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Estimation of Daily Exposure to 3-Monochloropropane-1,2-diol from Commercial Soy Sauces in Korea

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Abstract To assess the dietary exposure to 3-monochloropropane-1,2-diol (3-MCPD) from soy sauces, the levels of 3-MCPD in commercial soy sauces were analyzed with gas chromatography/mass spectrometry. Sixty nine out of the 72 soy sauces tested contained a level of 3-MCPD below 0.3 μg/g, the maximum limit in Korea. The average concentration of the 72 samples was 0.080 μg/g and the highest concentration was 3.131 μg/g. On the basis of the consumption data, 3-MCPD concentration and body weight, the estimated daily exposures to 3-MCPD were ranged from 0.037 to 0.146 μg/kg body weight (bw)/day for 95th percentile exposed population groups. Our estimated daily exposures are significantly lower than the provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bw/day, which was established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA).

Keywords: 3-MCPD, soy sauce, dietary intake

Introduction

3-Monochloropropane-1,2-diol (3-MCPD), which is produced by acid-hydrolysis of proteins in the presence of a small amount of lipid, is a well-known contaminant of foodstuffs associated with food processing. The UK's Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment concluded that 3-MCPD has no significant genotoxic potential *in vivo* (1). However, in a chronic study of rats, oral exposure to 3-MCPD resulted in chronic progressive nephropathy as well as tubular hyperplasia and adenomas. Dose-related alterations in the incidence of hyperplasia and tumors were observed in all groups, with increases in the kidney, the testis, and mammary gland (2). In 2001 the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) established a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight (bw)/day based on the tubule hyperplasia in the kidney as the most sensitive end point for deriving a tolerable intake (2).

Among food groups, acid-hydrolyzed soy sauces as well as acid-hydrolyzed vegetable proteins are the major sources of exposure to 3-MCPD contamination. Acid hydrolysis has the advantage of being more rapid and cost effective than fermentation in the production of soy sauce. Because of the lack of essential flavors in acid-hydrolyzed soy sauce, however, both types of hydrolysate are used in the same products (3). In Korea, efforts were made in the early 2000s to increase the production of fermented soy sauce but the commercial soy sauces containing acid-hydrolyzed soy sauces still comprised 86.8% of the total soy sauce production of 2003 (4). In 2002, Korea set its maximum limit of 3-MCPD in soy sauces to 0.3 μg/g. A survey on residual 3-MCPD levels of soy sauces in 2002 reported that 8.3% of 24 commercial products, which were collected before the maximum limit had been set, contained 3-MCPD levels above the maximum limit, and the highest concentration reported was 2.04 μg/g (5). In another report, 3.2% of 157 samples showed 3-MCPD levels above 0.3 μg/g, with a maximum level of 0.82 μg/g (6). Few reports on the current residual levels of 3-MCPD in soy sauces are available.

On the basis of consumption data, the JECFA compared the estimated 3-MCPD intake from soy sauce in various countries. The JECFA estimates show that in Japan the 3-MCPD intake from soy sauce is 2.7 to 3.8 times higher than in Australia and USA because of the higher consumption of soy sauces in Japan (7). Koreans may be at risk of high levels of exposure to 3-MCPD from soy sauces because soy sauce is a major condiment in Korea as well as Japan. However, as yet, there has been no assessment in Korea of the dietary intake of 3-MCPD from soy sauces.

We therefore analyzed residual levels of 3-MCPD in commercial soy sauces in Korea. To assess the dietary exposure to 3-MCPD from soy sauces, we relied on the contamination levels in analyzed samples and the consumption data for soy sauces. Next, we compared the estimated dietary exposure to 3-MCPD with the PMTDI to evaluate the level of 3-MCPD intake from soy sauces in Korea.

Materials and Methods

Sample collection In August and September 2005, we purchased 72 samples of soy sauce from retail outlets and markets in Seoul, Korea for analysis of 3-MCPD. The soy sauces are collected so that their brands would not overlap. We stored the samples at 4°C until analysis.

Chemicals and reagents All chemicals and reagents used were of the highest grade that was commercially available. We purchased 3-MCPD from Wako Chemicals

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(Tokyo, Japan), and *d5*-3-MCPD (98 atom %D) from CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). In addition, we obtained N-heptafluoro-n-butyrylimidazole (HFBI) from Pierce (Rockford, IL, USA), and Extrelut NT refill packs from Merck Chemical Co. (Darmstadt, Germany).

