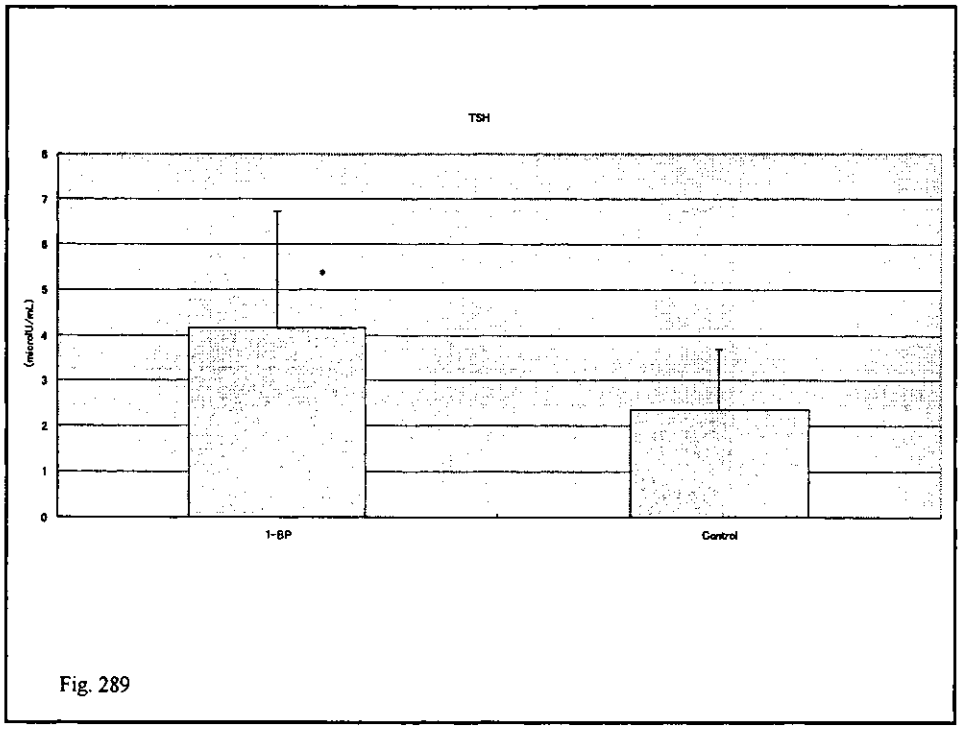


Fig. 289

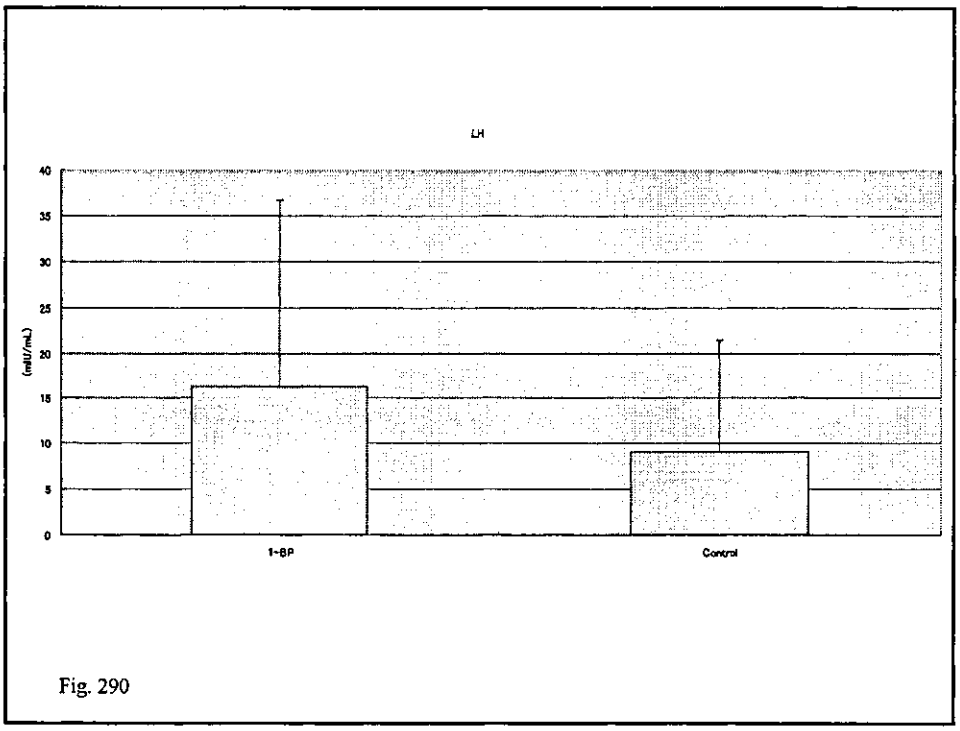


Fig. 290

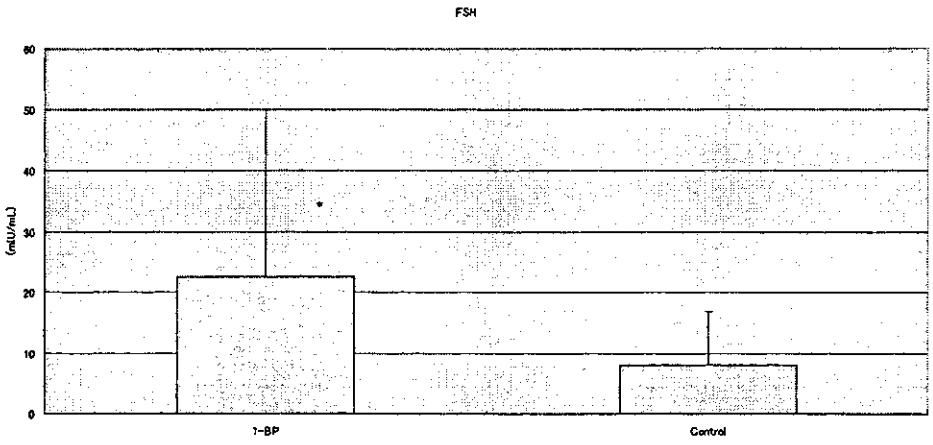


Fig. 291

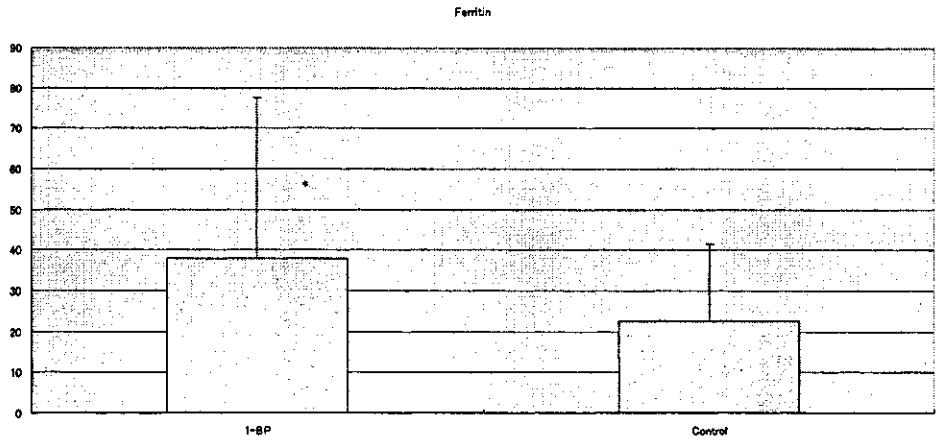


Fig. 292

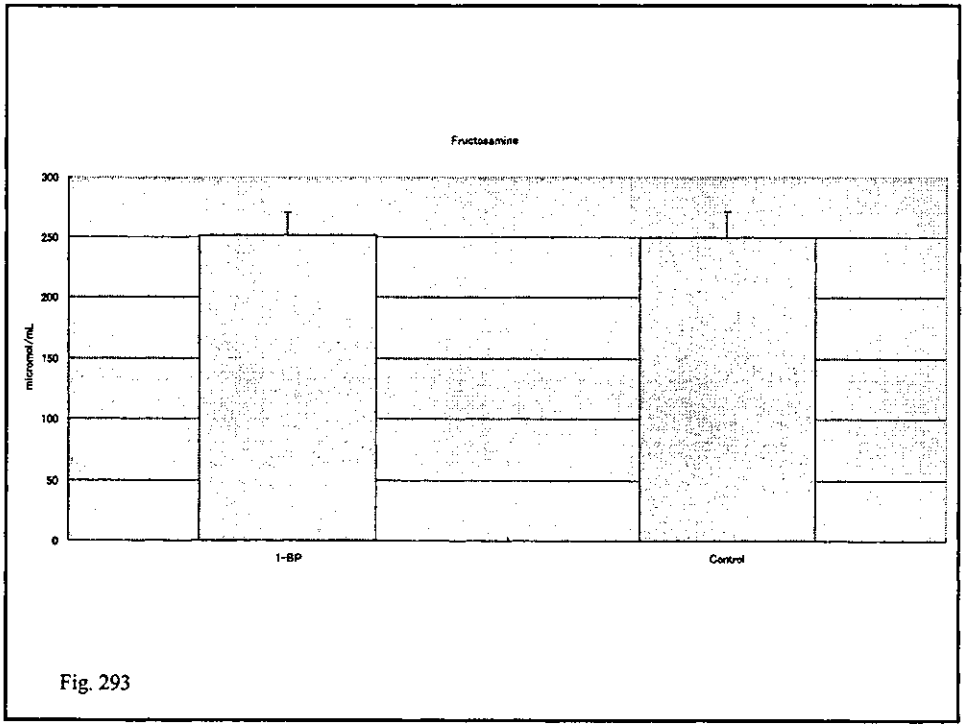


Fig. 293

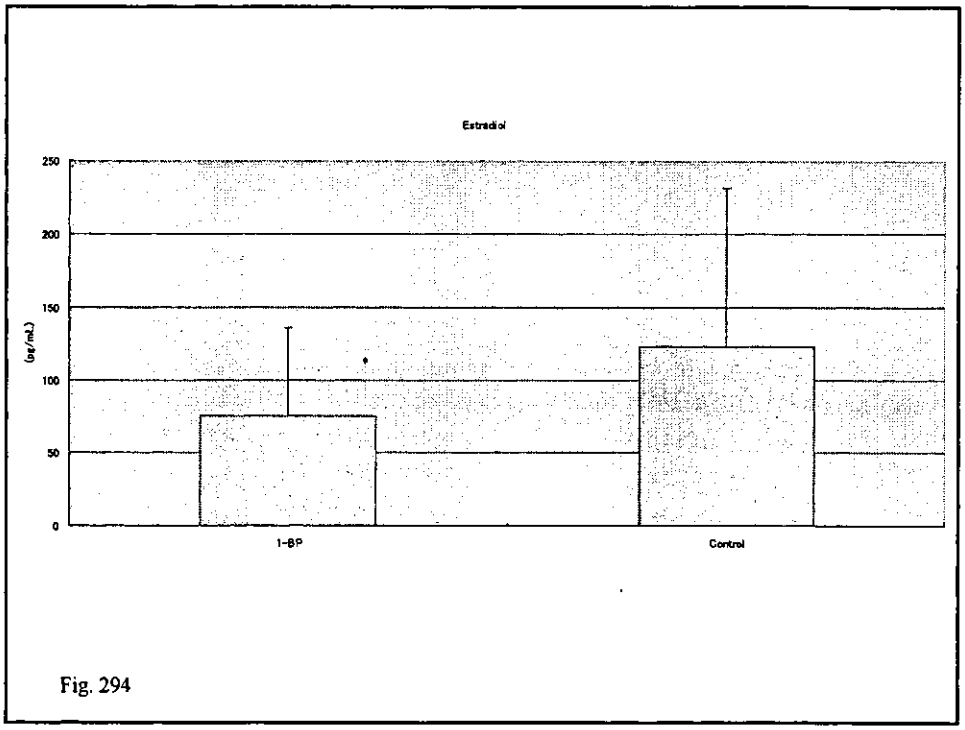


Fig. 294

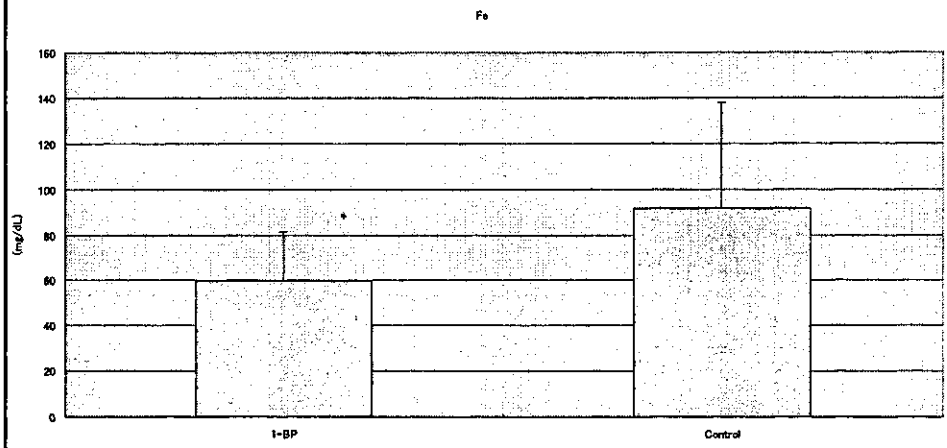


Fig. 295

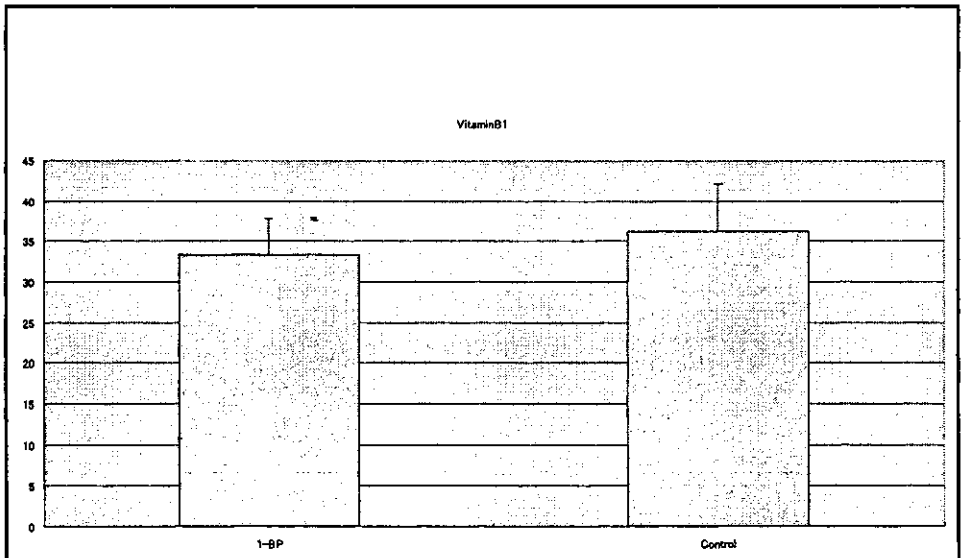


Fig. 296

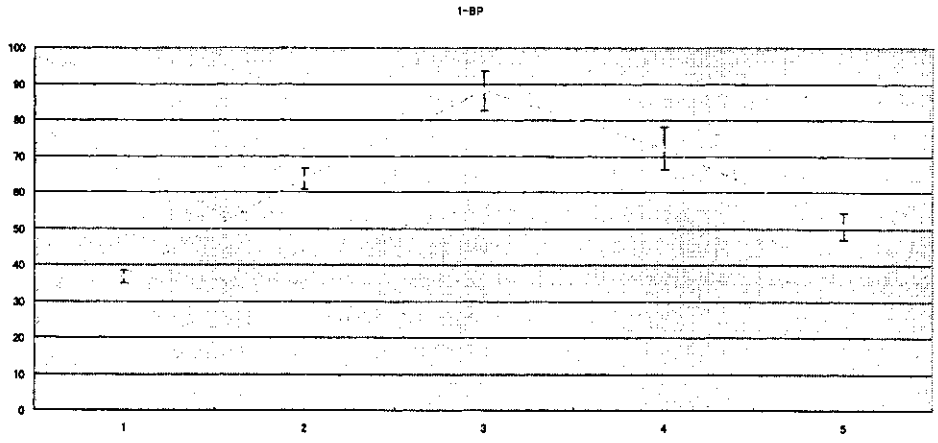


Fig. 297

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REVIEW

Gaku Ichihara

# Neuro-reproductive toxicities of 1-bromopropane and 2-bromopropane

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**Abstract** 2-Bromopropane was used as an alternative to chlorofluorocarbons in a Korean electronics factory and caused reproductive and hematopoietic disorders in male and female workers. This causality was revealed by animal studies, and target cells were identified in subsequent studies. After identification of 2-bromopropane toxicity, 1-bromopropane was introduced to the workplace as a new alternative to ozone-depleting solvents. 1-Bromopropane was considered less mutagenic than 2-bromopropane, but, in contrast, animal experiments revealed that 1-bromopropane is a potent neurotoxic compound compared with 2-bromopropane. It was also revealed that 1-bromopropane has reproductive toxicity, but the target cells are different from those of 2-bromopropane. Exposure to 1-bromopropane inhibits spermiation in male rats and disrupts the development of follicles in female rats, in contrast to 2-bromopropane, which targets spermatogonia and oocytes in primordial follicles. After the first animal study describing the neurotoxicity of 1-bromopropane, human cases were reported. Those cases showed decreased sensation of vibration and perception, paresthesia in the lower extremities, decreased sensation in the ventral aspects of the thighs and gluteal regions, stumbling and headache, as well as mucosal irritation, as the initial symptoms. The dose–response of bromopropanes in humans and mechanism(s) underlying the differences in the toxic effects of the two bromopropanes remain to be determined.

**Keywords** Bromopropane · Neurotoxicity · Reproductive toxicity · Ozone-depleting solvents · Global warming · Alternative

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## Introduction

Bromopropanes have become alternatives to chlorofluorocarbons (CFCs) and 1,1,1-trichloroethane, which are known to deplete the ozone layer [United Nations Environment Programme (UNEP) 2001]. CFCs and 1,1,1-trichloroethane were used extensively as cleaning agents for metal parts, and thus their substitutes are required in many workplaces. Alkaline cleaning methods and usage of hydrocarbons have also been introduced (Ahmed 1995), but their application in the workplace is limited. The former has the problem of possible induction of rust and requires copious amounts of rinsing water. The latter requires an expensive facility to prevent explosion. Many workplaces use washing baths and, therefore, need nonflammable alternatives such as hydroxychlorofluorocarbon (HCFC) that can be used in such baths to clean delicate electronics parts. However, consumption of HCFC will be banned in the next decades; partially by 2020, excluding servicing the remaining refrigeration and air conditioning equipment, and completely by 2030 (UNEP 2000). In addition, the use of other alternative hydrofluorocarbons (HFCs) will be regulated because of its global warming effects, as stated in the Kyoto Protocol (UNEP 1999). Under such circumstances, bromopropanes are considered promising alternatives to ozone-depleting solvents, because they are nonflammable, have a moderate vapor pressure and are fairy stable with stabilizers (UNEP 2001). The nonflammable property is important for any metal cleaning agent because heating is required when the metal is washed with vapor for optimal efficiency of cleaning.

