Testicular and Hematopoietic Toxicity of 2-Bromopropane, a Substitute for Ozone Layer-Depleting Chlorofluorocarbons

Gaku ICHIHARA¹, Nobuyuki ASAEDA², Toshihiko KUMAZAWA², Yoshiaki TAGAWA², Michihiro KAMIJIMA¹, Xiaozhong YU¹, Hidetaka KONDO¹, Tamie NAKAJIMA³, Junzoh KITO⁴, Il Je YU⁵, Young Hahn MOON⁵, Naomi HISANAGA⁶ and Yasuhiro TAKEUCHI¹

¹Department of Hygiene, Nagoya University School of Medicine, ²Safety Assessment Laboratory, Sanwa Kagaku Kenkyusho Co., Ltd., ³Department of Hygiene, Shinshu University School of Medicine, ⁴Institute for Laboratory Animal Experiment, Nagoya University School of Medicine, ⁵Industrial Health Research Institute, Korea Industrial Safety Corporation, and ⁶National Institute of Industrial Health

Abstract: Testicular and Hematopoietic Toxicity of 2-Bromopropane, a Substitute for Ozone Layer-Depleting Chlorofluorocarbons: Gaku ICHIHARA, *et al.* Department of Hygiene, Nagoya University School of Medicine—In 1995, unexpected amenorrhea, oligozoospermia and anemia were discovered in

Korean workers exposed to solvents containing 2-bromopropane which was a substitute for chlorofluorocarbon. We aimed to determine experimentally the testicular and hematopoietic toxicity of 2-bromopropane in male rats. Thirty-six Wistar male rats were divided into four groups of nine each. The rats were exposed to 300, 1,000 and 3,000 ppm 2-bromopropane or only fresh air, respectively, 8 hr a day, 7 days per week. The 300 ppm and 1,000 ppm groups were exposed for 9 weeks, but the 3,000 ppm group's exposure was discontinued and three rats in this group were dissected after 9–11 days' exposure because of serious illness. The others were dissected at the end of the experiment. At 300 ppm or over, the testicular and epididymal weights per body weight, epididymal sperm count, motile sperm percentage and the number of erythrocytes and platelets had decreased compared to the control. Histopathologically, all types of germ cells decreased in the 300 ppm group. Germ cells were absent but Sertoli cells still remained in the 1,000 ppm and 3,000 ppm groups at the end of the experiment. Spermatogonia were absent and the number of spermatocytes decreased in the 3,000 ppm group

rats sacrificed after 11 days' exposure. Sertoli cell vacuolations were marked in two of these three rats. Bone marrow was hypocellular in the 1,000 ppm group and in all the rats in the 3,000 ppm group. These results clearly showed that 2-bromopropane had a testicular and hematopoietic toxicity in male rats.

(J Occup Health 1997; 39: 57-63)

Key words: 2-bromopropane, Testicular toxicity, Hematopoietic toxicity, Chlorofluorocarbon substitute, Sertoli cell-only syndrome

The production of chlorofluorocarbons (CFCs) is now going to be drastically diminished all over the world, because these chemicals have a great potential to destroy the ozone layer and to warm the earth's environment. This countermeasure to CFCs has, on the other hand, resulted in the introduction of various alternatives into the workplace. In July, 1995 unexpected health disorders were discovered in Korean workers exposed to solvents containing 2-bromopropane (CAS No. 75-26-3) in an electronic machine components factory. An epidemiological survey¹⁾ conducted by the Korea Industrial Safety Corporation in August of that year, disclosed that (1) amenorrhea occurred in 17 (68%) of 25 females and oligozoospermia in six (75%) of eight males, accompanied by anemia in six females and one male; (2) amenorrhea appeared shortly after the introduction of 2-bromopropane as a substitute for 1,1,2-trichloro-1,2,2-trifluoroethane, one of the CFCs, and (3) these health disorders were not found among reference workers. The above evidence suggested that 2-bromopropane might be the

