



2004

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Cheng, W.-C.; Chen, H.-C.; Lin, Y.-P.; Lee, H.-F.; Chang, P.-C.; and Chou, S.-S. (2004) "Survey on 3-monochloro-1,2-propandiol (3-MCPD) contents of soy sauce products during fiscal year 2002 in Taiwan," *Journal of Food and Drug Analysis*: Vol. 12 : Iss. 4 , Article 6.
Available at: <https://doi.org/10.38212/2224-6614.2619>

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Survey on 3-Monochloro-1,2-propandiol (3-MCPD) Contents of Soy Sauce Products during Fiscal Year 2002 in Taiwan

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(Received: February 5, 2004; Accepted: July 2, 2004)

ABSTRACT

A survey of 3-monochloro-1,2-propandiol (3-MCPD) content in soy sauce products is reported for determining the distributions of 3-MCPD concentrations. Two hundred and fourteen samples of soy sauce products, including 188 domestic and 26 imported soy sauce products were analyzed using a GC/MS method (detection limit 0.01 ppm) that was validated and promulgated by the Department of Health to check for compliance with the government's recommended limit of 1 ppm for 3-MCPD in 2002. Results indicated that 3-MCPD was undetectable in 87 samples of domestic soy sauce products and 23 samples of imported soy sauce products 3-MCPD. Ninety-one samples of domestic soy sauce products contained 3-MCPD at concentrations in the range of 0.01 ppm to 1 ppm while 10 samples of domestic soy sauce products were found to contain 3-MCPD at concentrations greater than 1 ppm. Only 3 samples of imported soy sauce products contained 3-MCPD at concentrations in the range of 0.01 ppm to 0.03 ppm. 3-MCPD contents of domestic soy sauce products in 2002 decreased significantly in comparison with the surveyed contents in 2000. Ten domestic soy sauce products containing 3-MCPD over the regulation limits set by the government were sampled and reanalyzed again after improvements made by the manufacturers. 3-MCPD concentrations ranged from 0.02 ppm to 0.11 ppm in 5 samples and were undetectable in 4 samples, while 1 sample was no longer produced after the survey.

Key words: soy sauce, 3-monochloro-1,2-propandiol, 3-MCPD, GC/MS

INTRODUCTION

3-monochloro-1,2-propandiol (3-MCPD) is a member of a group of chemical contaminants known as chloropropanols. It is formed as a product of acid hydrolysis of vegetable proteins during the manufacturing of food seasonings when ingredients hydrolyze vegetable proteins⁽¹⁾. Several studies reported that 3-MCPD could be detected as contaminants in several food and food ingredients, including soy sauce products, and hydrolyzed vegetable protein (HVP)^(2,3). 3-MCPD could also be formed in other food products during storage and processing⁽⁴⁾. Soy sauces or acid HVPs exported from some Asian countries found to contain high levels of 3-MCPD⁽⁵⁾ have been rejected from European countries.

Toxicological studies had shown that 3-MCPD is carcinogenic in rat and has genotoxic activity *in-vitro*. 3-MCPD may be metabolized to genotoxic intermediates, particularly glycidol. The available evidence indicates that 3-MCPD is not a genotoxic carcinogen, but rather a chemical that causes tumors in rats through nongenotoxic mechanisms⁽⁶⁾. 3-MCPD has been identified by the European Union Scientific Committee for Food as a potential genotoxic carcinogen in 1994⁽⁴⁾. The EC committee set a Tolerable Daily Intake (TDI) for 2 $\mu\text{g}/\text{kg}$ bw based on the threshold-based approach in 2001⁽⁷⁾. The

UK Food Advisory Committee recommended that the industry take all steps to ensure that 3-MCPD is undetectable in food⁽⁸⁾. Recently, the UK Committee on Mutagenicity and Carcinogenicity concluded that 3-MCPD was an *in-vitro* mutagen but had no significant genotoxic potential *in-vivo* after reviewing all the available mutagenicity and carcinogenicity data^(9,10).