2-Bromopropane was first used as an alternative to ozone-depleting solvents in Korea and Japan (Takeuchi et al. 1997). However, after the identification of its reproductive and hematopoietic toxicities, the isomer 1-bromopropane was used as a new alternative. In this review, I outline the toxicity of 1-bromopropane and 2-bromopropane and compare their toxicities in order to understand their specificities.

## Reproductive and hematopoietic disorders in Korean workers

In 1995, oligospermia, amenorrhea and anemia were identified in Korean workers engaged in manufacturing tactile switches in a large scale electronics company and exposed to 2-bromopropane (Kim et al. 1996; Park et al. 1997). Among eight male workers, two showed azoospermia, while the other four showed oligospermia or reduced sperm motility. Of the 25 examined female workers, 16 showed amenorrhea, with high serum levels of follicle-stimulating hormone (FSH). Laparoscopy performed in six of these female workers showed the ovary to be either atrophic, small or nearly normal (Koh et al. 1998). Histopathological examination of biopsies taken from four of the women showed focal or diffuse fibrosis of the ovarian cortex, disappearance of follicles at various stages of development, and low numbers of primary follicles and corpus albicans. Primary follicles were atrophic and irregularly shaped, and medullary blood vessels were hyalinized. After 2 years follow-up, amenorrhea persisted in most affected workers. However, one worker became hCG-test positive after 7 months without any hormonal therapy and finally delivered a healthy baby. Another worker resumed her regular menstruation cycle 12 months after cyclic estrogen–progesterone therapy (Koh et al. 1998).

In this Korean workplace, a new cleaning solvent was introduced to replace Freon (CFC) 113. The new solvent consisted of mainly 2-bromopropane (97.4%) and others, *n*-heptane (0.33%), 1,2-dibromopropane (0.2%) and 1,1,1-trichloroethane (0.01%). Since 2-bromopropane is the major ingredient in the solvent, it was considered to be the potential causative agent. However, at that time there was no published information about the reproductive or hematopoietic toxicity of 2-bromopropane. We thus investigated the toxicity of 2-bromopropane in relation to other brominated hydrocarbons. In the late 1970s to the early 1980s, it was well known that dibromochloropropane (DBCP) (Egnatz et al. 1980; Kluwe 1981a, 1981b; Marquez 1978; Potashnik et al. 1979; Whorton et al. 1979) and ethylene dibromide (EDB) (Short et al. 1979) have testicular effects, that their extrahepatic toxicities are mediated through the glutathione pathway (MacFarland et al. 1984; Omichinski et al. 1988), and that their genotoxic effects are mediated through the formation of the episulfonium ion, a highly active molecule, by conjugation with glutathione at the position of two halogens (Guengerich 1994). 2-Bromopropane has only one halogen within its molecule, hence it cannot be activated through glutathione conjugation, like DBCP and EDB are. Thus, the activity of dihalogenated hydrocarbons can be enhanced with glutathione, but little was known about the activity of monobrominated hydrocarbons. This paucity of information prompted us to perform animal studies to investigate the pathomechanisms of 2-bromopropane toxicities on the reproductive and hematopoietic systems.