Received Aug 19, 1996; Accepted Oct 22, 1996

Correspondence to: G. Ichihara, Department of Hygiene, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan

A part of this article has appeared in this journal as a rapid communication, Ichihara G, *et al.* Testicular Toxicity of 2-Bromopropane. J Occup Health 1996; 38: 205-206.

causative agent. 2-Bromopropane is an existing chemical used as an intermediate in the production of various chemicals. The toxicity of 2-bromopropane, however, has not been reported except in the acute toxicity test²⁾, and its threshold limit value has not been proposed by the Korean Government, the Japan Society for Occupational Health or the American Conference of Governmental Industrial Hygienists Inc. (ACGIH).

The aim of this study is to determine experimentally the testicular and hematopoietic toxicity of 2-bromopropane. We examined the effects of 2-bromopropane on the weights and morphology of organs, the sperm parameters and hematological indices in male rats. Some of the data have already been reported in a rapid communication³⁾.

Materials and Methods

2-Bromopropane (99.4% purity) was kindly supplied by Tosoh Co., Japan. Thirty-six Wistar male rats (specific pathogen-free, 9 weeks) were purchased from SLC (Shizuoka Laboratory Animal Center, Japan). The animals were housed and adapted to the circumstances for 4 weeks and divided into four groups of nine rats each. The environment was kept on a 12 hr light-dark cycle (lights on at 9:00 and off at 21:00), and maintained at 22–25°C and 57–60% relative humidity. Three groups were then exposed to 300, 1,000 or 3,000 ppm 2-bromopropane, respectively, in stainless steel chambers (80×80×58 cm). The maximum concentration of 3,000 ppm was set to be less than the reported LC₅₀ 36,000 mg/m³ (7,159 ppm)²⁾ or 31,171 ppm, 4 hr⁴⁾. The common ratio was put at about 3. The control group was given only fresh air in a similar chamber. The inhalation exposure was performed eight hours a day, 7 days per week. The 300 ppm and 1,000 ppm groups were exposed for 9 weeks. After 9–11 days' exposure, all the rats in the 3,000 ppm group became seriously ill, then their exposure at 3,000 ppm was discontinued. Three in the 3,000 ppm group were sacrificed and the testes and femur were dissected from them 16–17 hr after the cessation of 11 days' exposure. The other six rats in the 3,000 ppm group were exposed to fresh air until the end of the experiment. Food and water were provided *ad libitum*. The inhalation exposure system was described elsewhere in detail^{5, 6)}. The regulated volume of solvents was evaporated and mixed with a larger volume of clean air to achieve the desired concentration. The vapor concentrations in the chamber were measured by gas chromatography and controlled to within ±5% of the target concentration by means of a personal computer, but the inhalation system was modified for the present

experiment in the following three ways: To prevent hydrolysis of 2-bromopropane into isopropyl alcohol and hydrogen bromide, (1) 2-bromopropane was evaporated at room temperature without heating, and (2) compressed air which evaporated 2-bromopropane was dried with an electronic air dryer (EAD-70, Yaezaki Kuatsu Co., Ltd.). Hydrogen bromide was not detected in a chamber with a detector tube (detection limit, 0.05 ppm). To prevent the reaction of 2-bromopropane with aluminium, (3) the aluminium evaporator was replaced with one made of stainless steel.

The testes, epididymides, prostate, seminal vesicle, femur, liver and kidneys were dissected out from the remaining six rats in the 3,000 ppm group and the rats in the other groups at the end of the experiment. The rats were weighed and anesthetized with sodium pentobarbital (50 mg/kg). They were exsanguinated and the blood was collected with a syringe by aortic puncture.

The dissected organs were weighed, and the left testis and epididymis were fixed in Bouin's solution and the prostate, seminal vesicle, femur, liver and kidneys were fixed in 10% neutral buffered formalin for histopathological studies. The femur was decalcified after the fixation. The specimens were processed and embedded in paraffin. Tissue sections of testis and epididymis were stained with periodic acid-Schiff's reagent (PAS) and those of other organs with hematoxylin and eosin (H-E).