Determination of 3-MCPD levels The 3-MCPD levels were analyzed according to the AOAC method (8). To extract 3-MCPD, we added *d5*-3-MCPD as an internal standard to a known amount of a sample, after which we added a saline solution and blended the mixture to a homogeneous consistency. Next, we thoroughly mixed the 3-MCPD extract and the Extrelut NT and transferred the resultant mixture to a manually compacted glass chromatography column. To elute the nonpolar compounds, we used a mixture of hexane and diethyl ether (90:10) and we extracted the residual 3-MCPD in the column with diethyl ether. The 3-MCPD extract was then concentrated to a small volume on a water bath at 50 to 70°C. Next, we used HFBI to derivatize a volume of the concentrated extract, which we analyzed using gas chromatography/mass spectrometry (GC/MS, Hewlett Packard, GC-6890, MS-5973; Wilmington, DE, USA) and an Ultra 2 column (0.32 mm × 50 m × 0.52 μm). For the carrier gas, we used helium at a flow rate of 2.0 mL/min. A sample with a volume of 1 μL was injected with a split ratio of 1:20.

The chromatographic conditions were as follows: the injection temperature was 270°C; the initial oven temperature was kept at 50°C for 2 min, before being raised to 260°C at 15°C/min with a hold time of 5 min; finally, the oven temperature was raised to 300°C at 20°C/min with a final hold time of 7 min. To identify the 3-MCPD, we compared a full scan mass spectrum of the sample chromatographic peak with a mass spectrum of the HFBI-derivatized 3-MCPD. The ions for selected ion monitoring (SIM) are 169, 253, 275, 289, and 453 *m/z* for 3-MCPD and 169, 257, 275, 289, and 453 *m/z* for *d5*-3-MCPD (Fig. 1). The quantification was based on a comparison of the chromatographic peak areas of 3-MCPD at *m/z* 253 and of *d5*-3-MCPD at *m/z* 257. By directly injecting standard solutions, we determined the limit of detection of 3-MCPD to be 0.005 μg/g at a signal-to-noise ratio of 3, and the limit of quantification (LOQ) to be 0.02 μg/g. 3-MCPD levels below the LOQ were the mean of duplicate results in a single independent experiment. Otherwise, the results would have represented the mean of two independent results. Any samples that were found to contain 3-MCPD at levels beyond the calibration range were diluted and reanalyzed. The results of samples with 3-MCPD levels beyond the calibration range represent the mean of three independent analytical trials.

Estimation of daily exposure to 3-MCPD The daily exposure to 3-MCPD from soy sauce intake was calculated as follows:

Daily exposure to 3-MCPD = residual level of 3-MCPD in soy sauce (μg/g) × consumption of soy sauce (g) / body weight (kg).

Our calculation is based on the 3-MCPD concentrations in the samples and the average daily consumption of commercial soy sauces for the corresponding population groups of different age and gender, as indicated in the 2001 National Health and Nutrition Survey (9). Population groups for food consumption data are categorized as male and female age groups of 3-6, 7-12, 13-19, 20-29, 30-49, 50-64, and over 65 years old. We used the body weight data of the population groups provided by Korea Food & Drug Administration (KFDA) (10).

For point estimation of daily exposure, we used the average and maximum concentration of 3-MCPD and average body weight of the each corresponding population groups. On the other hand, to estimate the percentile exposure, we applied the distribution of the 3-MCPD concentrations and the distribution of the body weight of the corresponding population groups (10). The percentage of commercial soy sauces containing acid-hydrolyzed soy sauces (86.8%) and fermented soy sauces (13.2%) of the total soy sauce production of 2003 (4) was reflected in estimation of percentile exposure to 3-MCPD. Because the 2001 National Health and Nutrition Survey did not provide the distribution of food consumption, we used the average consumption of commercial soy sauces to estimate the percentile daily exposure. Our percentile estimation was based on a Monte Carlo simulation using the @Risk program (Palisade, Australia) with iteration number of 50,000. We select the distribution functions under the hypothesis that any number of possible 3-MCPD concentrations has an equal probability of each outcome occurring and body weight of each population groups are lognormally distributed.

