainly 1-bromopropane, she became unable to stand up by herself. The second case, a 30-yr-old female worker developed a staggering gait, paresthesia, urinary incontinence, slurred voice, dysphagia, numbness or paresthesia in the hands, legs, lower back, hips and perineum, six months after beginning the same task. The third case, a 50-yr-old female worker showed signs of staggering, and numbness and paresthesias in the feet, thighs, lower back and hips, and headache, two months after starting the work. The daily time-weighted average of exposure concentrations ranged from 60 to 261 ppm (mean 133, N=11) after the ventilation was improved. The common signs in the three workers were staggering, numbness with paresthesia/dysesthesia, as well as a remarkable decrease in vibration sense in the legs and various symptoms in the central nervous system. Abnormal sensation was distributed to the area covered by pantyhose, rather than glove-stocking. Not only peripheral nerves, but also the spinal cord or brainstem was suspected to be impaired, given the paresthesia/dysesthesia and the distribution of sense deficits. Their diarrhea, incontinence of urination and abnormal sweating also suggested disorders in the autonomous nervous system. 1-Bromopropane might induce neurological disorders in the peripheral nerves and/or the central nervous system in humans.
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Key words: Intoxication cases, Neurological disorders, 1-Bromopropane, Alternative solvent to ozone-layer depleting, Intoxication

1-Bromopropane is a new alternative to ozone-depleting solvents. It is less destructive to the ozone layer, but nonflammable and moderately volatile, which are important properties for metal cleaning agents. Other alternatives have been phased out because of their ozone-depleting potency or toxicity, so the demand for 1-bromopropane as a solvent is now increasing in industry, but it was recently revealed to have neurotoxicity and reproductive toxicity in animal experiments[1–4]. Although one case report suggests the neurotoxicity of 1-bromopropane in a worker[5], its neurotoxicity in humans is generally not well understood. We report neurological disorders in three workers exposed to 1-bromopropane in a cushion company, because we consider these cases very important in understanding the neurotoxicity of 1-bromopropane in humans.

Case Reports

Three workers, who used 1-bromopropane as a solvent with a spray gun, showed abnormal neurological symptoms or signs. In a cushion company in North Carolina in the U.S., 1-bromopane was introduced in the workplace to replace dichloromethane as a solvent in glue in July 1999. The workers sprayed glue on polyurethane foam parts, then pressed them together by hand to form a bond as a part of the cushion production process. Fifteen workers were engaged in spraying glue in the same workroom at the time that case 1 became ill in June of

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2000. The workbenches for the fifteen workers were arranged along one long side and one short side of a rectangular room. Each worker, including both case 1 and case 2, did their jobs, while facing a wall equipped with a ventilator, but the ventilators were operated only 15 min per hour in the summer of June, 2000. The ventilator in front of case 3 was not operating at all up through June. Some workbenches had no ventilators from the very start for other workers. The workers used spray guns, and the glue sometimes spattered on their faces. They wore only thin latex gloves for work, but no proper gloves or masks able to protect them from the solvents. The Material Safety Data Sheet (MSDS) indicated that the glue contained 1-bromopropane 55%, ethyl acetate 8% and aliphatic petroleum distillates 2%.

