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ScienceDirect
Food and Chemical Toxicology 46 (2008) 1666–1673
www.elsevier.com/locate/foodchemtox

Subchronic toxicity study of 3-monochloropropane-1,2-diol administered by drinking water to B6C3F1 mice

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Received 9 October 2006; accepted 30 December 2007

Abstract

3-Monochloropropane-1,2-diol (3-MCPD) is a food processing contaminant in a wide range of foods and ingredients and is a suspected cause of cancer. In this study, the 13-week toxicity of 3-MCPD was examined in B6C3F1 mice (10/sex/group) administered 3-MCPD doses of 0, 5, 25, 100, 200 and 400 ppm dissolved in their drinking water over a 13-week period. All the mice survived to the end of study. The mean body weight gains in the males and females given 400 ppm were significantly lower than those of the controls. The relative kidney weights of the males and females given 200 and 400 ppm were significantly higher than those of the controls without any corresponding histopathological changes. The sperm motility was lower in the 400 ppm group than the control, and there was a significant increase in the incidence of germinal epithelium degeneration in the 200 and 400 ppm groups. A delayed total estrus cycle length was observed in the 400 ppm group without any histopathological changes. Based on these results, the target organ was determined to be kidney, testis, and ovary. The no-observed-adverse-effect level (NOAEL) was found to be 100 ppm (18.05 mg/kg/day for males and 15.02 mg/kg/day for females).

Keywords: 3-monochloropropane-1,2-diol; B6C3F1 mice; Toxicity; No-observed-adverse-effect level

1. Introduction

3-Monochloropropane-1,2-diol (3-MCPD) is a member of a group of contaminants known as chloropropanols, which includes known genotoxic animal carcinogens such as 1,3-dichloro-2-propanol (Olsen, 1993; Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, 2001). 3-MCPD might be formed as a result of a reaction between a chlorine source (e.g. chlorinated water or salt) in the food or a food contact material and a lipid source (Food Standards Agency, 2001). This reaction is accelerated during the heat processing of foods, including the roasting of cereals and malts used for brewing. In addition, the reaction occurs in acid-hydrolyzed vegetable protein (HVP) when produced using hydrochloric acid (Collier et al., 1991). The contaminant is usually present in trace amounts (<1 mg/kg) but individual samples may contain high levels (up to a few hundred mg/kg). The actual mechanisms for its formation in some of these cases are not completely understood (Scientific Committee on Food, 2001). 3-MCPD has also been reported in soy sauces (Macarthur et al., 2000) as well as

Abbreviations: AAALAC International, association for assessment and accreditation of laboratory animal care international; 3-MCPD, 3-monochloropropane-1,2-diol; KFDA, Korea food and drug administration; NOAEL, no-observed-adverse-effect level; OECD, organization for economic cooperation and development.

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doi:10.1016/j.fct.2007.12.030

in some food contact materials (Pesselmann and Feit, 1988).

Several studies have evaluated toxicological changes that occur as a result of 3-MCPD exposure. 3-MCPD has immunomodulatory effects on mice both *in vitro* and *in vivo* (Lee et al., 2004; Byun et al., 2006). In addition, 3-MCPD does not produce neurotoxicity *in vitro* or neuromotor deficits *in vivo* (Kim et al., 2004). 3-MCPD is genotoxic in most *in vitro* assays (Stolzenberg and Hine, 1980; Silhankova et al., 1982; Zeiger et al., 1988) but *in vivo* assays produce negative results (El Ramy et al., 2007; Robjohns et al., 2003). Four long-term animal carcinogenicity experiments for 3-MCPD have been reported; two in mice and two in rats (Van Duuren et al., 1974; Weisburger et al., 1981; Sunahara et al., 1993). However, three of these studies (Van Duuren et al., 1974; Weisburger et al., 1981) were carried out between 1970 and 1981 with inadequate protocols compared with OECD Test Guideline 451 for 'Carcinogenicity Studies' (OECD, 1981). In a chronic study of F344 rats, a high dose of 3-MCPD induced Leydig cell, mammary and preputial gland tumors in males and benign kidney tumors in both genders (Sunahara et al., 1993). Although 3-MCPD is considered to be a non-genotoxic carcinogen (Scientific Committee on Food, 2001) based on a carcinogenicity study of 3-MCPD in F344 rats, its ability to cause tumors should be reconsidered. Kidney tumors might be secondary to chronic progressive nephropathy and testis and mammary gland tumors are due to the species and strain dependent mechanisms including chronic changes in the hormonal balance. Therefore, additional experiments such as a carcinogenicity study of 3-MCPD in SD rats and mice may be necessary to confirm the carcinogenicity and potential as an endocrine disruptor. This study evaluated the toxicity profile of 3-MCPD in drinking water given to B6C3F1 mice according to the test guidelines from the Korea Food and Drug Administration (KFDA) and the OECD Test Guideline 408 for 'Repeated dose 90-day oral toxicity study in rodents' (OECD, 1998).