Sperm were collected as quickly as possible after the rat was killed. Sperm suspensions were prepared by mincing the right cauda epididymidis in 2.5 ml of Hank's solution kept at 37°C, pipetting the suspension and filtering it through gauze. A fraction of the suspension was diluted with Hank's solution kept at 37°C. Active sperm per hundred sperm were counted with an erythrocytometer (Neubauer type) under a light microscope. Another fraction of the sperm suspension was diluted with saline containing 0.5% formalin for determination of the sperm count. Sperm shape was examined according to the method of Wyrobek and Bruce (1975)⁷⁾ and Eyden and Maisin (1978)⁸⁾.

The following hematologic parameters were determined: erythrocyte counts, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leucocyte count and platelet count (with an F-800 Toa Microcell Counter, Toa Medical Electronics).

Statistical comparisons of the exposed groups with the control were performed by analysis of variance (ANOVA) followed by Dunnett's multiple

Table 1. Body weight and organ weights in rats exposed to 2-bromopropane at the end of the experiment

Group		Control	300 ppm	1,000 ppm	3,000 ppm
Number of rats		9	9	9	6
Body weight	(g)	491±21	458±31.4*	402±19.7**	468±12.1
Absolute organ weight					
Epididymides	(g)	1.18±0.11	0.91±0.06**	0.69±0.04**	0.70±0.04**
Testes	(g)	3.30±0.24	1.54±0.12**	1.36±0.09**	1.27±0.17**
Prostate	(g)	0.90±0.16	0.72±0.19*	0.44±0.10**	0.67±0.07*
Seminal vesicles	(g)	2.06±0.18	1.72±0.14**	1.24±0.21**	1.74±0.21**
Liver	(g)	15.43±1.48	14.48±1.22	13.36±1.16**	14.71±0.81
Kidneys	(g)	2.95±0.21	2.70±0.16*	2.45±0.17**	2.82±0.14
Relative organ weight					
Epididymides	(mg/g body)	2.41±0.20	1.99±0.15**	1.72±0.12**	1.49±0.09**
Testes	(mg/g body)	6.72±0.45	3.37±0.36**	3.39±0.31**	2.70±0.32**
Prostate	(mg/g body)	1.85±0.41	1.57±0.43	1.10±0.23**	1.44±0.18
Seminal vesicles	(mg/g body)	4.21±0.36	3.76±0.36	3.09±0.51**	3.71±0.46
Liver	(mg/g body)	31.39±2.12	31.61±1.01	33.16±1.43	31.42±1.44
Kidneys	(mg/g body)	6.02±0.36	5.91±0.45	6.10±0.34	6.02±0.29

The exposure in the 3,000 ppm group was discontinued after 9–11 days' exposure. Six rats in the 3,000 ppm group were then given only fresh air until the end of the experiment. The weights of epididymides, testes and kidneys are expressed as the sum of right and left organs. Values are the means±standard deviations for each group. Asterisks indicate that the value was significantly different from the control (* $p<0.05$, ** $p<0.01$, Dunnett's multiple comparison).

comparison method. The numbers of abnormal sperm in the 300 ppm and control groups were compared by Student's *t*-test, because the number of abnormal sperm could not be estimated in the 1,000 ppm and 3,000 ppm groups.

Results

The mean and standard deviations of daily exposure concentrations of 2-bromopropane were 302 ± 17 ppm, $1,035\pm35$ ppm, and $2,964\pm165$ ppm (mean±SD), respectively. Body weight gain was suppressed in a dose-dependent manner by 2-bromopropane exposure (Fig. 1). Mean body weight in the 3,000 ppm group fell throughout the exposure period, but it recovered after the exposure ended. It became comparable to that in the 300 ppm group or the control at the end of the experiment (Fig. 1 and Table 1).