Results and Discussion

Table 1 summarizes the 3-MCPD concentrations in the 72 commercial soy sauce brands including 69 domestic products and 3 imported products from Japan. Of the collected samples, 41 were fermented soy sauces, 30 were mixtures of acid-hydrolyzed soy sauces and fermented soy sauces, and one contained only acid-hydrolyzed soy sauce. We used the isotope dilution method, which is appropriate for quantifying a large range of foods with different matrixes, to analyze the residual level of 3-MCPD by adding the *d5*-3-MCPD as an internal standard prior to extraction (11). Figure 1 shows a representative GC/MS chromatogram for 3-MCPD and *d5*-3-MCPD in samples and representative SIM mass spectrum. The average residual level of 3-MCPD in all samples was 0.080 μg/g, which is lower than the maximum limit of 0.3 μg/g in soy sauces. Of the 72 analyzed soy sauce brands, 69 had 3-MCPD levels of less than 0.3 μg/g. Four % of samples contained 3-MCPD levels above the maximum limit, and the values detected in the three high-concentration samples ranged between 0.306-3.131 μg/g.

The level of contamination we found is higher than previously reported levels of the maximum contamination in commercial soy sauces, i.e., 0.82 and 2.04 μg/g (5, 6). High chloropropanol levels are generally the result of poor manufacturing control, direct acid treatment of the soy sauce ingredients, or high levels of residual lipids in the soy protein ingredients before acid hydrolysis (12). The 3-MCPD-contaminated samples that were above the maximum limit contained a mixture of acid-hydrolyzed

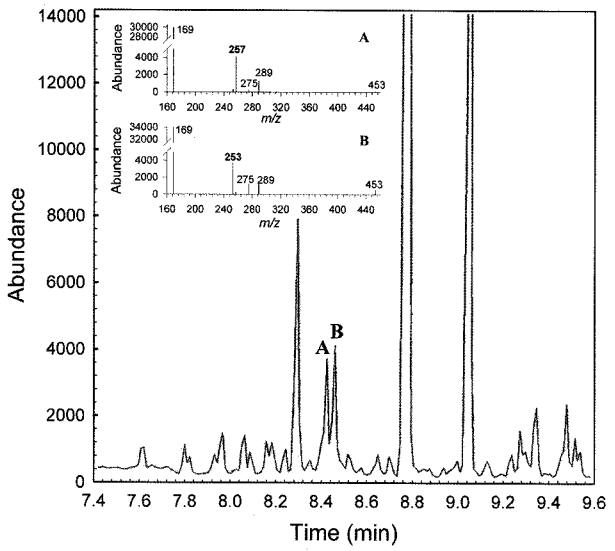

Fig. 1. Gas chromatography/mass spectrometry chromatogram (GC/MS) of 3-MCPD and *d5*-3-MCPD in sample. Inlet figures showed SIM mass spectrum of *d5*-3-MCPD (A) and 3-MCPD (B).

soy sauce and fermented soy sauce. The percentage of acid-hydrolyzed soy sauce in each sample was 70, 95, and 100%. Acid-hydrolyzed soybeans are the most likely source of contamination in these samples but the 3-MCPD levels are disproportional to the percentage of acid-hydrolyzed soy sauce in the products. As shown in Fig. 2, there is a very low correlation ($R^2 = 0.13$) between the 3-MCPD concentrations and the percentage of acid-hydrolyzed soy sauce in commercial soy sauces, which contain acid-hydrolyzed soy sauce in the products. The correlation between 3-MCPD levels and the percentage of acid-hydrolyzed soy sauce in all the collected samples, including fermented soy sauces, was $R^2 = 0.24$. Of 41 samples of fermented soy sauces, 38 had 3-MCPD levels below the LOQ and three had 3-MCPD levels above the LOQ with a maximum level of 0.021 μg/g. The detection of 3-MCPD in fermented soy sauces might be due to the intentional addition of acid-hydrolyzed soy sauce. Because we collected soy sauces of non-overlapping brands, the numbers of samples for each manufacturing company are not identical due to their different market share and production scale. It also explains the large standard