2. Materials and methods

2.1. Animal husbandry and maintenance

4-week old male and female B6C3F1 mice were obtained from a specific pathogen-free colony at Charles River Japan Inc. and quarantined for 14 days before the study. The animals were approximately six weeks old on the first day of the study. The female mice were housed five per cage and the male mice were housed individually. Water and feed were available *ad libitum*. The cages were changed twice weekly and the racks were changed every two weeks. The environmental conditions (temperature, 23 ± 1 °C; relative humidity, $55 \pm 5\%$; 12-h light/dark cycle) were monitored at ~4-h cycles for 24-h/day and maintained within the acceptable ranges through the study.

The mice 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).

2.2. Test chemical and treatment

3-MCPD (Cas No. 96-24-02), 98% pure, was purchased from Sigma Aldrich Inc. (St. Louis, MO, USA). Drinking water solutions of 3-MCPD were prepared using deionized water. The dose formulations were prepared every 2 weeks and stored at 4 °C in glass vessels that were protected from the light. The stability of 3-MCPD in drinking water and the dose formulations of animal room samples were analyzed by LabFrontier Co. Ltd., Korea. The analytical results for all dose formulations were within 10% of the theoretical concentrations.

2.3. Experimental design

Groups of 10 male and 10 female mice were exposed *ad libitum* to 0, 5, 25, 100, 200 or 400 ppm 3-MCPD in their drinking water. Feed was available *ad libitum* except for a one-night fast prior to the scheduled sacrifice. The body weight, and water and food consumption were recorded every week, and animals were observed daily for any clinical signs and mortality. The amount of supplied and residual water or diet was weighed weekly in order to calculate the average daily consumption each week. The overall mean throughout the treatment period was calculated from the measured weekly water or food consumption. At the end of the study, all the animals were anesthetized with ether, weighed, and blood samples were collected from the retroorbital sinus for hematology and blood chemistry. The animals were then sacrificed by exsanguinations from the abdominal aorta.

The study protocol was 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. Hematology and blood chemistry

Blood for the hematology determinations was placed in tubes containing potassium EDTA as an anticoagulant. The hematology determinations, including the erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count and differential leukocytes count were performed on an Advia 120 hematology analyzer. The serum biochemistry parameters including alkaline phosphatase, total protein, albumin, blood urea nitrogen, and creatinine were evaluated using an autoanalyzer (Prestige 24i, Tokyo Boeki Medical System, Japan).

2.5. Sperm motility and vaginal cytology evaluation

For 12 consecutive days prior to the scheduled sacrifice, the vaginal vaults of the females were moistened with saline if necessary, and the samples of vaginal fluid and cells were stained. The relative leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to determine estrus cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). The sperm motility was assayed using a modification of the method described by Kwack et al. (2004). The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymis body (corpus epididymis) and weighed. Modified M199 (pH 7.4, 0.5% BSA, 37 °C) was applied to the slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides and the sperm motility was determined using a TOX IVOS sperm analyzer (Hamilton Thorne Research, Beverly, MA).

2.6. Histopathology

A complete necropsy was performed on all animals. The liver, kidneys, testes, ovaries, heart, and spleen were then weighed. These and the following organs and tissues were removed: brain, clitoral glands, esophagus, eyes, femur with marrow, gallbladder, harderian glands, heart and aorta, small and large intestine, lungs and mainstem bronchi, mandibular and

mesenteric lymph nodes, mammary gland with adjacent skin, muscle, nasal cavity and nasal turbinate, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate, salivary glands, seminal vesicle, skin, spinal cord and sciatic nerve, spleen, stomach, epididymis, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina. All organs/tissues were fixed in 10% neutral buffered formalin, except for 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, nasal cavity, nasal turbinate, 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.

2.7. Statistical analysis

The variance in the data for the body weights, food and water consumption, hematology, blood chemistry, absolute and relative organ weights, sperm motility and vaginal cytology data was checked for homogeneity using Bartlett's procedure. If the variance was homogeneous, the data were assessed by one-way analysis of variance. If not, the Kruskal–Wallis test was applied. When statistically significant differences were indicated, a Dunnett's multiple test was used to compare the control and treatment groups. Data comparisons were considered significant if $P < 0.05$. For histopathological data analysis, the incidences were compared using a Fischer's exact probability test. A $P < 0.05$ was considered significant.