At 300 ppm and over, absolute weights of testes, epididymides, prostate and seminal vesicle decreased in comparison with the control (Table 1). Because body weight was significantly lower in the 300 and 1,000 ppm groups than in the control, the relative weights of organs to body weight are shown in Table 1. 2-Bromopropane reduced the relative weights of the genital organs, but not those of liver and kidneys.

Exposure of the six surviving rats in the 3,000 ppm group was discontinued after 9–10 days' exposure. This short-term exposure resulted in a weight loss in the testes comparable to or more than that

Fig. 1. Time course of body weight (Means±S.D.). The exposure in the 3,000 ppm group was discontinued after 9–11 days' exposure. Three rats in the 3,000 ppm group were killed 16–17 hr after cessation of exposure and the six surviving rats were given only fresh air until the end of the experiment.

at 1,000 ppm for 9 weeks' exposure, but decreased to a lesser extent the weight of the prostate and seminal vesicle.

The sperm count and percentage of motile sperm decreased and the percentage of tailless sperm in-

creased dose-dependently (Table 2). Exposure of 2-bromopropane at 1,000 ppm for 9 weeks and 3,000 ppm for 9–10 days resulted in complete loss of motile sperm and overwhelming predominance of

tailless sperm at the end of the experiment. The frequency of abnormal sperm could not be assessed in the 1,000 and 3,000 ppm groups, because very few intact sperm were detected in these groups.

Table 2. The sperm count and the percentage of motile sperm, tailless sperm and abnormal sperm in the rats exposed to 2-bromopropane

Group	Control	300 ppm	1,000 ppm	3,000 ppm
Number of rats	9	9	9	6
Sperm count ($\times 10^6/\text{g cauda}$)	568.78 $\pm$ 204.78	357.89 $\pm$ 117.28*	326.44 $\pm$ 139.90**	151.17 $\pm$ 34.39**
Motile sperm (%)	86.16 $\pm$ 2.51	15.83 $\pm$ 6.49**	0.00 $\pm$ 0.00**	0.00 $\pm$ 0.00**
Tailless sperm (%)	9.52 $\pm$ 3.09	56.06 $\pm$ 12.19**	98.28 $\pm$ 1.30**	99.39 $\pm$ 0.65**
Abnormal sperm (%)	6.94 $\pm$ 2.29	20.57 $\pm$ 7.76**	—a	a
hook up head (%)	1.54 $\pm$ 0.97	5.19 $\pm$ 1.72**	—a	—a
reflexed head (%)	3.96 $\pm$ 1.68	12.63 $\pm$ 5.41**	—a	—a
angular head (%)	0.11 $\pm$ 0.33	0.83 $\pm$ 1.24	—a	a
recurvant flagellum (%)	1.33 $\pm$ 0.41	1.93 $\pm$ 1.25	—a	—a

Values are the means $\pm$ standard deviations for each group. Asterisks indicate that the value was significantly different from the control (* $p < 0.05$, ** $p < 0.01$, Dunnett's multiple comparison for sperm count, motile sperm and tailless sperm and Student's *t*-test for abnormal sperm, hook up head, reflexed head, angular head and recurvant flagellum). a The frequency of abnormal sperm could not be assessed in the 1,000 and 3,000 ppm groups, because very few intact sperm were detected in these groups.

Fig. 2. Photomicrograms of testes of rats exposed to 2-bromopropane. (A) Control: The stage is VII. No abnormality was found. (B) 300 ppm for 9 weeks: The stage is VII. The seminiferous tubule was moderately atrophied and the number of all types of germ cells decreased. (C) 1,000 ppm for 9 weeks; All types of germ cells were absent, defined as Sertoli cell-only syndrome. Interstitial cells (Leydig) were hyperplastic. Seminiferous tubules were severely atrophied. (D) 3,000 ppm for 10 days at the end of the experiment: The findings were the same as in (C). Periodic acid-Schiff (PAS). Scale bar indicates 100 μm . The magnifications of all photographs (A), (B), (C) and (D) are the same.

The percentage of abnormal sperm with hook up head and reflexed head was significantly larger in the 300 ppm group than in the control.