## Animal studies confirm 2-bromopropane is toxic to reproductive organs and hematopoietic tissue

### Male reproductive toxicity

Male reproductive toxicities of 2-bromopropane are summarized in Table 1. The inhalation study (Ichihara et al. 1996, 1997) using Wistar rats showed exposure to 2-bromopropane decreased epididymal sperm count and motility, normal morphology of the sperm, atrophy of the seminiferous tubules and reduction of spermatogenic cells, leading to near-complete sterilization, though not affecting Sertoli cells. The loss of spermatogonia and spermatocytes without affecting Sertoli cells was also confirmed by other experimental studies in which the compound was injected intraperitoneally (Yu et al. 1997) or subcutaneously (Wu et al. 1999). It seems that such reducing effects on sperm or damage to spermatogenic cells resulted in a reduction of the rates of pregnancy, fertility, the number of implantations and the number of viable fetuses per litter, and increase in resorption after males were exposed subcutaneously to 2-bromopropane with untreated females (Wu et al. 1999).

Ichihara and co-workers (1997) also examined the testes of rats soon after 11 days of exposure and found no spermatogonia, suggesting that spermatogonia are a target of 2-bromopropane. However, they could not clarify whether only spermatogonia are the target because they also found reduced spermatocytes and degenerated spermatocytes with pyknotic nuclei. Furthermore, in that study, no quantification of spermatogenic cells and differentiation of stages of seminiferous tubules was conducted. Omura et al. investigated the primary target of 2-bromopropane in the testis (Omura et al. 1997). They observed reduction of pachytene spermatocytes at stages I and V but not at stages VII, X and XII after 2-week repetitive s.c. injection of 1-bromopropane. Since it takes 2 weeks for preleptotene spermatocytes (the earliest stage spermatocytes) to become stage VII pachytene spermatocytes, the target would be the spermatogonia, assuming that the developmental speed does not change by exposure to 1-bromopropane. Their second study (Omura et al. 1999) further investigated the target of 2-bromopropane by 1 or 2–5 repetitive injections. They found that stage I spermatogonia were likely the target and cell division of spermatogonia B was delayed. Omura and colleagues suggested that this delay in division was due to possible delaying effects of 2-bromopropane, an alkylator, on DNA synthesis. DNA damage is known to correlate with toxicity toward other organs such as necrotic effects on kidney or testis in DBCP-induced toxicity (Lag et al. 1991). DNA-damaging effects of 2-bromopropane was suggested by the Comet assay using cultured Leydig cells (Wu et al. 2002) and the ability to produce DNA adduct by exposure to 2-bromopropane in vitro.

Son et al. (1999) detected degeneration of spermatogonia in stages I–IV 1 day after the treatment, but no

**Table 1** Summary of male reproductive toxicity of 2-bromopropane (↑ increase, ↓ decrease, → no change, *i.p.* intraperitoneal injection, *s.c.* subcutaneous injection, *SD* Sprague–Dawley)

| Exposure condition and animals | Result | Reference |
|---|---|---|
| Inhalation 0, 300, 100, 3,000 ppm, 8 h/day, for 9 weeks (stopped after 9–11 days at 3,000 ppm) Four groups of 9 Wistar male rats (three rats at 3,000 ppm killed after 11 days, the others of 3,000 ppm group remained until the end of the experiment) | Debilitation after 9–11-days' exposure (3,000 ppm)<br>Macroscopic testicular atrophy (≥300 ppm)<br>Atrophy of seminiferous tubules (≥300 ppm)<br>↓All types of spermatogenic cells (300 ppm)<br>Almost complete sterilization of spermatogenic cells with remaining Sertoli cells (1,000 and 3,000 ppm)<br>↓ [a]epididymal sperm count and motility (≥300 ppm)<br>↑ [a]Morphologically abnormal sperm (300 ppm[b])<br>Hyperplasia of Leydig cells (≥300 ppm)<br>Almost loss of spermatogonia and ↓spermatocytes soon after 11-days' exposure (3,000 ppm) | Ichihara et al. (1996, 1997) |
| i.p. 0, 125, 250, 500 mg/kg per day; 28 days; four groups of ten SD male rats | ↓ [a]Weight of testis (≥250 mg/kg)<br>Depleted and atrophied seminiferous tubules (≥250 mg/kg)<br>Necrosis of spermatogonia and spermatocytes (500 mg/kg)<br>Hyperplasia or hypertrophy of Leydig cell (500 mg/kg)<br>Atrophy of epididymis with vacuolation of epithelium (500 mg/kg)<br>Exfoliation of epithelial cells with sperm aggregation in epididymal duct (500 mg/kg) | Yu et al. (1997) |
| s.c. 0, 200, 600, 1,800 mg/kg, 5 days/week for 5–7 weeks; four groups of six mature SD male rats; four groups of six immature SD male rats | ↓ [a]Testicular weight (≥600 mg/kg) in mature and immature rats<br>↓ [a]Weight of epididymis, prostate, seminal vesicle, pituitary gland (1,800 mg/kg) in mature and immature rats<br>↓ [a]Epididymal sperm count and viability and ratio of abnormal sperm (≥200 mg/kg) in mature and immature rats<br>↑ [a]Morphologically abnormal sperm in epididymides (≥200 mg/kg) in mature and immature rats<br>Atrophy of seminiferous tubules (≥200 mg/kg) in mature and immature rats<br>↓All types of germ cells (≥200 mg/kg) and complete loss of germ cells (1,800 mg/kg) in mature and immature rats<br>↓ [a]Serum testosterone (≥200 mg/kg) in mature rats<br>↓ [a]Serum testosterone (≥600 mg/kg) in immature rats<br>↑ [a]Beta-LH gene expression (1,800 mg/kg) in mature rats<br>↓ [a]Mating, pregnancy and fertility indices (≥600 mg/kg) after mating with untreated female rats<br>↓ [a]Number of implantations, viable fetuses per liter (1,800 mg/kg)<br>↑ [a]Resorption rate (1,800 mg/kg) | Wu et al. (1999) |
| Oral 3.5 g/kg per day for three consecutive days; perfusion at 1, 3, 5, 7, 14, 28, 42 and 70 days after treatment; five treated and three control rats at each time point; SD male rats | Degeneration of spermatogonia at stage I–IV on day 1 after treatment<br>Spermatid retention at stage IX–XI on day 7<br>Depletion of spermatocytes and spermatid over time<br>Regeneration of germ cells after day 42<br>Hyperplasia of Leydig cell on day 70<br>↑Proliferating cell nuclear antigen-positive Leydig cell<br>↓Ratio of diploid and tetraploid cells to haploid cells until day 28 by flow cytometry, ↑the ratio on day 42 and subsequently, ↓ on day 70 | Son et al. 1999 |
| s.c. 0 or 1.355 mg/kg per day; 5 days a week for 2 weeks; killed 20 h after the last injection; two groups of four Wistar male rats | ↓ [a]spermatogonia at stage X, XII, I, V<br>↓ [a]preleptotene spermatocytes at stage VII, leptotene spermatocytes at stage X, zygotene spermatocytes at stage XII<br>↓ [a]pachytene spermatocytes at I and V<br>→ pachytene spermatocytes at stages VII, X and XII.<br>→ round spermatids at stage I, V and VII | Omura et al. (1997) |

**Table 1** (Contd.)

| Exposure condition and animals | Result | Reference |
|---|---|---|
| s.c. 1,355 mg/kg per day for 1–5 days; killed 6 h after injection on each day; five groups of four treated Wistar male rats; one control of five Wistar male rats | ↓ [a] Stage I spermatogonia after the first injection<br>↓ [a] Sum of stage II and III spermatogonia after the second injection.<br>↓ [a] Stage XII spermatogonia after the third injection<br>↓ [a] Stage V spermatogonia after the fifth injection<br>↓ [a]Stage I pachytene spermatocytes after the first, third, fourth and fifth injection<br>A delay in mitotic division of type B spermatogonia after the fifth injection | Omura et al. (1999) |
| s.c. 0, 135, 405, 1,355 mg/kg per day; 28 days; four groups of 20 rats each; five rats from each group killed at each time point, after 7, 14, 21, 28 days' treatment | ↓ [a]Testicular weight after 14-days treatment (1,355 mg/kg)<br>↓ [a]Weight of testis, epididymis, seminal vesicle, prostate after 28 days (1,355 mg/kg)<br>↓ [a]Daily sperm production (≥405 mg/kg/day after 28 days treatment)<br>Degeneration of germ cells (1,355 mg/kg/day) after 14 days<br>Degeneration of spermatocytes, spermatid and vacuolization of Sertoli cell, multinuclear giant cell, Sertoli cell only tubule (1,355 mg/kg) after 28 days<br>Exfoliated germ cells in the lumen of epididymal duct (≥405 mg/kg) after 28 days<br>↓ [a]Spermatogenic cell at all the stages (≥405 mg/kg/day or 1,355 mg/kg/day after 14 and 28 days)<br>↑ [a]TUNEL-positive apoptotic cell in seminiferous epithelium (≥405 mg/kg/day) after 14 days<br>Electron microscopy: Marked nuclear chromatin condensation, cytoplasmic condensation, preservation of the integrity of organelles (1,355 mg/kg/day) after 14 days<br>Degeneration of many round spermatids and elongated spermatids after 14, 21 and 28 days<br>Dead elongated spermatid in seminiferous epithelium on day 21 or 28 (1,355 mg/kg) | Li et al. (2001) |
| Percutaneous injection; 1,350 mg/kg per day for 1– 5 days; killed at 6 or 12 h after one dose, 6 h after 2, 3 or 5 doses, and 2 or 9 days after the final treatment; six rats (light microscopy and protein extraction) and two rats (electron microscopy) for each time point; Wistar male rats | Degeneration of spermatogonia (nuclear chromatin condensation, DNA ladder by electrophoresis, TUNEL-positive) and DNA electrophoresis after 2-day treatment<br>↑ [a]Time-dependently the ratio of apoptotic-positive tubules and apoptotic index (percentage of tubules containing apoptotic germ cells multiplied by the number of apoptotic germ cells per tubule) after 3-day treatment<br>Immediate morphological alteration of spermatogonia<br>Secondary apoptosis of spermatocytes 9 days after the final treatment<br>Downregulation of Bcl-2 after the first or second injection of 2-bromopropane<br>Upregulation of Bax after the first treatment<br>Inhibition of FasL expression<br>↑Expression of Fas (↑ twofold until day 3, ↑ sixfold by 9 days after the final injection) | Yu et al. (2001b) |

[a]Statistically significant
[b]Evaluation of sperm count and motility (≥1,000 ppm) was impossible because of very few sperm

degeneration of other spermatogenic cells by 3 days. This study also suggests that 2-bromopropane targets the spermatogonia in the testis. In the study by Omura and colleagues (1999) a slight reduction of spermatocytes was noted, but the authors argued that such change had little biological meaning because further repetitive injections did not cause further falls in spermatocyte count. Apart from this, two groups (Yu et al. 1999a, 1999b and Son et al. 1999) found a delayed decrease in spermatocytes after reduction of spermatogonia. In the studies designed to detect the initial target in spermatogenic cells by anti-cancer drugs (Ettlin et al. 1984), it was suggested that the delayed decrease in spermatocytes was due to lack of supply from the damaged ancestral spermatogenic cells. However, two studies (Li et al. 2001; Yu et al. 2001b) confirmed apoptosis of spermatocytes, suggesting that the delayed decrease in spermatocytes was caused by apoptosis of spermatocytes rather than by lack of supply from spermatogonia, though they also confirmed that spermatogonia are the primary target. Yu et al. (2001b) proposed that secondary apoptosis of spermatocytes subsequently occurs after apoptosis of spermatogonia. Their observation might be in agreement with the initial findings of degenerated spermatocytes with pyknotic nuclei after subacute exposure (Ichihara et al. 1997).

