Food and Chemical Toxicology 46 (2008) 3172–3177

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Carcinogenicity study of 3-monochloropropane-1,2-diol in Sprague–Dawley rats

Wan-Seob Cho, Beom Seok Han, Ki Taek Nam, Kidae Park, Mina Choi, Seung Hee Kim, Jayoung Jeong, Dong Deuk Jang *

Department of Toxicological Research, Korea Food and Drug Administration, National Institute of Toxicological Research, Seoul 122-704, Republic of Korea

ARTICLE INFO

Article history:
Received 23 August 2007
Accepted 11 July 2008

Keywords:
3-Monochloropropane-1,2-diol
SD rats
Carcinogenicity
Renal tubule carcinoma
Leydig cell tumor

ABSTRACT

3-Monochloropropane-1,2-diol (α-chlorohydrin, 3-MCPD) is a well-known contaminant, which has been detected in a wide range of foods and ingredients, and is also a suspected cause of cancer. In this study, the carcinogenicity of 3-MCPD in SD rats was investigated. Groups of 50 male and 50 female rats were exposed for two years to drinking water containing 0, 25, 100 or 400 ppm 3-MCPD. The body weights and water consumptions of the male and female rats given 400 ppm 3-MCPD were significantly lower than those of the controls. The incidences of renal tubule adenomas or carcinomas and Leydig cell tumors occurred with dose-related positive trends in male rats. The incidences of renal tubule carcinomas and Leydig cell tumors were significantly increased in male rats given 400 ppm 3-MCPD. The incidence of renal tubule adenomas showed a positive trend in female rats, which was significant in 400 ppm 3-MCPD group. In conclusion, there was clear evidence of the carcinogenic activity of 3-MCPD in male SD rats, based on the increased incidences of renal tubule carcinomas and Leydig cell tumors. There was some evidence of the carcinogenic activity of 3-MCPD in female SD rats, based on the increased incidence of renal tubule adenomas.

1. Introduction

3-Monochloropropane-1,2-diol (α-chlorohydrin, 3-MCPD) is a well-known contaminant, which has been detected in a wide range of foods and ingredients. It can be found in foods prepared by hydrochloric acid hydrolysis, such as acid-hydrolyzed vegetable protein. 3-MCPD has also been reported in flavor enhancer (Crews et al., 2002), some soy sources (Macarthur et al., 2000), roasted cereals, fermented sausages and toasted breads, as used in domestic cooking (Crews et al., 2001), and other ingredients, such as fat, salt, emulsifiers, sugar, and baking agents, which can lead to the formation of 3-MCPD (Breitling-Utzmann et al., 2005). The amount of 3-MCPD in foods, especially in acid-HVP, has markedly decreased from approximately 100 mg/kg to less than 1 mg/kg due to technological processes (FSA, 2001, 2005; Hamlet et al., 2002). Therefore, 3-MCPD is usually present in trace amounts (<1 mg/kg), but individual samples may contain high levels (up to a few hundreds mg/kg).

Several toxicological studies have shown that 3-MCPD induces infertility in rats (Jackson et al., 1977; Kwack et al., 2004) and causes suppression of the immune function (Lee et al., 2004, 2005). It does not produce neurotoxicity or neuromotor deficits in rats (Kim et al., 2004), but has been shown to cause small vacuolated lesions in the brainstem area of mice (Cavanagh et al., 1993). It has been found to be genotoxic in most *in vitro* assays (Silhankova et al., 1982; Stolzenberg and Hine, 1980; Zeiger et al., 1988), but *in vivo* assays produce negative results (El Ramy et al., 2007; Epstein et al., 1972; Frei and Wurgler, 1997; Robjohns et al., 2003). Based on the available results in 2001, the European Scientific Committee on Food (SCF) concluded that the genotoxic activity of 3-MCPD observed *in vitro* was not expressed *in vivo* (SCF, 2001). Four long-term bioassays on 3-MCPD have been conducted; two carcinogenicity studies that investigated both the subcutaneous and intra-peritoneal routes in Swiss mice (Van Duuren et al., 1974), as well as two dietary studies conducted on SD (Weisburger et al., 1981) and F344 rats, respectively (Sunahara et al., 1993). However, these studies (Van Duuren et al., 1974; Weisburger et al., 1981) were performed with inadequate protocols compared with the OECD Test Guideline 451 for 'Carcinogenicity studies' (OECD, 1981). In a chronic study on F344 rats, a high dose of 3-MCPD induced Leydig cell and mammary gland tumors in males and benign kidney tumors in both genders (Sunahara et al.,

Abbreviations: 3-MCPD, (3-monochloropropane-1,2-diol); SD, (Sprague–Dawley); OECD, (Organisation for Economic Co-operation and Development); SCF, (scientific committee on food); JECFA, (joint expert committee on food additives); KFDA, (Korea Food and Drug Administration); MTD, (maximum tolerated dose); CPN, (chronic progressive nephropathy).

