Post-print version
Comprehensive Reviews in Food Science and Food Safety 14 (2015) 48-66

3-Chloropropane-1,2-diol (3-MCPD) in Soy Sauce: A Review on the Formation, Reduction, and Detection of This Potential Carcinogen

Bai Qin Lee and Sook Mei Khor

Abstract: Soy sauce, a dark-colored seasoning, is added to enhance the sensory properties of foods. Soy sauce can be consumed as a condiment or added during the preparation of food. There are 3 types of soy sauce: fermented, acid-hydrolyzed vegetable protein (acid- HVP), and mixtures of these. 3-Chloropropane-1,2-diol (3-MCPD) is a heat-produced contaminants formed during the preparation of soy sauce and was found to be a by-product of acid-HVP-produced soy sauce in 1978. 3-MCPD has been reported to be carcinogenic, nephrotoxic, and reproductively toxic in laboratory animal testing and has been registered as a chemosterilant for rodent control. 3-MCPD is classified as a possible carcinogenic compound, and the maximum tolerated limit in food has been established at both national and international levels. The purpose of this review is to provide an overview on the detection of 3-MCPD in soy sauce, its toxic effects, and the potential methods to reduce its concentration, especially during the production of acid-HVP soy sauce. The methods of quantification are also critically reviewed with a focus on efficiency, suitability, and challenges encountered in analysis.

Keywords: acid-HVP soy sauce, carcinogen, heat process contaminants, 3-monochloropropane-1, 2-diol (3-MCPD)

Introduction: 3-MCPD and Its Importance

3-MCPD is one of the chloropropanol compounds which consist of majorly 5 substances (Figure 1): 2-monochloropropane-1,3-diol (2-MCPD), 2,3-dichloropropan1-ol (2,3-DCP), 1,3-dichloropropan-2-ol (1,3-DCP), 3-monochloropropan-1-ol, and 3-chloropropane-1,2-diol (3-MCPD). 3-MCPD is a 3-carbon compound with 2 functional alcohol groups and a chloride and has the molecular formula $C_3H_7ClO_2$ and a relative molecular mass of 110.54 g/mol. 3-MCPD is a colorless liquid but has a tendency to turn straw-yellow and is soluble in water, alcohol, diethyl ether, and acetone (IARC 2013). Industrially, 3-MCPD has been used to lower the freezing point of dynamite, as a dye intermediate, as a rodent chemosterilant, and as a solvent for cellulose acetate (NJDHSS 1999). 3-MCPD was listed as a rodenticide under the name "alpha-chlorohydrin" by the U.S. Environmental Protection Agency (EPA 2013). In the food industry, 3-MCPD is a by-product of acid-hydrolyzed vegetable protein (acid-HVP) production. The acid hydrolysis of vegetable protein is a process used to mass-produce artificial soy sauce in a short period of time, without the fermentation process. Medium-high and high concentrations of 3-MCPD consumed in a short period have been found to cause kidney and reproductive organ failure. In some cases, especially in rats fed with high doses of 3-MCPD for a prolonged period, 3-MCPD has been reported to cause hyperplasia and tumors in kidneys and reproductive organs. With the available toxicology reports, International Agency for Research on Cancer (IARC) has classified 3-MCPD as a Group 2B carcinogen, which means that it is possibly carcinogenic to humans, and the OEHHA has also characterized it as Proposition 65 (Prop. 65), which refers to a substance that can cause cancer, birth defects, and other reproductive harm (OEHHA 2010a; IARC 2014).

Besides 3-MCPD, 1,3-DCP is another genotoxic carcinogen from the chloropropanol group. Unlike 3-MCPD, 1,3-DCP has only 1 alcohol functional group, but it has 2 chloride ions. 1,3-DCP is widely used in several industries as a key chemical in the synthesis of polymers, fumigants, synthetic glycerol, and dye fixatives in detergents (NTP 2005; OEHHA 2010b). Besides playing an important role in industrial organic synthesis, 1,3-DCP is also found in low concentrations in acid-HVP, albeit lower than the concentration of 3-MCPD (European Commission 2004). The ratio range of 1,3-DCP to 3-MCPD is between 1:2 and 1:3630 (European Commission 2004). 1,3-DCP is formed from 3-MCPD in the presence of acetic acid (HOAc) (Collier and others 1991). Intermediate and high dosages of 1,3-DCP were reported to be having carcinogenic effects in the liver, kidney, oral epithelium and tongue, and thyroid glands of laboratory rats (JECFA 2002). Available toxicity results have shown that 1,3-DCP is a genotoxic, hepatotoxic, and cancer-inducing agent, and it has been classified as being possibly carcinogenic to humans (group 2B) and placed on the Prop. 65 list (OEHHA 2010b; IARC 2014).

MS 20141267 Submitted 22/7/2014, Accepted 6/10/2014. *Authors are with Dept. of Chemistry, Faculty of Science, Univ. of Malaya, 50603, Kuala Lumpur, Malaysia. Direct inquiries to author Khor (E-mail: naomikhor@um.edu.my).*

doi: 10.1111/1541-4337.12120

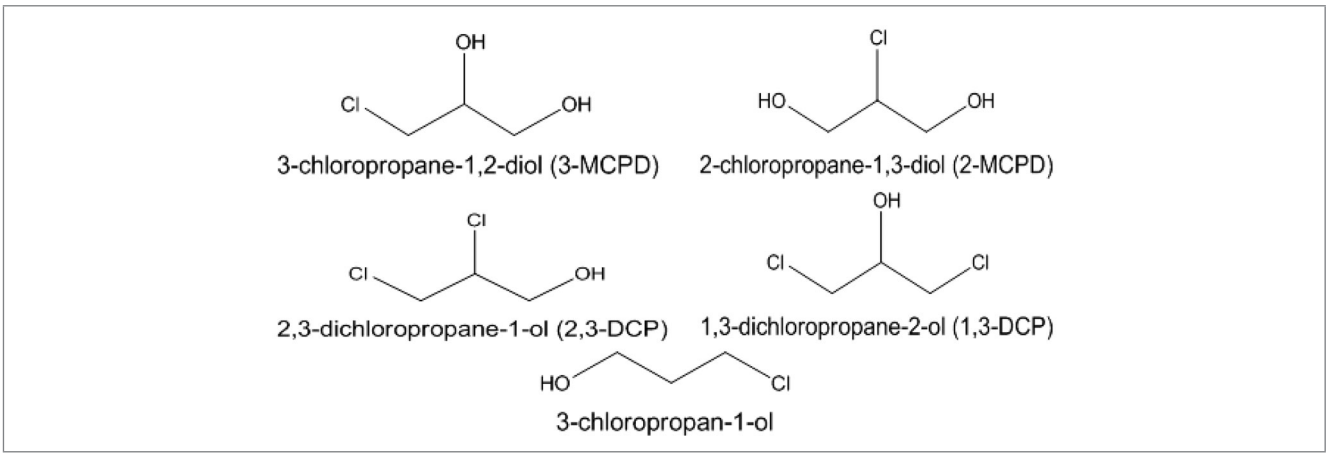

Figure 1–Chloropropanols.

The detection of 3-MCPD in soy sauce is vital, as it is one of the most widely used seasonings in Asian food preparation. Soy sauce consumption worldwide is estimated at 10 billion liters (L). Soy sauce consumption per capita in the USA is 0.8 L, while in Japan it is 9 L a year (Tokyo 2004). In China, soy sauce consumption is estimated to be 9 mL per person per day (Huo and others 2013). These data show that the consumption of soy sauce is quite high, especially in Asian regions. The main exporter of soy sauce in 2011 was China (94143 tons), followed by The Netherlands (34673 tons). The main importer for soy sauce in 2011 was the USA (59298 tons), followed by Hong Kong (22519 tons) and the United Kingdom (19576 tons) (FAOSTAT 2011), illustrating that soy sauce is consumed worldwide. Soy sauce has also been used as a flavor enhancer in ready-to-eat foods such as sausages and instant noodles. These foods are likely contaminated with 3-MCPD if 3-MCPD contaminated acid-HVP soy sauce is a raw ingredient. In addition to 3-MCPD, there are other contaminants found in soy sauce that are detrimental to health, for example, ethyl carbamate can be found in fermented soy sauce (Matsudo and others 1993). However, the main concern is that 3-MCPD found in acid-HVP soy sauce can cause kidney and reproductive organ failure. Acid-HVP can be produced rapidly compared with traditional fermented soy sauce. Research on 3-MCPD in acid-HVP soy sauce is important to ensure that the soy sauce added to foods is safe. The method of detection plays an important role in reducing the risk of 3-MCPD contamination in soy sauce. Fast, accurate, and reliable quantification of 3-MCPD will assist in the creation of Hazard Analysis and Critical Control Points (HACCP) in the production of soy sauce.

3-MCPD is formed in heat-processed foods in the presence of lipids and chloride ions (Figure 2). Formation of 3-MCPD is found to be highest when chloride reacts with lecithin, followed by the reaction of diacylglycerols and glycerol (Velisek and others 2003). Acid hydrolysis is the process known to produce acid-hydrolyzed soy sauce without bacterial fermentation. Traditional brewing of soy sauce involves 2 steps, namely, koji and brine/moromi fermentation. 3-MCPD will not be formed in fermented soy sauce, as there is no high temperature treatment involved.

Formation of 3-MCPD in Acid-HVP Soy Sauce

Acid-HVP soy sauce is made without microbial fermentation, while traditionally made soy sauce requires fermentation to break down the soy protein into aromatic compounds that give rise to the aroma and taste of the sauce. It requires a fermentation period of approximately 4 mo, depending on the type of soy sauce being produced and the bacteria used. Fermentation does not involve high-temperature treatment, as that would kill the microbes. In contrast, acid-HVP soy sauce can be produced in just a few days. Production begins with mixing defatted soy beans, wheat gluten, and/or corn meal (Figure 3). Then, the mixture undergoes hydrolysis with 4 to 9 molar (M) aqueous hydrochloric acid (HCl). The hydrolysis requires prolonged heating (20 to 35 h) at high temperatures (103 to 110 °C). The prolonged heating in high temperature is believed to be responsible for the formation of 3-MCPD in the production of acid-HVP soy sauce, likely due to the presence of glycerol, lecithin, and other glycerides in the soy sauce itself. The mixture subsequently undergoes neutralization with sodium carbonate (Na_2CO_3) or sodium hydroxide (NaOH) to remove any excess HCl. It is then refined by sedimentation, and filtered to remove undesirable aromatic compounds (FAO 2012).

Reduction of 3-MCPD during HVP Soy Sauce Production

Modifications can be made to reduce the formation of 3-MCPD in acid-HVP soy sauce. The FAO (2012) have proposed 3 methods for 3-MCPD reduction: careful control of acid hydrolysis, alkaline treatment after acid hydrolysis, and the substitution of HCl with sulfuric acid (H_2SO_4) (Figure 3). To further reduce the concentration of 3-MCPD in the final acid-HVP product, an enzymatic removal process can be introduced to the production line. 3-MCPD formation can also be completely prevented by using alkaline hydrolysis instead of acid hydrolysis of vegetable protein (Hall 1946). The reduction of 3-MCPD will also directly reduce the concentration of 1,3-DCP in the soy sauce, since 1,3-DCP is formed in the presence of 3-MCPD and HOAc (Collier and others 1991; Huang and others 2013).

Careful control of the acid hydrolysis step is crucial for the reduction of 3-MCPD, as this is when the production of 3-MCPD occurs. In conventional acid-HVP production, high temperatures and concentrated HCl are present for long periods of time. The concentration of HCl can be reduced to minimize the introduction of chloride ions through the addition of HCl. However, the reduction in HCl concentration will also reduce the efficiency of acid hydrolysis. To optimize the low HCl concentration acid hydrolysis process, the temperature of the reaction must be increased gradually with a particular holding time. The gradual increase in temperature increases the efficiency of HCl at low concentrations (FAO 2012).

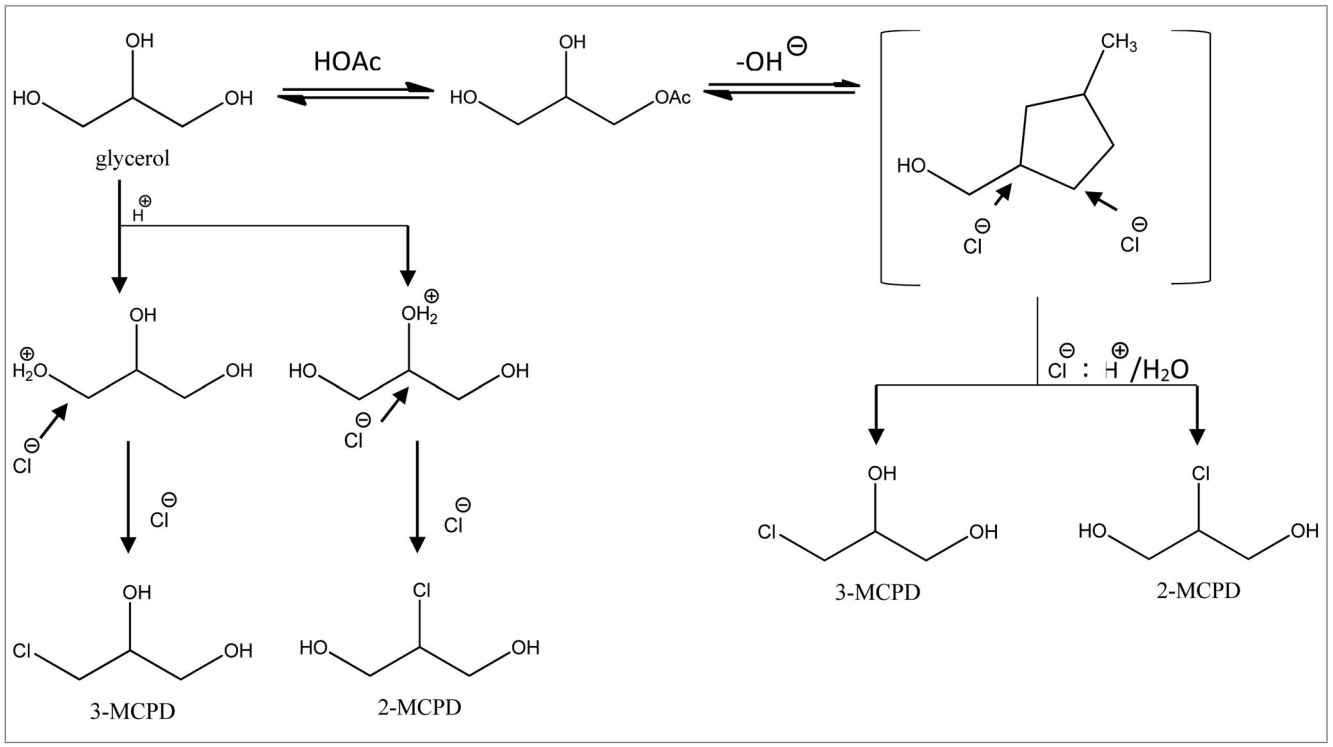

Figure 2–Formation of 3-MCPD and 2-MCPD from glycerol (Collier and others 1991).