Case 1: The patient, a 35-yr-old female was carried to the local emergency Room on June 30, 2000, because she could not walk by herself in the morning. She had dystaxia and dysesthesia with a burning sensation or tingling with numbness in both feet, legs, thighs and lower back. On admission she could not walk without assistance. It was difficult for her to use stairs. Dysesthesia was noted by touching. Her vaginal area felt numb both inside and outside, as if she had been given local anesthesia. She walked with a wide spread gait. She had been engaged in spraying glue in a cushion factory since 1998. From April to June, just prior to developing the above mentioned neurological symptoms, she went to the emergency room four times for a sore throat, earache, sinus irritation and hoarseness. Urinary incontinence was also noted during this period. She usually worked eight hours a day from Monday to Friday, but in June 2000, she worked nine hours per day. At the beginning of that month, she experienced difficulty in swallowing as if something was stuck in her throat. On June 23, she began to stumble, feel dizzy and lose her balance. She stayed home from work for two days, but the symptoms worsened. On June 26, she consulted her primary care doctor, because of the dizziness, sore throat, eyeache and numbness in the perineum. She was prescribed antibiotics and anti-allergic agents (Nasonex, mometasone furoate monohydrate) for ten d. On June 27–29, she worked for eight or nine h per d. The stumbling worsened, and her sore throat, earache and malaise in the sinuses failed to improve. She felt numbness and muscle pain in her feet, legs, thighs and lumbar area. Numbness in the thighs was more remarkable at the front than at the back, but in the legs, conversely, more remarkable at the back than at the front. She also sometimes had headaches. She found it more difficult to walk with each passing day. She lost 11 pounds (about 5 kg) during the four days from 26 to 30 June, 2000.

Neurologic examination on admission revealed that the patient was alert and oriented with clear speech and sensorium. No intranuclear ophthalmoplegia was identified. The pupils were round and reactive to light. The left pupil seemed slightly larger than the right one, and there was some hippus noted bilaterally. Both tympanic membranes appeared clear. Tongue movements were intact. No carotid bruit was noted and the heartbeat was regular. Tone did not increase and deep tendon reflexes were equal, symmetric and active throughout. There was no clear weakness in her upper and lower extremities. Plantar responses were flexor bilaterally. Her prick sensory level was about T6, and her vibration sense was distally diminished. The patient did not show any sings of spastic gait, but she was unsteady and had to hang onto the wall. Romberg sign was negative. There was no abnormality in the nose-finger-nose, heel-shin, line-drawing or pronation-supination test. Her spouse said that the patient was originally cheerful and active, but her mood had been changed to be depressive. The patient felt tired even after working for a short time. She was depressive but sometimes irritated and tended to get angry.

Past and family history: Hypertension, migraine headaches. The patient was allergic to penicillin and codeine. She had had a tonsillectomy, bunionectomy, cholecystectomy and tubal ligation. Her family history included diabetes mellitus (grandfather and sister), heart attack (father) and ovarian cancer (sister). Drinking history: one or two glasses of beer per two wk. Smoking history: 20 cigarettes per d for 18 years. She felt recently that she had become unable to drink much. Her cholesterol was 224 with HDL of 29 (mg/d*l*). Her respiratory rate was sedate and 12. ANA was negative. The MRIs of the brain and cervical and thoracic portions of the spinal cord did not show any particular findings. No Lhermitte's phenomenon was observed.

On July 3 (3 days after admission), her walking had improved a little, but she still stumbled and her throat remained sore. On July 4, serum vitamin B12 was 442 mg/d*l* and Lyme disease antibody, IgG and IgM were negative. No urinary lead or mercury was detected and urinary arsenic was within normal limits (48.3 $\mu g/l$, 150.9 μg/g creatinine). On July 6, lumbar puncture produced clear, colorless fluid. Cell counts of cerebrospinal fluid (CSF) were all within normal limits (RBC 2, WBC 2, Lymphocyte 70, Monocyte-macrophage 30 but no other cells were found). The serum protein pattern was normal (total protein 7.2, albumin 4.19, alpha-1 0.46, alpha-2 0.75, beta 0.74, gamma 1.06 g/d*l*). On July 19, a gynecologist found mild cystocele and rectocele, which suggested mild pelvic relaxation. In July, her menstruation began 2 wk earlier than usual. On August 23, an electrophysiological study showed values within normal limits in motor nerve conduction velocity on both sides of the peroneal nerve and left posterior tibial nerve, and sensory nerve conduction velocity on both sides of

the sural nerve. On November 21, MRI of lumbar portions of the spinal cord showed only degenerative change with facet hypertrophy and mild central bulging of the intervertebral disc at L5-S1.