* Corresponding author. Tel.: +82 2 380 1821; fax: +82 2 388 6451.
E-mail addresses: ddjang@kfda.go.kr, chows77@kfda.go.kr (D.D. Jang).

0278-6915/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2008.07.003

1993). However, the kidney tumors might be secondary to chronic progressive nephropathy, and the Leydig cell and mammary gland tumors due to species- and strain-specific mechanisms, including chronic changes associated with a hormonal imbalance (JECFA, 2002; Lynch et al., 1998). Therefore, additional experiments may be necessary to confirm the carcinogenicity and potential of hormonal imbalance. The purpose of this study was to clarify the possible involvement of the species- and strain-specific non-genotoxic carcinogenicity of 3-MCPD. Thus, the carcinogenicity of 3-MCPD when administered to SD rats in the drinking water was investigated according to the test guidelines from Korea Food and Drug Administration (KFDA) and the OECD Test Guideline 451 for 'carcinogenicity study' (OECD, 1981).

2. Materials and methods

2.1. Test material

3-MCPD (Cas No. 96-24-02), 98% pure, was purchased from Sigma Aldrich Inc. (St. Louis, MO, USA). The structure and purity was verified prior to use, with its stability confirmed at all concentrations. The dose formulations were prepared every two weeks by dissolving 3-MCPD in deionized water and stored in glass vessels protected from the light at 4 °C. The solutions given to rats in the water bottles were replaced every three days. Periodic analyses of the dose formulations were conducted by LabFrontier Co. Ltd., Korea. The analytical results for all dose formulations were within 10% of the theoretical concentrations.

2.2. Study design

As a prelude to conducting the carcinogenicity study on 3-MCPD, a 13-week toxicity study was performed on SD rats to survey target organs and select doses to be used for a two-year study (Shin et al., 2002). As a result, the MTD was limited to 40 mg/kg/day, as higher doses had adverse effects on the body weights, organ weights and clinical chemistry.

Groups of 50 males and female rats were exposed *ad libitum* to 0, 25, 100 or 400 ppm 3-MCPD, administered in their drinking water over a two-year period. The body weights and water and food consumptions were recorded every week, with the animals observed at least once daily for their general appearance, behavior, signs of toxicity, morbidity and mortality. The average daily consumptions of 3-MCPD were calculated from the measured weekly water consumptions.

2.3. Animal husbandry and maintenance

Four-week old male and female SD rats were obtained from a specific pathogen-free colony at Charles River Japan Inc. and quarantined for 14 days prior to commencement of the study. The environmental conditions (temperature, 23 ± 1 °C; relative humidity, 55 ± 5%; 12-h light/dark cycle) were monitored at ~4-h cycles for 24-h, and maintained within acceptable ranges through the study. Rats were fed Purina Certified Rodent Chow #5002 (Richmond, IN mill of Ralston Purina Co., St. Louis, MO) *ad libitum*, with the exception of a one-night fast prior to their scheduled sacrifice. The rats were housed two per cage, with the cages changed twice weekly and the racks changed every two weeks. The animals were handled in an accredited Korea Food and Drug Administration animal facility in accordance with the AAALAC International Animal Care Policies (accredited unit – Korea Food and Drug Administration; unit number – 000996). Furthermore, all the study protocols were reviewed and approved by the Animal Care and Use Committee of the National Institute of Toxicological Research, Korea Food and Drug Administration, Korea.