Several analytical methods for 3-MCPD determination have been demonstrated in many studies^(11,12,13). Gas chromatography with Mass was frequently used as the qualitative and quantitative analysis method. We also set up the method for the 3-MCPD determination in soy sauce products by ethyl acetate extraction and direct derivatization with heptafluorobutyl imidazole (HFBI). The method was announced by the Department of Health in 2001⁽¹⁴⁾.

Soy sauce is one of the most important seasonings in Chinese society. As a result, monitoring the 3-MCPD contents is an important job for improving people's health. For the determination of 3-MCPD contents in soy sauce products, the Department of Health set a safety regulation on 3-MCPD soy sauce products at 1 ppm⁽¹⁵⁾. The aim of this study was to understand the distributions of 3-MCPD contents in soy sauce products sold in Taiwan. The study also compared results from different cities, years, countries and 3 different kinds of soy sauce products. The result could provide information for government regulations and apply pressure on the manufacturers to improve the quality of soy sauce products by decreasing the 3-MCPD content.

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MATERIALS AND METHODS

I. Sample Collection

Two hundred and fourteen samples of soy sauce products, including 188 domestic and 26 imported soy sauce products were sampled from manufacturers or purchased from retail outlets by the local hygiene bureaus in 18 cities and prefectures.

II. Reagents and Chemicals

Ethyl acetate and *n*-hexane were both pesticide residue grade; anhydrous sodium sulfate and sodium chloride were AR grade, and Extrelut[®] NT20 column were purchased from Merck; 3-chloro-1,2-propanediol was from Aldrich. Heptafluorobutyl imidazole (HFBI) was from Sigma and *p*-dichlorobenzene (PDCB) was from Chem Service.

III. Instrument and Apparatus

- (I) Gas chromatograph / electron impact mass spectrometer
- (II) Rotary evaporator
- (III) Gas-tight syringe
- (IV) Shaker bath
- (V) Ultrasonic vibrator
- (VI) Micro reaction vessels (screw cap septum vials, 5 mL)

IV. Methods

(I) Internal standard solution preparation

One hundred milligram of PDCB was weighed to a 100-mL volumetric flask and made up to volume with *n*-hexane. The stock solution was diluted with *n*-hexane to 0.05 $\mu\text{g/mL}$ to be served as the internal standard solution.

(II) Standard solution preparation

One hundred milligram of 3-MCPD was weighed to a 100-mL volumetric flask and made up to volume with ethyl acetate. The stock 3-MCPD solution was diluted with *n*-hexane to 0.025, 0.05, 0.1, 0.2, 0.5 and 1.0 $\mu\text{g/mL}$, each with an internal standard of 0.05 $\mu\text{g/mL}$.

(III) Sample preparation

Five gram of soy sauce was weighed into a glass beaker; 15 mL of 5 M sodium chloride solution was added, mixed well and sonicated in an ultrasonic bath for 15 min. Prepared samples were then applied to an Extrelut[®] NT20 column and allowed the samples to spread over the inert matrix as a thin layer for 15 min. The column was then eluted with 250 mL of ethyl acetate. The liquid elute was shaken well with 30 g of anhydrous sodium sulfate, allowed to stand for 15 min, then filtered through a glass funnel containing 50 g of anhydrous sodium sulfate. The sodium sulfate was rinsed with 50

mL of ethyl acetate and the volume of the combined extract was reduced to 3 mL with a rotary evaporator at 40°C. The extract was quantitatively transferred to a graduated tube and made up to 5 mL with ethyl acetate.

(IV) Derivatization

For derivatization, 1 mL of the sample extract was transferred to a micro reaction vessel. Sample extract was blown to dryness under a gentle stream of nitrogen before 1 mL of internal standard solution was added into the micro reaction vessel. Fifty microliter of HFBI was added and the vessel was capped, shaken and incubated at 70°C for 20 min. The reaction mixture was cooled to room temperature, 2 mL of deionized water was added and the vessel was shaken for 30 sec. The organic phase was collected and dried with 300 mg of anhydrous sodium sulfate and was then applied to GC/MS analysis.