An alkaline treatment can also be applied after acid hydrolysis to reduce the concentration of 3-MCPD (Figure 3). 3-MCPD was found to be unstable at alkaline pH (Hamlet and others 2003; Reece 2005; FAO and WHO 2007), and a pH higher than 6 would be sufficient to cause the degradation of 3-MCPD. A combination of alkaline and heat treatment after acid hydrolysis would efficiently reduce the concentration of 3-MCPD formed during acid hydrolysis.

The chloride ion is important for the formation of 3-MCPD from fatty acids. Removal of chloride ions in acid hydrolysis would eliminate the formation of 3-MCPD. In the acid hydrolysis process, acid is added to the system as a catalyst. H_2SO_4 can be used instead of HCl to perform the acid hydrolysis, but is not preferable as H_2SO_4 will not result in a high yield of amino acids in the end product. This is because the second proton in H_2SO_4 is not utilized in the hydrolysis and it likely affects the stability of amino acids after they had been hydrolyzed (Flork 1989). Thus, in order to produce H_2SO_4-hydrolyzed vegetable proteins with the required taste profile, flavorings such as monosodium glutamate, caramel, disodium inosinate, disodium guanylate, and lactic acid will have to be added to the final product (FAO 2012).

3-MCPD can also be removed through an enzymatic reaction. Bornscheuer and Hesseler (2010) reported on the enzymatic removal of 3-MCPD and its ester from oils. 3-MCPD can be removed by the enzyme halohydrin dehalogenase (HHD) extracted from *Arthrobacter* sp. AD2. The end product, glycidol, would then be hydrolyzed to glycerol by epoxide hydrolase (Figure 4). However, this reaction requires a long time for degradation and the 3-MCPD is not completely degraded, even after 24 h. The pilot-scale study was conducted with a basic buffer at 30 °C. Therefore, with the current technology, the enzymatic removal method is not suitable for large-scale implementation in the industry because it requires optimization and immobilization of enzymes. In addition to the enzyme from *Arthrobacter* sp. AD2 reported by Bornscheuer and Hesseler (2010), there are also a number of other HHDs that can be applied to degrade 3-MCPD. The efficiencies of HHDs from different bacterial species were well reviewed by You and others (2013). HHD has also been found to effectively degrade halogenated organic compounds (HOCs), which include 1,3-DCP. In fact, some HHDs were found to be more effective in the removal of 1,3-DCP than in removing 3-MCPD.

Alkaline hydrolysis would be an alternative to acid hydrolysis, but it is carried out less often in industry-scale production because it requires continued cooking of the amino acids, rendering the product to be partially racemized and thus undesirable (Borkenhagen 1953). Generally, the process of generating alkaline-HVP starts with heating to dissolve the proteins, and then an alkaline agent such as calcium, sodium, or potassium hydroxide is added. The temperature is then increased to a certain point between 27 °C and 54 °C, and hydrolysis is carried out for several hours until the desired end products meet the required amino acid profiles (Pasupuleti and Braun 2010). Alkaline-HVP contains an unacceptable flavor profile and unbalanced amino acid content as compared with acid-HVP (Reineccius 2006). The advantage of using alkaline hydrolysis is that there will be less or little humin formed after the hydrolysis process (Hall 1946).

Maximum Tolerable Limits and the Occurrence of 3-MCPD in Soy Sauce and Related Products

The European Union (Commission Regulation 2001) has set a maximum limit for 3-MCPD of 0.02 mg/kg for soy sauce and 0.05 mg/kg (dry weight) for other foods containing acid-HVP (Table 1) and recommended a tolerable daily intake (TDI) of 0.002 mg/kg bw (European Commission 2006). The TDI was established on the basis of toxicity information available for 3-MCPD. Malaysia has also set a limit of 0.02 mg/kg in liquid food

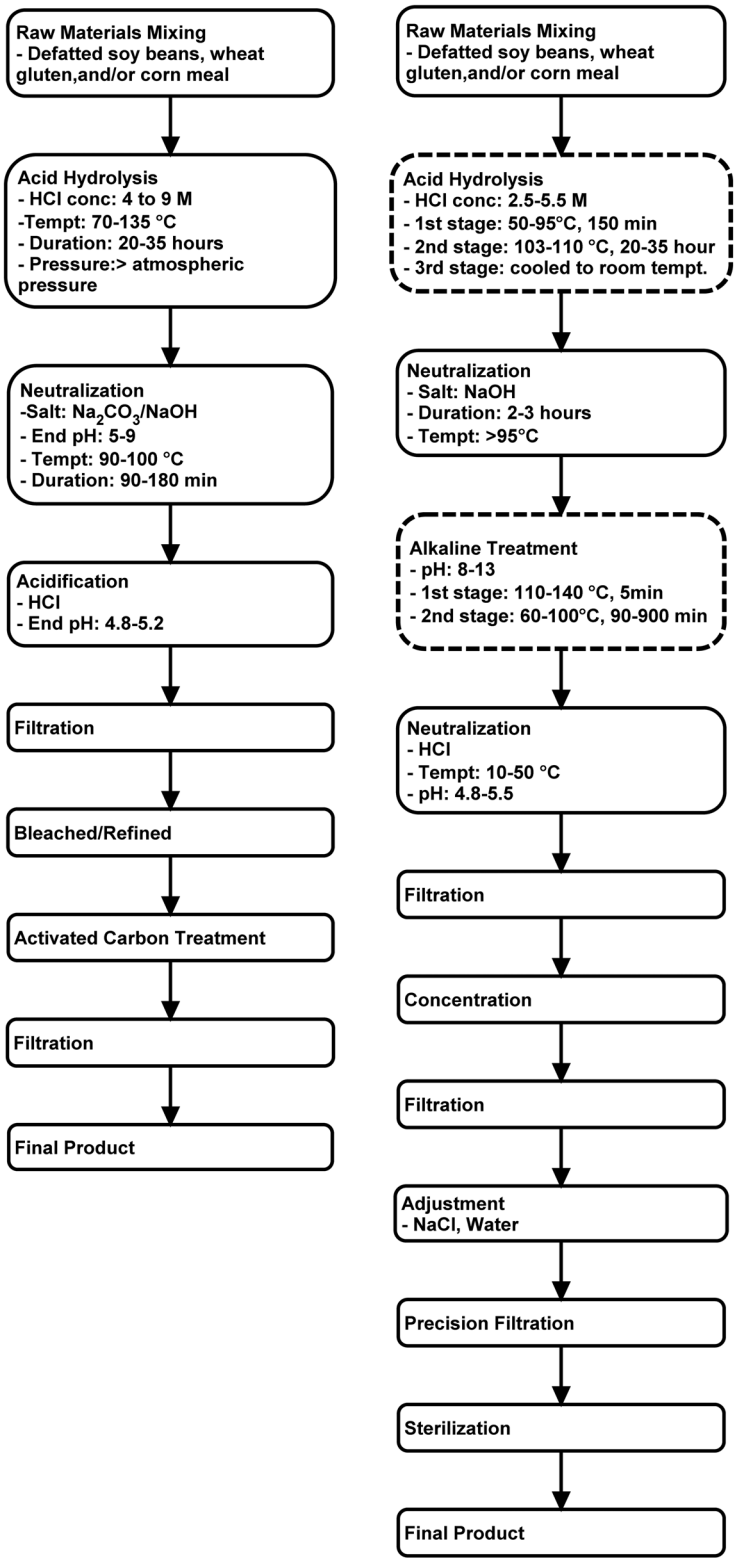

Figure 3–(Left) Conventional production of acid-HVP. (Right) Dotted box, proposed modification for reduction of 3-MCPD production (FAO 2012).

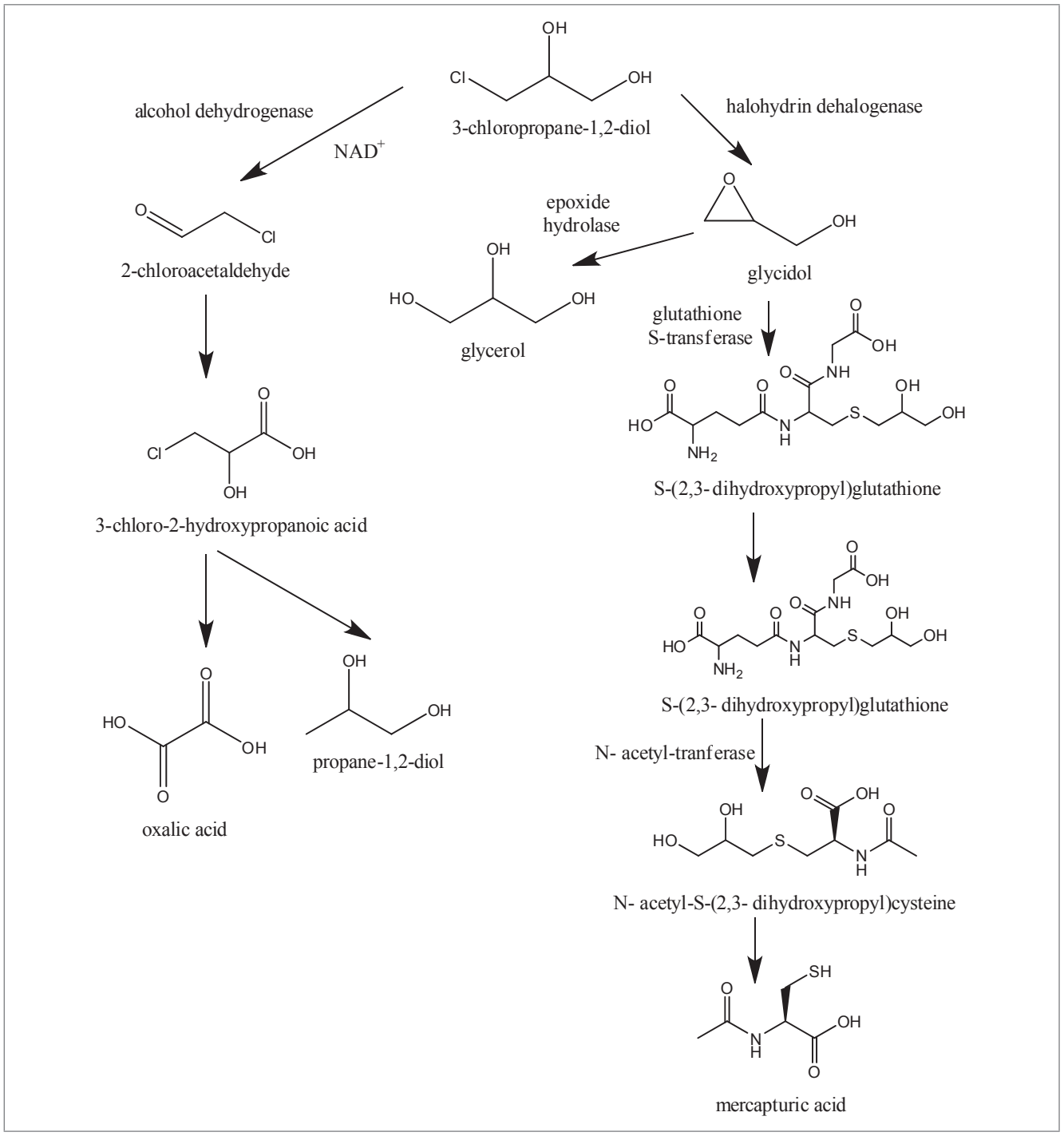

Figure 4–Proposed metabolic pathway for 3-MCPD based on bacterial and putative mammalian pathways (Lynch and others 1998).

with acid HVP and 1.00 mg/kg for acid HVP (Laws of Malaysia 2012). The United States (FDA 2008) and Canada (Canadian Standards 2012) have both set the limit for detectable 3-MCPD in foods at 1.00 mg/kg. Australia and New Zealand have set a maximum of 0.20 mg/kg for 3-MCPD in soy and oyster sauces (Food Standards Australia New Zealand Act 1991). Previous studies have reported that 3-MCPD has been detected in soy sauce and related products (Table 2). Surveys and case studies have been conducted in the United Kingdom (Macarthur and others 2000), Taiwan (Cheng and others 2004), New Zealand (MAF 2011), Hong Kong (Chung and others 2008), the United States (Nyman and others 2003), Singapore (Wong and others 2006), Spain (Leon and others 2008), Brazil (Vicente and others 2011), and Belgium (Christova-Bagdassarian and others 2013). The WTO Committee on Sanitary and Phytosanitary Measures (WTO 2002) ensures that 3-MCPD-contaminated products in international trade are within the required limits.

Cheng and others (2004) conducted a survey on the 3-MCPD contents of soy sauce in products in Taiwan during the 2002 fiscal year. A total of 214 samples were collected, in commercial areas of

Table 1–International maximum tolerable amount of 3-MCPD in foods.