Table 1. The range of 3-MCPD levels in commercial soy sauces

Company	No. of collected products	No. of detected samples	3-MCPD levels (μg/g)		Presence of acid-hydrolyzed soy sauce (%)
			Mean[1)]	Detection range	
A	1	1	0.010±0.003	0.010	0
B	6	0	ND[2)]	ND	0
C	1	1	0.306 ±0.046	0.306	100
D	3	2	0.039±0.051	ND-0.097	0-85
E	1	1	0.281±0.008	0.281	90
F	2	2	0.014±0.010	0.007-0.021	0
G	12	5	0.002±0.004	ND-0.011	0-93
H	1	0	ND	ND	0
I	1	1	0.018±0.013	0.018	61
J	6	2	0.019±0.034	ND-0.085	0-70
K	1	0	ND	ND	0
L	1	0	ND	ND	0
M	12	4	0.025±0.045	ND-0.140	0-80
N	3	1	0.001[3)]±0.001	ND-0.002	0-95
O	2	0	ND	ND	0-80
P	2	0	ND	ND	0
Q	2	0	ND	ND	0
R	2	2	0.067±0.046	0.035-0.100	70-80
S[4)]	2	0	ND	ND	0
T[4)]	1	0	ND	ND	0
U	2	2	2.163±1.370	1.195-3.131	70-95
V	1	0	ND	ND	70
W	5	2	0.024±0.034	ND-0.072	0-95
X	1	0	ND	ND	90
Y	1	0	ND	ND	95
Total	72	26	0.080±0.394	ND-3.131	0-100

[1)]To calculate the mean values for the 3-MCPD level, we assigned samples with a 3-MCPD level below the LOQ to a level of 0 μg/g.
[2)]Not detected. Below the limit of detection.
[3)]The LOQ of 3-MCPD is 0.02 μg for 8 g of soy sauce. Therefore, the LOQ of 3-MCPD in soy sauce corresponds to 0.0025 μg/g.
[4)]Imported soy sauces.

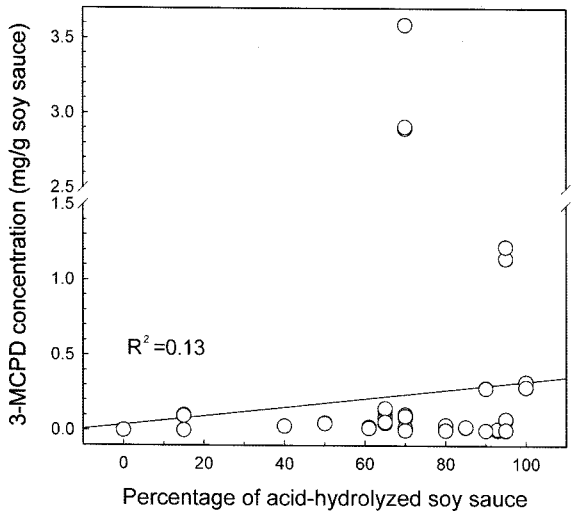

Fig. 2. Correlation between 3-MCPD concentrations and percentage of acid-hydrolyzed soy sauces in collected samples. Each data point represents the result of an independent determination.

deviation of mean values for 3-MCPD levels in Table 1. We analyzed only a limited number of samples for each company, so, further analysis of 3-MCPD concentrations in highly contaminated products will be needed to risk management of 3-MCPD in commercial soy sauces.

For point estimation of dietary intake of 3-MCPD from commercial soy sauces, we used 3-MCPD levels of 0.080 mg/g as the average concentration and 3.131 μg/g as the maximum residual concentration. When we applied an average contamination of 0.080 μg/g, the estimated average daily exposures to 3-MCPD ranged from 0.003 to 0.011 μg/kg bw/day, which corresponds to a PMTDI of 0.1 to 0.6% for each population group. The population groups that were most vulnerable to 3-MCPD exposures were the 3 to 6 year-old male and female groups followed by the 7 to 12 year-old male and female groups (Table 2). Children less than 12 years old are vulnerable because of their physiological immaturity and because their food intake per body weight is higher than adults (13). Considering the maximum observed contamination level, the estimated dietary intake of 3-MCPD for male group of 3-6 years increased to 0.439 μg/kg bw/day and the % PMTDI was 22.0%. The daily exposure for females in the over 65 year-old group was 0.113 μg/kg bw/day with a PMTDI of 5.7%.