(V) Gas chromatography-mass spectrometry analysis

A Hewlett-Packard 5890 gas chromatograph/5970B mass selective detector (GC/MS) equipped with an HP-G1034C Chemstation software was used for this study. The gas chromatograph was equipped with a 30 m \times 0.25 mm i.d. HP-5MS column with a 0.25 μm film thickness. The oven temperature was programmed at 50°C, 5°C/min to 100°C, 30°C/min to 280°C, hold for 5 min; injector 250°C. The following parameters were used for injecting samples into the GC-MS system⁽¹⁴⁾: sample size, 1 μL ; injection mode, split; injector purge-off duration, 0.75 min. The carrier gas was helium and the head pressure was set at 10 psi. The ionization energy was set at 70eV.

(VI) Quantitative analysis

Quantitative analysis was carried out by monitoring characteristic ions at *m/z* 453, 277 and 275 for 3-MCPD; 146, 148 and 113 for PDCB. 3-MCPD was calculated by the internal standard method. The ratios of the peak area response for 3-MCPD (*m/z* 453, 277 and 275) to the peak area response for PDCB (*m/z* 146, 148 and 113) was determined. 3-MCPD were determined from standard curve constructed by plotting the peak area ratios against the concentrations of 3-MCPD.

(VII) Quality assurance and quality control

Quality assurance: The standard curve should be linear with $R^2 \geq 0.995$. The recovery of spiked sample should range between 90% and 110%.

Quality control: The sample peak area ratios should fall within the range of those of the calibration standards for that batch. If not, reduce the suitable volume to less than 1 mL for derivatization. The 3-MCPD-HFBI spectrum of sample should match the 3-MCPD-HFBI spectrum of standard reference.

RESULTS AND DISCUSSION

I. Survey Results

Two hundred and fourteen samples of soy sauce products, including 188 domestic and 26 imported soy sauce products were analyzed by the GC/MS method for 3-MCPD contents. Among the imported soy sauce products, 24 samples were made in Japan and 2 samples were made in Hong Kong.

3-MCPD was undetectable in 87 samples of domestic soy sauce products, but 101 samples of domestic soy sauce products were detected with 3-MCPD. Ten samples (5%) contained 3-MCPD in the concentration range of 0.01 to 0.02 ppm. Fifty-three (28%) samples contained 3-MCPD in the range of 0.03 to 0.10 ppm. Twenty-eight (15%) samples contained 3-MCPD in the range 0.11 to 1 ppm. Ten (5%) samples contained 3-MCPD in the range of 1.01 to 10 ppm. The investigation results of Table 1 show that the 3-MCPD contents in 10 domestic soy sauce products violated the 3-MCPD limitation (1 ppm) set by the government. The highest level of 3-MCPD in the survey samples is 6.63 ppm (Table 2).

3-MCPD was undetectable in 23 samples of imported soy sauce products. Only 3 samples of imported soy sauce products were detected with 3-MCPD. Two (8%) samples contained 3-MCPD at the concentration in range from 0.01 to 0.03 ppm. One (4%) sample contained 3-MCPD in the range of 0.03 to 1.0 ppm (Table 1).

Table 3 shows the comparisons of the 3-MCPD contents of three kinds of soy sauce products in the fiscal year of 2002. In 118 samples of soy sauce products, 65 (55%) samples were not found to contain 3-MCPD, 47 (40%) sample were found to contain 3-MCPD not over the limitation set by the government, and 6 samples were found to contain 3-MCPD exceeding the limitation. In 90 samples of soy sauce paste products, 41 (46%) samples were not found to contain 3-MCPD, 45 (50%) sample were found to contain 3-MCPD which the amounts did not exceed the limitation set by the government, and 4 samples were found to contain 3-MCPD with amounts over the limitation. In 6 samples of reduced salt soy sauce products, 4 (67%) samples were not found to contain 3-MCPD, and 2 sample (33%) were found to contain 3-MCPD but the amounts were not over the limitation set by the government.