Country	Maximum limit	Scope	Reference
Australia/New Zealand	0.20 mg/kg	Soy and oyster Sauce (40% dry matter content)	(Food Standards Australia New Zealand Act 1991)
Canada	1.00 mg/kg	Soy and oyster Sauce	(Canadian Standards 2012)
China	1.00 mg/kg	Acid HVP seasoning	(National Standard of the People's Republic China 2000)
European Union (EU)	0.02 mg/kg	HVP and soy sauce	(Commission Regulation 2001)
Malaysia	0.02 mg/kg	Liquid food with acid HVP	(Laws of Malaysia 2012)
	1.00 mg/kg	Acid HVP	
Singapore	0.02 mg/kg	Soy and related sauces	(Wong and others 2006)
United States	1.00 mg/kg	Acid HVP	(FDA 2008)

Taiwan, of which 118 were domestic and 26 were imported. The limit of detection (LOD) method used was 0.01 mg/kg. There were 87 domestic samples and 23 imported samples that contained undetected concentrations of 3-MCPD, 91 domestically manufactured soy sauces that contained 3-MCPD levels between 0.01 and 1.00 mg/kg, and 10 samples that contained 3-MCPD concentrations of more than 1.00 mg/kg, which is over the limit required by the Taiwanese government. Meanwhile, for imported sauces, 3 samples in which 3-MCPD was detected contained between 0.01 mg/kg and 0.03 mg/kg.

3-MCPD levels in 421 soy and oyster sauces in Singapore were investigated in a case study by Wong and others (2006). The reporting limit and lowest calibration point for the method used were 0.01 mg/kg and 0.05×10^{-1} mg/kg, respectively. The national maximum limit of 3-MCPD for Singapore is 0.02 mg/kg. In total, 376 samples were found to contain 3-MCPD levels at or below 0.02 mg/kg, and 3 out of the 58 domestically manufactured and 42 out of the 363 imported sauces contained 3-MCPD levels over the recommended limit. The highest quantified levels of 3-MCPD were in a soy sauce from Thailand and an oyster sauce from Taiwan.

Nyman and others (2003) conducted a survey on chloropropanols in soy sauces and related products in the United States. The LOD for the survey was 0.05×10^{-1} mg/kg. A total of 55 samples were purchased from retail markets in Baltimore and Washington, and the results showed that there were 19 samples that contained levels of 3-MCPD over the limit of 1.0 mg/kg. All of the samples that did not meet the requirements were imported from Asia: Hong Kong, Vietnam, China, the Philippines, and Thailand. All of the domestically manufactured samples met the requirement. The highest 3-MCPD quantified in the study was 876 mg/kg in a sample from Hong Kong.

Crews and others (2003) conducted a survey of chloropropanols in soy sauce and related products purchased in the United Kingdom in 2000 and 2002. The detection method used was the standard AOAC method. One hundred samples of soy sauce and related products were purchased from retail outlets in the United Kingdom in 2000, and in 2002, 99 samples were purchased from 5 areas in the United Kingdom. The results from 2000 were compared with those of 2002. In 2000, 32% of the samples contained levels of 3-MCPD over the limit (0.02 mg/kg) and 16 samples contained more than 1 mg/kg. However, the majority of samples had levels of 3-MCPD below 0.10 mg/kg. In 2002, only 8 of the 99 samples contained levels of 3-MCPD more than 0.01 mg/kg. Soy sauce and related products containing more than 0.01 mg/kg 3-MCPD were greatly reduced from 2000 to 2002.

Vicente and others (2011) conducted a survey of chloropropanols (3-MCPD and 1,3-DCP) in soy sauce and similar products from Brazil. A total of 45 samples of soy sauce and 16 products containing soy sauce were collected from the Brazilian marketplace. The LOD for the method used was 0.09×10^{-1} mg/kg. Seven samples of soy sauce were found to be positive for 3-MCPD, and all of the samples containing 3-MCPD ranged from undetectable to 4.405 mg/kg. 3-MCPD was undetected in all of the soy-containing special sauces.

A survey of 3-MCPD in soy sauce from Bulgaria was conducted by Christova-Bagdassarian and others (2013). The LOD reported in the survey was 0.23×10^{-2} mg/kg. A total of 21 soy sauce samples were collected from the Bulgarian marketplace. The majority of samples where the levels of 3-MCPD did not comply with EU regulations originated from Bulgaria, but the soy sauces imported from China did comply with EU requirements.

There are also technical reports available on the occurrence of 3-MCPD in soy sauce reported by local food safety enforcement groups. FSANZ (2003) surveyed the occurrence of 3-MCPD in foods and included soy sauce and soy products. The method of detection for the survey was the official AOAC method with an LOD of 0.01 mg/kg. A total of 39 samples of soy sauce and soy products were collected and 18 samples were reported to contain levels of 3-MCPD above the LOD, and among these, 14 samples contained 3-MCPD concentrations higher than the limit of 0.02 mg/kg. The highest concentration of 3-MCPD detected was 148.2 mg/kg in a soy seasoning sauce. Out of the 8 soy seasoning sauces, 7 contained levels of 3-MCPD above the limit of 0.02 mg/kg. In a newspaper article, the Malaysian government was said to have recalled 22 sauces and seasoning products of 11 different brands imported from 5 Asian countries that were found to have concentrations of 3-MCPD higher than the 0.02 mg/kg limit (Sennyah 2001).

In the "Report of Experts for Scientific Cooperation Task 3.2.9," the European Union collected data on the levels of 3-MCPD and related substances in foodstuffs (European Commission 2004). The report contains data of chloropropanol in soy sauces and foods other than soy sauces. There were a total of 10 countries involved in the cooperative study. In Austria, out of 316 samples of soy sauces, there were 130 samples that contained a quantifiable level of 3-MCPD, and the highest concentration of 3-MCPD detected was 104 mg/kg. In Denmark, out of 43 samples, there were a total of 27 samples with quantifiable concentrations of 3-MCPD, and the highest concentration of 3-MCPD detected was 90.0 mg/kg. In Finland, 53 out of 163 samples collected contained quantifiable concentrations of 3-MCPD, and the highest concentration reported was 940 mg/kg. In France, 39 of 73 samples collected contained quantifiable concentrations of 3-MCPD. In Germany, it was reported that 198 out of 692 samples collected contained quantifiable levels of 3-MCPD. In Ireland, 47 out of 178 samples were reported to contain quantifiable concentrations of 3-MCPD, and the highest concentration of 3-MCPD reported was 1779 mg/kg detected in light soy sauce samples. The Netherlands reported that out of 273 samples, there were a total of 77 samples that contained quantifiable concentrations of 3-MCPD, and the highest concentration of 3-MCPD was

Table 2–Survey of 3-MCPD in countries.

Country	Instrument	LOD	Type of food analyzed	Number of samples	Detected with 3-MCPD	Range	Reference
Taiwan	GC-MS	0.01 μg/mL	Domestic soy sauce	188	101	0.01 to 10.00 mg/kg	(Cheng and others 2004)
			Imported soy sauce	26	3	0.01 to 0.10 mg/kg	
Singapore	GC-MS	0.01 mg/kg	Soy sauce	317	44*	>0.01 to >3.00 mg/kg	(Wong and others 2006)
			Oyster sauce	104	18*	>0.01 to 3.00 mg/kg	
United States	GC-MS	0.005 mg/kg	Domestic and Canadian soy sauce	9	–	–	(Nyman and others 2003)
			Asia soy sauce	45	18	2.40 to 876.00 mg/kg	
			Unknown country origin soy sauce	1	–	–	
United Kingdom	GC-MS	0.01 mg/kg	Soy sauce and related product	100	100	0.01 to 93.10 mg/kg	(Crews and others 2003)
			Soy sauce and related product	99	99	0.01 to 21.20 mg/kg	
	–	–	Soy sauce and soy sauce based Products	170	65	LOD- 93.10 mg/kg	(European Commission 2004)
Australia	GC-MS	0.01 mg/kg	Soy and oyster sauces	39	18	<0.01 to 150.00 mg/kg	(FSANZ 2003)
Malaysia	–	–	Sauce and seasoning products	–	22	–	(Sennyah 2001)
Brazil	GC-MS	0.9 μg/kg	Soy sauce	45	7	–	(Vicente and others 2011)
			Special sauce contain soy sauce	16	–	–	
Bulgaria	GC-MS	2.3 μg/kg	Special sauce contain soy sauce	16	–	–	(Christova-Bagdassarian and others 2013)
Austria	–	–	Soy sauce and soy sauce based products	316	130	LOD- 104.00 mg/kg	(European Commission 2004)
Denmark	–	–	Soy sauce and soy sauce based products	43	27	LOD- 90.00 mg/kg	(European Commission 2004)
Finland	–	–	Soy sauce and soy sauce based products	163	53	LOD- 145.00 mg/kg	(European Commission 2004)
France	–	–	Soy sauce and soy sauce based Products	73	39	LOD- 145.00 mg/kg	(European Commission 2004)
Germany	–	–	Soy sauce and soy sauce based products	198	692	LOD- 158.00 mg/kg	(European Commission 2004)
Ireland	–	–	Soy sauce and soy sauce based Products	178	47	LOD- 1779.00 mg/kg	(European Commission 2004)
The Netherlands	–	–	Soy sauce and soy sauce based Products	273	77	LOD- 108.00 mg/kg	(European Commission 2004)
Norway	–	–	Soy sauce and soy sauce based Products	51	47	LOD- 146.00 mg/kg	(European Commission 2004)
Sweden	–	–	Soy sauce and soy sauce based Products	76	31	LOD- 79.90 mg/kg	(European Commission 2004)

151 mg/kg. Norway reported that 47 out of 51 samples collected contained quantifiable concentrations of 3-MCPD, and the highest 3-MCPD quantified was 146 mg/kg. Sweden reported that out of 76 samples, 31 contained quantifiable concentrations of 3-MCPD. The United Kingdom reported that 65 of 170 samples contained quantifiable concentrations of 3-MCPD, and the highest concentration of 3-MCPD reported was 93.1 mg/kg. In these reports, there were samples contaminated with high concentrations of 3-MCPD, some up to 100 mg/kg. Though the EU enforced the maximum tolerable limit of 0.02 mg/kg for 3-MCPD, there were still soy sauces and related products with 3-MCPD concentrations above the permitted limits.

Surveys conducted on 3-MCPD in soy sauces and related products in locally available markets have had a significant impact on the enforcement of maximum tolerances of 3-MCPD in food samples. Crews and others (2003) compared survey data with those of Macarthur and others (2000) for differences in 3-MCPD in soy sauce between 1999, 2000, and 2002. For samples containing more than 0.10 mg/kg 3-MCPD, there was a decreasing trend, from 48% in 1999 to 31% in 2000 to 8% in 2002. Samples containing more than 1 mg/kg 3-MCPD decreased from 23% in 1999 to 17% in 2000 and to 2% in 2002. In Taiwan, Cheng and others (2004) showed that the number of samples with nondetectable 3-MCPD had increased from 29% to 46%. There was also a decrease in the maximum detected 3-MCPD in samples. This demonstrates that after publication of results, actions were taken by local authorities to ensure that soy sauce and related products in the local market are within the limits of maximum allowance. In certain countries, manufacturers produced a combination of fermented and HVP soy sauce (Luh 1995), and this type of combination can reduce the contents of 3-MCPD while still increasing the sensory properties of the products.

Genotoxicity of 3-MCPD

Genotoxicity refers to a chemical agent that damages genetic information within a cell, causing mutations that may lead to cancer. All mutagens are genotoxic, but not all genotoxic agents are mutagens. 3-MCPD is categorized as a potentially carcinogenic compound, and, thus, a review to evaluate the genotoxic potential of 3-MCPD is important. Genotoxicity of 3-MCPD had been reported in both *in vitro* and *in vivo* studies, and the findings show that 3-MCPD is a genotoxic agent *in vitro* but is a nongenotoxic agent *in vivo* (Schlatter and others 2002).

For *in vitro* genotoxicity, testing on reverse mutation has been reported in *Salmonella* strains. Table 3 shows that positive genotoxicity was reported with both the presence and absence of Aroclor 1254-induced rat liver homogenate (S9) (Stolzenberg and Hine 1980; Silhankova and others 1982; Zeiger and others 1988; Ohkubo and others 1995). In the presence of the S9 factor, 3-MCPD has been shown to be genotoxic in bacteria. However, there have also been reports of negative genotoxicity in the presence of S9 (Stolzenberg and Hine 1979; Silhankova and others 1982; Majeska and Matheson 1983; Ohkubo and others 1995). The difference between these 2 findings is the species and strains of bacteria used. For positive results, in either the presence or absence of S9, the bacteria and strain selected were *Salmonella* TA100 (Stolzenberg and Hine 1980; Zeiger and others 1988; Ohkubo and others 1995) and TA1535 (Silhankova and others 1982; Zeiger and others 1988). For negative results, in either the presence or absence of S9, the bacteria selected were *Salmonella* TA98 (Stolzenberg and Hine 1979; Silhankova and others 1982; Zeiger and others 1988; Ohkubo and others 1995) and *E. coli* (Silhankova and others 1982; Ohkubo and others 1995). It can be concluded that 3-MCPD shows genotoxic potential only in certain types and strains of bacteria. Different strains of *Salmonella* resulted in different outcomes for the genotoxicity of 3-MCPD. However, selected strains of *E. coli* showed consistent results for positive genotoxicity with or without S9.

Yeast has also been used to evaluate the genotoxic potential of 3-MCPD. The results showed that 3-MCPD exerted potential genotoxicity with the absence of S9 (Rossi and others 1983). This is consistent with the findings from the reverse mutation in the bacteria *Salmonella* TA98 (Zeiger and others 1988; Ohkubo and others 1995). With the presence of S9, the genotoxic potential of 3-MCPD is either reduced or removed from the test subject system.