Along with changes in spermatogenic cells, the interstitial Leydig cells may also be changed. Ichihara et al. (1997) demonstrated hyperplastic Leydig cells, but it was not clear if this reflected a decrease in the absolute cell number as the apparent distribution of these cells could be affected by shrinkage of interstitial space with atrophy of seminiferous tubules. Son et al. (1999) found an increase in proliferating cell nuclear antigen (PCNA)-positive Leydig cells, by immunohistochemistry. They hypothesized that exposure to 2-bromopropane induced compensatory hyperplasia of Leydig cells with a loss of tubule diameter and tissue shrinkage. In the study by Wu et al. (1999) serum testosterone level decreased in both mature and immature rats, but, in contrast, beta LH gene expression was enhanced at the highest level in mature rats. High LH gene expression might be a compensatory phenomenon in response to the decrease in testosterone and might be related to hyperplasia of Leydig cells, which was reported in other studies (Ichihara et al. 1997; Son et al. 1999). Nevertheless, the exact mechanism or involvement of hormonal change remains poorly defined.

## Female reproductive toxicity

Inhalation study (Kamijima et al. 1997) and intraperitoneal injection (i.p.) study (Lim et al. 1997) revealed that exposure to 2-bromopropane at 100, 300 and 1,000 ppm for 8 h/day, 7 days/week, for 9 weeks or at 300, 600 and 900 mg/kg per day for 14 days prolongs the estrous cycle only when the concentration was ≥300 ppm or 900 mg/kg. Histopathologically, the number of primordial follicles, growing follicles and antral follicles decreased dose-dependently after 9-weeks' exposure to 2-bromopropane for 8 h/day, 7 days/week at 100, 300 and 1,000 ppm (Yu et al. 1999b). Pre-mating i.p. exposure to 2-bromopropane of only female rats also decreased, dose-dependently, fertility and litter size after mating with non-exposed male rats, but there was no influence on duration of gestation (Lim et al. 1997).

The most susceptible process was investigated in a time-course study. A single inhalation exposure to 2-bromopropane at 3,000 ppm for 8 h induced apoptosis of oocytes, which was confirmed by morphological changes and terminal-deoxynucleotidyl transferase-mediated d-UTP nick end labeling (TUNEL) staining, in primordial follicles 5 and 17 days after the exposure, as well as a significant decrease in the number of primordial follicles 17 days after the exposure (Yu et al. 1999b). The control rats did not show oocyte apoptosis although they did in the granulose cells of the atretic follicles. Other researchers found that i.p. administration of 2-bromopropane inhibited ovulation induced by PMSG-hCG treatment in both mice (Sekiguchi and Honma 1998) and rats (Sekiguchi et al. 2001) but did not reproduce that by inhalation exposure at 50, 200 and 1,000 ppm for 8 h/day for at least 20 consecutive days (Sekiguchi et al. 2002). Those results suggested that ovulation is not the most susceptible process to 2-bromopropane by the normal exposure route of inhalation; thus, the main target cell or process is considered to be oocytes in the primordial follicles (Yu et al. 1999b).

## Hematotoxicity

Ichihara and colleagues (1996, 1997) also demonstrated reduction of hematocrit (significant decrease, ≥1,000 ppm) and counts of all types of blood cells (erythrocyte and platelet ≥300 ppm and leucocyte ≥1,000 ppm) in the same study that investigated the reproductive toxicity. The study showed that bone marrow was hypoplastic and replaced by adipose tissue. Both processes were quantified subsequently in another study (Nakajima et al. 1997). Jeong et al. (2002) found an overall decrease in immunological cells in the spleen and thymus as well as peripheral circulating blood cells after immunization with sheep blood cells, which was performed 4 days prior to killing, after a 28-day oral administration with 2-bromopropane at 100, 300 or 1,000 mg/kg/day. Although thymocytes seemed to be more sensitive (significant decrease, ≥330 mg/kg) to 2-bromopropane than splenocytes (1,000 mg/kg), the immunological suppression may be considered as a part of the overall reduction of bone marrow cells, which was observed in the initial studies (Ichihara et al. 1996, 1997; Nakajima et al. 1997).

## Other toxicities of 2-bromopropane

Other than reproductive and hematopoietic ones, no toxicities were reported in human (Kim et al. 1996; Park

et al. 1997; Ichihara et al. 1997) or animal studies (Ichihara et al. 1997, Yu et al. 1997, Kamijima et al. 1997), apart from genotoxicity, neurotoxicity and developmental toxicities.

### Effects of low-dose 2-bromopropane in humans

The investigation was conducted on 25 workers (11 male and 14 female) in a Chinese factory producing 2-bromopropane (Ichihara et al. 1999). The time-weighted average of individual exposure level was less than 16.18 ppm. The study could not find cases with severe toxicity similar to the Korean study but showed a possible association between hematological indices and individual exposure levels of 2-bromopropane. However, this study was limited, since the number of workers was small and the exposure levels were measured for only one shift and may not represent long-term average exposure levels. Further studies are needed to confirm this association at low dose.

### Shift from 2-bromopropane to 1-bromopropane and their genetic toxicities

Following the description of toxicity of 2-bromopropane in humans and animals, the use of 2-bromopropane has declined quickly and its isomer 1-bromopropane has been introduced as a new alternative. This change seemed to be reasonable, because of the studies that had suggested that 1-bromopropane had lower mutagenic activity than 2-bromopropane (Kim et al. 1998; Maeng and Yu 1997), and it was also expected that genetic toxicity of 2-bromopropane might be involved with its reproductive and hematopoietic toxicities. Wu et al. (2002) found that exposure to 2-bromopropane induced DNA damage of Leydig cells isolated from the rat testis, as well as increased malondialdehyde (MDA), GSH-PX (glutathione peroxidase) but decreased superoxide dismutase (SOD-PX). The authors considered DNA damage, impaired anti-oxidant defenses and lipid peroxidation to be responsible for the testicular toxicity of 2-bromopropane. Possible DNA damage was also suggested by DNA adduct formation by 2-bromopropane in vitro (Zhao et al. 2002).

Even if it is plausible that 1-bromopropane is less mutagenic than 2-bromopropane, this does not mean that 1-bromopropane has no mutagenic activity. Studies on genetic toxicities of bromopropanes are summarized in Table 2. Two studies (Barber et al. 1981; Elf Atochem 1996) demonstrated the mutagenicity of 1-bromopropane using a *Salmonella* sp, in a closed incubation system or mouse lymphoma cells, although there was another experiment (Elf Atochem 1994) using the same bacteria in a closed incubation system that contradicted the findings of Barber et al.

A few studies did not find an increase in micronucleus formation in bone marrow cells in rats exposed to 2-bromopropane (Maeng and Yu 1997) or 1-bromopropane (Elf Atochem 1995; Kim et al. 1998), but only Ishikawa et al. (2001) found an increase in micronucleus formation, by exposure to 2-bromopropane using mouse embryos. It might be difficult to compare the effects of the two bromopropanes on micronucleus formation at present because of the studies' different experimental conditions.

## Neurotoxicity of 1-bromopropane: comparison of 2-bromopropane with 1-bromopropane leads to discovery of neurotoxicity of 1-bromopropane

In the process of discovery of 1-bromopropane neurotoxicity, the neurotoxicity of 2-bromopropane was first noticed because neuropathy due to 2-bromopropane was also suspected in Korean workers who suffered from reproductive and hematopoietic disorders. Our group examined the dose-dependent effects of 2-bromopropane on the tail nerve of rats and used 1-bromopropane as a negative control (Yu et al. 1998, 1999a, 2001a). We expected that 1-bromopropane might be less toxic by extending its low mutagenicity to general toxicity, and also based on the results of a 13-week clinical trial study that showed only subtle changes in the liver (mild centrilobular vacuolation at 397 and 596 ppm) (Clin Trials 1997a). However, in contrast to our prediction, 1-bromopropane showed more potent neurotoxicity than 2-bromopropane did (Yu et al. 2001a). The 36 Wistar rats were divided into four equal groups and exposed to 2-bromopropane (100 or 1,000 ppm), 1-bromopropane (1,000 ppm) or fresh air (Yu et al. 2001a). In the 1-bromopropane group the distal latency was prolonged and motor nerve conduction velocity decreased after 4 weeks, while no changes were observed in the 2-bromopropane groups. Histopathological examination showed ovoid or bubble-like debris of myelin sheath in the common peroneal nerve, axonal swelling in the gracile nucleus in the medulla oblongata, and pyknotic shrinkage of Purkinje cells in the cerebellum of 1-bromopropane-exposed rats. Ohnish et al. (1999) also reported that exposure to 1-bromopropane at 1,500 ppm at 6 h/day, 5 days a week for 4 weeks induced degeneration of Purkinje cells in the cerebellum of rats.