Case 2: The patient, a 30-yr-old female, worked in the same cushion company from February to June 30, 2000. On June 30, she developed a staggering gait as if she were in a drunken stupor. The patient was seen at the same local emergency room and was noted to have paresthesias. She had difficulty in noticing the need to defecate or void and could tell only by a sense of discomfort in her abdomen. She had numbness in the perineum and lacked sensation during sexual intercourse. At first, there was some numbness in her hand, but it resolved later. She had some lower back pain, but no trouble with fever or chills. She had some difficulty in speaking, and her words were somewhat slurred. Her lower extremities usually felt tired. She lost 5 pounds (about 2 kg) in the last week of June.

Past illness: kidney infection (1998), cholelithotomy (1993), Family history: heart attack (grandmother), brain attack (grandfather), diabetes mellitus (mother) Smoking: forty cigarettes per week. Drinking: 2 times per yr. The former work: packing mail staples for four yr. She had a Cesarean section in the past. She was not allergic to any medications, nor was she taking any medications on a daily basis.

Neurological examination revealed her to be alert and oriented with clear speech and sensorium. The pupils were round and reactive to light. Extraocular muscle movements and tongue movements were intact. The patient had the full range of motion of the cervical spine. She had no Lhermitte's phenomenon. There was no definite focal motor weakness in her upper or lower extremities. Plantar responses were flexor bilaterally. Deep tendon reflexes were equal, symmetric and active throughout. There was peripheral diminution to vibratory and pin prick sensation on the right lower extremity to the knee. The sensation was less impaired up toward the waist. It was less severe on the left side than the right side both in the legs and in the thighs. There was no spasticity, no fasciculations nor clear muscle atrophy. Finger-nose and heel-shin testing was normal. Gait and station were normal. Tandem gait was done without any difficulty. Romberg sign was negative. Rectal exam showed fair rectal tone. On July 19, 2000, the patient noted at times that her feet sensation easily changed from hot to cold. On August 9, 2000, the symptoms had improved, but she was still having paresthesias in both her lower extremities. No focal motor weakness was identified. MRIs of cervical, thoracic and lumbar spine did not show any particular findings. On August 24, 2000, she still had urinary incontinence. She had difficulty in swallowing solid food. She had a very poor appetite and abdominal pain. She felt hot and sometimes cold in her feet. She felt hot but not really hot and just flushed. On 6 September 2000, the patient had some pain in her lower back, as well as in her stomach. She felt hot at night and her stomach sometimes felt hot in the morning. She felt her stomach bloated and also felt as if she were pregnant. She also had some repeated diarrhea and nausea/vomiting. Although there was nothing to void, she found herself repeatedly trying to do so. She suffered somewhat from insomnia. The patient also had bifrontal headache and at times her head felt like popping. Higher cortical functioning was normal. Funduscopic examination showed no particular findings. There was no abnormality in the line-drawing or pronation-supination test. Menstruation began one wk later than usual in June 2000. The patient tired easily and was misunderstood as lazy by her spouse. The patient was depressive, but sometimes irritated and tended to get angry. In June this patient fought with the former patient in case 1, though she had never done so before.

Case 3: The patient, a 50-yr-old female, started the work in March, 2000. She became aware of weight loss, staggering, numbness in the feet and pain in her wrists, elbows and shoulders from May to June, 2000. She also felt pain in the front of her thighs at night. During the week, she felt better on Monday, but on Tuesday she felt pain in her feet and shoulders, headache, and sore throat. On Wednesday and Thursday, rhinorrhea, and stumbling occurred, and her headaches became more severe. These symptoms were reduced over the weekend holidays. This change in symptoms was repeated every week. She could call up words, but sometimes felt difficulty in speaking.