In mammalian cells *in vitro*, the evidence for the genotoxic potential of 3-MCPD is inconsistent. Henderson and others (1987) showed negative genotoxic results with the absence of S9 and positive results with the presence of S9. Their research was conducted on the mouse lymphoma TK locus. Sister chromatid exchange conducted on Chinese hamster V79 cells showed that 3-MCPD was genotoxic either in the presence or absence of S9 (May 1991). These findings are unpublished; thus, differences in the variables of these investigations cannot be evaluated. Additionally, Painter and Howard (1982), with related end point DNA synthesis inhibition (HeLa cells), reported that 3-MCPD is not genotoxic in the presence and absence of S9.

There is no consistency of data to prove the genotoxicity of 3-MCPD *in vitro*. *In vivo* data, however, consistently revealed that 3-MCPD is not genotoxic in the presence and absence of S9 (Jones and others 1969; Epstein and others 1972; Jones and Jackson 1976; Jaccaud and Aeschbacher 1989; Frei and Wurgler 1997; Fellows 2000; Marshall 2000). 3-MCPD exerts genotoxic potential in certain selected organisms and strains *in vitro*, but the results are not as convincing in *in vivo* genotoxic studies; *in vivo* genotoxic studies show that 3-MCPD is a nongenotoxic chemical.

Metabolism of 3-MCPD

The genotoxic potential of 3-MCPD has been found to be inconclusive and species-related. This can be further explained through the metabolism of 3-MCPD in physiological systems. Two pathways of 3-MCPD metabolism were proposed by Jones (1983): the microbial metabolic pathway and the mammalian metabolic pathway. Both pathways involve the enzyme dehalogenase, where the chloride ion in 3-MCPD is removed. The end product of the microbial pathway can be mercapturic acid or glycerol; for the mammalian pathway, the end product is oxalic acid (Figure 4).

There is no conclusive evidence to prove the metabolic pathway of 3-MCPD in microbes. Van Den Wijngaard and others (1989) proposed that microbes utilize the enzyme HHD to oxidize 3-MCPD to glycidol but did not mention specifically which microbes utilized the aforementioned pathway (they stated that the microbes were Gram-positive). Glycidol is a genotoxic carcinogen (Lee and others 2012) that has been classified as Group 2A (IARC 2014). Glycidol can be hydrolyzed to glycerol and, conversely, glycidol can be deconjugated and acetylated to form mercapturic acid (Lynch and others 1998).

Jones (1975) hypothesized that 3-MCPD has the same metabolic pathway in mammals and microbes ending with mercapturic acid, though the nephrotoxic and reproductive toxicity exhibited by 3-MCPD contradicts this idea. In the mammalian pathway, 3-MCPD is oxidized to 2-chloroacetaldehyde by alcohol

Table 3–Genotoxicity of 3-MCPD, adopted from Lynch and others (1998) and Schlatter and others (2002).

Test end point	Test subjects	Dosage	Findings +S9	Findings –S9	Reference
Bacteria					
Reverse Mutation	*Salmonella* TA100	10 to 1000 μmol/plate	+	+	(Stolzenberg and Hine 1980)
	Salmonella TA1535	2 to 200 μmol/plate	+	+	(Silhankova and others 1982)
	Salmonella TA100, TA1535	100 to 10000 μg/plate	+	+	(Zeiger and others 1988)
	Salmonella TA 100	10 to 1250 μg/plate	+	+	(Ohkubo and others 1995)
	Salmonella TA 1535, TA100	100 to 1000 μmol/plate	+	–	(Stolzenberg and Hine 1979)
	Salmonella TA98	10 to 10000 μg/plate	–	+	(Zeiger and others 1988)
	*Salmonella*TA677, TA98	10 to 1250 μg/plate	–	+	(Ohkubo and others 1995)
	Salmonella TA98	101 to 1000 μmol/plate	–	–	(Stolzenberg and Hine 1979)
	Salmonella TA1537, TA1538, TA98	3 to 200 μmol/plate	–	–	(Silhankova and others 1982)
	E.coli WP2, TM930, TM1080	2 to 200 μmol/plate	–	–	(Ohkubo and others 1995)
	E.coli WP2, TM930, TM1080	2 to 200 μmol/plate	–	–	(Silhankova and others 1982)
	Salmonella TA 100	Not Reported	–	nd	(Majeska and Matheson 1983)
	Salmonella TA 97	10 to 10000 μg/plate	–	nd	(Zeiger and others 1988)
Yeast					
Forward Mutation	*Schizosaccharomyces pombe*	100 to 300 mM	–	+	(Rossi and others 1983)
Mammalian cells					
In vitro (Mutation)	Mouse Lymphoma TK locus	2 to 9mg/ml	+	–	(Henderson and others 1987)
	Related end point DNA synthesis inhibition (HeLa cell)	Not Reported	–	–	(Painter and Howard 1982)
	Cell transformation (M2 fibroblasts)	100 to 2000 μg/mL	nd	+	(Piasecki and others 1990)
	Chinese hamster V79 cells HPRT	0.3 to 70 mM	weak at >70 mM		(Gorlitz 1991)
In vitro (Sister Chromatid Exchange)	Chinese hamster V79 cells	700 to 2800 μg/mL	+	+	(May 1991)
In vivo (Dominant Lethal)	Male Mice	5 to 10 mg/kg bw/day, 5 d (oral)	–		(Jones and others 1969)
	Male ICR/Ha Swiss Mice	125 mg/kg bw/day or 20mg/kg bw/day, 5 d (oral)	–		(Epstein and others 1972)
	Male Wistar Rats	5, 10, 20 mg/kg bw/day, 5 d (oral)	–		(Jones and Jackson 1976)
	Bone marrow micronuclues OF1 Mice	40 to 120 mg/kg bw	–		(Jaccaud and Aeschbacher 1989)
	Chinese Hamster Ovary (CHO)-K1 cells	0.5 to 5 mg/mL	nd	+	(El Ramy and others 2007)

dehydrogenase, which is converted to 3-chloro-2-hydroxypropanoic acid (also known as chlorolactic acid). Jones and others (1981) reported that chlorolactic acid has been shown to inhibit respiration and lactate metabolism (playing an important role in causing nephrotoxicity). Lee and others (2005b) showed that chlorolactic acid could induce immunotoxic effects *in vitro*. Chlorolactic acid suppressed T-lymphocytes, B lymphocytes, and the production of cytokines. This explains why rats fed with high and medium concentrations of 3-MCPD showed signs of morbidity and mortality (Lee and others 2005b). Jones and others (1978) showed that glycidol is not the major metabolite of 3-MCPD *in vivo*. The end product in the mammalian pathway, oxalic acid, has been found to cause kidney failure through the formation of crystals of calcium oxalate. Calcium oxalate has been associated with focal necrosis, mineralization, and impairment of kidney function (EAEMP 2004). The pathway ending with oxalic acid or propane-1,2-diol is the better explanation for the metabolism of 3-MCPD *in vivo*, with intermediate products as the causative agents of nephrotoxicity and reproductive toxicity.

Biomarkers of 3-MCPD

Biomarkers are characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Atkinson and others 2001). When the physiological system is exposed to foreign substances, it will respond by releasing biomarkers. Biomarkers are useful tools for disease detection, prevention of further exposure to contaminants and evaluation of the severity of exposure.

Jones (1975) investigated the metabolism of 3-MCPD in rats and mice and isolated and identified 2 biomarkers from the animals' urine: S-(2,3-dihydroxypropyl)cysteine (VII), and the corresponding mercapturic acid N-acetyl-S-(2,3-dihydroxypropyl)cysteine (VIII). Jones and Fakhouri (1979) reported the urinary metabolites 1,3-DCP, N-acetyl-S-(2,3-dihydroxypropyl)cysteine, and N,N-bis-acetyl-S,S'-(1,3-bis-cysteinyl)propan-2-ol in 3-MCPD-exposed animals. Jones and others (1978) exposed labeled 3-MCPD to male rats to investigate the oxidative metabolism of 3-MCPD, and isolated β-chlorolactic acid (IV) and oxalic acid (V) from the exposed rats' urine. All the biomarkers found in the urine of 3-MCPD-exposed rats were either from the bacterial or putative mammalian pathways of 3-MCPD metabolism (Figure 4). Since the exact metabolic pathway of 3-MCPD is yet to be determined, there is no definitive way to prove that any of the metabolites reported is directly related to 3-MCPD exposure.

In a study of the toxicological effects of chloropropanols on rats, Li and others (2003) found that there was an increase in N-acetyl-beta-D-glucosaminidase (NAG) activity for rats exposed to 3-MCPD. They proposed that NAG in urine and in sperm counts are sensitive biomarkers for 3-MCPD. Unfortunately, NAG in urine is not a specific biomarker for 3-MCPD; NAG can also be used to detect renal injury (Skalova 2005), which can be due to various causes such as injury or dysfunction due to diabetes mellitus, nephrotic syndrome, inflammation, vesicoureteral reflux, urinary tract infection, hypercalciuria, urolithiasis, nephrocalcinosis, perinatal asphyxia, hypoxia, hypertension, heavy metal poisoning, and treatment with aminoglycosides, valproate, or other nephrotoxic drugs. Li and others (2010) narrowed down the specific biomarker for 3-MCPD, using the metabonomic analysis for specific biomarkers in male Wistar rats. The biomarker was determined and measured from the urine with ultra-performance liquid chromatography/mass spectrometry (UPLC-MS). The study found that high concentrations of galactosylglycerol were found in urine of rats exposed to high concentrations of 3-MCPD, and this biomarker can be detected as early as 10 d following exposure. To increase the validity of the biomarkers found, this research could be extended to other species of animals such as mini-pigs. The relationship between the concentration of the biomarker, exposure concentration, and exposure period would provide important information. With a relationship established, exposure of humans to 3-MCPD could be measured and would enable further validation of the adverse effects of 3-MCPD on the human biological system.

Toxicity of 3-MCPD

Prior to 3-MCPD being reported to be potentially carcinogenic, it was widely used as an antifertility treatment for rodents. 3-MCPD was the active ingredient in Epibloc, registered trademark of Gametrices (Ericsson 1982), an effective rodenticide targeting a specific rodent, *Rattus norvegicus* (Norway or common brown rat). It renders the male rodent temporarily or permanently infertile, depending on the concentration ingested. Jones (1983) reported that the reproductive toxicology of 3-MCPD is species-dependent. It will be effective for rats, rams, boars, guinea pigs, hamsters, rhesus monkeys, and ejaculated human sperm but ineffective in mice and rabbits. The mechanism of 3-MCPD antifertility is the blockage of the glycolysis pathway (Stevenson and Jones 1984). Glycolysis is an important pathway for sperm mobility, as it compensates for the lack of oxidative phosphorylation (Mukai and Okuno 2004; Miki 2007). The blockage of glycolysis due to the action of (s)-3-MCPD in tyrosine protein phosphorylation will impair the 3'-5'-cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway in sperm (Zhang and others 2012). It has been recently reported that 3-MCPD reduces progesterone production in R2C rat Leydig cells (Sun and others 2013), and this report also stated that 3-MCPD will induce morphological changes and DNA damage in Leydig cells, resulting in apoptotic cell death.

Lee and others (2004) investigated the potential immunotoxicity of 3-MCPD in female Balb/c mice. In this study, 3-MCPD was dissolved in water and administered by gavage for 14 d. The exposure concentrations used were 0 (control), 25, 50, and 100 mg/kg. Food and water were given freely. Hematological changes, histopathological changes, antigen-specific immunity (response to sheep erythrocyte), proliferative potential of splenic lymphocytes (T- and B-cell mitogens), and natural killer (NK) cell activity (nonspecific immunity) were evaluated. After 2 wk, no experiment-related mortality was observed. There were also no significant changes in weight gained observed between the control and exposed mice, though mice fed at the high dose were found to have reduced thymus and spleen weights. There were significant decreases of spleen and thymus cellularity for groups fed with high concentrations of 3-MCPD. 3-MCPD also found to significantly reduce the antibody-forming cell response in female Balb/c mice exposed to a dose of 100 mg/kg dose. The research concluded that 3-MCPD can disrupt the immune system of female mice exposed to a high-concentration dosage (100 mg/kg). The major immune system modulated was antibody-forming cell response, spleen and thymus cellularity, and NK cell activity. To further understand immunotoxicity response of 3-MCPD, Lee and others (2005a) investigated the effect of 3-MCPD on the thymic subset, delayed-type hypersensitivity, mixed-lymphocyte reaction, and peritoneal macrophage activity. The experimental settings were 3-MCPD dissolved with water and administered

through gavage to female Balb/c mice for 2 wk. The dosages were 0 (control), 25, 50, and 100 mg/kg. There were no significant changes for the mixed-lymphocyte reaction and delayed-type hypersensitivity. There were significant decreases in CD4+CD8+ thymic subset and activity of peritoneal macrophage, but significant increases in apoptosis of thymocytes in mice treated with high dose of 3-MCPD. The findings of this research support the findings of Lee and others (2004), 3-MCPD can reduce the immune system of female Balb/c mice fed with 100 mg/kg 3-MCPD for 2 wk.

Carcinogenicity Study in Mice

A dermal study was conducted for 19 mo with 50 female CHR/Ha Swiss mice. Fifty mice were subjected to solvent alone as a control. 3-MCPD was dissolved in 0.1 mL acetone for application in the study. A 2-mg dose was administered as a dermal application 3 times a week for 19 mo. At the end of the treatment period, no treatments related to neoplastic findings were reported (Van Duuren and others 1974).

Similarly, a subcutaneous study was conducted for 19 mo with 50 female CHR/Ha Swiss mice (Van Duuren and others 1974). Fifty mice were subjected to solvent alone as the control. 3-MCPD was dissolved in tricaprylin for application, and 1 mg was injected into each mouse once weekly. Local sarcoma was found at the site of injection in 1 dosed and 1 control mouse. This study concluded that 3-MCPD is not a carcinogenic compound.

