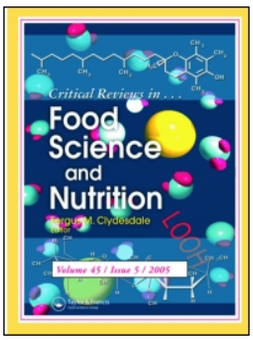

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3-MCPD: A Worldwide Problem of Food Chemistry

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3-Monochloropropane-1,2-diol (3-MCPD) is a heat-induced food contaminant that has been widely investigated for decades. This paper presents an overview of current knowledge about 3-MCPD, including its formation routes, occurrence in various foodstuffs, analytical approach, toxicological aspects, and future research perspectives. So far, 3-MCPD was determined in its free and bound form in thermally treated foods, edible oils and fats, and infant foods including human breast milk. Contaminants in infant foods and human breast milk were highlighted in this paper as a serious problem as they can pose a potential hazard for infants. The analytical approach of 3-MCPD determination has been modified for over a decade. Nowadays, the method based on determining the derivative of this compound by using gas chromatography and mass spectrometry is widely used. However, there is still a big need for developing new methods that would produce repeatable results. Some of the toxicologic aspects associated with 3-MCPD still remain unknown. A number of studies on the carcinogenicity and genotoxicity of 3-MCPD were carried out on rodents; however, no clinical studies on humans have been reported so far. Moreover, both detrimental effect on kidneys and antifertility activity have been widely reported. The knowledge of 3-MCPD absorption into body fluids and tissues and its metabolic pathways is based on sometimes conflicting data derived from different studies. In conclusion, although a lot of research has been carried out on 3-MCPD, there is still a need for further research in this area.

Keywords 3-MCPD, edible oils, infant foods, thermally treated foods, analytical approach

INTRODUCTION

Undesired compounds, which contaminate foodstuffs, are formed mostly during food processing. One such toxicant is a group of chemicals called chloropropanols, represented by 3-monochloropropane-1,2-diol (3-MCPD). 3-MCPD was first discovered in 1978 in its free form (Velisek et al., 1978), and its bound (esterified) form was identified in 1980 (Davídek et al., 1980); both forms were found in acid–hydrolyzed vegetable protein (HVP) used in soy sauce production. The carcinogenicity and genotoxicity of 3-MCPD emerged as a serious problem over 10 years ago. For this reason, in 2001, the experts of the Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the European Commission (EC) Scientific Committee on Food established a maximum tolerable daily intake (TDI) for 3-MCPD of 2 μg/kg body weight per day for HVP and soy sauce (Food Standards, 2003). In the same year, the EC set the regulatory limit of 0.02 mg/kg 3-MCPD for foodstuff with regard to HVP and soy sauce (European Commission Regulation, 2001). At that time, it was assumed that 3-MCPD occurs in many foodstuffs that human beings consume in high amounts daily. Therefore, the UK Food Advisory Committee suggested that the industry should make an effort to reduce the content of 3-MCPD in both foods and food ingredients to the lowest possible level (Food Advisory Committee, 2000). Since then, a lot of research with regard to the chemical properties, formation, occurrence, and analytics of 3-MCPD has been carried out in order to mitigate its presence in foodstuffs and the associated risk to human health.

Chemically, 3-MCPD is a glycerol chlorohydrin, formed when one hydroxyl group in a glycerol molecule is replaced by chlorine atom. This stereospecific reaction results in two enantiomers (Figure 1) exhibiting different biological activity (Hamlet et al., 2002).

3-MCPD is a non-volatile compound with a high boiling point at 213°C. It is readily soluble in water, and has high

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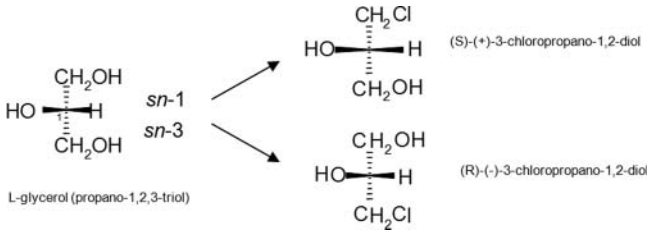

Figure 1 Fisher projection of monochloropropanediol isomers with relation to L-glycerol molecule.

solubility in fats and fatty matrices (National Toxicology Programme, 2013).