Our group also conducted fully designed experiments to examine the dose-dependent neurotoxicity of 1-bromopropane (Ichihara et al. 2000a). Wistar male rats exposed to 800 ppm 1-bromopropane showed myelin degeneration in peripheral nerves, pre-terminal axon swelling in the gracile nucleus in the medulla oblongata, cerebrum weight loss, limb muscle weakness, distal latency prolongation and decrease of motor nerve conduction velocity. In contrast, Sohn et al. (2002) did not find any abnormalities in peripheral nerves, spinal cord or brain in Sprague–Dawley (SD) rats exposed to 0, 200, 500 and 1,250 ppm for 6 h per day, 5 days per week, for 13 weeks. The difference between the above

**Table 2** Genotoxicity of 2-bromopropane and 1-bromopropane (↑ increase, ↓ decrease, → no change, *GD* gestation day, *PE* polychromatic erythrocyte, *NE* monochromatic erythrocyte)

| Exposure condition | Result | Reference |
|---|---|---|
| Mutagenecity | | |
| 2-Bromopropane (0, 50, 100, 500, 1,000, 5,000 μg per plate forpreliminary assay and 313, 625, 1,250, 2,500 and 5,000 μg perplate for second assay) | ↑ Number of revertants of TA100 with S9 mix and in TA1535 with or without S9 mix | Maeng and Yu (1997) |
| Incubation with or without S9 mix for 30 min at 37°C | No mutagenic activity in TA98, TA1537 or WP2 uvrA | |
| *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, WP2uvrA | | |
| 1-Bromopropane (0, 50, 100, 500, 1,000, 5,000 μg per plate for preliminary assay and 313, 625, 1,250, 2,500 and 5,000 μg per plate for second assay) | No increase in revertants | Kim et al. (1998) |
| Incubation with or without S9 mix for 20 min at 37°C | | |
| *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, WP2uvrA | | |
| 1-Bromopropane (0, 1.1, 2.3, 4.9, 9.0, or 20.3 μmol per plate) | ↑ Revertant in TA1535 and TA100 with or without S9 mix | Barber et al. (1981) |
| Closed incubation system with or without S9 mix | | |
| *Salmonella* sp, TA1535, TA 98, TA100 | | |
| 1-Bromopropane (0, 100, 500, 1,000, 5,000, 10,000 μg per plate) | No mutagenic with or without S9 mix | Elf Atochem (1994) |
| Closed incubation system with or without S9 mix | | |
| *Salmonella* sp. TA1535, TA1537, TA1538, TA98, TA100 | | |
| 1-Bromopropane | | Elf Atochem (1996) |
| First experiment (0, 125, 250, 500, 1,000, 1500 μg/ml) without S9 mix | ↑ Mutation frequency between 1,000 and 1,500 μg/ml | |
| Second experiment (0, 250, 500, 1,000, 1,250, 1,500 μg/ml) without S9 mix | ↑ Mutation frequency between 1,000 and 1,500 μg/ml | |
| First experiment (0, 125, 250, 500, 1,000, 1,500 and 2,000 μg/ml) with S9 mix | No increase in mutation frequency | |
| Second experiment (at 0, 500, 1,000, 1,500, 2,000 and 2,500 μg/ml) with S9 mix | ↑ Mutation frequency (1,500 and 2,000 μg/ml, all cells were dead at 2,500 μg/ml)[a] | |
| Mouse lymphoma cells L5178Y TK ± | | |
| Chromosomal aberration | | |
| 2-Bromopropane (0, 0.077, 0.154, 0.307, 0.615, 1.23 and 2.46 mg/ml) | No chromosomal aberration with or without S9 mix | Maeng and Yu (1997) |
| Incubation with S9 mix for 6 h or without S9 mix for 24 h | | |
| Chinese hamster lung (CHL) cells | | |
| Micronucleus formation | | |
| 2-Bromopropane (i.p. 0, 125, 250 and 500 mg/kg per day, 6 days per week for 28 days to male and non-pregnant female SD rats) | → Micronucleus frequency (micronucleus in polychromatic erythrocytes) | Maeng and Yu (1997) |
| Bone marrow cells from 20 rats (10 male and 10 female) for each test group | ↓ [a]Percentage of polychromatic erythrocytes[a] (≥125 mg/kg) | |
| 2-Bromopropane (i.p. 0, 300, 600, 900 and 1,800 mg/kg to pregnant female ICR mice on DG 3) | → Number of corpora lutea or degenerated embryos | Ishikawa et al. (2001) |
| Embryonic cells from 15 dams for each dose | → Cell numbers of embryos<br>↑ [a]Micronucleus per embryo[a] (≥900 mg/kg)<br>↑ [a]Percentage of embryos with micronucleus[a] (≥900 mg/kg) | |
| 1-Bromopropane | | |
| First experiment (i.p. 0, 100, 400, 800 mg/kg/day, 2 days)Bone marrow cells from 10 rats (5 male and 5 female) for dose of 0, 100 or 400 mg/kg/day and from 16 rats ( 8 male and 8 female) for 800 mg/kg/day | The results of the first experiment were not evaluated because the PE/NE ratio in the control (0 mg/kg/day) was lower than the usually obtained data | Elf Atochem 1995 |
| Second experiment (i.p. 0, 800 mg/kg/day, 2 days)<br>Bone marrow cells from 10 rats (5 male and 5 female) for 0 mg/kg/day and from 16 rats (8 male and 8 female) for 800 mg/kg/day | → micronucleated polychromatic crythrocytes (800 mg/kg/day) in female<br>Six of eight males died 24 h after the first injection at 800 mg/kg | |
| Third experiment (i.p. 0, 600 mg/kg/day, 2 days)<br>Bone marrow cells from 10 male rats for 0 mg/kg/day and from 8 male rats for 600 mg/kg/day | → micronucleated polychromatic erythrocytes (600 mg/kg/day) | |
| Swiss OF1/ICO:OF1(IOPS Caw) | | |
| 1-Bromopropane (0, 50, 300 or 1,800 ppm, 6 h/day, 5 days/week, 8 weeks, to SD male and female rats) | → Frequency of red blood cells having micronuclei among polychromatic erythrocytes (PCE) | Kim et al. (1998) |
| Bone marrow from 10 male rats and 10 female rats for each dose | | |

[a]Statistically significant

two studies, with clear alteration in the Ichihara et al. study might be attributed to the portion of peripheral nerve, longitudinal direction of sectioning, semi-thin sectioning in the gracile nucleus and/or rat strain.

On the other hand, exposure to 2-bromopropane temporarily reduced motor nerve conduction velocity after 8 weeks and prolonged the distal latency after 8 and 12 weeks' exposure in the rat tail nerve (Yu et al. 1999a, 2001a). Morphological studies found scattered ball-like swelling of myelin sheath near the Ranvier nodes in the common peroneal nerve after a 12-week exposure to 1,000 ppm of 2-bromopropane but no abnormal findings in the central nervous system. These results (Yu et al. 1998, 1999a, 2001a; Ichihara et al. 2000b) demonstrate that 1-bromopropane is more toxic to the nervous system than 2-bromopropane, based on comparison of the serial changes in distal latency (DL) and motor nerve conduction velocity (MCV) and the extent of morphological changes. In contrast to Yu et al. (1998, 1999a, 2001a) and Ichihara et al. (2000a), Zhao et al. (1999) did not find differences in toxicities between 1-bromopropane and 2-bromopropane in rats treated subcutaneously with these agents. Whether the differences in the two studies are due to differences in the route of administration or other factors remain to be investigated.

The morphological studies of Ichihara et al. (2000a) did not show the pyknotic shrinkage of cerebellar Purkinje cells that were found following exposure to 1,000 ppm (Yu et al. 1998) or 1,500 ppm (Ohnishi et al. 1999). It is possible that the morphological alteration in the cerebellum appear only at extremely high concentrations. The question of whether the cerebellum could be a target of 1-bromopropane will be also discussed in the section on human data later in this review.

Although the clinical trials 4-week study (Clin Trials 1997b) showed vacuolation of the brain and spinal cord, this vacuolation might be artificial, because the rat cells were fixed by immersion but not perfused. However, even when such vacuolation might be considered as an artifact occurring after the killing of the rats, it is possible that the appearance of 1-bromopropane-induced invisible changes might be enhanced by post-mortem effects. Thus, convincing histopathological evidence for 1-bromopropane toxicity on the central nervous system is limited, apart from the alteration of pre-terminal axons in the gracile nucleus. However, based on the result of decreased cerebrum weight in our study (Ichihara et al. 2000a), whether it is due to the decrease in the specific cell layer length or diffuse atrophy remains to be determined.

## Reproductive toxicity of 1-bromopropane

Following the discovery of the neurotoxicity of 1-bromopropane it was considered that this compound may target mainly the nervous system while, in contrast, 2-bromopropane targets reproductive organs, because the initial histopathological study of the testis did not demonstrate obvious abnormality (Yu et al. 1998). However, the 12-week study (Ichihara et al. 2000b) revealed decreased epididymal sperm count and motility. A multigeneration study (WIL Research Laboratories 2001) also showed, in F0 male rats, changes in epididymal sperm indices and reproductive organ weight that were essentially similar to those reported in the study by Ichihara and colleagues (Ichihara et al. 2000b) (Table 3).

As opposed to 2-bromopropane, exposure to 1-bromopropane did not cause atrophy of seminiferous tubules or decrease spermatogenic cells from spermatogonia to spermatids at stage VII (Ichihara et al. 2000b). On the other hand, the retained elongated spermatids (step 19) increased dose-dependently in the seminiferous epithelium in post-spermiation stages, suggesting disruption of spermiation from the epithelium. This failure may be related to Sertoli cell dysfunction (Creasy 1997), based on the close structural relationship between Sertoli cells and elongated spermatids. Thus, the reproductive toxicity of 1-bromopropane is different from that of 2-bromopropane in terms of primary target. However, exposure to a higher concentration of 1-bromopropane [1,590 ppm, 6 hourly over 5 days for 4 weeks (Clin Trials 1997b) or 1,500 ppm, 6 hourly over 5 days for 4 weeks (Ohnishi et al. 1999)] resulted in atrophy of the testes. Therefore, the last theory might be valid on condition that the exposure level was 1,000 ppm or less. In the female rats, it was also revealed that exposure to 1-bromopropane at ≥400 ppm resulted in prolongation of the estrous cycle (WIL Research Laboratories 2001; Yamada et al. 2003) and decreased ovary weight in F0 female rats in part of a multigeneration study (WIL Research Laboratories 2001). Histopathologically, exposure to 750 ppm resulted in a reduction in the corpora lutea and increase in the number of ovarian cysts (WIL Research Laboratories 2001). Yamada et al. (2003) quantified ovarian follicles at each developmental stage. The affected follicles were the antral (≥200 ppm) and developing follicles (400 ppm) rather than the primordial follicles (Yamada et al. 2003). This also contrasts with the effects of 2-bromopropane, which targets mainly the primordial follicles, by single exposure at 3,000 ppm for 8 h, and decreased all types of follicles after 9-week exposure with significant changes in primordial and growing follicles at 100, 300 and 1,000 ppm and in antral follicles at 300 and 1,000 ppm (Yu et al. 1999b). Thus, the major targets of 1-bromopropane and 2-bromopropane are different from each other in the reproductive organs, both in male and female rats under exposure at 800 ppm or below.

On the other hand, pre-mating exposure of both male and female rats to 1-bromopropane decreased implantation sites and litter size and increased the number of completely infertile rats (WIL Research Laboratories 2001). Those effects may be explained by the decrease in developed follicles or interruption of estrous cycle, as reported by Yamada et al. (2003).