In contrast, Cho and others (2008a) investigated the subchronic toxicity of 3-MCPD in drinking water on B6C3F1 mice for 13 wk. Treatments of 0 (control), 5, 25, 100, 200, and 400 ppm 3-MCPD were administered to 10 mice of each sex for all concentrations over a period of 13 wk. All mice survived the treatments, but the body weight gained for male and female mice administered with the 400 ppm dose was significantly lower compared to the control. The relative kidney weights of males and females mice treated with 200 and 400 ppm doses were significantly higher than the controls without any corresponding histopathological changes. A decrease in sperm motility was also reported for male mice treated with 400 ppm. There was a significant increase in the degeneration of the germinal epithelium for males given between 200 ppm and 400 ppm. A significant delay in the estrus cycle was reported for female mice treated with 400 ppm, but no histopathological changes in the reproductive organs were found. The study concluded that the target organs for 3-MCPD toxicity are the kidneys, testes, and ovaries.

Jeong and others (2010) conducted a carcinogenicity study on 3-MCPD administered in drinking water to B6C3F1 mice and showed no carcinogenic potential. Their investigation lasted 104 wk, with 3-MCPD concentrations of 0, 30, 100, and 300/200 ppm. There were a total of 50 mice (males and females) for each exposure concentration. The 300 ppm group was reduced to a 200 ppm dose during the research due to the toxic effects of 3-MCPD. In the report, the weight of the high-dosage mice had significantly decreased and food and water consumption were lower compared to the control mice. There was no histopathological evidence to support differences in hematology and serum biochemistry. No treatment-related neoplastic findings were reported, and it was concluded that there was no evidence of carcinogenic potential. The research was conducted in accordance with OECD and ICH requirements for a satisfactory carcinogenicity study (ICH 2008; OECD 2008). There are no conclusive results regarding the carcinogenic potential of 3-MCPD in a mouse test model (Table 4).

Table 4–Carcinogenic potential of 3-MCPD.

Target animals and amount	Exposure	Durations	Findings	Reference
Mice CHR/HA (50 female)	Dermal (2 mg)	19 mo	No neoplastic findings	(Van Duuren and others 1974)
Mice CHR/HA (50 female)	Injection (1 mg)	19 mo	No neoplastic findings	(Van Duuren and others 1974)
Mice B6C3F1 (10 male and female for each concentration)	Drinking water (5, 25, 100, 200, 400 ppm)	3 mo	Target organs: kidney, testis, and ovary	(Cho and others 2008a)
Mice B6C3F1 (50 male and female for each concentration)	Drinking water (30, 100, 300/200 ppm)	24 mo	No carcinogenic potential	(Jeong and others 2010)
Rats Sprague-Dawley (26 male and female for each concentration)	Galvage (20/35, 60/70 mg/kg)	17 mo	Weight lost in exposure to high concentration, and no neoplastic findings	(Weisburger and others 1981)
Rats Fisher 344 (50 male and female for each concentration)	Drinking water (25, 100, 400 ppm)	24 mo	CPN, hyperplasia and/or tumors in kidney, testis, mammary gland, and pituitary gland	(Sunahara and others 1993a)
Rats Sprague-Dawley (50 male and female for each concentration)	Drinking water (20, 100, 500 mg/L)	24 mo	CPN, kidney renal tubule carcinomas, and Leydig cell tumors	(Cho and others 2008b)
Rats Wistar (50 male and female for each concentration)	Corn oil oral galvage (0.92, 3.68, 14.75 mg/mL)	3 mo	Renal hyperplasia, and testicular toxicity. Signs of morbidity in male and female and mortality in female.	(Barocelli and others 2011)

Carcinogenic Study in Rats

Weisburger and others (1981) reported a gavage study with Sprague-Dawley (SD) rats for 72 wk and observation for 104 wk. Twenty-six males and females were used for the study, but only 20 were used as controls. The dosage fed was 30/35 and 60/70 mg/kg-body weight. The dosage was increased after 10 wk. It was reported that all of the rats fed with high concentrations of 3-MCPD had lower weights compared with the controls. There was no treatment-related neoplasticity found in any of the research subjects.

Sunahara and others (1993), as reported by the WHO (Schlatter and others 2002), studied the carcinogenicity of 3-MCPD in drinking water with Fisher 344 rats. In the study, 4 groups of 50 pathogen-free Fisher rats of each sex (male and female) were given 0 (control), 20, 100, and 500 mg/L 3-MCPD (98% purity) doses of 3-MCPD for 104 wk. There was a significant decrease in food intake and water consumption in both males and females treated with 500 mg/L 3-MCPD, which contributed to a significant body weight reduction for the high-dosage treatment. The mortality rate was unaffected by treatment, with more than 42% of rats terminated at the conclusion of the experiment. No treatment-related clinical signs were noted by the hematological and blood clinical parameters. Chronic progressive nephropathy (CPN) was found in all 3-MCPD-exposed rats where female rats were found to be more severely affected than male rats. Dose-related incidence of hyperplasia and/or tumors was observed in all 3-MCPD-treated groups, with increases in kidney, testis, mammary gland, and pituitary gland. The research concluded that 3-MCPD increases the incidence of renal and testicular Leydig-cell tumors in a dose-dependent pattern.

Another toxicity study was conducted by Cho and others (2008b) on 50 male and female SD rats. The dosages involved in this research were 0, 25, 100, and 400 ppm administered for 2 y. The water intake for rats treated with 400 ppm was significantly lower than for the controls, contributing to a significantly lower weight for the high dosage groups (both genders). At the end of the research, there was no significant difference in the survival rate between males and females. The rate of survival for both genders was less than 50% due to spontaneous pituitary tumors in both sexes. CPN were observed in all 3-MCPD exposed groups, consistent with the findings of Sunahara and others (1993), though different in species of rats in the experiment settings. The authors concluded that 3-MCPD is a carcinogen as there were incidences of kidney renal tubule carcinomas in both male and female rats and Leydig cell tumors in male SD rats.

Barocelli and others (2011) conducted a 90-d toxicity study of 3-MCPD and 3-MCPD palmitic ester, but only the toxicity of 3-MCPD is discussed here. The toxicology study used Wistar rats treated with 3 concentrations of 3-MCPD: high (14.75 mg/kg), medium-high (3.68 mg/kg), and low (0.92 mg/kg). For each concentration, there were 10 rats as a control and 20 rats for the treatment. The 3-MCPD dosage in the research was diluted with corn oil. Dosages of different concentrations of 3-MCPD were administered via oral gavage, and food and water consumption was freely. At the conclusion of the research, all male rats survived, but there were 7 deaths in the high concentration-treated female rats and 1 death in the medium-high female group. The Wistar rat model shows that short-term, high dosages of 3-MCPD are fatal to female rats. All surviving test subjects, male and female, also showed signs of morbidity. In contrast with the findings of Sunahara and others (1993) and Cho and others (2008b), the rats in this study had a weight increase. The research shows that 3-MCPD will cause renal and testis damage.

According to results from short-term testing with rats, 3-MCPD has been shown to be nephrotoxic and reproductively toxic. Additionally, long-term toxicity tests with rats showed that 3-MCPD can induce adenoma and/or carcinoma. Although the test results are animal-specific, it certainly cannot be ignored that 3-MCPD could potentially be carcinogenic to the human physiological system. Toxicity research on animals such as mini-pigs will give a better understanding of the mechanisms and the effect of 3-MCPD on the human physiological system, as mini-pigs share basic physiology with humans (OECD 2012). Postmortem information from these studies will further confirm the target organs, genotoxicity, and carcinogenic mechanisms of 3-MCPD.

Methods of Detection—Advantages and Disadvantages

At present, several techniques have been developed and reported for the quantification of 3-MCPD. Most of these techniques require a process to change 3-MCPD into its derivatives and analyze with gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). Chloropropanols, including 3-MCPD, were first detected by Velisek and others (1978) in protein hydrolysate by isolation of neutral fractions using GC. There was no LOD reported in the literature. After this research was published, several quantification methods followed and most of them involve using a derivatization agent such as phenylboronic acid (PBA), bis(trimethylsiyl)trifluoroacetamide (BSTFA), heptafluorobutyrylimidazole (HFBI), heptafluorobutyric anhydride (HFBA), ketal/acetonide formation, and periodate oxidation (Table 5). There are also methods that do not utilize the derivatization process, but a majority of these have a higher LOD than the requirement set by the EU (Table 1).

Owing to the complexity of food matrices and the low concentration of 3-MCPD, prior to derivatization, a procedure to extract or clean up the 3-MCPD is required in order to obtain a cleaner chromatogram and a higher recovery, and to lower the required quantity of derivatization agent. To derivatize in an anhydrous environment, solid-phase extraction will be required to extract 3-MCPD from the aqueous sample into the anhydrous solvent. 3-MCPD in food samples will first be extracted into saline water. The 3-MCPD-extracted saline water will then be loaded onto a chromatography column, with diatomaceous earth as the stationary phase and a protic solvent as the mobile phase. The purpose of using a protic solvent to extract 3-MCPD from saline water is due to the polarity of 3-MCPD. A solid-phase extraction technique is based on liquid–liquid extraction, but possesses major advantages over conventional liquid–liquid extraction, such as the absence of emulsion formation, the gain of higher yields and cleaner extracts, and savings on the uses of solvent and time. The collected eluate is dried to dryness or near dryness and subjected to derivatization. The derivatized 3-MCPD is then dissolved in an organic solvent and injected into a GC column. There are also prepacked disposable supported liquid extraction columns available on the market, such as Chem Elut (Agilent Technologies 2014) and EXtrelut (Merck Milipore 2014), which can be used to extract 3-MCPD. Only PBA derivatization and periodate oxidation can be done in an aqueous environment. Divinová and others (2004) and Breitling-Utzman and others (2005) reported the derivatization of 3-MCPD with PBA in an aqueous environment. Divinová and others (2004) first extracted 3-MCPD from food samples with a hexane: acetone mixture. The 3-MCPD in

Table 5–Methods developed to quantify free 3-MCPD.

Derivatization agent	Advantage	Disadvantage	Challenge	LOD	Instruments	References
–	No derivatization agent	High limit of detection	To determine a suitable polar column for better separation due to high polarity of 3-MCPD	0.13 to 1.00 mg/kg	GC, CE-ECD	(Spyres 1993; Xing and Cao 2007)
Phenylboronic acid (PBA)	Work in aqueous and organic medium, intensive characteristic ions	Require GC with sensitive detector, lack of confirmation ions	To determine the suitable alkaline to neutralize the acidity of end product	0.03×10^{-1} – 1.00 mg/kg	GC-MI-FIR, GC-FID, GC-MS-SIM, GC-MS/MS MRM,	(Rodman and Ross 1986; Plantinga and others 1991; IARC 1994; Anon 1995; Kuballa and Ruge 2003; Divinová and others 2004; Huang and others 2005)
N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA)	Better heat stability of derivatized analytes	Require anhydrous derivatizing environment	Silicone residue can be accumulated in detector	0.16×10^{-2} – 1.12×10^{-2} mg/kg	GC-FID, GC-MS-SIM, GC-MS	(Kissa 1992; Bodén and others 1997; Gonzalez and others 2011)
N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA)	Better stability of derivatized analytes	Require anhydrous derivatizing environment	Silicone residue can be accumulated in detector	3.91×10^{-3} mg/kg	GC-MS	(Lee and others 2007)
Acetone in toulene-4-sufonic acid	React with diols in 3-MCPD specifically, intensive characteristic ions	Require anhydrous derivatizing environment, lack of confirmation ions	Normal acetone require pretreatment with Epsom salt, removal of acidic catalyst from end product	0.01×10^{-1} – 0.12×10^{-2} mg/kg	GC-MS	(Meierhans and others 1998; Rétho and Blanchard 2005; Dayrit and Niñonuevo 2004)
Cyclohexanone	Applicable to free and bound 2- and 3-MCPD, solid catalyst can be removed easily	Require anhydrous derivatizing environment, lack of confirmation ions	Cyclohexanone will require pretreatment to remove moisture	0.01×10^{-1} – 0.03×10^{-1} mg/kg	GC-MS	(Becalski and others 2013)
Heptafluorobutyryl imidazole (HFBI)	Wide range of samples	Require anhydrous derivatizing environment	Require heat incubation, HFBI is expensive	0.05×10^{-1} – 0.10 mg/kg	GC-ECD, GC-MS, GC-MS/MS MRM	(van Bergen and others 1992; Hamlet and Sutton 1997; Brereton and others 2001; Bel-Rhlid and others 2004; Robert and others 2004)
Heptafluorobutyric acid (HFBA)	Cheaper as compared to HFBI	Require anhydrous derivatizing environment	Require a catalyst for optimum derivatization process and neutralization of acidic end product	0.07×10^{-2} – 0.05×10^{-1} mg/kg	GC-MS, GC-ECD, GC-MS SIM	(Matthew and Anastasio 2000; Chung and others 2002; Abu-El-Haj and others 2007)
Fluorescence Derivatization	Fast and alternative to GC	Require periodate oxidation as pretreatment	Require color removal pretreatment to quantify 3-MCPD in dark color samples	0.36×10^{-3} μg/kg	HPLC-FLD	(Hu and others 2013)

hexane: acetone was then extracted into water with liquid–liquid extraction. The aqueous 3-MCPD was dried to dryness and derivatized with acetone/water-diluted PBA. Breitling-Utzman and others (2005) simplified the extraction method by directly extracting 3-MCPD from food samples into saline water and derivatizing it with acetone: water-diluted PBA. After the derivatization, the dioxaborolane products were dissolved in hexane and injected into a GC column. As compared with the extraction for anhydrous derivatization, the PBA extraction method is much simpler and faster, and does not use harmful organic solvents.

For detection of 3-MCPD without derivatization, Spyres (1993) directly quantified 3-MCPD with gas chromatography coupled with electrolytic conductivity detection (GC-ECD), but only achieved a 1 mg/kg detection limit. Xing and Cao (2007) reported that instead of using GC, capillary electrophoresis with electrochemical detection (CE-ECD) was used and an LOD of 0.13 mg/kg was achieved. Although a different instrument was utilized, the LOD without derivatization was still high. Leung and others (2003) developed a molecular imprinted polymer (MIP) that is able to function as a potentiometric chemosensor for 3-MCPD in aqueous samples. Unfortunately, the limited range of quantification has restricted the use of this method, and it will require further improvements. However, the 3-MCPD MIP developed can be used to extract 3-MCPD from samples, followed by derivatization and quantification by GC-MS.