2.4. Necropsy and histopathology

Male and female rats were necropsied at 100 and 104 weeks, respectively, in accordance with the OECD Test Guideline 451 for 'Carcinogenicity studies' (OECD, 1981). Complete necropsies were performed on all rats, including those that died or became moribund. All organs/tissues were fixed in 10% neutral buffered formalin, with the exception of the testes, which were fixed in Bouin's solution, and the eyes and Harderian glands, which were fixed in Davidson's AFA fixative. Tissues that required decalcification, such as the femur and spinal cord with bone, were treated with 7.5% nitric acid for approximately 4–5 h. All the organs and tissues were processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 μm, and stained with hematoxylin and eosin for the microscopic examination. Histopathological diagnosis was performed according to the standardized system of nomenclature and diagnostic criteria (SSNDC). The pathology data were peer reviewed by two pathologists (Forth Tox Ltd., UK, and North Carolina State University, USA).

2.5. Statistical analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for possible dose-related effects on survival were performed using Cox's (1972) method, for testing two groups for equality, and Tarone's (1975) life table test to identify dose-related trends. The body weight, food and water consumption data were analyzed using an analysis of variance followed by the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971). The Poly-*k*-test (Bailer and Portier, 1988) was used to assess the incidences of neoplastic and non-neoplastic lesions.

3. Results

3.1. In-life observations

The survival rates of the rats administered 0, 25, 100 and 400 ppm 3-MCPD by the end of study were 28%, 34%, 18% and 26% for males and 30%, 44%, 22% and 32% for females, respectively. The survival of all the dosed groups was similar to that of the control (data not shown). The body weights of both sexes in the 400 ppm groups were significantly decreased throughout the study compared to those of the control (Fig. 1). The water consumptions of the male and female rats administered 400 ppm 3-MCPD were significantly lowered throughout the study than those of the control (Fig. 2). Concentrations of 25, 100 or 400 ppm 3-MCPD in the drinking water resulted in average daily consumptions of approximately 1.97, 8.27 and 29.50 mg/kg 3-MCPD for males and 2.68,

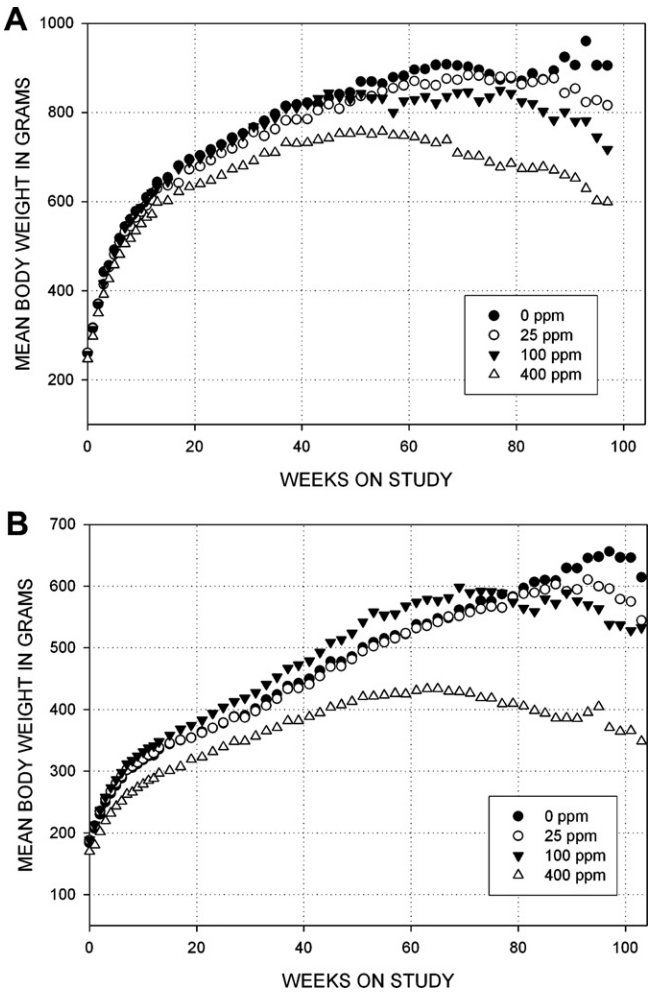

Fig. 1. Body weights of (A) male and (B) female rats administered 3-MCPD in drinking water for two years. The body weights of both sexes in the 400 ppm groups were significantly decreased throughout the study compared to those of the control.