3. Results

3.1. General observations

Table 1 shows the survival and body weights, as well as the mean water consumption and daily 3-MCPD intake data. There was no mortality or deterioration in the general conditions observed in any of the groups. Concentrations of 5, 25, 100, 200 or 400 ppm 3-MCPD in the drinking water resulted in average daily consumptions of approximately 0.94, 4.59, 18.05, 36.97 and 76.79 mg/kg 3-MCPD

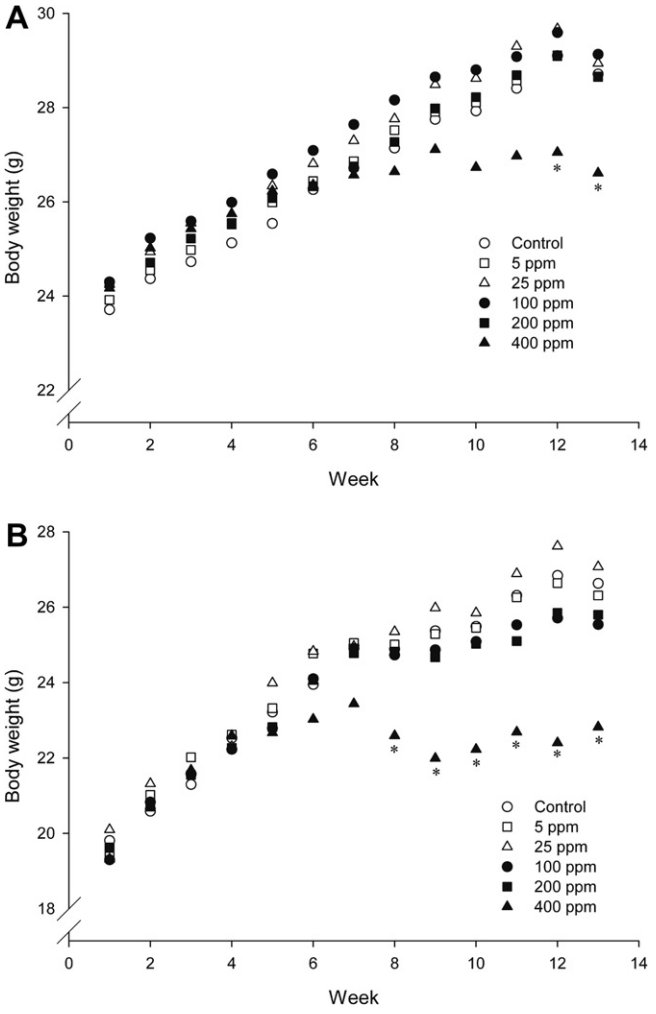

Fig. 1. Body weight curve for male (A) and female (B) B6C3F1 mice in the 13-week drinking water study of 3-MCPD. $^{*}P < 0.05$, as compared with the untreated controls.

Table 1
Survival, body weight and mean daily intakes of water and test chemical for B6C3F1 mice in the 13-week drinking water study of 3-MCPD

Dose level (ppm)	Survival[a]	Final body weights (g)[b]	Mean water consumption (g/mouse/day)	Daily chemical intake (mg/kg/day)
Male				
0	10/10	28.71 ± 1.61	4.93	0
5	10/10	28.66 ± 1.51	5.04	0.94
25	10/10	28.94 ± 1.42	5.00	4.59
100	10/10	29.13 ± 1.23	4.94	18.05
200	10/10	28.65 ± 1.51	4.96	36.97
400	10/10	26.61 ± 2.37*	5.03	76.79
Female				
0	10/10	26.63 ± 1.96	3.69	0
5	10/10	26.31 ± 1.54	3.80	0.79
25	10/10	27.07 ± 1.95	3.85	3.94
100	10/10	25.54 ± 1.13	3.55	15.02
200	10/10	25.80 ± 1.59	3.57	30.23
400	10/10	22.82 ± 2.05**	3.40	61.34

*,**Significantly different from the control group at the level of $P < 0.05$ and $P < 0.01$, respectively.
[a] Number of animals surviving at 13-week/number initially in group.
[b] Data are expressed as mean ± S.D.

for males and 0.79, 3.94, 15.02, 30.23 and 61.34 mg/kg 3-MCPD for females, respectively. The body weights were suppressed in the 400 ppm group from week 12 in males and week 8 in females (Fig. 1). There were no significant treatment-related effects on the survival rate, food or water consumption over the 13-week exposure period.