PBA can be used to derivatize 3-MCPD, as it reacts specifically with 1,2-diol and 1,3-diol. PBA has been widely used as a derivatizing agent for glucose detection and for the development of a biomimetic sensor to determine blood glucose levels. This derivatizing agent reacts with the 1,2-diol of 3-MCPD and forms a dioxaborolane derivative, which dissolves in a nonpolar solvent. The detection of 3-MCPD with PBA derivatization was first reported by Rodman and Ross (1986). The PBA derivatization of 3-MCPD was reported to be done in an anhydrous environment and detected with gas chromatography-matrix isolation-Fourier transform infrared spectrometry (GC-MI-FIR). Pesselman and Feit (1988) successfully derivatized 3-MCPD with PBA in an aqueous environment and extracted the derivatives with hexane prior to identification with GC-ECD. This had proven that derivatization of 3-MCPD with PBA can be done in aqueous condition. Plantinga and others (1991) and Anon (1995) applied PBA as a derivatization agent but quantified with gas chromatography-flame ionization detection (GC-FID). The detection limit established was between 0.5 and 1 mg/kg. The method was not sensitive enough to detect 3-MCPD as required by the EU. To increase the sensitivity of the method of derivatization by PBA, IARC (1994) detected the derivative of 3-MCPD with gas chromatography-mass spectrometry-selective ion monitoring (GC-MS-SIM). The detection limit improved to between 0.03×10^{-1} and 0.01 mg/kg. Both Divinová and others (2004) and Huang and others (2005) using GC-MS-SIM had detection limits of 0.03×10^{-1} and 3.87×10^{-3} mg/kg, respectively. Kuballa and Ruge (2003) quantified 3-MCPD with gas chromatography-tandem triple quadrupole mass spectrometry (GC-MS/MS MRM). The detection limit was in accordance with that required by the EU. Derivatization with PBA has been proven to be effective with the conditions that it must be quantified using GC coupled with a sensitive detector. PBA-derivatized 3-MCPDs produce intense characteristic ions, which is good for low-concentration detection but lack of confirmation of the ions (Table 6). The other drawback of PBA as a derivatizing agent is that it is required in high concentration. The excess PBA is detrimental to the chromatography system and has to be removed prior to injection (Weisshaar and Perz 2010). Breitling-Utzman and others (2005) removed the excess PBA through precipitation by storing the vials at −18 °C for 12 h prior to GC injection. The removal of PBA cannot be done through neutralization with an alkaline agent because PBA is a diol, not an acid.

Table 6–Characteristic ions of PBA derivatized 3-MCPD and 3-MCPD$_{d5}$ (Hamlet and Sadd 2009).

Analytes	MW	$[M]^{+-}$	$[M\text{-}CH_2Cl]^+$	Other structurally significant ions
3-MCPD	196	196/198[a]	146/147[b]	103/104[b] $[Ph\text{-}BO]^{+-}$ 91 $[C_7H_7]^+$
3-MCPD$_{d5}$	201	201/201[a]	149/150[b]	103/104[b] $[Ph\text{-}BO]^{+-}$ 93 $[C_7H_5D_2]_+$

[a] Isotopic chlorine cluster ion.
[b] Isotopic boron cluster ion.

Meierhans and others (1998) developed a sensitive method for the determination of 3-MCPD with a capillary GC-MS using derivatization by acetone in toluene-4-sulfonic acid monohydrate. The LOD was 0.01×10^{-1} mg/kg. Diols functional group in the 3-MCPD will react with acetone and result in the formation of cyclic acetals and ketals. The reaction had to be performed in an anhydrous environment, as the formed acetals are moisture sensitive and will undergo hydrolysis into aldehyde or ketone and alcohol (Carey 2000). Owing to the sensitivity of the derivatization method to moisture, 3-MCPD extraction for acetone derivatization has to be done in anhydrous organic conditions, preferably by solid-phase extraction, unlike the PBA extraction method where 3-MCPD can be extracted into water prior to derivatization. Dayrit and Niñonuevo (2004) utilized acetone in toluene-4-sulfonic acid monohydrate to quantify 3-MCPD, with 3-MCPD$_{d5}$ as the internal standard. The LOD achieved was 1.20×10^3 mg/kg. Rétho and Blanchard (2005) modified the procedure of Meierhans and others (1998) by filtering the derivative 3-MCPD with a basic aluminum oxide cartridge, achieving an LOD of 0.01×10^{-1} mg/kg. The disadvantage of this method is that acetone used in the derivatization contains moisture and an additional step will be required to remove it. It is very important to ensure that all glassware used in this method is dry, as the end products will react with water. The advantage of acetone-derivatized 3-MCPDs is the intense ions of the analytes that are helpful for low-concentration detection, but the limited characteristics of the ions are a drawback to their confirmation (Table 7). The catalyst toluene-4-sulfonic acid monohydrate also has to be removed from the end products. Becalski and others (2013) improved the method by replacing the aliphatic ketones with cyclic ketones (cyclohexanone), which provided more resistant characteristic ions. They also replaced the toluene-4-sulfonic acid monohydrate with a solid catalyst (Nafion and Amberlyst), which could be easily removed at the end of derivatization. The 1,3-dioxolane of 3-MCPD was quantified by GC-MS, where the LOD achieved was between 0.01×10^{-1} and 0.03×10^{-1} mg/kg.

A small number of studies have utilized BSTFA as the derivatization agent. 3-MCPD has to be extracted using a solid-phase extraction method, prior to the silylation derivatization by BSTFA. This idea was first published by Kissa (1992), where 3-MCPD was derivatized by BSTFA and quantified with GC-FID. The LOD for the method used was 5.00 mg/kg, which was too high compared with the requirements of the EU. Bodén and

Table 7–Characteristic ions of dioxolane/dioxane derivatized 3-MCPD and 3-MCPD$_{d5}$ (Hamlet and Sadd 2009).

Derivatizing agent	Analytes	MW	[M-C_nH_{2n+1}]+	Other structurally significant ions
Acetone	3-MCPD	150	135/137[a]	43[C_2H_3O]$^+$
	3-MCPD$_{d5}$	155	140/142[a]	43[C_2H_3O]$^+$
3-Pentanone	3-MCPD	178	149/151[a]	57[C_3H_5O]$^+$
	3-MCPD$_{d5}$	183	154/156[a]	57[C_3H_5O]$^+$
4-Heptanone	3-MCPD	206	163/165[a]	71[C_4H_7O]$^+$
	3-MCPD$_{d5}$	211	168/170[a]	71[C_4H_7O]$^+$
Cyclohexanone	3-MCPD	262	191/193[a]	99[$C_6H_{11}O$]$^+$
	3-MCPD$_{d5}$	267	196/198[a]	99[$C_6H_{11}O$]$^+$

[a] Isotopic chlorine cluster ion.

Table 8–Characteristic ions of HFB derivatized 3-MCPD and 3-MCPD$_{d5}$ (Hamlet and Sadd 2009).

Analytes	MW	[M-CH_2Cl]$^+$	[M-$C_3F_7CO_2$]$^+$	[M-$C_3F_7CO_2CH_2$]$^+$	[M-$C_3F_7CO_2$-HCl]$^+$	[M-$C_3F_7CO_2$-$C_3F_7CO_2H$]$^+$
3-MCPD	502	453	289/291[a]	275/277[a]	253	75/77[a]
3-MCPD$_{d5}$	507	456	294/291[a]	278/280[a]	257	79/81[a]

[a] Isotopic chlorine cluster ion.

others (1997) derivatized with BSTFA coupled with GC MS-SIM. The detection limit was 0.04 mg/kg. Gonzalez and others (2011) combined solid-phase extraction with GC-MS to compare the efficiency of the derivatizing activities of BSTFA and HFBI. It was found that the LOD for BSTFA is 1.12×10^{-2} mg/kg, which is low enough to quantify 3-MCPD according to the EU. The research also found that BSTA was a better derivatization agent compared to HFBI, as derivatization by BSTFA demonstrated greater stability over time compared to HFBI. Racamonde and others (2011) determined the levels of chloropropanols in food using BSTFA as the derivatization agent and achieved a limit of quantification (LOQ) of 0.16×10^{-2} mg/kg. Lee and others (2007) determined the concentration of 3-MCPD using solid-phase micro-extraction (SPME) derivatized with N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) and quantified with GC-MS. 3-MCPD was extracted with an 85-μm polyacrylate-coated fiber and head-spaced derivatized. The LOD achieved was 3.91×10^3 mg/kg. BSTFA and MSTFA are better alternatives than HFBI and HFBA, as the end product is stable. The disadvantage of BSTFA and MSTFA as derivatizing agents is that silicone residues might accumulate in the detector. However, BSTFA and MSTFA will not harm the GC column as they are evaporated prior to GC injection. The derivatives of BSTFA and MSTFA have ions of low mass, thus resulting in a reduced sensitivity of the method.

The method based on the heptafluorobutyrate derivative is the only method that allows the control of a wide range of samples (Rétho and Blanchard 2005). 3-MCPD will have to be extracted with solid-phase extraction on diatomaceous earth before the acylation derivatization process. Brereton and others (2001) extensively studied this method with the utilization of 3-MCPD$_{d5}$ as an internal standard. Most if not all method of quantification for 3-MCPD utilized the deuterated 3-MCPD internal standard. The method became the European Standard (European Standard 2005) and was adopted by the AOAC (AOAC 2002). The first research performed using HFBI was by van Bergen (1992), where GC-ECD and GC-MS were used to quantify chloropropanols, including 3-MCPD. The LOD is between 0.01 and 0.10 mg/kg. Hamlet and Sutton (1997) combined HFBI derivatization with GC-MS/MS MRM and achieved a detection limit of 0.05×10^{-1} mg/kg, which was low enough to meet the EU requirement. Robert and others (2004) and Bel-Rhlid and others (2004) researched the combination of HFBI and GC-MS, and the LOD was 0.05×10^{-1} mg/kg. The advantage of HFBI is that it is not acidic, and thus will not harm the column. Besides this, the excess HFBI in the derivatization system will also protect the column, as it will react with co-extracted compounds that could harm the column. HFBI-derivatized 3-MCPD produces a number of characteristic ions that are useful for confirmation of the analytes, making it more favorable for the derivatization of 3-MCPD (Table 8). The challenge of HFBI is that the derivatization has to be performed in an anhydrous environment. HFBI is also an expensive derivatizing agent. HFBA is a cheaper derivatization reagent that can replace HFBI and still provide HFB-derivatized characteristic ions.

Derivatization with HFBA to quantify 3-MCPD was conducted by Chung and others (2002) using a combination HFBA with GC-MS, and the LOD obtained was 0.05×10^{-1} mg/kg. Matthew and Anastasio (2000), with the same derivatization agent and GC-ECD, achieved a lower and more sensitive LOD between 0.07×10^{-2} and 0.17×10^{-2} mg/kg. Abu-El-Haj and others (2007), using HFBA coupled with GC-MS-SIM, developed a method with an LOD of 0.01×10^{-1} mg/kg. HFBA can provide almost the same sensitivity as HFBI with a suitable catalyst. Unfortunately, HFBA derivatization also must be performed in an anhydrous environment. The acidic characteristic of HFBA was one of the concerns, as it may reduce the lifespan of the GC column. However, this can be solved by the washing step with water before injection.

There is also a novel approach using periodate oxidation to derivatize 3-MCPD and quantification with HPLC-FLD as reported by Hu and others (2013). The fluorescence derivatization procedure was modified from Huang and Waxman (1999) for the determination of chloroacetaldehyde. The method was reported to be able to quantify 3-MCPD up to 0.36×10^{-3} mg/kg. However, this method was found not to be suitable for quantification of 3-MCPD in soy and soy-related sauces, as the dark color of the sample matrix will render the reading of fluorescence ineffective.

Conclusions

Research on 3-MCPD has been intensive in the past 10 y, and many countries already have regulations on the levels of 3-MCPD allowed in food, yet foods with a high concentration of 3-MCPD are still widely available to consumers. Better enforcement of regulations and more efficient removal methods must be introduced to ensure that the concentrations of 3-MCPD in foods are within the required levels. Improvement in the analytical field would be an advantage to food safety; food quality would be compromised

if the quantification of 3-MCPD is too time-consuming. Most analytical methods used for quantifying 3-MCPD require sample clean-up coupled with a sensitive instrument such as a GC-MS. Therefore, a method based on a chemosensor or biosensor would be an advantage, as sensors are robust and specific. The development of user intervention-free sensors for the rapid determination of 3-MCPD will provide an alternative to the existing standard methods, allowing faster quantification of 3-MCPD, which, in turn, would allow immediate actions to be taken to ensure the safety of food in the marketplace.

Acknowledgments

This work was financially supported by the Univ. of Malaya Research Grant (UMRG) (RG159-12SUS, RP012C-14SUS), the Fundamental Research Grant Scheme (FRGS) from the Ministry of Higher Education of Malaysia (MOHE) (FP014-2013A), and Univ. of Malaya Postgraduate Research Grant (PG030-2014A). Bai Qin thanks Univ. of Malaya Fellowship Scheme for providing scholarship to support his study.

Author Contributions

Bai Qin Lee prepared the literature overview regarding to 3-MCPD, all the documents related and drafting the manuscript. Dr. Sook Mei Khor contributed to the manuscript revision and supervised Bai Qin Lee's research activity.

References

Abu-El-Haj S, Bogusz MJ, Ibrahim Z, Hassan H, Al Tufail M. 2007. Rapid and simple determination of chloropropanols (3-MCPD and 1,3-DCP) in food products using isotope dilution GC–MS. Food Control 18:81–90.

Agilent Technologies. 2014. Supported liquid extraction: SLE cartridges and plates. Available from: http://www.chem.agilent.com/en-US/products-services/Columns-Sample-Preparation/Sample-Preparation/Supported-Liquid-Extraction/SLE-Cartridges-and-Plates/Pages/default.aspx. Accessed 2014 Sep 23.