In the 300 ppm group, seminiferous tubules were moderately atrophied and all types of germ cells decreased to about a half of the control (Fig. 2B). In the 1,000 ppm group and the six surviving rats in the 3,000 ppm group, germ cells were completely lost and seminiferous tubules severely atrophied (Figs. 2C and 2D). On the other hand, Sertoli cells still remained in these exposed rats (Figs. 2B, 2C and 2D). Leydig's interstitial cells were hyperplastic to a slight extent in the 300 ppm group and to a moderate extent in the 1,000 ppm group and the six surviving rats in the 3,000 ppm group (Figs. 2C and 2D).

In three rats in the 3,000 ppm group sacrificed after 11 days' exposure, spermatogonia was absent and the number of spermatocytes decreased (Fig. 3). In two of the three rats, some seminiferous epithelial cells (probably spermatocytes) had pyknotic nuclei. Sertoli cell vacuolation was much more marked in these two rats than in the rats in all exposed groups sacrificed at the end of the experiment. Multinucleate giant cell-like structures were found in the 300 ppm group.

The number of sperm decreased in the epididymal duct in all the exposed groups. Prostate and seminal vesicles showed only slight atrophic change in the exposed groups. No abnormal findings were observed in the liver or kidney in any exposed groups.

Exposure to 2-bromopropane decreased erythrocytes, hemoglobin, hematocrit, platelets and leucocytes (Table 3). These indices decreased to a lesser degree in the six surviving rats exposed to 3,000 ppm for 9–10 days than in the 1,000 ppm group (Table 3). In the 300 ppm group, erythrocytes and platelets significantly decreased in comparison with the control, but leucocytes, hematocrit and hemo-

Fig 3. Photomicrogram of testes in 3,000 ppm group sacrificed 16–17 hours after 11 days' exposure. Spermatogonia were not found and Sertoli cells were vacuolated. Some seminiferous epithelial cells (probably spermatocytes) had pyknotic nuclei. Periodic acid-Schiff (PAS). Scale bar indicates 100 μ m.

globin did not (Table 3). The anemia was classified as macrocytic. Histopathological examination showed hypocellular and fatty bone marrow in the 1,000 ppm and 3,000 ppm groups at the end of the experiment and in the three rats in the 3,000 ppm group which were sacrificed after 11 days' exposure.

Discussion

Oligozoospermia and anemia found in human cases were reproduced in the present animal experiment. The changes in sperm indices were accompanied by weight loss of the testis and epididymis and loss of testicular germ cells. The changes in hematological indices were accompanied by hypocellular bone marrow at 1,000 ppm or over. It should be noted that the testicular and epididymal changes mentioned above and changes in some hematological indices were caused by inhalation exposure to 300 ppm or higher concentrations

Table 3. The hematological indices in rats exposed to 2-bromopropane

Group		Control	300 ppm	1,000 ppm	3,000 ppm
Number of rats		9	9	9	6
Erythrocyte	($\times 10^4/\mu l$)	920.33 $\pm$ 24.17	882.78 $\pm$ 20.63**	806.67 $\pm$ 21.77**	833.50 $\pm$ 18.75**
Hemoglobin	(g/dl)	16.08 $\pm$ 0.39	16.10 $\pm$ 0.33	15.22 $\pm$ 0.36**	15.67 $\pm$ 0.40
Hematocrit	(%)	46.03 $\pm$ 0.92	46.20 $\pm$ 0.65	44.02 $\pm$ 1.17**	45.50 $\pm$ 1.39
MCV	(fl)	50.03 $\pm$ 0.66	52.34 $\pm$ 0.82**	54.60 $\pm$ 1.35**	54.58 $\pm$ 0.75**
MCH	(pg)	17.47 $\pm$ 0.25	18.24 $\pm$ 0.30**	18.89 $\pm$ 0.43**	18.80 $\pm$ 0.30**
MCHC	(%)	34.93 $\pm$ 0.33	34.84 $\pm$ 0.41	34.58 $\pm$ 0.37	34.43 $\pm$ 0.35*
Platelet	($\times 10^4/\mu l$)	90.38 $\pm$ 3.45	78.99 $\pm$ 7.01**	65.72 $\pm$ 7.14**	82.33 $\pm$ 6.56
Leucocyte	($\times 10^2/\mu l$)	117.44 $\pm$ 34.46	128.56 $\pm$ 33.67	72.22 $\pm$ 9.65**	91.33 $\pm$ 20.07