We calculated the percentile daily exposure to simulate the real exposure to 3-MCPD. The estimated daily intake of 3-MCPD for males in the 95th percentile exposed group ranged from 0.053 to 0.146 μg/kg bw/day. The estimated levels correspond to a PMTDI of 2.7% for the over 65 year-old group and 7.3% for the 3-6 year-old groups. The female group had a lower exposure level than the male group. The %PMTDI of the 95th percentile exposed population is far below that of the point estimation based on the highest observed level of 3.131 μg/g. In our estimation, dietary exposure for the 20 to 29 year-old age group and the 30-49 year-old group contain higher uncertainty than other population groups because we used the body weight data categorized as male and female age groups of 3-6, 7-12, 13-18, 19-44, 45-64, and over 65 years old. We used the body weight data provided by the

Table 2. Estimated daily exposure to 3-MCPD from soy sauce

Gender	Age (years)	Consumption of soy sauce (g)	Body weight (kg)	Level of 3-MCPD used in the calculation of dietary exposure[1]				95th percentile daily exposure (μg/kg bw/day)	95th percentile %PMTDI
				0.080 μg/g		3.131 μg/g			
				Daily exposure (μg/kg bw/day)	%PMTDI[2]	Daily exposure (μg/kg bw/day)	%PMTDI		
Male	3-6	2.7	19.25	0.011	0.6	0.439	22.0	0.146	7.3
	7-12	4.1	40.65	0.008	0.4	0.316	15.8	0.105	5.3
	13-19	4.2	58.43	0.006	0.3	0.225	11.3	0.074	3.7
	20-29	6.0	70.61[3]	0.007	0.3	0.266	13.3	0.088	4.4
	30-49	6.4	70.61[3]	0.007	0.4	0.284	14.2	0.094	4.7
	50-64	5.3	68.62[4]	0.006	0.3	0.242	12.1	0.079	4.0
	>65	3.4	66.58	0.004	0.2	0.160	8.0	0.053	2.7
Female	3-6	2.5	18.77	0.011	0.5	0.417	20.9	0.137	6.8
	7-12	4.1	36.37	0.009	0.5	0.353	17.6	0.118	5.9
	13-19	4.1	51.31	0.006	0.3	0.250	12.5	0.082	4.1
	20-29	4.7	54.66[3]	0.007	0.3	0.269	13.5	0.089	4.4
	30-49	5.0	54.66[3]	0.007	0.4	0.286	14.3	0.094	4.7
	50-64	4.1	56.75[4]	0.006	0.3	0.226	11.3	0.075	3.7
	>65	2.2	60.79	0.003	0.1	0.113	5.7	0.037	1.9

[1]The observed mean and maximum residual level of 3-MCPD in commercial soy sauces.
[2]For the PMTDI of 3-MCPD, we used the JECFA's recommended value of 2 μg/kg bw/day.
[3]Mean body weight of 19-44 year old group for males and females.
[4]Mean body weight of 45-64 year-old group for males and females.

KFDA 2004 as an exposure factor for risk assessment in spite of the apparent discrepancies because it provides standard deviation values needed for estimation of body weight distribution of population groups.

The point estimation and the percentile estimation are both significantly lower than the JECFA's recommended PMTDI of 2 μg/kg bw/day. This result shows that the Korean population is not at risk of 3-MCPD contamination from soy sauces, even with conservative assumptions based on the highest observed 3-MCPD levels. The maximum limit for soy sauce in Korea is higher than the EU's limit of 0.02 μg/g (40% dry basis) but lower than the interim guideline of 1 ppm in sauces in Canada and the established limit of 1 ppm 3-MCPD in acid-hydrolyzed vegetable proteins (on a dry basis) in the United States (14).

On the basis of the maximum limit, we estimate the dietary intake of 3-MCPD from soy sauces for the highest risk group, namely the group of 3 to 6 year-old males to be 0.042 μg/kg bw/day with a %PMTDI of 2.1%. An exposure level far below the PMTDI for the highest risk group shows that the Korean limit for 3-MCPD is effective in protecting public health in Korea in case of soy sauces.

We used the mean consumption of commercial soy sauces for each population group to estimate the 3-MCPD exposures because the full distributions of soy sauce consumption are not provided. The intake of 3-MCPD by extreme consumers in the USA, Japan, and Singapore is reported as approximately twice that of the average consumer (2, 15) and the intake by extreme consumers in Australia is reported to be about three times that of the average consumer (16). In Korea, the mean consumption of extreme consumers (the 95th percentile consumers) is reportedly 4.4 times higher than that of average consumers (17). Thus, we cannot exclude the possibility of 3-MCPD risks for extreme consumers who regularly consume highly contaminated soy sauce products, especially for children less than 12 years. Further research is therefore needed on extreme consumers among children less than 12 years and the special consideration for risks of these groups will be needed.

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