Anon. 1995. Bestimmung von 3-Chlor-1, 2-Propandiol (3-MCPD) in Speisewürzen (Eiweißhydrolysate). Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. L 52.02-1.

AOAC. 2002. Determination of 3-chloro-1,2-propanediol in foods and food ingredients, gas chromatography/mass spectrometric detection. AOAC Official Method 2000.01. AOAC Intl. 48.1.06.

Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, Oates JA, Peck CC, Schooley RT, Spilker BA, Woodcock J, Zeger SL. 2001. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69:89–95.

Barocelli E, Corradi A, Mutti A, Petronini P. 2011. Comparison between 3-MCPD and its palmitic esters in a 90-day toxicological study. Scientific report submitted to EFSA, CFP/EFSA/CONTAM/2009/01.

Becalski A, Zhao T, Sit D. 2013. Cyclohexanone/sulfonated polymer catalyst: a new simple derivatizing procedure for GC-MS determination of 2- and 3-monochloropropanediols. Food Energy Secur 2:157–65.

Bel-Rhlid R, Talmon JP, Fay LB, Juillerat MA. 2004. Biodegradation of 3-chloro-1,2-propanediol with *Saccharomyces cerevisiae*. J Agr Food Chem 52:6165–9.

Bodén L, Lundgren M, Stensiö K-E, Gorzynski M. 1997. Determination of 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol in papers treated with polyamidoamine-epichlorohydrin wet-strength resins by gas chromatography-mass spectrometry using selective ion monitoring. J Chromatogr A 788:195–203.

Borkenhagen LK, inventors. 1953. Process for preparing amino acids. U.S. Patent 2657232 A.

Bornscheuer UT, Hesseler M. 2010. Enzymatic removal of 3-monochloro-1,2-propanediol (3-MCPD) and its ester from oils. Eur J Lipid Sci Technol 112: 552–6.

Breitling-Utzmann CM, Hrenn H, Haase NU, Unbehend GM. 2005. Influence of dough ingredients on 3-chloropropane-1,2-diol (3-MCPD) formation in toast. Food Addit Contam 22:97–103.

Brereton P, Kelly J, Crews C, Honour S, Wood R, Davies A. 2001. Determination of 3-chloro-1,2-propanediol in foods and food ingredients by gas chromatography with mass spectrometric detection: collaborative study. J AOAC Int 84:455–65.

Canadian Standards. 2012. Maximum levels for various chemical contaminants in foods. Available from: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php#share. Accessed 2014 May 5.

Carey FA. 2000. Organic chemistry. 4th ed. United States: McGraw-Hill Higher Education.

Cheng W-C, Chen H-C, Lin Y-P, Lee H-F, Chang P-C, Chou S-S. 2004. Survey on 3-monochloro-1, 2-propandiol (3-MCPD) contents of soy sauce products during fiscal year 2002 in Taiwan. J Food Drug Anal 12:336–41.

Cho WS, Han BS, Lee H, Kim C, Nam KT, Park K, Choi M, Kim SJ, Kim SH, Jeong J, Jang DD. 2008a. Sub chronic toxicity study of 3-monochloropropane-1,2-diol administered by drinking water to B6C3F1 mice. Food Chem Toxicol 46:1666–73.

Cho WS, Han BS, Nam KT, Park K, Choi M, Kim SH, Jeong J, Jang DD. 2008b. Carcinogenicity study of 3-monochloropropane-1,2-diol in Sprague-Dawley rats. Food Chem Toxicol 46:3172–7.

Christova-Bagdassarian V, Tishkova JA, Vrabcheva TM. 2013. 3-monochloro-1,2-propandiol (3-MCPD) in soy sauce from the Bulgarian market. Food Addit Contam B 6:163–67.

Chung WC, Hui KY, Cheng SC. 2002. Sensitive method for the determination of 1,3-dichloropropan-2-ol and 3-chloropropane-1,2-diol in soy sauce by capillary gas chromatography with mass spectrometric detection. J Chromatogr A 952:185–92.

Chung SWC, Kwong KP, Yau JCW, Wong AMC, Xiao Y. 2008. Chloropropanols levels in foodstuffs marketed in Hong Kong. J Food Compos Anal 21:569–73.

Collier PD, Cromie DDO, Davies AP. 1991. Mechanism of formation of chloropropanols present in protein hydrolysates. J AOAC 68:785–90

Commission Regulation. 2001. Setting maximum levels for certain contaminants in foodstuffs, Commission regulation (EC) Nr 466/2001. Official Journal of the European Communities, L77/1 -13.

Crews C, Hasnip S, Chapman S, Hough P, Potter N, Todd J, Brereton P, Matthews W. 2003. Survey of chloropropanols in soy sauces and related products purchased in the UK in 2000 and 2002. Food Addit Contam 20:916–22.

Dayrit FM, Niñonuevo MR. 2004. Development of an analytical method for 3-monochloropropane-1,2-diol in soy sauce using 4-heptanone as derivatizing agent. Food Addit Contam 21:204–9.

Divinová V, Svejkovská B, Doležal M, Velíšek J. 2004. Determination of free and bound 3-chloropropane-1,2-diol by gas chromatography with mass spectrometric detection using deuterated 3-chloropropane-1,2-diol as internal standard. Czech J Food Sci 22:182–9.

EAEMP. 2004. European agency for the evaluation of medicinal products, oelxalic acid. Summary report EMEA/MRL/891/03-FINAL. Available from: http://www.emea.eu.int/pdfs/vet/mrls/o89103en.pdf. Accessed 2014 May 5.

El Ramy R, Ould Elhkim M, Lezmi S, Poul JM. 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 and Chemical 45:41–8.

EPA. 2013. Registration review: conventional cases schedule: 2012-2015. Available from: www.epa.gov/oppsrrd1/registration_review/fy12-fy15-conventional-sched.pdf. Accessed 2014 July 22.

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

Ericsson RJ. 1982. Alpha-chlorohydrin (Epibloc®): a toxicant-sterilant as an alternative in rodent control. Proceedings of the Tenth Vertebrate Pest Conference. February 23, 1982, Univ. of Nebraska-Lincoln.

European Commission. 2004. Report of experts participating in task 3.2.9, collection and collation of data on levels of 3-monochloropropanediol (3-MCPD) and related substances in foodstuffs. Directorate-General Health and Consumer Protection, Brussels. Available from: http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-9_final_report_chloropropanols_en.pdf. Accessed 2014 May 5.

European Commission. 2006. Nr 1881/2006 of 19 September 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union, 9. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2006.364.01.0005.01.ENG. Accessed 2014 July 3.

European Standard. 2005. Foodstuffs-determination of 3-monochloropropane-1,2-diol by GC/MS. Report Nr. EN 14573:2004. Berlin, Germany: Beuth Verlag.

FAO, WHO. 2007. Discussion paper on chloropropanols derived from the manufacture of acid-HVP and the heat processing of food. Proc 1st Session of Codex Committee on Contaminants in Foods. Beijing, China. p 16–20.

FAO. 2012. Reduction of 3-monochloropropane-1, 2-diol (3-MCPD) during the production of acid-hydrolyzed vegetable protein (Acid-HVPs) and products that contain acid-HVPs. In: Prevention and reduction of food and feed contamination. Rome: World Health Organization. p 148–54.

FAOSTAT. 2011. Available from: http://faostat.fao.org/site/535/default.aspx#ancor. Accessed 2014 May 5.

FDA. 2008. CPG Sec 500.500 guidance levels for 3-MCPD (3-chloro-1,2-propanediol) in acid-hydrolyzed protein and Asian-style sauces. Available from: http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074419.htm. Accessed 2014 May 5.

Fellows M. 2000. 3-MCPD: measurement of unscheduled DNA synthesis in rat liver using an *in vitro/in vivo* procedure. Report nr. 1863/1-D5140. York: Convance Laboratories Ltd.

Flork M, inventor. 1989. Using sulfuric acid and specific conditions. U.S. 4874893 A.

Food Standards Australia New Zealand Act. 1991. Issue Nr. 149, New Zealand Gazette Office. Available from: https://www.dia.govt.nz//Pubforms.nsf/NZGZT/NZGazette149Nov01.pdf/$file/NZGazette149Nov01.pdf. Accessed 2014 May 5.

Frei H, Wurgler FE. 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.

FSANZ. 2003. Chloropropanols in food, an analysis of the public health risk. Technical Report Series Nr. 15. October 2003. Available from: http://www.foodstandards.gov.au/publications/documents/Chloropropanol%20Report%20(no%20appendices)-%2011%20Sep%2003b-2.pdf. Accessed May 5, 2014.

Gonzalez P, Racamonde I, Carro AM, Lorenzo RA. 2011. Combined solid-phase extraction and gas chromatography-mass spectrometry used for determination of chloropropanols in water. J Sep Sci 34:2697–704.

Gorlitz BD. 1991. *In vitro* mammalian cell HPRT-test with 3-chloro-1,2-propanol. In: Unpublished report Nr. G91/3 from Fraunhofer-Institute für Toxicologie und Aerosolforschung, Hannover, Germany.

Hall LA. 1946. Protein hydrolysates; flavor ingredients for foods. Food Ind 18:4808–16.

Hamlet CG, Sadd PA. 2009. Chloropropanols and chloroesters. In: Stadler RH, Lineback DR, editors. Process-induced food toxicants: occurance, formation, mitigation and health risks. NJ: John Wiley and Sons. p 175–214.

Hamlet CG, Sutton PG. 1997. Determination of the chloropropanols, 3-Chloro-1,2-propandiol and 2-Chloro-1,3-propandiol, in hydrolysed vegetable proteins and seasonings by gas chromatography/ion trap tandem mass spectrometry. Rapid Commun Mass Sp 11:1417–24.

Hamlet CG, Sadd PA, Gray DA. 2003. Influence of composition, moisture, pH and temperature on the formation and decay kinetics of monochloropropanediols in wheat flour dough. Eur Food Res Technol 216:122–28.

Henderson L, Bosworth H, Ransome S, Banks S, Brabbs C, Tinner A. 1987. An assessment of the mutagenic potential of 1,3-dichloro-2-propanol, 3-chloro-1,2-propanediol and a cocktail of chloropropanols using the mouse lymohoma TK locus assay. Unpublished report nr. ULR 130 ABC/861423, Huntingdon Research Centre, England.

Hu Z, Cheng P, Guo M, Zhang W, Qi Y. 2013. A novel approach of periodate oxidation coupled with HPLC-FLD for the quantitative determination of 3-chloro-1,2-propanediol in water and vegetable oil. J Agr Food Chem 61:6614–21.

Huang Z, Waxman DJ. 1999. High-performance liquid chromatographic-fluorescent method to determine chloroacetaldehyde, a neurotoxic metabolite of the anticancer drug ifosfamide, in plasma and in liver microsomal incubations. Anal Biochem 273:117–25.

Huang M, Jiang G, He B, Liu J, Zhou Q, Fu W, Wu Y. 2005. Determination of 3-chloropropane-1,2-diol in liquid hydrolyzed vegetable proteins and soy sauce by solid-phase micro extraction and gas chromatography/mass spectrometry. Anal Sci 21:1343–7.

Huang MQ, Wu JH, Sun PP, Sun BG, Zhang JL. 2013. Studies on formation of 3-monochloropropanediols in the models consisted of monoolein, sodium chloride and water. International Conference on Frontiers of Energy, Environmental Materials and Civil Engineering, 21-22 November 2013. Shanghai, China: DEStech Publications.

Huo J, Sun J, Chen J. 2013. Iron-fortified soy sauce in China-an assessment of 10 years of policy and business development. Micronutrient Fortification-Science and Strategy for Public Health Improvements in Asia Symposium, IUNS 20th International Congress of Nutrition. September 2013, Spain.

IARC. 1994. IARC monographs on the evaluation of carcinogenic risks to humans: some industrial chemicals. International Agency for Research on Cancer, Vol. 60, France.

IARC. 2013. Some chemicals present in industrial and consumer products, food and drinking water. International Agency for Research on Cancer, Vol. 101, France.

IARC. 2014. Agents classified by the IARC monographs, Volumes 1–109. Available from: http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf. Accessed 2014 May 5. p. 15.

ICH. 2008. Guidance for industry SIC (R2) dose selection for carcinogenicity studies. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm074-919.pdf. Accessed 2014 May 5.

Jaccaud E, Aeschbacher H. 1989. Evaluation of 3-chloro-1,2-propanediol (3-MCPD) in the bone marrow and colonic micronucleus test in mice. Unpublished report nr 1265, p 1–57, Nestec Ltd, Research Centre.

JECFA. 2002. 1,3-dichloro-2-propanol. Safety evaluation of certain food additives and contaminants. In: WHO food addit ser 48. Prepared by the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland.

Jeong J, Han BS, Cho WS, Choi M, Ha CS, Lee BS, Kim YB, Son WC, Kim CY. 2010. Carcinogenicity study of 3-monochloropropane-1,2-diol (3-MCPD) administered by drinking water to B6C3F1 mice showed no carcinogenic potential. Arch Toxicol 84:719–29.

Jones AR. 1975. The metabolism of 3-chloro-, 3-bromo- and 3-iodopropan-1,2-diol in rats and mice. Xenobiotica 5:155–65.

Jones AR. 1983. Antifertility actions of alpha-chlorohydrin in the male. Aust J Biol Sci 36:333–50.

Jones AR, Fakhouri. 1979. Epoxides as obligatory intermediated in the metabolism of α-halohydrins. Xenobiotica 9:595–9.

Jones P, Jackson H. 1976. Antifertility and dominant lethal mutation studies in male rats with dl-alpha-chlorohydrin and an amino-analogue. Contraception 13:639–46.

Jones AR, Davies P, Edwards K, Jackson H. 1969. Antifertility effects and metabolism of alpha and epi-chlorhydrins in the rat. Nature 224:83.

Jones AR, Milton DH, Murcott C. 1978. The oxidative metabolism of alpha-chlorohydrin in the male rat and the formation of spermatocoeles. Xenobiotica 8:573–82.