Table 4 shows the results of 3-MCPD contents in soy

sauce products sampled in different cities and counties. Soy sauce products from the soy sauce factories or supermarkets were sampled from 18 cities or counties. Pingtung county, Tainan county and Taipei city hygiene bureaus sampled contributed 47 (22%), 34 (16%) and 31 (14%) samples, respectively. All imported soy sauce products were sampled from Taipei city. The 3-MCPD contents did not violate the limitation set by the government. Products containing 3-MCPD were not confined to any single city or county. All the soy sauce products sampled from Taoyuan county were not detected with the 3-MCPD.

II. 3-MCPD Distributions of Soy Sauce Products

Many countries have set the maximum limit for 3-MCPD content in soy sauce products, including USA, Canada, Finland, and Austria (1 ppm), Australia and New Zealand (0.2 ppm), European Union, the Netherlands, Greece, Portugal, Malaysia, and Sweden (0.02 ppm). The

Table 1. Distributions of 3-MCPD contents in soy sauce products of the fiscal year 2002

3-MCPD (ppm)	Domestic soy sauce products (%)	Imported soy sauce products (%)	Total
ND ^a	87 (46)	23 (88)	110
0.01-0.02	10 (5)	2 (8)	12
0.03-0.10	53 (28)	1 (4)	54
0.11-1.00	28 (15)	0 (0)	28
1.01-10.00	10 (5)	0 (0)	10
Total	188	26	214

^aND: not detected.

Table 2. The survey results of domestic soy sauce products with 3-MCPD contents not in compliance with the sanitary regulation (not > 1 ppm) set by the Department of Health

Sample no.	3-MCPD ^a (ppm)	Sample type	Sampling city
1	6.63	Black bean sauce	Hsinchu county
2	4.48	Soy bean sauce	Nantou county
3	4.64	Soy bean sauce	Pingtung county
4	3.68	Soy bean sauce	Pingtung county
5	2.82	Soy bean sauce paste	Hsinchu city
6	2.27	Soy bean sauce	Taipei county
7	1.73	Black bean sauce	Hsinchu city
8	1.45	Soy bean sauce	Yunlin county
9	1.37	Soy bean sauce	Pingtung county
10	1.25	Black bean sauce	Chia-I county

^aAccording to sanitary regulation set by Dept. of Health (2002), the 3-MCPD contents in soy sauce products shall not exceed 1 ppm.

Table 3. Comparisons of 3-MCPD contents within three kinds of soy sauce products during the fiscal year 2002

Sample type	Total surveyed samples	No. of samples (%)		
		> 1 ppm ^b	≤ 1 ppm	undetectable
Soy bean sauce	118	6 (5)	47 (40)	65 (55)
Soy bean sauce paste ^a	90	4 (4)	45 (50)	41 (46)
Reduced salt sauce	6	0 (0)	2 (33)	4 (67)
Total	214	10	94	110

^aSoy bean sauce paste products including soy bean sauce paste, black bean sauce, and black bean sauce paste.

^bAccording to a sanitary regulation set by Dept. of Health (2002), the 3-MCPD contents in soy sauce products shall not exceed 1 ppm.

Table 4. The results of 3-MCPD contents in soy sauce products sampled by local hygiene bureaus in the fiscal year 2002

Sampling district	Total surveyed samples	No. of samples (%)		
		> 1 ppm ^a	≤ 1 ppm	undetectable
Keelung city	2	0 (0) ^b	2 (100)	0 (0)
Taipei city	31	0 (0)	7 (23)	24 (77)
Taipei county	10	1 (10)	3 (30)	6 (60)
Taoyuan county	2	0 (0)	0 (0)	2 (100)
Hsinchu city	4	2 (50)	2 (50)	0 (0)
Hsinchu county	3	1 (33)	2 (67)	0 (0)
Nantou county	4	1 (25)	1 (25)	2 (50)
Taichung city	4	0 (0)	2 (50)	2 (50)
Taichung county	16	0 (0)	14 (88)	2 (12)
Changhua county	20	0 (0)	8 (40)	12 (60)
Yunlin county	16	1 (6)	9 (56)	6 (38)
Chia-I county	10	1 (10)	1 (10)	8 (80)
Tainan city	2	0 (0)	2 (100)	0 (0)
Tainan county	34	0 (0)	20 (59)	14 (41)
Kaohsiung city	4	0 (0)	1 (25)	3 (75)
Kaohsiung county	4	0 (0)	2 (50)	2 (50)
Pingtung county	47	3 (6)	17 (36)	27 (57)
Hualien county	1	0 (0)	1 (100)	0 (0)
Total	214	10	94	110