3.2. Hematology and blood chemistry

Tables 2 and 3 list the data for hematology and blood chemistry at study termination, respectively. There were no definite dose-related changes in any of the hematological parameters in either gender. There was a slight decrease in MCV observed in the males given 400 ppm and in the females given 100 and 200 ppm. Furthermore, a minimal increase in the MCHC was observed in the males given 100 and 400 ppm and in the females given 25 ppm but there was no corresponding decrease in the erythrocyte counts. The leukocyte, lymphocyte and monocyte counts were higher in the males given 100 ppm. There were no significant changes in the blood chemistry between the groups.

3.3. Organ weights

Tables 4 and 5 summarize the data for the final body weights and absolute and relative organ weights, respectively. A significant decrease in final body weights was observed in the males given 400 ppm and in the females given 100, 200 and 400 ppm. In the males, a decrease in the absolute weights of the liver was observed at 400 ppm, while an increase in the relative weight of the kidneys was observed at 200 and 400 ppm. In females, an increase in the relative weights of the liver was observed at 400 ppm and the relative kidney weight was higher in the groups given 200 and 400 ppm than the control.

3.4. Sperm motility and vaginal cytology evaluation

Table 6 summarizes the sperm motility and vaginal cytology evaluation results. The percentage of motile sperm in the males given 400 ppm was significantly lower than those of the controls. The length of the estrus cycle in the females given 400 ppm was significantly longer than those of the controls because females in the 400 ppm group spent more time in diestrus than the control females.

3.5. Histopathology

Table 7 gives a summary of the histopathological changes in the tissues from the mice exposed to 3-MCPD in drinking water. In the testes, there was an increase in the incidence and severity of degeneration of the germinal

Table 2
Hematological findings for B6C3F1 mice in the 13-week drinking water study of 3-MCPD

	Dose level (ppm)					
	0	5	25	100	200	400
Males						
Erythrocyte (10^3cells/μl)	10.7 ± 0.2	10.7 ± 0.6	10.9 ± 0.4	10.7 ± 0.2	10.8 ± 0.7	10.6 ± 0.6
Hemoglobin (g/dl)	16.9 ± 0.8	17.1 ± 1.1	17.2 ± 0.7	17.0 ± 0.3	17.0 ± 0.7	16.6 ± 0.9
Hematocrit (%)	60.4 ± 4.0	60.3 ± 5.4	59.6 ± 2.7	57.7 ± 2.8	58.1 ± 3.2	56.1 ± 2.7
MCV (fl)	56.3 ± 2.7	56.2 ± 1.9	54.9 ± 1.9	54.0 ± 2.0	54.1 ± 2.1	53.0 ± 1.4*
MCH (pg)	15.8 ± 0.5	15.9 ± 0.2	15.8 ± 0.1	15.9 ± 0.2	15.9 ± 0.5	15.7 ± 0.3
MCHC (g/dl)	28.1 ± 0.6	28.4 ± 0.8	28.7 ± 0.9	29.5 ± 1.0*	29.4 ± 0.7	29.5 ± 0.4*
Platelet count (10^4/μl)	104 ± 28.8	96.0 ± 30.8	111.5 ± 15	115.7 ± 16.4	129.2 ± 14.2	120.1 ± 18.1
Leukocytes (10^2cells/μl)	13.8 ± 7.1	18.3 ± 5.4	20.8 ± 5.2	30.0 ± 11.0**	10.2 ± 3.0	12.3 ± 8.6
Neutrophils (10^2cells/μl)	1.98 ± 0.67	4.42 ± 1.73	3.89 ± 2.07	3.82 ± 1.56	1.43 ± 0.65	2.00 ± 1.00
Lymphocytes (10^2cells/μl)	8.05 ± 3.68	12.5 ± 4.1	13.4 ± 2.51	20.5 ± 6.70**	10.9 ± 0.80	15.6 ± 3.30
Monocytes (10^2cells/μl)	0.05 ± 0.06	0.1 ± 0.07	0.14 ± 0.05	0.22 ± 0.19*	0.06 ± 0.05	0.06 ± 0.05
Eosinophils (10^2cells/μl)	0.85 ± 0.21	2.04 ± 1.17	2.17 ± 1.91	0.98 ± 1.42	1.52 ± 0.87	2.43 ± 2.94
Basophils (10^2cells/μl)	0.1 ± 0.08	0.1 ± 0.07	0.09 ± 0.07	0.13 ± 0.05	0.04 ± 0.05	0.17 ± 0.20
Females						
Erythrocyte (10^3cells/μl)	9.85 ± 0.66	10.7 ± 0.1	10.2 ± 0.5	10.7 ± 0.4	10.2 ± 1.2	10.5 ± 0.3
Hemoglobin (g/dl)	15.8 ± 0.8	16.9 ± 0.3	16.3 ± 0.8	17.0 ± 0.5	17.0 ± 0.5	16.6 ± 0.9
Hematocrit (%)	55.6 ± 3.2	59.2 ± 1.6	54.2 ± 3.5	57.0 ± 2.6	57.7 ± 3.5	56.7 ± 2.8
MCV(fl)	56.5 ± 0.6	55.3 ± 1.4	53.3 ± 1.8	53.1 ± 1.1*	52.4 ± 3.6*	54.0 ± 1.3
MCH (pg)	16.1 ± 0.3	15.8 ± 0.3	16.0 ± 0.3	15.9 ± 0.3	15.9 ± 0.2	15.8 ± 0.4
MCHC (g/dl)	28.7 ± 0.7	28.5 ± 0.3	30.1 ± 0.9*	29.9 ± 0.7	29.6 ± 1.0	29.3 ± 0.2
Platelet count (10^4/μl)	92.3 ± 25.2	111.1 ± 20.9	96.6 ± 11.8	103.8 ± 28.2	119.6 ± 6.7	121.7 ± 8.7
Leukocytes (10^2cells/μl)	2.11 ± 0.49	2.32 ± 2.01	2.34 ± 0.26	3.56 ± 1.86	2.52 ± 1.17	1.68 ± 1.04
Neutrophils (10^2cells/μl)	2.38 ± 0.30	4.65 ± 4.03	4.33 ± 1.27	4.13 ± 1.30	3.28 ± 1.68	3.3 ± 0.42
Lymphocytes (10^2cells/μl)	17.8 ± 2.1	22.7 ± 8.3	14.3 ± 2.5	21.1 ± 8.2	24.2 ± 8.2	13.9 ± 0.4
Monocytes (10^2cells/μl)	0.1 ± 0.00	0.13 ± 0.15	0.16 ± 0.11	0.20 ± 0.06	0.18 ± 0.13	0.13 ± 0.12
Eosinophils (10^2cells/μl)	2.33 ± 0.79	0.95 ± 0.78	2.65 ± 1.15	2.60 ± 1.34	1.58 ± 1.28	4.17 ± 1.27
Basophils (10^2cells/μl)	0.05 ± 0.06	0.07 ± 0.12	0.09 ± 0.04	0.33 ± 0.44	0.22 ± 0.38	0.23 ± 0.12