Neurological examination revealed her to be alert and oriented with clear speech and sensorium. The pupils were round and reactive to light. Extraocular muscle movements and tongue movements were intact. She had no definite focal motor weakness in her upper or lower extremities. Plantar responses were flexor bilaterally. Deep tendon reflexes were equal, symmetric and active throughout. There was peripheral diminution of response to vibratory and pin prick sensation in both lower extremities to about the level of the thighs, hips and lower back, but tactile sense was lowered only in the toes and was comparatively well preserved in the upper levels. She could not maintain a standing position when her knees were bent even slightly by others. Romberg sign was negative. There was no abnormality in the nose-finger-nose, heel-shin, line-drawing or pronation-supination test. She said that she could stare but could not concentrate.

The exposure level of the worker to 1-bromopropane was estimated with a passive sampler (passive gas tube for organic solvents, Sibata Scientific Technology Ltd., Tokyo, Japan). A passive sampler was attached to the worker for eight h during work on October 23–26, 30 and 31, 2000. Immediately after each day's work, the sampler was sealed in an aluminum bag and kept at 4°C

Table 1. Worker symptoms while using organic solvent

Working period	Case 1 12 months (99.7–00.6)	Case 2 5 months (00.2–00.6)	Case 3 9 months (00.3–00.12)
Eye pain	+	–	–
Nose stimulation	+	+	–
Sore throat	+	+	–
Strange taste	+	+	–
Hot face	+	+	–
*Vertigo or dizziness	+	+	+
*Light-headedness	+	+	+
*Feel intoxicated	+	+	+
Heavy-headedness	+	–	–
*Headache	+	+	+

*Items common to all three workers. No worker complained of "dim eyesight" or "strange smell" in answer to the questions regarding symptoms while using organic solvent.

until analysis. On analysis, coal particles were taken from the samplers and then immersed in 2 m*l* carbon disulfide (Wako Pure Chemicals, Osaka, Japan) in a glass tube with a screw cap. The tube was shaken for 5 min and left for 2 h and the supernatant liquid was injected into GC-MS (GCD system G1800A, Hewlett Packard). 1-Bromopropane was quantified by the single-ion mode at 43.1 (m/z) with a qualifier at 41.15 (m/z). The value of 0.134 was used in calculation as the sampling rate for 1-bromopropane. The daily time-weighted average ranged from 60 ppm (min.) to 261 ppm (max.). The average of the daily values and the standard deviation (S.D.) for eleven d (N=11) was 133 ± 67 (mean ± S.D.) ppm.

Questionnaire to three workers

Three workers were interviewed based on the check up form for organic solvent intoxication at Nagoya University School of Medicine (Tables 1 and 2). The common symptoms or signs of the three workers were staggering, weakness in legs, and numbness and paresthesia with a similar distribution in their feet, legs, thighs, lower back and hips, along with various symptoms in the central nervous system such as headache, dizziness, nausea, insomnia, anxiety, irritation, light headedness, muddle-headedness, forgetfulness, difficulty in concentrating and listlessness. They also complained of weakness in the legs, tremor in the legs, dizziness when standing up, thirst, indigestion, diarrhea, weight loss, rough skin, a strange feeling in throat and frequent cough.

Discussions

The three workers showed the common symptoms of staggering, numbness and paresthesia/dysesthesia with a similar distribution in their feet, legs, thighs, lower back and hips as well as a remarkable decrease in vibration sense, along with various symptoms of the central nervous system and autonomic symptoms. There were no laboratory data supporting Lyme disease, multiple sclerosis, Guillan-Barre syndrome, lack of Vitamin B12 or metal intoxications in Case 1, but no such data were available in the other cases. Although other causes were not completely ruled out, common work-related causes were suspected in the three cases. This is because common symptoms and signs were found in these three among the fifteen workers engaged in the same work almost at the same time and during the busiest months. The weekly changes in neurological symptoms in case 3 also strongly suggested solvent involvement.