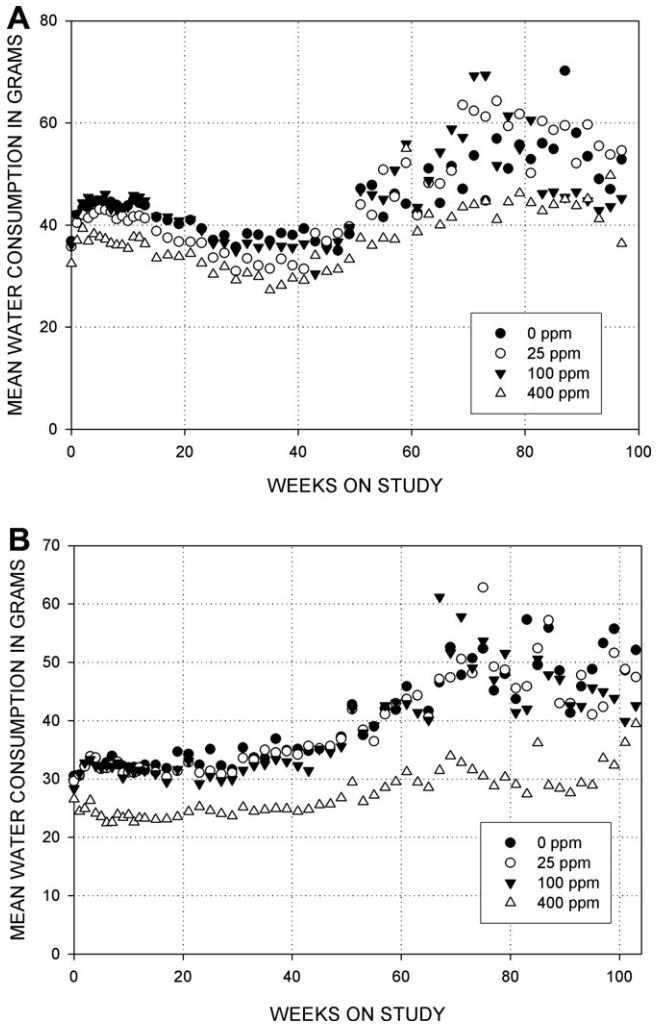

Fig. 2. Water consumption of (A) male and (B) female rats administered 3-MCPD in drinking water for two years. The water consumptions of the male and female rats administered 400 ppm 3-MCPD were significantly lowered throughout the study compared to control.

10.34 and 37.03 mg/kg 3-MCPD for females, respectively. The dietary consumptions in the male and female rats exposed to 25, 100 and 400 ppm 3-MCPD were generally similar to those of the control throughout the study (data not shown).

3.2. Histopathology

Tables 1 and 2 summarize the non-neoplastic and neoplastic lesions in the rats exposed to 3-MCPD in their drinking water. There were positive trends in the incidence of renal tubule neoplasm (adenoma or carcinoma) and renal tubule adenoma in the male and female rats, respectively. Renal tubule carcinomas and renal tubule adenomas of the males and females in the 400 ppm groups, respectively, were significantly increased compared to those of the controls. Higher incidences of renal tubule carcinomas were observed in the males in the 400 ppm groups than in the respective female group, suggesting that males more sensitive to 3-MCPD than females. The incidences of renal tubule adenoma or carcinoma in both sexes were higher than in historical SD rat controls from Charles River Laboratories (Giknis and Clifford, 2001). Renal tubule neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia and chronic progressive nephropathy (CPN). In males, the incidences of renal tubule hyperplasia and CPN in all exposed groups were significantly increased in a dose dependent manner (Table 1). In females, the incidences of renal tubule hyperplasia in the 100 and 400 ppm groups were significantly increased compared to those in the control and those of CPN in the 400 ppm group were significantly increased compared to the control group.

There was a dose-related increasing trend of Leydig cell tumors in the testes of males administered 400 ppm 3-MCPD. Non-neoplastic changes, such as seminiferous tubular atrophy and arteritis or periarteritis, were also identified in the 25, 100 and 400 ppm groups.

4. Discussion

In the current two-year carcinogenicity study, all surviving male rats were sacrificed at 100 weeks, as the survival of male control rats reached 28%. According to the OECD Test Guideline 451 for 'Carcinogenicity studies', termination of the study is acceptable when the numbers of survivors on the lower doses or in the control group reach 25%; if there is an apparent sex difference in the response, each sex should be considered separately. The overall survival of rats exposed to 3-MCPD for two years was similar to that of the controls. However, the survival was lower than 50% in all groups. The mortality was increased in all SD rat groups, largely due to the high rate of moribund sacrifice caused by naturally occurring pituitary gland tumors.