*,**Significantly different from the control group at the level of $P < 0.05$ and $P < 0.01$, respectively.
MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 3
Blood chemistry data for B6C3F1 mice in the 13-week drinking water study of 3-MCPD

	Dose level (ppm)					
	0	5	25	100	200	400
Males						
Alkaline phosphatase (IU/I)	268 ± 21	251 ± 21	263 ± 23	255 ± 12	263 ± 24	251 ± 22
Total protein (g/dl)	7.03 ± 0.88	7.63 ± 0.21	7.22 ± 0.63	7.18 ± 0.76	7.05 ± 0.54	6.81 ± 0.37
Albumin (g/dl)	2.07 ± 0.20	2.10 ± 0.28	2.14 ± 0.13	2.14 ± 0.23	2.05 ± 0.11	2.01 ± 0.11
Blood urea nitrogen (mg/dl)	26.3 ± 3.3	21.8 ± 1.7	23.7 ± 1.15	25.0 ± 1.3	23.4 ± 3.6	26.1 ± 4.7
Creatinine (mg/dl)	0.48 ± 0.05	0.40 ± 0.14	0.47 ± 0.12	0.40 ± 0.06	0.43 ± 0.05	0.41 ± 0.06
Females						
Alkaline phosphatase (IU/I)	310 ± 53	333 ± 39	327 ± 27	337 ± 42	316 ± 46	315 ± 46
Total protein (g/dl)	6.53 ± 0.65	7 ± 0.49	7.72 ± 0.79	7.10 ± 0.44	7.34 ± 0.91	7.64 ± 1.18
Albumin (g/dl)	2.04 ± 0.09	2.15 ± 0.14	2.20 ± 0.16	2.12 ± 0.10	2.23 ± 0.23	2.15 ± 0.17
Blood urea nitrogen (mg/dl)	20.7 ± 1.6	18.9 ± 3.1	20.3 ± 1.2	22.0 ± 1.5	20.7 ± 1.5	24.4 ± 5.0
Creatinine (mg/dl)	0.48 ± 0.05	0.48 ± 0.08	0.42 ± 0.18	0.38 ± 0.11	0.43 ± 0.05	0.43 ± 0.05

*,**Significantly different from the control group at the level of $P < 0.05$ and $P < 0.01$, respectively.