The aim of this work is to present the current knowledge about 3-MCPD including its formative mechanism, occurrence in thermally treated foods, edible oils and fats, infant foods and human breast milk, toxicology, and analytical approach, and to discuss the issues that should be further investigated in the future. In addition, this paper focuses, to a great extent, on analytical perspectives in order to emphasize the need for developing new analytical methods and to highlight the problem of the presence of 3-MCPD in infant foods and human breast milk. The aforementioned topics should undoubtedly be considered as novel among already published review and research papers with regard to this chloropropanol.

FORMATION ROUTES

The formation routes of 3-MCPD can be divided into three groups. The first pathway is acid hydrolysis (used in HVP production), which is the reaction of hydrochloric acid with residual vegetable oil. The second route is heat processing, which is independent of the presence of acid–HVP. In this way, 3-MCPD is formed from lipids and sodium chloride, which can be present in the material naturally or may be added during processing. Heat can affect food not only during industrial production but also during domestic cooking, such as baking or frying. The two pathways were described in detail by Baer et al. in 2010.

The third and recently most investigated pathway is the release of free 3-MCPD from its bound (esterified) form. 3-MCPD can occur as a mixture of mono- and diesters, usually of palmitic, oleic, or stearic fatty acids. The formative mechanism of 3-MCPD esters has not been fully understood until now. An earlier hypothesis, published in 1991 by Collier et al., proposed a mechanism based on triacylglycerol reaction wherein a key step is nucleophilic substitution of the acyl group by the chloride anion at positions activated by neighboring ester groups that results in a chloropropanediol diester. According to a more recent assumption, the formation of 3-MCPD esters progresses through the cyclic acyloxonium ion as an intermediate, which derives from the elimination of hydroxyl groups from mono- and diacylglycerols (MAG and DAG) (Bakhiya et al., 2011). Rahn and Yaylayan (2011) provided further evidence for this hypothesis by monitoring this intermediate-ion formation in palmitin systems using infrared (IR) spectroscopy and an isotope labeling technique. Both the above assumptions take into consideration the presence of chloride ions, which seem to have a strong influence on the formation of 3-MCPD esters (Shimizu et al., 2013).

3-MCPD can be released from its esterified form via lipase-catalyzed hydrolysis in the human gastrointestinal tract during digestion. In 2004, Robert et al. simulated the formation of chloropropanols using a model consisting of mammalian, plant and fungal lipases, vegetable oil or fat, water, and sodium chloride. Three years later, Seefelder et al. (2008) used a simple intestinal model to quantify the level of 3-MCPD release from the ester form. Mono- and diesters were incubated with pancreatic lipase and porcine bile extract. The monoesters were almost completely hydrolyzed after one minute. The 3-MCPD release from diesters was slower, reaching 45% after one minute, 65% after two minutes, and almost 100% after approximately one hour. Moreover, the authors proposed that 3-MCPD esters have the same metabolic pathway as the one known for acylglycerols during human digestion, where pancreatic lipases release glycerol only from 1- and 3-monoacylglycerols. Triacylglycerols are hydrolyzed to diacylglycerols and incorporated into lipoproteins. However, this hypothesis appeared to be speculative, mainly because their studies have already proven a partial release of free 3-MCPD from the diester form. Moreover, lipoproteins can be bioavailable through the lymphatic system (Chon et al., 2007). According to recent investigations on lipolytic enzymes, there are three enzymes that can completely hydrolyze triacylglycerols (Lass et al., 2011). Thus, it can be assumed that these enzymes may release free 3-MCPD from the diester form (Abraham et al., 2013). Until now, no in vivo research has been delivered to verify the aforementioned postulates; therefore, the assumption of 100% hydrolysis of 3-MCPD esters should be taken into account while assessing the human health risk.

Besides the formative pathways of 3-MCPD described above, other routes were also proposed. Myszkowski and Zielinski (1965) assumed that monochloropropanediol can form from allyl alcohol, chlorine, and water. Collier et al. (1991) reported the possibility of 3-MCPD formation from carbohydrates (pentosan and pectin) and hydrochloric acid. Cerbulis et al. (1984) demonstrated that small but significant amounts of 3-MCPD diesters were isolated in raw goat's milk; therefore, the authors assumed these compounds occur naturally in food. However, these postulates have not been proved in later studies.