^aAccording to a sanitary regulation set by Dept. of Health (2002), the 3-MCPD contents in soy sauce products shall not exceed 1 ppm.

^bValues in the parenthesis are the percentage relative the number of samples.

Table 5. The 3-MCPD contents in soy sauce products surveyed in the fiscal year 2002 in comparison with the 3-MCPD maximum limits set by different countries

Sample type	Total samples	No. of samples (%) ^a		
		> 0.2 ppm ^b	> 0.1 ppm ^c	> 0.02 ppm ^d
Domestic	188	33 (18)	38 (20)	91 (48)
Imported	26	0 (0)	0 (0)	1 (4)
Total	214	33 (15)	38 (18)	92 (43)

^a(%) = (no. of detected samples / no. of samples)100%.

^bThe allowable maximums of 3-MCPD in soy sauce in Australia and New Zealand is 0.2 ppm.

^cA sanitary regulation set by Dept. of Health (2002), the 3-MCPD contents in soy sauce products shall not exceed 1 ppm. By 2003, the upper limits will be further reduced to 0.1 ppm⁽¹⁶⁾.

^dThe allowable maximums of 3-MCPD in soy sauce in European Union, the Netherlands, Greece, Portugal, Malaysia, and Sweden is 0.02 ppm.

comparison with the 3-MCPD maximum limits set by different countries was shown in Table 5. The domestic soy sauce products had a higher proportion likely to be detected with 3-MCPD compared to imported soy sauce products in exceeding the maximum limit set by different countries. The proportion of 3-MCPD contents in domestic soy sauce products exceeding the government regulation⁽¹⁶⁾ and European Union maximum limits were 20% and 48% separately. Domestic soy sauce producers still have to closely monitor the 3-MCPD content during production process.

III. Comparison of the Survey Results in the Fiscal Year of 2000 and 2002

The comparison of 3-MCPD contents of domestic soy sauce products in the fiscal year 2000 and 2002 is shown in Figure 1. Fourteen samples of soy sauce products were detected by the GC/MS method in the fiscal year 2000.

Twenty nine percent of the soy sauce products were detected to contain 3-MCPD in the range of 1.01 to 10 ppm and 29% products were found to contain over 10 ppm. Fifty eight percent of soy sauce products surveyed in 2000 were over the 1 ppm maximum limit set by the government in fiscal year 2002. But only 5% of soy sauce products were detected to contain 3-MCPD contents over the maximum limit. Most of the soy sauce products, i.e. 95%, surveyed in 2002 contained 3-MCPD lower than 1 ppm. The 3-MCPD contents of domestic soy sauce products in 2002 are decreasing significantly in comparison with the surveyed contents in 2000.

IV. Comparison of the Survey in Taiwan and UK

We analyzed the data obtained in fiscal year 2002 according to the distributions of 3-MCPD content surveyed in UK⁽²⁾ (Figure 2). The results showed that the proportion of the undetectable sample for the 3-MCPD analysis were