Table 4
Organ weights for male B6C3F1 mice in the 13-week drinking water study of 3-MCPD

	Dose level (ppm)					
	0	5	25	100	200	400
B.W. (g)	25.0 ± 1.1	24.1 ± 1.4	24.4 ± 1.4	24.7 ± 0.8	23.9 ± 1.7	21.9 ± 2.3*
Absolute (g)						
Liver	1.29 ± 0.07	1.25 ± 0.11	1.24 ± 0.09	1.28 ± 0.10	1.30 ± 0.06	1.19 ± 0.09*
Kidneys	0.265 ± 0.016	0.258 ± 0.025	0.264 ± 0.019	0.271 ± 0.022	0.285 ± 0.019	0.266 ± 0.026
Testes	0.104 ± 0.009	0.104 ± 0.008	0.111 ± 0.010	0.101 ± 0.019	0.106 ± 0.014	0.100 ± 0.017
Heart	0.167 ± 0.025	0.224 ± 0.092	0.176 ± 0.033	0.190 ± 0.028	0.200 ± 0.042	0.166 ± 0.012
Spleen	0.052 ± 0.007	0.049 ± 0.007	0.054 ± 0.005	0.050 ± 0.011	0.049 ± 0.007	0.056 ± 0.055
Relative (g/100 gB.W.)						
Liver	5.18 ± 0.273	5.19 ± 0.43	5.06 ± 0.30	5.17 ± 0.32	5.44 ± 0.40	5.43 ± 0.29
Kidneys	1.06 ± 0.06	1.07 ± 0.07	1.08 ± 0.08	1.10 ± 0.08	1.19 ± 0.07**	1.22 ± 0.08**
Testes	0.418 ± 0.038	0.432 ± 0.038	0.455 ± 0.043	0.407 ± 0.074	0.444 ± 0.058	0.460 ± 0.077
Heart	0.668 ± 0.106	0.930 ± 0.357	0.718 ± 0.136	0.768 ± 0.107	0.839 ± 0.143	0.758 ± 0.065
Spleen	0.209 ± 0.025	0.203 ± 0.019	0.219 ± 0.019	0.206 ± 0.043	0.206 ± 0.017	0.257 ± 0.251

*,**Significantly different from the control group at the level of $P < 0.05$ and $P < 0.01$, respectively.

Table 5
Organ weights for female B6C3F1 mice in the 13-week drinking water study of 3-MCPD

	Dose level (ppm)					
	0	5	25	100	200	400
B.W. (g)	24.0 ± 2.2	23.0 ± 1.4	23.5 ± 2.3	21.9 ± 0.9*	21.7 ± 1.3*	19.3 ± 1.8**
Absolute (g)						
Liver	1.12 ± 0.08	1.19 ± 0.18	1.21 ± 0.11	1.10 ± 0.09	1.13 ± 0.14	1.04 ± 0.13
Kidneys	0.173 ± 0.010	0.179 ± 0.018	0.183 ± 0.017	0.180 ± 0.012	0.187 ± 0.022	0.170±0.033
Ovaries	0.013 ± 0.002	0.014 ± 0.003	0.013 ± 0.003	0.015 ± 0.006	0.012 ± 0.006	0.012 ± 0.007
Heart	0.150 ± 0.016	0.149 ± 0.012	0.150 ± 0.021	0.142 ± 0.019	0.142 ± 0.021	0.137 ± 0.034
Spleen	0.079 ± 0.012	0.083 ± 0.014	0.078 ± 0.014	0.067 ± 0.010	0.074 ± 0.010	0.068 ± 0.021
Relative (g/100 gB.W.)						
Liver	4.67 ± 0.50	5.16 ± 0.49	5.14 ± 0.37	5.01 ± 0.32	5.21 ± 0.52	5.42 ± 0.56*
Kidneys	0.727 ± 0.055	0.776 ± 0.051	0.780 ± 0.037	0.824 ± 0.066	0.860 ± 0.071*	0.891 ± 0.186**
Ovaries	0.050 ± 0.006	0.059 ± 0.013	0.055 ± 0.013	0.069 ± 0.030	0.054 ± 0.025	0.064 ± 0.044
Heart	0.625 ± 0.082	0.648 ± 0.049	0.636 ± 0.114	0.651 ± 0.081	0.655 ± 0.078	0.711 ± 0.189
Spleen	0.330 ± 0.036	0.359 ± 0.050	0.332 ± 0.058	0.306 ± 0.052	0.341 ± 0.040	0.352 ± 0.110

*,**Significantly different from the control group at the level of $P < 0.05$ and $P < 0.01$, respectively.

epithelium in the males given 200 and 400 ppm (Fig. 2). Microgranulomas in the liver, interstitial nephritis, basophilic tubules, and mineralization in the kidneys were detected as spontaneously occurring lesions in the males. In females, microgranulomas in the liver, interstitial nephritis, basophilic tubules, hyaline cast, and cystic