Jones AR, Porter K, Stevenson D. 1981. The renal toxicity of some halogenated derivatives of propane in the rat. Naturwissenschaften 68:98–9.

Kissa E. 1992. Determination of 3-chloropropanediol and related dioxolanes by gas chromatography. J Chromatogr A 605:134–8.

Kuballa T, Ruge W. 2003. Nachweis und Bestimmung von 3-Monochlorpropan-1,2-diol (3-MCPD) mit GC-MS/MS. Lebensmittelchem 57:57–8.

Laws of Malaysia. 2012.Malaysia food act 1983 and regulations 1985. The Commissioner of Law Revision. Malaysia.

Lee JK, Byun JA, Park SH, Kim HS, Park JH, Eom JH, Oh HY. 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 JK, Byun JA, Park SH, Choi HJ, Kim HS, Oh HY. 2005a. Evaluation of the potential immunotoxicity of 3-monochloro-1, 2-propanediol in Balb/c mice: II. Effect on thymic subset, delayed-type hypersensitivity, mixed-lymphocyte reaction, and peritoneal macrophage activity. Toxicology 211:187–196.

Lee JK, Ryu MH, Byun JA. 2005b. Immunotoxic effect of β-chlorolactic acid on murine splenocyte and peritoneal macrophage function *in vitro*. Toxicology 210:175–87.

Lee MR, Chiu TC, Dou J. 2007. Determination of 1,3-dichloro-2-propanol and 3-chloro-1,2-propandiol in soy sauce by headspace derivatization solid-phase microextraction combined with gas chromatography-mass spectrometry. Anal Chim Acta 591:167–72.

Lee Y, Choi I-K, Sheen Y, Park S, Kwon H. 2012. Moesin is a biomarker for the assessment of genotoxic carcinogens in mouse lymphoma. Mol Cells 33:203–10.

Leon N, Yusa V, Pardo O, Pastor A. 2008. Determination of 3-MCPD by GC-MS/MS with PTV-LV injector used for a survey of Spanish foodstuffs. Talanta 75:824–31.

Leung MKP, Chiu BKW, Lam MHW. 2003. Molecular sensing of 3-chloro-1,2-propanediol by molecular imprinting. Analytica Chimica Acta 491:15–25.

Li N, Liu Z, Jia X, Cui W, Wang W, Zhang X, Han C, Chen J, Wang M. 2003. Study on the toxicological effect of chloropropanols on rats. J Hyg Res 32:349-52.

Li Y, Liu S, Wang C, Li K, Shan YJ, Wang XJ, Sun CH. 2010. Novel biomarkers of 3-chloro-1,2-propanediol exposure by ultra-performance liquid chromatography/mass spectrometry based metabonomic analysis of rat urine. Chem Res Toxicol 23:1012–7.

Luh BS. 1995. Industrial production of soy sauce. J Ind Microbiol 14:467–71.

Lynch BS, Bryant DW, Hook GJ, Nestmann ER, Munro IC. 1998. Carcinogenicity of monochloro-1,2-propanediol (α-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 17:903–6.

MAF. 2011. Survey of chloropropanols in soy sauce: imported food monitoring. Available from: http://www.foodsafety.govt.nz/elibrary/industry/soy-sauce-survey-report2011.pdf. Accessed 2014 May 5.

Majeska J, Matheson DW. 1983. Quantitative estimate of mutagenicity of tris-[1,3-dichloro-2 propyl]-phosphate (TCPP) and its possible metabolites in *Salmonella*. Environ Mol Mutagen 5:478.

Marshall RM. 2000. 3-MCPD: induction of micronuclei in bone-marrow of treated rats. Unpublished Report Nr 1863/2-D5140, Covance Laboratories.

Matsudo T, Aoki T, Abe K, Fukuta N, Higuchi T, Sasaki M, Uchida K. 1993. Determination of ethyl carbamate in soy sauce and its possible precursor. J Agr Food Chem 41:352–56.

Matthew BM, Anastasio C. 2000. Determination of halogenated mono-alcohols and diols in water by gas chromatography with electron-capture detection. J Chromatogr A 866:65–77.

May C. 1991. *In Vitro* sister chromatid exchange assay in mammalian Cells. Unpublished report Nr. 91/4 CM, Fraunhofer- Institute für Toxicologie und Aerosolforschung, Hannover, Germany.

Meierhans DC, Bruehlmann S, Meili J, Taeschler C. 1998. Sensitive method for the determination of 3-chloropropane-1,2-diol and 2-chloropropane-1,3-diol by capillary gas chromatography with mass spectrometric detection. J Chromatogr A 802:325–33.

Merck Milipore. 2014. Liquid-liquid extraction. Available from: http://www.emdmillipore.com/MY/en/products/analytics-sample-prep/chromatography-for-analysis/sample-preparation-for-chromatography/liquid-liquid-extraction/QpGb.qB._b0AAAE_Hg93.Lxj,nav?CategoryName=0000002600002c8f800010023&CategoryDomainName=Merck-MerckMillipore#Benefits. Accessed 2014 Sep 23.

Miki K. 2007. Energy metabolism and sperm function. Soc Reprod Fertil Suppl 65:309–25.

Mukai C, Okuno M. 2004. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. Biol Reprod 71:540–7.

National Standard of the People's Republic of China. 2000. Acid hydrolyzed vegetable protein seasoning. SB10338-2000, File Nr. 7296-2000. Available from: http://down.gb99.cn:8080/gb1309/sb/20040514_sb10338-2000.rar. Accessed 2014 May 5.

NJDHSS. 1999. Glycerol-alpha-monochlorohydrin. Hazardous Substance Fact Sheet. Available from: http://nj.gov/health/eoh/rtkweb/documents/fs/2453.pdf. Accessed 2014 May 5.

NTP. 2005. 1,3-dichloro-2-propanol [CAS no. 96-23-1]: review of toxicological literature. National Toxicology Program, Research Triangle Park, NC.

Nyman PJ, Diachenko GW, Perfetti GA. 2003. Survey of chloropropanols in soy sauces and related products. Food Addit Contam 20:909–15.

OECD. 2008. Test no. 451: carcinogenicity studies. Available from: http://www.oecd.org/chemicalsafety/testing/41753121.pdf. Accessed 2014 May 5.

OECD. 2012. Guidance document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452, and 453 (2nd ed.). Available from: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=ENV/JM/MONO(2011)47&doclanguage=en. Accessed 2014 May 5.

OEHHA. 2010a. 3-monochloropropane-1,2-diol (3-MCPD; α-chlorohydrin). California Environmental Protection Agency. Available from: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/123mcpd.pdf. Accessed 2014 May 5.

OEHHA. 2010b. 1,3-dichloro-2-propanol (1,3-DCP; α, γ-dichlorohydrin). California Environmental Protection Agency. Available from: http://www.oehha.ca.gov/Prop65/hazard_ident/pdf_zip/13dcp.pdf. Accessed 2014 Sep 18.

Ohkubo T, Hayashi T, Watanabe E, Endo H, Goto S, Endo O, Mizoguchi T, Mori Y. 1995. Mutagenicity of chlorohydrins. Nippon Suisan Gakk 61:596–601.

Painter RB, Howard R. 1982. The Hela DNA-synthesis inhibition test as a rapid screen for mutagenic carcinogens. Mutat Res 92:427–37.

Pasupuleti VK, Braun S. 2010. State of the art manufacturing of protein hydrolysates. In: Pasupuleti VK, Demain AL, editors. Protein hydrolysates in biotechnology. New York: Springer. p 11–32.

Pesselman RL, Feit MJ. 1988. Determination of residue epichlohydrin and 3-chloropropanediol in water by gas chromatography with electron capture detection. J Chromatogr 439:448–52.

Piasecki A, Ruge A, Marquardt H. 1990. Malignant transformation of mouse M2-fibroblasts by glycerol chlorohydrines contained in protein hydrolysates and commercial food. Arzneimittel-Forsch 40:1054–5.

Plantinga WJ, Van Toorn WG, Stegen GHD. 1991. Determination of 3-chloropropane-1,2-diol in liquid hydrolyzed vegetable proteins by capillary gas chromatography with flame ionization detection. J Chromatogr A 555:311–4.

Racamonde I, Gonzalez P, Lorenzo RA, Carro AM. 2011. Determination of chloropropanols in foods by one-step extraction and derivatization using pressurized liquid extraction and gas chromatography-mass spectrometry. J Chromatogr A 1218:6878–83.

Reece P. 2005. The origin and formation of 3-MCPD in foods and food ingredients. FSA Project: C03017, 18, 19. Available from: http://www.foodbase.org.uk//admintools/reportdocuments/43_84_FINAL_REPORT.pdf. Accessed 2014 May 5.

Reineccius M. 2006. Changes in food flavor due to processing. In: Flavor chemistry and technology. 2nd ed. Boca Raton, Fla.: CRC Press. p 261–94.

Rétho C, Blanchard F. 2005. Determination of 3-chloropropane-1,2-diol as its 1,3-dioxolane derivative at the $\mu g\,kg^{-1}$ level: application to a wide range of foods. Food Addit Contam 22:1189–97.

Robert MC, Oberson JM, Stadler RH. 2004. Model studies on the formation of monochloropropanediols in the presence of lipase. J Agric Food Chem 52:5102–8.

Rodman LE, Ross RD. 1986. Gas-liquid chromatography of 3-chloropropanediol. J Chromatogr A 369:97–103.

Rossi AM, Migliore L, Lascialfari D, Sbrana I, Loprieno N, Tortoreto M, Bidoli F, Pantarotto C. 1983. Genotoxicity, metabolism, and blood kinetics of epichlorohydrin in mice. Mutat Res 118:213–26.

Schlatter J, Baars AJ, DiNovi M, Lawrie S, Lorentzen R. 2002. Safety evaluation of certain additives and contaminants, 3-chloro-1,2-propanediol. In: WHO food additives series 48, 57th meeting of the joint FAO/WHO, Rome.

Sennyah P. 2001. 22 Sauces withdrawn: foreign products found to contain excessive cancer-causing chemical. The New Straits Times, Malaysia. Available from: http://www.aboutsafety.com/article.cfm?id=1061. Accessed 2014 May 5.

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

Skalova S. 2005. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. Acta Med (Hradec Kralove) 48:75–80.

Spyres G. 1993. Determination of 3-chloropropane-1, 2-diol in hydrolysed vegetable proteins by capillary gas chromatography with electrolytic conductivity detection. J Chromatogr A 638:71–4.

Stevenson D, Jones A. 1984. The action of (R)-and (S)-α-chlorohydrin and their metabolites on the metabolism of boar sperm. Int J Androl 7:79–86.

Stolzenberg SJ, Hine CH. 1979. Mutagenicity of halogenated and oxygenated three-carbon compounds. J Toxicol Env Health 5:1149–58.

Stolzenberg SJ, Hine CH. 1980. Mutagenicity of 2- and 3-carbon halogenated compounds in the *Salmonella*/mammalian-microsome test. Environ Mol Mutagen 2:59–66.

Sun J, Bai S, Bai W, Zou F, Zhang L, Su Z, Zhang Q, Ou S, Huang Y. 2013. Toxic mechanisms of 3-monochloropropane-1,2-diol on progesterone production in R2C rat Leydig cells. J Agr Food Chem 61:9955–60.

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

Tokyo AP. 2004. Global trade dispute brewing over soy sauce. The Taipei Times, Taiwan. Available from: http://www.taipeitimes.com/News/worldbiz/archives/2004/09/27/2003204609. Accessed 2014 May 5.

Van Bergen CA, Collier PD, Cromie DDO, Lucas RA, Preston HD, Sissons DJ. 1992. Determination of chloropropanols in protein hydrolysates. J Chromatogr A 589:109–19.

Van Den Wijngaard AJ, Janssen DB, Witholt B. 1989. Degradation of epichlorohydrin and halohydrins by bacterial cultures isolated from freshwater sediment. J Gen Microbiol 135:2199–208.

Van Duuren BL, Goldschmidt BM, Katz C, Seidman I, Paul JS. 1974. Carcinogenic activity of alkylating agents. J Natl Cancer I 53:695–700.

Velisek J, Davidek J, Hajslova J, Kubelka V, Janicek G, Mankova B. 1978. Chlorohydrins in protein hydrolysates. Z Lebensm Unters For 167: 241–4.

Velisek J, Calta P, Crews C, Hasnip S, Dolezal M. 2003. 3-chloropropane-1,2-diol in models simulating processed foods: precursors and agents causing its decomposition. Czech J Food Sci 21:153–61.

Vicente E, Arisseto AP, Monteiro V, Furlani RPZ, Toledo MCF. 2011. A survey of chloropropanols (3-MCPD and 1,3-DCP) in soy sauces and similar products from Brazil. Toxicol Lett 205:S145–6.

Weisburger EK, Ulland BM, Nam J, Gart JJ, Weisburger JH. 1981. Carcinogenicity tests of certain environmental and industrial chemicals. J Natl Cancer I 67:75–88.

Weisshaar R, Perz R. 2010. Fatty acid ester of glycidol in refined fats and oil. Eur J Lipid Sci Technol 112:158–65.

Wong KO, Cheong YH, Seah HL. 2006. 3-Monochloropropane-1,2-diol (3-MCPD) in soy and oyster sauces: occurrence and dietary intake assessment. Food Control 17:408–13.

WTO. 2002. Committee on sanitary and phytosanitary measures. Report G/SPS/GEN/204/Rev.2.

Xing X, Cao Y. 2007. Determination of 3-chloro-1,2-propanediol in soy sauces by capillary electrophoresis with electrochemical detection. Food Control 18:167–72.

You ZH, Liu ZQ, Zheng YG. 2013. Properties and biotechnological applications of halohydrin dehalogenase: current state and future perspectives. Appl Microbial Biotechnol 97:9–21

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 11:1–157.

Zhang H, Yu H, Wang X, Zheng W, Yang B, Pi J, He G, Qu W. 2012. (S)-α-chlorohydrin inhibits protein tyrosine phosphorylation through blocking cyclic AMP - protein kinase A pathway in spermatozoa. PLoS One 7:e43004.