**Table 3** Reproductive toxicity of 1-bromopropane. Male (female) mating index = no. of males (females) with evidence of mating (or confirmed pregnancy) per total no. of males (females) used for mating × 100. Male fertility index = no. of males' string per litter per total no. of males used for mating × 100. Female fertility index = no. of females with confirmed pregnancy per total no. of females used for mating × 100. ↑ increase, ↓ decrease, → no change, *GD* gestation day, *PND* postnatal day

| Exposure condition and animals | Result | Reference |
|---|---|---|
| Inhalation; 0, 200, 400, 800 ppm<br>8 h/day, 7 days/week; 12 weeks<br>Four groups of nine Wistar male rats | → Testicular weight<br>↓ Weight of epididymis (≥400 ppm), prostate (800 ppm), seminal vesicle (≥200 ppm)[a]<br>↓ [a]Epididymal sperm count (≥400 ppm) and motility (≥400 ppm)[a]<br>↑ [a]Sperm with morphologically abnormal head (800 ppm)[a]<br>↑ [a]Elongated spermatid (step 19) in the seminiferous epithelium in post-spermiation stages IX–XI (≥400 ppm)[a]<br>→ Spermatogonia, preleptotene spermatocytes, pachytene spermatocytes or round spermatid at stage VII | Ichihara et al. 2000b |
| Inhalation; 0, 200, 400, 800 ppm,<br>8 h/day, 7 days/week; 12 weeks<br>Four groups of ten Wistar female rats | Serious illness of all rats in the 800 ppm group after 7 weeks<br>↑ Irregular estrous cycle with extended diestrus (≥400 ppm)[a]<br>↓ Antral follicle (≥200 ppm[a])[b]<br>↓ Growing follicle (400 ppm[b])[b]<br>No newly formed corpus luteum (800 ppm)<br>Most follicles were atresic (800 ppm) | Yamada et al. (2003) |
| Inhalation; 0, 100, 250, 500,<br>750 ppm for 6 h/day,<br>7 days/week<br>Male: for at least 70 days before mating, throughout mating until the day before it was killed<br>Female: for at least 70 days before mating, throughout mating until GD 20 and from PND 5 until the day before it was killed<br>F0 were necropsied after F1 weaning<br>CD (SD) rats; 25 male and 25 female for each dose | F0 Male and female: 100% infertility (750 ppm)<br>↓ Mating indices (750 ppm)[a]<br>↓ Fertility indices (500 ppm)[a]<br>F0 male:<br>↓ Cumulative body weight gain throughout week 0–1 to 0–19[a]<br>↓ Epididymal sperm number (750 ppm)[a]<br>↓ Sperm motility (≥500 ppm)[a]<br>↓ Morphologically normal sperm (≥250 ppm)[a]<br>↓ Epididymal weight (Right ≥500 ppm, Left 750 ppm)[a]<br>↓ Prostate (≥250 ppm)[a],<br>↓ Seminal vesicle (750 ppm)[a]<br>↓ Pituitary (750 ppm)[a]<br>↓ Brain (≥250 ppm)[a]<br>↓ Adrenal gland (750 ppm)[a]<br>↑ Number of rats that showed vacuolation of hepatocells (≥250 ppm) or increase in glycogen (750 ppm) in liver[a]<br>No correlating macroscopic or microscopic changes in the brain tissue of the exposed rats<br>F0 Female:<br>↓ Cumulative body weight gain throughout week 0–1 to 0–10[a]<br>↑ Length of estrous cycle (statistically not significant)<br>↑ Pre-coital interval (statistically not significant)<br>→ Length of gestation<br>↓ Ovary weight (750 ppm)[a]<br>↓ Number of implantation sites (500 ppm)[a]<br>↓ Number born (500 ppm)[a]<br>↓ Brain (≥500 ppm)[a]<br>↑ Number of rats that showed vacuolation of hepatocells (≥500 ppm) or increase in glycogen (750 ppm) in liver[a]<br>↑ Number of rats that showed decrease in corpora lutea (750 ppm) and increase in follicular luteinized cyst (750 ppm)[a]<br>↓ Number of corpora lutea (750 ppm)[a] | WIL Research Laboratories (2001) |

[a]Statistically significant
[b]Statistical comparison was not performed because the time of killing was different for the 800 ppm group and other groups

**Table 4** Developmental toxicity of 1-bromopropane and 2-bromopropane (↑ increase, ↓ decrease, → no change, *GD* gestation day, *PND* postnatal day, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyl transferase)

| Exposure condition and animals | Result | Reference |
|---|---|---|
| 1-Bromopropane | F1 male and female | WIL Research Laboratories (2001) |
| Exposure of F0 is described in Table 3 | ↓ Number born (500 ppm)<br>↓ Live litter size on PND0 (500 ppm)[a] | |
| F1: inhalation; 0, 100, 250, 500 ppm for 6 h/day, 7 days/week | F1 male | |
| Male: for at least 70 days before mating, throughout mating until the day before being killed | ↓ Body weight on PND 1 (500 ppm) and PND 28 (≥250 ppm)[a] | |
| Female: for at least 70 days before mating, throughout mating until GD 20 and from PND 5 until the day before being killed | ↓ Weekly body weight gains, cumulative body weight gains in males (500 ppm)[a] | |
| F1 were necropsied after F2 weaning | ↓ Food consumption (500 ppm) throughout the majority of gestation and into the lactation period[a]<br>↑ Day of acquisition from balanopreputial separation (500 ppm)[a]<br>↓ Sperm motility (≥250 ppm)[a]<br>↓ Morphologically normal sperm (500 ppm)[a]<br>↓ Weight of brain (≥100 ppm)[a]<br>↓ Weight of kidney (100 and 500 ppm)[a]<br>↓ Weight of cauda epididymidis (500 ppm)[a]<br>↑ Weight of thymus gland (500 ppm)[a]<br>↓ Weight of pituitary gland (500 ppm)[a]<br>F1 Female<br>↓ Body weight on PND 1 and 4 (500 ppm)[a]<br>↓ Body weight gain from the first week of exposure throughout the pre-mating period in females (500 ppm)[a]<br>↓ Food consumption (500 ppm) throughout the majority of gestation and into the lactation period[a]<br>↑ Length of estrous cycle (≥250 ppm, statistically not significant)<br>↓ Number of implantation sites (500 ppm)[a]<br>↓ Weight of brain (500 ppm)[a]<br>↑ Number of primordial follicle (500 ppm)[a] | |
| F2 necropsied on PND 21 | F2 male and female:<br>↓ Number of pups born and live litter size on PND 0 (500 ppm)<br>↓ Body weight and body weight gain (500 ppm)[a]<br>↓ Spleen weight (500 ppm)[a]<br>↓ Absolute weight of brain on PND 21 (500 ppm)[a] | |
| 1-Bromopropane | F0: Higher incidence of red stains on the snout or head, bilateral or unilateral lacrimation, and excessive salivation immediately after exposure (996 ppm) | Huntingdon Life Sciences (2001) |
| Inhalation; 0, 100, 498, 996 ppm, 6 h/day on GD 6–19<br>A cesarean section on GD 20 | ↓ Body weight change (≥498 ppm)[a] | |
| Four groups of 25 pregnant CD rats | F1<br>↓ Body weight (≥498 ppm) in male (≥100 ppm) in female[a]<br>↓ Litter incidence of reduced ossification of the skull (≥498 ppm)[a]<br>↓ Litter incidence of rib(s) bent (996 ppm)[a] | |
| 1-Bromopropane | F0: salivation and lacrimation after exposure (996 ppm) | Huntingdon Life Sciences (1999) |
| Inhalation; 0, 100, 199, 598, 996 ppm on GD 6–19 for 6 h/day and LD 4–20<br>Five groups of ten pregnant CD rats | ↓ AST (≥199 ppm)[a] | |
| F1: inhalation on post-weaning days 1–7 (PND 22–29) | F1 Male<br>↓ Body weight on PND 25 and 29 (996 ppm)[a]<br>↓ Body weight gain on PND 22–25 (≥598 ppm) and on PND 25–29 (996 ppm)[a]<br>↓ Platelet (996 ppm)[a]<br>↓ Glucose (996 ppm)[a]<br>↓ ALT (≥598 ppm)[a]<br>↑ GGT (996 ppm)[a]<br>↓ Brain weight (996 ppm)[a] | |

Table 4 (Contd.)

| Exposure condition and animals | Result | Reference |
|---|---|---|
| | F1 Female<br>↓ Body weight gain on PND 22–25 (996 ppm)[a]<br>↓ Platelets (≥598 ppm)[a]<br>↓ Glucose (996 ppm)[a]<br>↓ ALT (≥199 ppm)[a]<br>↑ GGT (996 ppm)[a]<br>↓ Brain weight (996 ppm)[a] | |
| 2-Bromopropane<br><br>s.c. 0, 135, 405, 1,215 mg/kg per day, on GD 6 to PND 20 | F0<br><br>↓ Body weight on GD 20 and PND 21 (≥405 mg/kg)[a]<br><br>↓ Dams littering (≥405 mg/kg)<br>↓ Rate of delivery and surviving pups (1,215 mg/kg)<br>F1 male:<br>↓ Body weight on PND1, 21, 33, 49, 63 (1,215 mg/kg)[a]<br>↓ Relative weights of testes to brain on PND 33 and 63 (≥405 mg/kg), and on PND 90 (1215 mg/kg)[a]<br>↓ Daily sperm production (≥405 mg/kg)[a]<br>↓ Epididymal sperm count (≥405 mg/kg)[a]<br>Atrophy of seminiferous tubules (≥405 mg/kg),, absent germ cells on PND33 (1,215 mg/kg)<br>↑ Number of Leydig cells on PND 63 and 90 (1,215 mg/kg)[a]<br>F1 female<br>↓ All three types of ovarian follicles (primordial, growing, antral) (1,215 mg/kg/day)[a] | Kang et al. (2002) |
| 2-Bromopropane<br><br>i.p. 0, 300, 600, 900, 1,800 mg/kg on GD 10 78 (14, 15, 15, 16, 18, respectively) of pregnant female ICR mice | → Number of corpora lutea, implantation sites<br><br>→ Percentage of dead fetuses or resorbed fetuses<br>→ Fetal weight (male and female)<br><br>→ External malformations | Ishikawa and Yamanchi (2003) |

[a]Statistically significant

## Developmental studies on bromopropanes

Subcutaneous administration of 2-bromopropane in female rats at 405 and 1,215 mg/kg per day during gestation and lactation produced early death at the implantation sites and a decrease in the rate of dams littering, in a dose-dependent way (Kang et al. 2002), although single i.p. administration of 2-bromopropane to the pregnant mice on gestation day 10 did not affect pregnancy or malformations of offspring (Ishikawa and Yamauchi 2003) . On the other hand, exposure to 1-bromopropane ranging from 100 to 996 ppm only during the gestational period did not decrease implantation or litter size (Huntingdon Life Sciences 1999, 2001). Gestational exposure to 2-bromopropane could interrupt continuation of pregnancy. However, there are currently no studies that show that gestational exposure to 1-bromopropane could produce the same results (Table 4).