The target organs of 3-MCPD were the kidneys and testes, as shown by the significant neoplastic responses in these organs. In a previous long-term carcinogenicity study using F344 rats, 3-MCPD was also reported to increase the incidences of renal tubule adenomas in both sexes when administered at a level of 500 ppm (Sunahara et al., 1993). However, the kidney benign tumors were classified as early adenoma, which is a borderline lesion between hyperplasia and adenoma; therefore, it was not associated with early death and malignancy (renal tubule carcinoma). Furthermore, no adenomas were observed before week 100 after the initiation of the experiment in rats treated with 3-MCPD. In another study on SD rats, where 3-MCPD was administered only twice weekly for 72 weeks (instead of 104 weeks as period of carcinogenicity study recommended by the OECD), no increased incidence of kidney tumors was observed (Weisburger et al., 1981). However, in our study, the incidences of renal tubule neoplasms (adenoma or carcinoma) were significantly increased in both sexes of the 400 ppm groups. In addition, the incidences of renal tubule adenoma or carcinoma were higher than those of the historical control data of Charles River Laboratories (Giknis and Clifford, 2001). Moreover, incidences of renal tubule carcinomas were observed in both males (5/50) and in females (3/50) groups administered 400 ppm 3-MCPD. In males, renal tubule adenomas and carcinomas were observed earlier than at 100 weeks of study, with the first incidences of renal tubule adenoma and carcinoma observed at 78 and 74 weeks, respectively. Therefore, as the incidence of spontaneous renal tubule neoplasm is extremely rare, their incidences in this study may have been related to the administration of 3-MCPD.

In addition, renal tubule neoplasms were accompanied by significantly increased incidences of chronic progressive nephropathy (CPN) and renal tubule hyperplasia. The incidences of CPN and renal tubule hyperplasia in both the male and female treatment groups were significantly greater than those of the controls. In a previous study, CPN, renal tubule hyperplasia, adenomas and carcinomas were thought to represent a continuum in the progression of proliferative lesions of the renal tubule epithelium (Hard et al., 1993). Therefore, our study demonstrated that 3-MCPD can accel-

Table 1
Incidences of non-neoplastic lesions in SD rats exposed to 3-MCPD in drinking water for two years

Dose (ppm)	Males				Females			
	0	25	100	400	0	25	100	400
Number examined	50	50	50	50	50	50	50	50
Kidney								
Renal tubule, hyperplasia	1	11*	21*	36*	1	0	1	10*
Nephropathy, chronic progressive	15	27*	39*	41*	6	8	23*	42*
Mineralization	0	0	1	1	12	6*	3*	4*
Testes								
Atrophy	6	16*	13*	34*	–	–	–	–
Arteritis/periarteritis	3	15*	9*	11*	–	–	–	–
Epididymis								
Atrophy	0	0	0	5*	–	–	–	–
Seminal vesicle								
Atrophy	36	41	49*	44	–	–	–	–
Adrenal gland, cortex								
Hemangiectasis	8	8	5	3	39	42	34	33*
Necrosis	0	5*	2	1	0	0	1	0
Vacuolation, fat	11	13	19*	11	8	7	6	10
Heart								
Cardiomyopathy, progressive	24	24	26	20	28	20*	12*	8*
Liver								
Cellular foci, basophilic	6	0*	0*	2	5	5	11	7
Degeneration, cystic	5	9	14*	6	1	1	1	0
Inflammation	12	17	17	4*	11	19	9	7
Fatty change	15	13	12	9	21	13*	13	6*
Lymph node, cervical								
Plasmacytosis	12	17	17	9	23	25	23	16*
Pancreas								
Atrophy	8	10	19*	4	5	5	2	3
Parathyroid gland								
Hyperplasia	19	21	28*	26	5	12	6	7
Pituitary gland, pars distalis								
Hyperplasia	5	3	4	2	7	1*	4	1*
Thymus								
Hyperplasia, epithelial component	0	4	4*	2	6	14	6	13
Cyst, epithelial	0	0	1	0	0	0	1	9*
Thyroid gland, C-cell								
Hyperplasia	4	7	5	1	17	24	13	9*

* Significantly different ($p < 0.05$) from the control group by the poly-3 test.