Our previous animal experiments showed 1-bromopropane exposure induced myelin degeneration in the peripheral nerves, swelling of preterminal axons in the gracile nucleus in the medulla oblongata at 800 ppm or over[1, 3, 4] and pyknotic shrinkage of Purukinje cells in the cerebellum at 1000 ppm[3], as well as electrophysiological changes in the peripheral nerves and weight loss of the brain at 800 ppm[1]. Recent animal studies also revealed a decrease in neuro-specific γ-enolase in the brain[6, 7]. These animal studies, which provide definite evidences of the neurotoxicity of 1-bromopropane, suggest the involvement of 1-bromopropane in the cases under study.

The present cases provided some implications as to vulnerable regions in the nervous system, which had not been found in previous animal studies. The neurological signs and symptoms suggesting abnormal regions are summarized from our three cases and the previous case[5] in Table 3. The present three cases showed abnormal sensation not only in distal portions of the lower extremities, but also in the thighs, back, hips and genital area. The present cases were not the typical glove-stocking type neuropathy observed in hexane intoxication[8, 9], but as it were, pantyhose type. The paresthesia and dysesthesia like a burning sensation could

Table 2. Recent symptoms and signs after 1-bromopropane exposure in three workers

Working period	Case 1 12 months (99.7–00.6)	Case 2 5 months (00.2–00.6)	Case 3 9 months (00.3–00.12)
Heavy-headedness	+	+	−
*Headache	+	+	+
*Vertigo or dizziness	+	+	+
*Nausea	+	+	+
Vomitting	−	+	−
*Insomnia	+	+	+
Frequent nightmares	−	+	+
*Anxious about various things	+	+	+
*Irritated by various things	+	+	+
*Light-headed	+	+	+
*Feel muddle-headed	+	+	+
*Forgetful	+	+	+
*Difficulty in concentrating	+	+	+
*Tremor in hands or legs	+ (leg)	+ (leg)	+ (leg)
Cramp in hands or legs	+ (leg)	−	+ (leg)
*Feel dizzy when standing up	+	+	+
Palpitation	−	−	+
Feel as though chest is compressed	+	−	−
Feel as though choking or suffocating	+	+	−
*Listlessness	+	+	+
*Hands or legs feel heavy	+	+	+
No appetite	+	+	−
Abdominal pain (Stomachache)	−	+	−
*Thirsty	+	+	+
*Indigestion (digestive trouble)	+	+	+
*Likely to have diarrhea	+	+	+
*Lost weight	+	+	+
Changed the way you sweat	+	−	−
Irregular periods	+	+	−
Lost sexual desire	+	+	−
Dim eyesight	−	−	+
Eye strain	+	−	+
Difficulty in hearing	−	−	+
Difficulty in speaking	−	−	+
Taste strange	−	+	−
*Pain in joints or elsewhere	+	+	+
*Numbness in hands and legs	+	+	+
Chilly hands and legs	+	+	−
*Smarting or abnormal feeling in hands and legs	+ (leg)	+ (leg)	+ (leg)
Weakened grip	+	+	−
*Weakness in the hands or legs	+	+	+
*Tend to stumble	+	+	+
*Rough skin	+	+	+
*Throat feels strange	+	+	+
*Frequent cough	+	+	+
Unable to drink much	+	+	−

*Items common to all three workers. No worker complained of "Sometimes lose consciousness", "Have convulsive fits", "Likely to have constipation", "Have a slight fever", "Ringing or buzzing in my ears", "Difficulty in smelling", "Difficulty in tasting" or "Gums bleed easily".