Exposure to a high dose of 2-bromopropane at 1,215 mg/kg per day resulted in atrophy of the seminiferous tubules, depletion of spermatogenic cells and decrease in all types of follicles in the ovary in F1 offspring. The effects of 2-bromopropane on the reproductive organs in male and female offspring seemed to be essentially similar to those described in adult rats (Ichihara et al. 1997; Kamijima et al. 1997; Son et al. 1999; Yu et al. 1997). On the other hand, one of the common findings in the multigeneration studies on 1-bromopropane was a decrease in fetal body weight (Huntingdon Life Sciences 1999, 2001; WIL Research Laboratories 2001). Other findings in the F1 offspring by maternal exposure to 1-bromopropane were increased incidence of bent ribs, reduced skull ossification and decreased brain weight. For the endpoint of reproductive organs in F1 and F2 offspring, WIL Research Laboratories (2001) also revealed prolongation of the estrous cycle, a decrease in implantation sites and litter sizes, normal sperm counts, sperm motility, weight of cauda epididymis, and weight of pituitary gland. Histopathological studies of F1 offspring showed no alteration of the testis, but an increase in the number of cysts and in interstitial cell hyperplasia. Noticeably, the number of primordial follicles showed an increase in rats exposed to 1-bromopropane at 500 ppm, which is the highest exposure level in F1 offspring. The authors did not consider the increase in primordial follicle is an adverse effect (WIL Research Laboratories 2001), but it might suggest inhibition of follicular development from primordial follicles to growing follicles that was also shown by tendency for an increase in primordial follicle and

decrease in growing and antral folicles in adult female rats exposed to 1-bromopropane (Yamada 2003).

## Other adverse effects of 1-bromopropane

Hematotoxicity

As 2-bromopropane had severe hematotoxic effects on humans and rats, we also investigated possible hematotoxic effects of the isomer 1-bromopropane. Several subchronic inhalation studies did not show any hematotoxic effects in rats at 200, 400 and 800 ppm for 8 h per day, 7 days per week for 12 weeks (Ichihara et al. 2000b; Kim et al. 1999) and humans (Ichihara et al. 2002; Sclar 1999). In the investigation on the 1-bromopropane factory, nine of 24 women and four of 13 men showed lower hemoglobin (Hb) or hematocrit (Ht) than the lower limit of reference values, but this study could not exclude confounding factors such as iron deficiency, habitual smoking and past exposure to 2-bromopropane (Ichihara et al. 2004b). Other experimental studies showed possible hematotoxic effects of 1-bromopropane at relatively high exposure levels. In the clinical trials (Clin Trials 1997b) rats were exposed to 1-bromopropane at 398, 993 or 1,590 ppm for 6 h per day, 5 days per week for 4 weeks. The surviving seven female rats at 1,590 ppm showed a decrease in erythrocyte count, hemoglobin concentration and hematocrit. Erythrocyte count and hemoglobin concentration were significantly decreased in both male and female rats exposed to 993 ppm 1-bromopropane, while hematocrit was decreased in female rats exposed to 993 ppm. In the developmental study by Huntington life Sciences (1999), platelet count decreased in female offspring exposed to 598 and 996 ppm of 1-bromopropane and in male offspring exposed to 996 ppm.

Hepatotoxicity

Ichihara et al. (2000a) showed the presence of scanty areas, suggestive of glycogen areas, in the cytoplasm of hepatocytes in rats exposed to 800 ppm of 1-bromopropane. Fat droplets around the central vein were smaller in size, number and population in the 800 ppm-exposed groups. An increase in liver glycogen in the exposed groups was also shown by WIL Research Laboratories (2001). The decrease in the fat droplets might be related to the decrease in serum total cholesterol (Ichihara et al. 2000b). Centrilobular vacuolation was reported in several studies (Clin Trials 1997a; Kim et al. 1999; WIL Research Laboratories 2001). Yamada et al. found scattered cytoplasmic degeneration in hepatocytes around the central veins, with nuclear pyknosis in female rats exposed to 800 ppm of 1-bromopropane. While these histopathological studies show the adverse effects of 1-bromopropane on the liver, there are no studies that show the release of hepatic enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Two studies showed a tendency for decreased activity of these enzymes (Ichihara et al. 2000a; Ishidao et al. 2002). While human data are limited, they also showed no abnormalities of hepatic enzymes (Ichihara et al. 2004b).

Renotoxicity

Female rats exposed to relatively high concentrations of 1-bromopropane (800 and 1,590 ppm) showed increased absolute kidney weight (Yamada et al. 2003; Clin Trials 1997b). Histopathologically, such increase was accompanied by mild dilation of the proximal tubules (Yamada et al. 2003), urinary dilatation and pelvic dilatation of the kidney and dilatation and hemorrhage in the urinary bladder (Clin Trials 1997b). On the other hand, Kim et al. (1999) reported increased relative weight of the kidney rather than its absolute weight. They could not confirm the above histopathological findings but identified tubular casts only at 1,800 ppm. Despite the inconsistent results, the increase in absolute kidney weight may be related to the dilatation of the proximal tubules or the renal pelvis, suggesting tubule dysfunction or post-renal disturbance.

Muscle toxicity

Ichihara et al. (2000a) showed irregular banding of the striated muscle fibers of soleus muscle. Electron microscopic observation revealed loss of regular linearity in the Z line and zigzag arrangement of the myofilaments. It is uncertain if this muscle degenerative change originates from the nerve or muscle.

## Human cases of 1-bromopropane toxicity

At the time that the neurotoxicity and reproductive toxicity of 1-bromopropane were identified in animal studies, there was no evidence for 1-bromopropane toxicity in humans. The first suspected case was reported by Sclar (1999). One worker presented weakness of the lower extremities and right hand, numbness and dysphagia and urinary difficulties following a 2-month exposure to solvents containing 1-bromopropane. Clinical tests showed prolongation of the distal motor latency in the lower extremities by more than 150% of the upper limit of normal range and only a mild decrease of motor conduction velocity in the peroneal segment below the knees. The significantly delayed distal latency and mild change of MCV were similar to the result noted at the early stage of 4 weeks in the 12-week animal study (Ichihara et al. 2000a). Gadolinium-enhanced magnetic resonance brain imaging studies of the same

patient showed patchy areas of increased T2 signal in the periventricular white matter, mostly outside the corpus callosum, with asymmetric distribution, and enhanced proximity of the nerve root ganglia at multiple thoracic and lumbar levels of the spinal cord. Somatosensory evoked potentials did not show any cortical potentials, suggesting a lesion at the dorsal column or lemniscal level. Subsequently, three patients that showed neurological signs were reported in North Carolina in United States of America (Ichihara et al. 2002). On 31 July 2000, I received an e-mail from one female worker (A) in North Carolina, USA. On 30 June 2000 she was taken to the emergency room of a regional hospital after exposure to 1-bromopropane for approximately 1 year. She was engaged in the manufacture of polyurethane foam parts and spraying of adhesives and solvents. The solvent used contained mainly 1-bromopropane, which had been introduced as an alternative for dichloromethane. The worker initially had headaches and nasal irritation and later developed numbness and burning sensations in her legs. Ventilators were installed in the workplace, but they were operating for only 15 min/h, in June 2000, to keep the air-conditioned air cool and to reduce electrical consumption. Finally, one day, she could not stand in the morning and was transferred to the emergency room. Neurological examination revealed reduced sensation of vibration in the toes and numbness of the feet, frontal thighs and buttocks. MRI and electrophysiological studies did not show any abnormal findings. No urinary mercury or lead was detected, and urinary arsenic concentrations were within the normal limits (48.3 μg/l, 150.9 μg/g creatinine). Initially, she was suspected to have multiple sclerosis, but a cerebrospinal fluid test for this proved negative. At almost the same time the physician saw another patient (B), a colleague of the first patient, who was suffering from numbness and stumbling. She also reported numbness of the feet, thighs, and buttocks and had reduced sensations of vibration in her feet. A third person (C) was also identified that had similar but milder symptoms of numbness and headache. The diagnosis of these three cases also suggests that 1-bromopropane has a deleterious effect on peripheral nerves and the central nervous system, although it does not allow establishment of the exact etiology. In these patients the distribution of numbness was limited to the legs, the ventral aspects of the thighs and the gluteal region. This distribution is different from the distal-dominant polyneuropathy with severe muscle atrophy in workers exposed to neurotoxic solvents such as hexane. Our patients also showed symptoms related to the autonomic nervous system, such as diarrhea, change in sweating pattern and urinary incontinence. No dysmetric signs were found in tests such as the nose–finger nose test. The dystaxia and stumbling could be due to diminished deep sensation or be of cerebellar origin, although there are no data in support of the latter. Various symptoms such as headache, dizziness, and memory loss suggested adverse effects on the central nervous system.

On 24 August 2000, I suggested that workers A and B contact the National Institute for Occupational Safety and Health (NIOSH), USA, for assessment of the workplace and workers' health. NIOSH received a letter from the workers on 28 August 2000 and visited the workplace in November 2000, April 2001 and July 2001 (Harney et al. 2002). Before the first NIOSH visit, on 23–26 October, we determined the time-weighted average of the exposure level of the third worker (C), using diffusive sampling, and the results showed 133 ppm, on average, ranging from 60 ppm to 261 ppm (Ichihara et al. 2002). On the other hand, during the first visit by NIOSH the airborne exposure level of 1-bromopropane was 65.9 ppm (range 41.3–143 ppm), but it was lower during the second and third visits. It should be noted that our estimation and the NIOSH determination (Harney et al. 2002) were performed after the ventilation had been improved in the autumn. Therefore, the exposure levels in the summer of 2000 might have been higher than those values. In the NIOSH study (Harney et al. 2002) the most often reported symptoms were headache (48%), trouble falling asleep or staying asleep (28%), dizziness or feeling "off balance" (25%) and blurred vision (24%). Blurred vision and dizziness or feeling "off balance" were significantly more frequent in the exposed group than in the control. The examined blood indices were within normal ranges. There was no difference, in the postural stability test, between the exposed and control groups. Among 16 neurobehavioral tests, only three items, Tremor Pen Right-Intensity ($m/s^2$) and Tremor Pen Right-Frequency (Hz) and Slow RF Finger Tap-Standard deviation, showed greater values in the exposed group than in the control. The authors suggested that the unilateral tremors were probably due to muscle fatigue, because if 1-bromopropane exposure had caused those abnormalities, they should have been bilateral. However, even if muscle fatigue is involved, it might be difficult to disprove a possible adverse effect of 1-bromopropane because human cases of intoxication with volatile chemicals such as vinyl chloride have not necessarily shown bilateral changes (Takeuchi and Mabuchi 1973).