Table 2
Incidences of neoplastic lesions in SD rats exposed to 3-MCPD in drinking water for two years[a]

	0 ppm	25 ppm	100 ppm	400 ppm	Historical data[b]
Males					
Number examined	50	50	50	50	1531
Kidney					
Renal tubule, adenoma	0 (0%)	0 (0%)	1 (2%)	4 (8%)	1.43–4.00
Renal tubule, carcinoma	0 (0%)	0 (0%)	0 (0%)	5* (10%)	1.67–4.00
Renal tubule, adenoma or carcinoma	0 (0%)	0 (0%)	1 (2%)	7*(14%)	
Testes					
Leydig cell tumor	1 (2%)	1 (2%)	4 (8%)	14* (28%)	1.43–7.14
Pituitary gland, pars distalis					
Adenoma	25 (50%)	26 (52%)	24 (48%)	13* (26%)	0.77–70.00
Females					
Number examined	50	50	50	50	1729
Kidney					
Renal tubule, adenoma	0 (0%)	0 (0%)	1 (2%)	6* (12%)	0
Renal tubule, carcinoma	1 (2%)	0 (0%)	1 (2%)	3 (6%)	0.77–1.85
Renal tubule, adenoma or carcinoma	1 (2%)	0 (0%)	2 (4%)	9* (18%)	

* Significantly different ($p < 0.05$) from the control group by the poly-3 test.
[a] The data are reported as the number of animals with a neoplasm per number of animals examined microscopically, the % incidence (in parentheses).
[b] The range of tumor incidences in control SD rats (Giknis and Clifford, 2001).

erate the progression of CPN and the subsequent stimulation of renal tubule hyperplasia and; finally, produce renal tubule adenomas and carcinomas.

The nephrotoxic mechanisms of 3-MCPD are thought to be due to the inhibition of glycolysis by metabolites associated with the β-chlorolactate pathway (Jones and Chantrill, 1989). Impairments of

the glycolytic pathway and energy production could contribute to permanent kidney damage (Jones and Fakhouri, 1979) and exacerbation of CPN (Sunahara et al., 1993). In addition to the effects of β-chlorolactate on glycolysis, the accumulation of oxalic acid in the kidney also could contribute to the progression of CPN (Jones et al., 1981). Our study showed that the renal tumors induced by 3-MCPD appeared to be secondary to CPN and renal tubule hyperplasia. These findings were different from those for renal tumors caused by classical carcinogens, such as dimethylnitrosamine, but consistent with a previous study (Sunahara et al., 1993). Furthermore, the occurrence of renal tubule carcinomas, which supports the carcinogenic effects of 3-MCPD, should be reconsidered in the evaluation of the carcinogenicity of 3-MCPD.

Our results show also a positive trend in the incidence of Leydig cell tumors, which significantly increased in the 400 ppm group. Atrophy and arteritis or periarteritis were considered toxic effects induced by the administration of 3-MCPD. However, there were no statistically significant pre-neoplastic changes, such as Leydig cell hyperplasia. In a previous carcinogenicity study using F344 rats, the incidences of Leydig cell tumors in the 100 and 500 ppm groups was significantly increased (Sunahara et al., 1993). In another study using SD rats, Leydig cell tumors were not found in rats treated with 3-MCPD for 72 weeks (Weisburger et al., 1981). Therefore, it was concluded that the increased incidence of Leydig cell tumors observed in F344 rats treated with 3-MCPD was the result of a species- and strain-specific non-genotoxic mechanism associated with a hormonal imbalance (Lynch et al., 1998). However, the results from our study showed that Leydig cell tumors can occur in SD rats; therefore, the carcinogenic potential to testis should be reconsidered.

In our study, there were no statistically significant mammary tumors or mammary gland associated toxicological changes in either sex. However, in a previous carcinogenicity study using F344 rats, the incidence of fibroadenomas was significantly greater than in the control and in excess of the historical control range (Sunahara et al., 1993). Another previous SD rats study using 3-MCPD reported no effects on the incidences of mammary tumors (Weisburger et al., 1981). Our results were consistent with those of a chronic gavage study in SD rats (Weisburger et al., 1981). Therefore, the mammary tumors induced by 3-MCPD are suggested to be a strain-specific phenomenon.