Table 6
Sperm motility and vaginal cytology evaluation of B6C3F1 mice in the 13-week drinking water study of 3-MCPD

	Dose level (ppm)					
	0	5	25	100	200	400
Sperm motility (%)[a]	73.00 ± 5.85	71.22 ± 6.42	72.20 ± 3.46	70.60 ± 3.20	74.33 ± 3.43	60.20 ± 6.53*
Vaginal cytology evaluation						
Estrus cycle length (days)	4.90 ± 0.74	5.50 ± 0.97	6.10 ± 1.10	5.30 ± 1.42	5.70 ± 1.49	6.30 ± 1.06*
Proestrus[b]	18.4	23.6	23.0	13.2	26.3*	15.9
Estrus[b]	38.8	38.2	34.4	43.4	28.1	36.5
Metaestrus[b]	12.2	10.9	9.8	13.2	8.8	11.1
Diestrus[b]	30.6	27.3	32.8	30.2	36.8	36.5*

* Significantly different from the control group at the level of $P < 0.05$.
[a] Data are expressed as mean ± S.D.
[b] Data are expressed as percent of cycle.

Table 7
Histopathological changes for B6C3F1 mice in the 13-week drinking water study of 3-MCPD

Organ/Findings	Males						Females					
Dose of 3-MCPD (ppm)	0	5	25	100	200	400	0	5	25	100	200	400
No. of mice examined	10	10	10	10	10	10	10	10	10	10	10	10
Liver												
Microgranuloma (±/+)[a]	4 (4/0)	3 (2/1)	2 (2/0)	2 (2/0)	2 (1/1)	1 (1/0)	1 (1/0)	0	0	0	0	2(2/0)
Centrilobular necrosis	0	0	0	0	0	1	0	0	0	0	0	1
Cellular swelling	0	0	0	0	1	0	0	0	0	0	0	0
Kidney												
Interstitial nephritis (±/+)	5 (5/0)	7 (7/0)	6 (6/0)	4 (4/0)	8 (7/1)	4 (2/2)	5 (5/0)	6 (6/0)	3 (3/0)	2 (2/0)	7 (7/0)	4 (3/1)
Basophilic tubules (+)	1	1	0	1	2	3	0	1	0	0	2	1
Tubular hyperplasia	0	0	0	0	0	0	0	1	0	0	0	0
Hyaline cast	0	0	0	0	0	0	1	1	0	1	0	1
Cystic tubules	0	0	0	0	0	0	1	1	0	0	0	0
Mineralization	0	0	0	0	0	1	0	0	0	0	0	0
Testes												
Degeneration of germinal epithelium (±/+)	0	0	1 (1/0)	2 (2/0)	5* (4/1)	8** (4/4)						
Atrophy	0	0	0	0	0	2						
Ovaries												
Pigmentation								1				
Cystic dilatation									1			

*,**Significantly different from the control group at the level of $P < 0.05$ and $P < 0.01$, respectively.
[a] Grade of change: (±) minimal; (+) mild.

tubules in the kidneys, and pigmentation and cystic dilatation in the ovaries were also observed. Other lesions, as shown in Table 7, were also detected sporadically, but there were no significant changes in the incidence between the control and treatment groups.

4. Discussion

The suppression of body weights at 400 ppm in both genders during the experiment, is associated with sweetish taste and is consistent with the results of the 2-year carcinogenicity study with F344 rats (Lynch et al., 1998). 3-MCPD has a direct mitotic activity in the liver of rats without any histopathological changes of hepatocytes (El Ramy et al., 2007). In our study, the absolute (male) and relative (female) liver weight changes are not considered to be toxicological changes because the changes in the liver weights were not consistent and there were no histopathological changes in both genders. The significant changes in the hematological findings are of doubtful toxicological significance because they were very slight and within the limits of the normal biological ranges except for the leukocyte and differential cell counts at 100 ppm in males. However, these changes are of no toxicological significance because it is not associated with other toxicological parameters including the histopathological changes and organ weights.

An increase in the relative kidney weights was observed at the final necropsy at 200 and 400 ppm in both genders. However, there were no statistically significant histopathological kidney lesions (Table 7). Therefore, the results suggest that 3-MCPD has toxic effects in the kidney. The nephrotoxic mechanisms of 3-MCPD are thought to be due to the inhibition of glycolysis by metabolites associated with β-chlorolactate pathway (Jones and Chantrill, 1989).