About 1 year before the above cases were detected, in March 1999, four workers engaged in another cushion-manufacturing company in North Carolina were hospitalized for similar neurological symptoms, lightheadedness and/or dizziness, weakness of lower extremities, varying degrees of difficulty in standing or walking (ataxia), and bilateral numbness or paresthesias in the lower extremities (Harney et al. 2003). Noticeably, these symptoms are exactly similar to those of three workers engaged in cushion manufacturing in a different company (Ichihara et al. 2002). One symptom reported only in this study was visual hallucination, which was described by a single worker (Harney et al. 2003). All four workers had elevated serum bromide (Br) (77, 53, 100 and 24 mg/dl) and three of the four had high urinary arsenic concentration (224, 200 and 318 μg/l). The fourth worker showed elevation of urinary arsenic level

(224 μg/l) after being discharged. The authors of the health hazard evaluation (HHE) report considered that none of these four subjects had a history or findings consistent with arsenic intoxication and that the symptoms, such as feeling drunk or anxious, headache or signs of walking problems (ataxia), could be attributed to Br intoxication. However, the available literature on Br intoxication could not explain the painful tingling/numbness/pins and needles feeling (paresthesia). If we look back at our previous cases (Ichihara et al. 2002) from the viewpoint of possible Br intoxication (Hanes and Yates 1938; Trump and Hochberg 1976), the irritation, forgetfulness, appetite loss, and slurred speaking found in the workers reported by Ichihara might be also explained by Br intoxication, at least partially. However, there is also another possibility: the alkylating ability of 1-bromopropane with a sulfhydryl base (Ichihara et al. 2004a; Wang et al. 2002, 2003) could also be involved in the neurotoxic effects of 1-bromopropane.

NIOSH (Harney et al. 2003) conducted health examinations on workers in the same factory but could not find any exposure-related effects on blood cell counts, male reproductive functions (sperm number, shape or motility), or nerve conduction velocity in these workers. However, they also increased the limitation of their study, because of such factors as the small sample number and the high rate of drop out from the initial workers, including the hospitalized workers.

NIOSH conducted another medical assessment of the workers involved in the manufacture of cushions using 1-bromopropane as a solvent (Reh et al. 2002). Having a headache at least once per week (22 workers), having painful tingling in hands or feet (18), reporting a tremor (16), and feeling drunk when not drinking (18) were reported by 43 workers exposed to 1-bromopropane. There was no significant relationship between symptoms or hematological parameters and personal exposure levels or job category based on the exposure. Interestingly, the reported symptoms are in accord with the case report in North Carolina and they might be typical symptoms of exposure to 1-bromopropane. In addition, infertility problems (in two of nine men and one of 32 women) were reported, but it was difficult to relate it directly to exposure to 1-bromopropane. Ichihara et al. (2004b) investigated a chemical plant producing 1-bromopropane. The major clinical symptoms among 24 workers were strange smell (14), nasal irritation (10), sore throat (9), painful eyes (9) and dizziness or vertigo (7), suggesting mucosal irritation and possible adverse effects on the central nervous system. However, the study could not find any serious neurological abnormalities as seen in rat models. It should be noted that this survey was originally designed to investigate a 2-bromopropane plant, but the product had been changed to 1-bromopropane. This means that the tests were not necessarily designed to detect neurotoxic effects but rather hematological changes. Some workers were found to be anemic, but we could not determine its cause including past exposure to 2-bromopropane. An interesting finding from the same study was the changing of the product that might reflect a change in demand from 2-bromopropane to 1-bromopropane in the world. Thus, few suspected cases were reported, but data suggestive of a link between dose of 1-bromopropane and effects in humans are insufficient.

## Neurological and neurobehavioral changes in rats and their significance for human cases

Ohnishi et al. (1999) reported ataxic gait in rats exposed to 1-bromopropane at 1,500 ppm, 6 h/day, 5 days a week for 4 weeks. Fueta et al. (2000, 2002b) also described ataxic gait and convulsions in rats exposed to 1-bromopropane, under similar conditions, at the end of the 4-week exposure. However, it might be difficult to extrapolate such "ataxic gait" in rats to humans, since the 4-footed walking rat is quite different from the bipedal walking human in terms of ataxia expression.

Fueta et al. showed hyperexcitability of the hippocampal CA1 and dental gyrus in the brain of rats exposed to 1-bromopropane in vivo (Fueta et al. 2000, 2002b), accompanied by modification of intracellular signaling cascades such as MAPK, CaMKII alpha and beta (Fueta et al. 2002a). Although the authors investigated hippocampal slices in relation to episodic memory and learning, it is difficult to interpret the above results at this time, and further epidemiological or animal studies on behavioral changes are needed.

The study by Honma et al. (2003) suggested that exposure to 1-bromopropane has excitatory effects on the central nervous system, as shown by spontaneous locomotor activity and open-field activity tests, and that exposure to this compound reduced the grip or muscle strength, as shown by traction test. Reported cases in the USA have shown depressed mood after exposure, but the patients were excited and sometimes quarrelsome, particularly those in whom the exposure level and frequency were highest (Ichihara et al. 2002). Workers exposed to 1-bromopropane complained of disturbance of memory, but experimental studies in rats did not show the same effects in the passive avoidance behavior test. There was also no evidence of motor coordination disturbance in human cases (Ichihara et al. 2002), in accordance with the animal study. On the other hand, in contrast to the decrease in defecation and urination in the study by Honma et al. (2003), human patients reported diarrhea or urinary incontinence (Ichihara et al. 2002). This discrepancy is not surprising, based on the different regulatory mechanisms that control urination and defecation in various species. While it is not simple to extrapolate the results of animal behavioral tests to humans, the above studies indicate that 1-bromopropane has neurobehavioral effects in humans.

## Biochemical approach to studying the effects of 1-bromopropane on the central nervous system

Generally speaking, alterations of the morphological structure of the central nervous system are more robust than other changes, such as biochemical indices. Therefore, biochemical markers or indices could have great meaning, especially in the assessment of the adverse effects of 1-bromopropane on the central nervous system. Wang et al. (2002) examined neuron-specific and glia-specific proteins in the central nervous system of rats exposed to 1-bromopropane for 7 days. The rats were exposed to 200, 400 or 800 ppm or fresh air, 8 h/day for 7 days. Exposure to 1-bromopropane resulted in a dose-dependent decrease in neuron-specific gamma-enolase in the cerebrum and cerebellum but did not change the level of glia-specific protein, beta-S100, in the cerebrum, cerebellum, brainstem or spinal cord. In the 12- week study (Wang et al. 2003) the decrease in gamma-enolase was reproduced only in the cerebrum. Since gamma-enolase is localized only in neurons, the observed decrease might represent a decrease in the amount of enzyme per cell or a decrease in the number of neurons. Taken together with the finding that reduced weight is limited to the cerebrum, the decrease in gamma-enolase in the cerebrum might represent 1-bromopropane adverse effects suggested by the weight loss. Both in the 7-day (Wang et al. 2002) and 12-week (Wang et al. 2003) studies we reported that exposure to 1-bromopropane also resulted in a dose-dependent decrease in creatinine kinase activity in all regions of the brain as well as spinal cord and plasma (Ichihara et al. 2000a). Other neurotoxic compounds, such as acrylamide and ethylene oxide, are also known to reduce blood creatinine kinase levels (Matsuoka et al. 1990a, 1990b, 1993, 1996), but the significance of this finding is not fully understood. Total glutathione level was dose-dependently decreased in the cerebrum and cerebellum at 7 days and 12 weeks, and in the brain stem at 12 weeks, but spinal cord showed the opposite trend of dose-dependent increase at both time intervals. Protein-bound sulfhydryl base was decreased in the cerebrum and cerebellum and brain stem by 12 weeks' exposure but not by 7 days' exposure. The above biochemical studies provided more sensitive indices than morphological alterations did and suggested the underlying mechanism of 1-bromopropane neurotoxicity.

## Kinetics and metabolism of 1-bromopropane and 2-bromopropane—is it possible to explain the difference in toxicities?

Kaneko et al. (1997) reported little difference between 1-bromopropane and 2-bromopropane with regard to their distribution rates in tissue/air and tissue/blood. Therefore, the different toxicities of these two compounds should be explained by factors other than those related to their distribution into tissues. Bromopropanes release bromium ions and covalently bind with nucleophils (Kemp and Vellaccio 1980). Previous studies have confirmed that the urinary excretion of bromium was bromopropane dose-dependent in workers and animals exposed to bromopropanes (Kawai et al. 1997, 2001; Ishidao et al. 2002). 1-Bromopropane can react with nucleophils by mainly SN2 mode, but 2-bromopropane does by SN1/SN2. The reactivity of SN2 and SN1 depends on the polarity of the solvent for reaction (Kemp and Vellaccio 1980). SN2 reaction occurs more easily in polar solvents, while in contrast, SN1 reaction occurs in non-polar solvents. Exactly how this difference in chemical reactivity is related to the difference in toxicities of both bromopropanes remains to be investigated.

The difference in reactivity between the two bromopropanes may be related to differences in metabolism and/or degradation. 1-Bromopropane conjugated with glutathione (Khan and O'Brien 1991) and excreted as mercapturates (Barnsley et al. 1966). Barnsley et al. (1966) showed that the number of mercapturates from 2-bromopropane was less than that from 1-bromopropane in the urine of rats exposed to the same doses of 2-bromopropane and 1-bromopropane. Kaneko et al. (1997) demonstrated that 2-bromopropane is less stable in water than 1-bromopropane, indicating also the tendency for SN1 reactions in polar solvents as mentioned above.

Zhao et al. (2002) investigated N7-adduct of guanine formed from 2-bromopropane and concluded that DNA adduct might be related to DNA damage although they did not examine other DNA adducts including O6-adduct of guanine. On the other hand, 1-bromopropane was found to reduce protein-bound sulfhydryl base (Wang et al. 2003) and could form a covalent bond with cysteine residues of globin and other proteins in the spinal cord (Ichihara et al. 2004a). However, no comparison has been reported between 1-bromopropane and 2-bromopropane with regard to the formation rates of these DNA and protein adducts, which might explain the differences between their toxicities.

1-Bromopropane can conjugate with glutathione directly (Khan and O'Brien 1991), but a proportion of up-taken 1-bromopropane could be hydrolyzed, forming propylene oxide. The presence of propylene oxide is supported by urinary *N*-acetyl-*S*-(2-hydroxypropyl)cysteine (Barnsley et al. 1966; Jones and Walsh 1979) and glycidol (Ishidao et al. 2002) in rats exposed to 1-bromopropane, and production of 1,2-propanediol and 2-hyroxypropylglutathione in microsomal and/or glutathione-added incubation (Tachizawa et al. 1982). While it is not clear whether metabolic activation plays a role in the toxicities of these compounds, the understanding of such a process might allow us to identify the reason for the difference in toxicities of the two bromopropanes.