Values are the means $\pm$ standard deviations for each group. Asterisks indicate that the value was significantly different from the control (* $p < 0.05$, ** $p < 0.01$, Dunnett's multiple comparison).

of 2-bromopropane. On the other hand, liver and kidneys showed little change in their weights and no pathological findings. These results show that the adverse effects of 2-bromopropane on the reproductive system and hematopoiesis may be organ-specific.

Cessation of exposure at 3,000 ppm, which was not planned beforehand, resulted in demonstrating the effects of 2-bromopropane 53–54 days after the short term exposure. In the six surviving rats in the 3,000 ppm group, the hematological indices seemed to recover to a moderate degree, whereas testicular and epididymal weights, sperm indices and the cellularity of testicular germ cells still remained almost equal to or a little less than those in the 1,000 ppm group. These results suggest that hematopoiesis may be less vulnerable to 2-bromopropane or more reversible than spermatogenesis. The results may coincide with the Korean report that showed some cases of oligozoospermia without anemia but no cases of anemia without oligozoospermia¹⁾.

The absence of germ cells with remaining Sertoli cells in the 1,000 ppm group and the surviving rats in the 3,000 ppm group is called the Sertoli cell-only syndrome. 2-Bromopropane may have a selective toxic effect on the spermatogonia, as demonstrated in the rats sacrificed shortly after cessation of 3,000 ppm exposure. The specific toxic effect of 2-bromopropane on spermatogonia and hematopoietic cells suggests that differentiation and frequent dividing of cells could be involved with the toxicity.

Sertoli cell vacuolations may be an early change accompanying progressive degeneration and exfoliation of germ cells, because this change was much more noticeable in the rats in 3,000 ppm group sacrificed after 11 days' exposure than in the six surviving rats in the 3,000 ppm group at the end of the experiment. The Sertoli cell vacuolations were also reported as an early change following the administration of other agents to rats^{9,15)}.

Hyperplasia of Leydig cell is generally observed as a compensatory reaction to disruption of seminiferous epithelium.

In the Korean case, it was reported that the workers could be exposed to 2-bromopropane at more than 4,000 ppm as short-term exposure¹⁾. Ambient concentrations of 2-bromopropane were detected from 9.2 ppm to 19.6 ppm in the workplace during regular operation after improvement of ventilation. The real exposure concentrations were not evaluated in the field survey, but 300 ppm to 3,000 ppm exposure could have been possible in the Korean workplace judging from the above data. Absorption through the skin may also be responsi-

ble for the uptake of 2-bromopropane into workers¹⁾, but this was not investigated in the present study.

Clinical information concerning the toxicity of 2-bromopropane has been lacking thus far. The reason for this may be that this chemical has been mainly used in a closed system, wherein it was almost always an intermediate in the production of other chemicals, and therefore workers were not exposed to large dose of 2-bromopropane to produce its specific toxicity. 2-Bromopropane came to be used as a substitute for chlorofluorocarbene, since this chemical is nonflammable and volatile like chlorofluorocarbons and is easily broken down in the environment and is less destructive to the ozone layer. As a large amount of 2-bromopropane is used now and in the future the amount used in industry may rapidly increase, urgent measures must be taken to minimize 2-bromopropane exposure in workplaces. It should also be noted that numerous kinds of harmful new or existing chemicals can be introduced into workplaces with the progress of technology¹⁶⁾ or by social regulation of chemical production, even if intended to protect the earth's environment or workers' health.

In conclusion, 2-bromopropane was revealed to have a testicular and hematopoietic toxicity in male rats. The oligozoospermia and anemia in human cases were experimentally confirmed in rats.