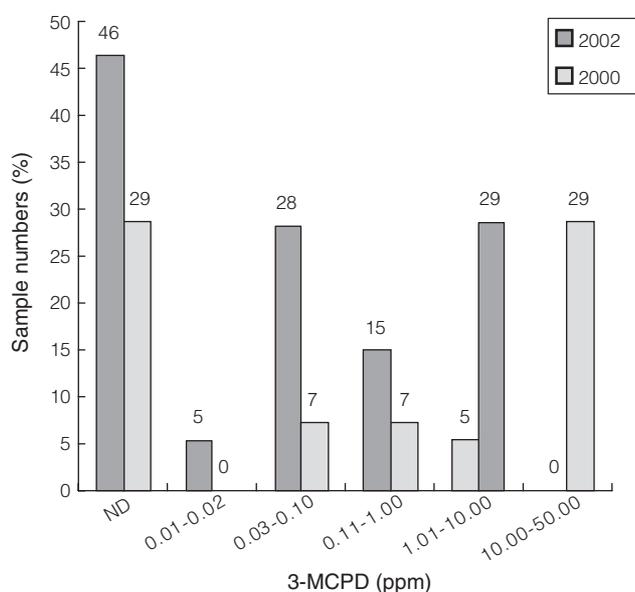


Figure 1. Comparisons of 3-MCPD contents of domestic soy sauce products in the fiscal year 2000 and 2002.

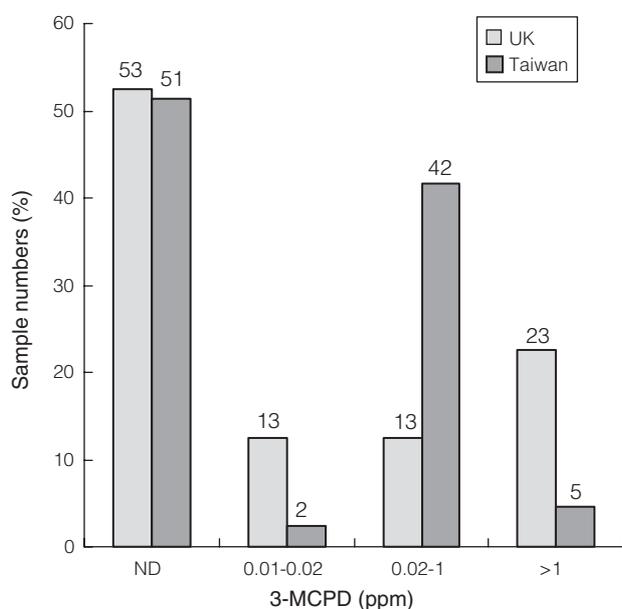


Figure 2. Comparisons of 3-MCPD contents of soy sauce products surveyed in Taiwan (2002) and UK.

53% and 51% in UK and Taiwan, respectively. In Taiwan only 5% of soy sauce products contained more than 1 ppm 3-MCPD, while in the UK there were 23% of soy sauce products that contained more than 1 ppm 3-MCPD. The majority (42%) of the soy sauce products in Taiwan were detected to contain 3-MCPD in the range of 0.02 to 1 ppm.

V. Improvement by Manufactures

Ten domestic soy sauce products where 3-MCPD content violated the sanitary regulation set by government

Table 6. The secondary survey results of domestic soy sauce products with 3-MCPD contents not in compliance with the sanitary regulation (not > 1 ppm) set by the Department of Health

Sample no.	3-MCPD (ppm)
1	ND.
2	0.03
3	N.D.
4	0.02
5	0.05
6	0.06
7	0.11
8	N.D.
9	N.D.
10	No longer produced

were sampled and reanalyzed again after improvement by the manufactures. 3-MCPD concentrations ranged from 0.02 ppm to 0.11 ppm in 5 samples and were undetectable in 4 samples, while 1 sample was no longer produced after the survey (Table 6).

CONCLUSIONS

The results of the survey showed that 3-MCPD was detected in several kinds, brands, and grades of soy sauce products. 3-MCPD contents in imported soy sauce products were not found to be in violation of the sanitary regulation set by the government in fiscal year 2002 but 10 samples of domestic soy sauce products contained amounts greater than 1 ppm. Although the 3-MCPD contents of domestic soy sauce products were lower than before, there is still room for the producer to improve the quality of soy sauce products.

ACKNOWLEDGMENTS

This research was supported by Department of Health, Taiwan, R.O.C. (project no. DOH 91-FD-2046)

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