In conclusion, there was clear evidence of the carcinogenic activity of 3-MCPD in male SD rats, based on the increased incidences of kidney renal tubule carcinomas and Leydig cell tumors. There was some evidence of the carcinogenic activity of 3-MCPD in female SD rats, based on increased incidences of kidney renal tubule adenomas.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgement

This research was supported by a Grant (05121KFDA455) from Korea Food and Drug Administration from 2002 to 2005.

References

Bailer, A.J., Portier, C.J., 1988. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics 44, 417–431.

Breitling-Utzmann, C.M., Hrenn, H., Haase, N.U., Unbehend, G.M., 2005. Influence of dough ingredients on 3-chloropropane-1,2-diol (3-MCPD) formation in toast. Food Addit. Contam. 22, 97–103.

Cavanagh, J.B., Nolan, C.C., Seville, M.P., 1993. The neurotoxicity of alpha-chlorohydrin in rats and mice: I. Evolution of the cellular changes. Neuropathol. Appl. Neurobiol. 19, 240–252.

Cox, D.R., 1972. Regression models and life-tables. J. Roy. Stat. Soc. B34, 187–220.

Crews, C., Brereton, P., Davies, A., 2001. The effects of domestic cooking on the levels of 3-monochloropropanediol in foods. Food Addit. Contam. 18, 271–280.

Crews, C., Hough, P., Brereton, P., Harvey, D., Macarthur, R., Matthews, W., 2002. Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999–2000. Food Addit. Contam. 19, 22–27.

Dunnett, C.W., 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1096–1121.

El Ramy, R., Ould Elhkim, M., Lezmi, S., Poul, J.M., 2007. Evaluation of the genotoxic potential of 3-monochloropropane-1,2-diol (3-MCPD) and its metabolites, glycidol and beta-chlorolactic acid, using the single cell gel/comet assay. Food Chem. Toxicol. 45, 41–48.

Epstein, S.S., Arnold, E., Andrea, J., Bass, W., Bishop, Y., 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23, 288–325.

Frei, H., Wurgler, F.E., 1997. The vicinal chloroalcohols 1,3-dichloro-2-propanol (DC2P), 3-chloro-1,2-propanediol (3CPD) and 2-chloro-1,3-propanediol (2CPD) are not genotoxic in vivo in the wing spot test of *Drosophila melanogaster*. Mutat. Res. 394, 59–68.

FSA, 2001. Survey of 3-monochloropropane-1,2-diol (3-MCPD) in soy sauce and related products (Number 14/01).

FSA, 2005. Analysis of 3-monochloropropane-1,2-diol (3-MCPD) in the UK diet: 2001 total diet study.

Giknis, M.L.A., Clifford, C.B., 2001. Complication of Spontaneous Neoplastic Lesions and Survival in Crl:CD (SD) BR Rats from Control Groups. Charles River Laboratories.

Hamlet, C.G., Jayaratne, S.M., Matthews, W., 2002. 3-Monochloropropane-1,2-diol (3-MCPD) in food ingredients from UK food producers and ingredient suppliers. Food Addit. Contam. 19, 15–21.

Hard, G.C., Rodgers, I.S., Baetcke, K.P., Richards, W.L., McGaughy, R.E., Valcovic, L.R., 1993. Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ. Health Perspect. 99, 313–349.

Jackson, H., Rooney, F.R., Fitzpatrick, R.W., Gibson, K.H., 1977. Characterization and antifertility activity in rats of S(+)alpha-chlorohydrin. Chem. Biol. Interact. 17, 117–120.

JECFA, 2002. 3-Chloro-1,2-propane-diol. In: Schlatter, J., Baars, A.J., DiNovi, M., Lawrie, S., Lorentzen, R. (Eds.), Proceedings of the Fifty-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the Safety Evaluation of Certain Food Additives Contaminants. WHO Food Additives Series, No. 48, pp. 401–432.

Jones, A.R., Chantrill, L.A., 1989. Oxidative metabolic activity of boar spermatozoa: a system for assessing anti-glycolytic activity of potential inhibitors in vitro. Reprod. Fertil. Dev. 1, 357–367.

Jones, A.R., Fakhouri, G., 1979. Epoxides as obligatory intermediates in the metabolism of alpha-halohydrins. Xenobiotica 9, 595–599.

Jones, A.R., Gadiel, P., Stevenson, D., 1981. The fate of oxalic acid in the Wistar rat. Xenobiotica 11, 385–390.

Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53, 457–481.

Kim, K., Song, C., Park, Y., Koh, S., Kim, J., Kim, S., Kim, Y., Kim, S.U., Jung, H., 2004. 3-Monochloropropane-1,2-diol does not cause neurotoxicity in vitro or neurobehavioral deficits in rats. Neurotoxicology 25, 377–385.

Kwack, S.J., Kim, S.S., Choi, Y.W., Rhee, G.S., Da Lee, R., Seok, J.H., Chae, S.Y., Won, Y.H., Lim, K.J., Choi, K.S., Park, K.L., Lee, B.M., 2004. Mechanism of antifertility in male rats treated with 3-monochloro-1,2-propanediol (3-MCPD). J. Toxicol. Environ. Health A 67, 2001–2011.

Lee, J.K., Byun, J.A., Park, S.H., Kim, H.S., Park, J.H., Eom, J.H., Oh, H.Y., 2004. Evaluation of the potential immunotoxicity of 3-monochloro-1,2-propanediol in Balb/c mice. I. Effect on antibody forming cell, mitogen-stimulated lymphocyte proliferation, splenic subset, and natural killer cell activity. Toxicology 204, 1–11.

Lee, J.K., Ryu, M.H., Byun, J.A., 2005. Immunotoxic effect of beta-chlorolactic acid on murine splenocyte and peritoneal macrophage function in vitro. Toxicology 210, 175–187.

Lynch, B.S., Bryant, D.W., Hook, G.J., Nestmann, E.R., Munro, I.C., 1998. Carcinogenicity of monochloro-1,2-propanediol (alpha-chlorohydrin, 3-MCPD). Int. J. Toxicol. 17, 47–76.

Macarthur, R., Crews, C., Davies, A., Brereton, P., Hough, P., Harvey, D., 2000. 3-Monochloropropane-1,2-diol (3-MCPD) in soy sauces and similar products available from retail outlets in the UK. Food Addit. Contam. 17, 903–906.

OECD, 1981. OECD Guidelines for the Testing of Chemicals/Section 4: Health Effects. Test No. 451: Carcinogenicity Studies. OECD Publishing.

Robjohns, S., Marshall, R., Fellows, M., Kowalczyk, G., 2003. In vivo genotoxicity studies with 3-monochloropropan-1,2-diol. Mutagenesis 18, 401–404.

SCF, 2001. Opinion of the Scientific Committee on Food on 3-monochloropropane-1,2-diol (3-MCPD). Updating the SCF opinion of 1994.

Shin, D.H., Oh, J.H., Kim, S.H., Hwang, M.S., Lim, C.J., Yum, Y.N., Yang, K.H., Cho, D.H., 2002. Studies on toxicity of 3-monochloro propane-1,2-diol. The Annn. Rep. KNTP 1, 47–55.

Silhankova, L., Smid, F., Cerna, M., Davidek, J., Velisek, J., 1982. Mutagenicity of glycerol chlorohydrins and of their esters with higher fatty acids present in protein hydrolysates. Mutat. Res. 103, 77–81.

Stolzenberg, S.J., Hine, C.H., 1980. Mutagenicity of 2- and 3-carbon halogenated compounds in the *Salmonella*/mammalian-microsome test. Environ. Mutagen 2, 59–66.

Sunahara, G., Perrin, I., Marchessini, M., 1993. Carcinogenicity study on 3-monochloropropane-1,2-diol (3-MCPD) administered in drinking water to Fischer 344 rats. Report RE-SR93003 submitted to WHO by Nestec Ltd., Research and Development, Lausane, Switzerland.

Tarone, R.E., 1975. Tests for trend in life table analysis. Biometrika, 62.

Van Duuren, B.L., Goldschmidt, B.M., Katz, C., Seidman, I., Paul, J.S., 1974. Carcinogenic activity of alkylating agents. J. Natl. Cancer Inst. 53, 695–700.

Weisburger, E.K., Ulland, B.M., Nam, J., Gart, J.J., Weisburger, J.H., 1981. Carcinogenicity tests of certain environmental and industrial chemicals. J. Natl. Cancer Inst. 67, 75–88.

Williams, D.A., 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27, 103–117.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., 1988. *Salmonella* mutagenicity tests: IV Results from the testing of 300 chemicals. Environ. Mol. Mutagen 11 (Suppl. 12), 1–157.