Acknowledgments: We gratefully acknowledge the helps of Mr. Hisakazu Iwai and Mr. Kazuhito Ichikawa (Safety Assessment Laboratory, Sanwa Kagaku Kenkyusho Co., Ltd., Japan) and the advice of Mr. Shunji Aman (Nanyo Laboratory, Tosoh Co., Japan). We also thank Tosoh Co., Japan for a kind gift of 2-bromopropane.

References

- 1) Industrial Health Research Institute, Korea Industrial Safety Corporation. Final report of epidemiological survey on Yangsan LG Electronics. 1995 (in Korean).
- 2) Liublina E, Rabotnikova L. Comparative evaluation of acute toxicity of certain brominated hydrocarbons. *Gigiena Truda i Professionalnye Zabollevaniia*, 1974 Apr 18(4): 55–7 (in Russian).
- 3) Ichihara G, Asaeda N, Kumazawa T, et al. Testicular toxicity of 2-bromopropane. *J Occup Health* 1996; 38: 205–206.
- 4) Kim H, Chung Y, Yi K, Kim J, Yu I. LC50 of 2-bromopropane. *Ind Health* 1996; 34: 403–407.
- 5) Takeuchi Y, Huang J, Shibata E, Hisanaga N, Ono Y. A trial to automatize an organic solvent exposure system for small animals. *Jpn J Ind Health* 1989; 31: 722 (in Japanese).
- 6) Huang J, Kato K, Shibata E, Hisanaga N, Ono Y, Takeuchi Y. Effects of subacute toluene exposure on

- neuronal and glial marker proteins in rat brain. *Toxicol* 1990; 61: 109–117.
- 7) Wyrobek A, Bruce W. Chemical induction of sperm abnormalities in mice. *Proc Natl Acad Sci USA* 1975; 72: 4425–4429.
- 8) Eyden B, Maisin J. Observations on the structure and levels of expression of murine spermatozoan abnormalities with special reference to tail deformations. *Arch Anat Microsc* 1978; 67: 19–30.
- 9) Flores M, Fawcett D. Ultrastructure effects of anti-spermatogenic compound WIN 18446 (bis dichloroacetyl diamine). *Anat Rec* 1972; 172: 310.
- 10) De Martino C, Stefanini M, Agrestini A, Cocchia D, Morrelli M, Scorza Barcellona P. Antispermatozoan activity of 1-p chlorobenzyl-1 H indazol-3-carboxylic acid (AF 1321/TS) in rats. III. A light and electron microscopic study after single oral doses. *Exp Mol Pathol* 1975; 23: 321–356.
- 11) Hoffer A, Carlton B, Brunengraber H. Ultrastructure and intercellular vacuolization of isolated perfused and control rat testes. *J Androl* 1983; 4: 361–370.
- 12) Chapin R, Morgan K, Bus J. The morphogenesis of testicular degeneration induced in rats by orally administered 2,5-hexanedione. *Exp Mol Pathol* 1983; 38: 149–169.
- 13) Creasy D, Beech L, Gray T, Butler W. An ultrastructural study of ethylene glycol monomethyl ether-induced spermatocyte injury in the rat. *Exp Mol Pathol* 1986; 45: 311–322.
- 14) Anderson R, Berryman S, Phillips J, Feathergill K, Zaneveld L, Russell L. Biochemical and structural evidence for ethanol-induced impairment of testicular development: apparent lack of Leydig cell involvement. *Toxicol Appl Pharmacol* 1989; 100: 62–85.
- 15) Creasy D, Ford GR, Gray TJ. The morphogenesis of cyclohexylamine-induced testicular atrophy in the rat: in vivo and in vitro studies. *Exp Mol Pathol* 1990; 52(2): 155–169.
- 16) Kaczmarczyk J, Paul M. Reproductive health hazards in the workplace: guidelines for policy development and implementation. *Int J Occup Environ Health* 1996; 2: 48–58.