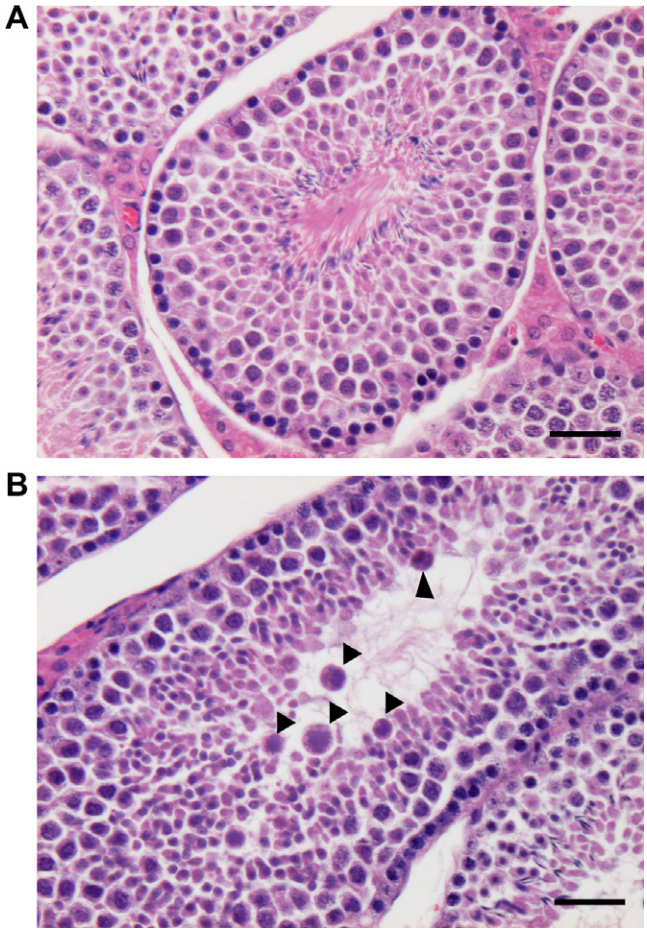

Fig. 2. Representative photographs of kidney sections from the negative control and highest dose groups stained with hematoxylin-eosin. (A) A control male rat, showing normal appearance. (B) Testis from male mice receiving the highest dose. Note the degeneration and loss of germinal epithelium within the seminiferous tubules (arrow head). Bar = 40 μm.

Impairments of glycolytic pathway and energy production could contribute to permanent kidney damage (Jones and Fakhouri, 1979). 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 kidney injury (Jones et al., 1981).

Decreases in the sperm motility were also observed at 400 ppm in this study (Table 6). Histopathologically, degeneration of the germinal epithelium was significantly higher at the 200 and 400 ppm groups than the control. These results suggesting that 3-MCPD reduces the sperm motility and affects the germinal epithelium in the testes, which is consistent with several reproductive toxicity studies (Gill and Guaraya, 1980; Kwack et al., 2004). Although the exact metabolites are unknown, the metabolites of 3-MCPD have an inhibitory activity on enzymes in the spermatozoa glycolysis, and the inhibition of sperm motility was suggested to be partly due to the alkylation of spermatozoa cysteine by 3-MCPD (Kalla and Bansal, 1997; Jones, 1983).

In females, a significantly delayed total estrus cycle length was observed at 400 ppm and more time was spent in proestrus (200 ppm) and diestrus (400 ppm) compared with the other stages. However, there were no treatment-related histopathological changes in the female reproductive organs (Table 7). 3-MCPD is suspected to have luteolytic and antioestrogenic effects in female rats (Lohika and Arya, 1979). Although more profound research about the toxicity of 3-MCPD in the female reproductive organs will be needed, the luteolytic and antioestrogenic effect of 3-MCPD can influence the total estrus cycle length.

In summary, a 13-week study of 3-MCPD in B6C3F1 mice showed that 3-MCPD suppressed the body weight gains in the 400 ppm group and increased the relative kidney weight in the 200 and 400 ppm groups in both genders. In addition, decreased sperm motility, degeneration of the germinal epithelium and a delayed total estrus cycle induced by the 3-MCPD treatments might be manifestations of the toxic effects of 3-MCPD. The no-observed-adverse-effect level (NOAEL) of 3-MCPD was considered to be 100 ppm (18.05 mg/kg/day for males and 15.02 mg/kg/day for females) under these experimental conditions in mice. Based on present 13-week subchronic study, the carcinogenicity study with B6C3F1 mice is in progress in our laboratory.

Conflict of interest statement

This paper has no conflict of interest (Financial, personal, or relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence the work submitted).

Acknowledegemet

This work was supported by a grant (05121KFDA455) from Korea Food & Drug Administration for the National Toxicology Program in Korea (KNTP).

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