Table 3. Neurological signs or symptoms suggesting abnormal regions of the nervous system

Working period	Case 1 12 months (99.7–00.6)	Case 2 5 months (00.2–00.6)	Case 3 9 months (00.3–00.12)	(Sclar's case) 2 months (98.1–98.2)
Weakness in limbs	+ (subjective)	+ (subjective)	+ (subjective)	+
Tremor in legs	+	+	+	?
*Numbness in low back	+	+	+	?
*Numbness in frontal thighs	+	+	+	?
*Decrease in temperature sensation in foot	+	+	+	?
*Decrease in vibration sense in feet	+	+	+	+
*Decrease in superficial sense in feet	+	+	+	+
Numbness in perineum	+	+	–	?
*Paresthesia or dysesthesia	+	+	+	?
Dysphagia	+	+	–	+
Difficulty in speaking	+	+	+	?
Urinary difficutly	+	+	–	+
*Diarrhea	+	+	+	?
*Dizziness when standing up	+	+	+	?
*Thirsty	+	+	+	?
*Memory loss	+	+	+	?
*Headache	+	+	+	?
Dizziness	+	+	+	?
Insomnia	+	+	+	?
Brain and cervical MRI	–	–	Not examined	+
Electrophysiology in legs	–	Not examined	Not examined	+

*Items common to cases 1–3.

indicate peripheral nerve, spinal cord or probably brainstem origin[10]. They also showed symptoms of the central nervous system such as memory loss, headache or mood changes. Sclar reported a patient[5] exposed to 1-bromopropane and with distal dominant neuropathy in the limbs, but there was no description of the presence of abnormal sensation in other areas or symptoms of the central nervous system. Nevertheless, the Sclar's case showed abnormality of neural formina near the root ganglion and periventricular white matter of the brain in MRI. Somatosensory-evoked potential studies did not produce any cortical potentials, suggesting a lesion in the dorsal column or lemniscal level. These findings in Sclar's case suggest possible deficits in more central regions of the nervous system than the distal peripheral nerve. Moreover, the abnormality near the root ganglion and the somatosensory-evoked potential studies might explain the distribution of abnormal sensation in the present cases. The three cases also suggested abnormality of the autonomic nervous system as indicated by diarrhea and the change in sweating. It was not clear if the urinary incontinence in cases 1 and 2 were the same as "urinary difficulty" in Sclar's case, but this also suggested deficits in the autonomic nervous system. However, there could be other impaired regions; deficits in the somatic sensory nerves in the bladder, for example, were suggested by the unawareness of urination in case 2.

Deep sensation was diminished but the Romberg sign was negative. Since a positive Romberg sign requires a severe loss of deep sensation, the negative Romberg sign does not necessarily rule out ataxia due to lesions in the spinal cord or peripheral nerves. The decrease in deep sensation suggested ataxia due to spinal cord or peripheral nerve rather than cerebellular ataxia, but the possibility of cerebellular involvement was not disproved completely. In rats, pyknotic atrophy of Purukinje cells in the cerebellum was found at 1000 ppm for 4–5 wk, although this change was not remarkable below 800 ppm. Extrapolation of this result in the animal experiment to humans is difficult at this point. Dysphagia in cases 1 and 2, which was also reported in Sclar's case, suggested a disorder in the glossopharyngeal or vagus nerve, or medulla oblongata. This disorder might also be suggested by articulatory disturbance in case 2 and possibly 3, though differential diagnosis was not completed.

Case 1 did not demonstrate any abnormality in distal latency or nerve conduction velocity, nor did it show significant MRI findings explaining her symptoms, as different from the previous case[5]. This might be due to the difference in the degree of impairment or the

observation time, or whether gadolinium enhancement was applied.

Menstrual cycles were disrupted temporarily in cases 1 and 2. This might accord with the previous animal experiment[11].

The daily individual exposure levels were estimated to range from 60 to 261 ppm in case 3. As this evaluation was done after the ventilation was improved, the workers might have been exposed to higher 1-bromopropane than the above levels in June.

In conclusion, 1-bromopropane might induce deficits in the peripheral nerves and/or the central nervous system in humans. The symptoms of diarrhea, abnormal sweating and urinary incontinence also suggested disorders in the autonomic nervous system.

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