OCCURRENCE IN FOOD

During the past decade, the development of new analytical methods as well as renewed interest in chloropropanols as dangerous food toxicants caused 3-MCPD to be determined in a number of different foodstuffs. The investigated foods can be

divided into three main groups, i.e. thermally treated foods, edible oils and fats, and infant and baby foods (including human breast milk). In the following sections, each group has been described in detail in the consecutive paragraphs.

Thermally Treated Foods

Crews et al. were the first researchers to examine a large number of foodstuffs marketed in the United Kingdom (2001). The main food group contaminated by 3-MCPD was cereal-derived products; the highest concentration of monochloropropanediol was determined in toasted bread (0.088 mg/kg) and cream crackers (0.087 mg/kg). The same group of foodstuffs was analyzed by Breitling-Utzmann et al. (2003). The highest concentration of 3-MCPD was also found in toasts (>0.500 mg/kg in a well-toasted bread) and breadcrumbs (>0.400 mg/kg). It seems that the exposure to high temperatures is a key step in 3-MCPD formation in cereal-derived foods. Breitling-Utzmann et al. (2005) also tested the influence of bread ingredients on 3-MCPD formation. It appeared that the addition of fat and baking agent (consisting of sugar, flour, soy flour, calcium sulfate, and mono- and diacylglycerols of edible fatty acids as emulsifiers) may influence the concentration of 3-MCPD in the final product, with emulsifiers and sugar having the strongest effect. With regard to the precursors of 3-MCPD, Hamlet et al. used model dough systems to estimate the production of chloropropanols in leavened (Hamlet et al., 2004a) and unleavened (Hamlet et al., 2004b) dough. In both cases, glycerol or compounds based on a glycerol skeleton, such as monoacylglycerols and phosphatidylglycerols, were the main 3-MCPD precursors.

Another foodstuff in which heat treatment seems to trigger 3-MCPD formation is malt-derived products, such as food-grade malted grains, malt flours, and malt extracts (color and flavor agents). The original components of these products can promote the formation of 3-MCPD; therefore, there is no need to add fat, acid, or chloride. However, significant amounts of 3-MCPD in this type of foods were detected only in dark brewing malt (0.247 mg/kg) [22]. The amount of free 3-MCPD present in beer is relatively low (10 μg/L) (IARC, 2000), but it seems that it may be bound to other beer components such as, acids, aldehydes. and alcohol, which can significantly exceed the free-form content (Divinová et al., 2007).

Smoked foods also contain significant amounts of 3-MCPD (>0.02 mg/kg). Kuntzer and Weisshaar (2006) analyzed the influence of food smoking on the formation of 3-MCPD in fermented sausages and ham. The smoking process appeared to be the main source of 3-MCPD, especially with regard to the type of wood and the duration of processing (Kuntzer and Weisshaar, 2006; FAO, WHO, 2007). In contrast to cereal- and malt-derived products, lipids are not considered precursors of 3-MCPD in smoked foods, and the formative mechanism from 3-hydroxyacetone during cracking of cellulose was proposed instead. Apart from this, Reece (2005) suggested that the concentration of salt in the brine used in the smoking process may also be an influential factor in the formation of 3-MCPD.

Furthermore, 3-MCPD was determined in heat-processed foodstuffs such as coffee, particularly roasted coffee after a prolonged roasting process, and instant coffee (Doležal et al., 2005); melted or grilled cheese prepared via domestic cooking (in which the most possible precursors are abundant components such as chloride ions and glycerol) (Crews et al., 2001); and meat such as salami, bacon, and hamburgers, where glycerol does not seem to be the direct precursor (Baer et al., 2010).

The survey of JECFA (2007) summarized the content of 3-MCPD in various foodstuffs consumed by adults and young children per kilogram body weight with regard to the estimates of exposure. Average dietary exposures ranged from 0.02 to 0.7 μg per kilogram body weight for a wide range of foods, including soy sauce and related foodstuffs in which the average concentration of 3-MCPD was the highest (8 mg/kg). The exposures estimated for young children, who constitute the highest percentage share among other consumer groups, ranged from 0.06 to 2.3 μg per kilogram body weight. Chung et al. (Chung et al., 2008) also reported a general overview on 3-MCPD concentration in foodstuffs marketed in Hong Kong that was based on the analysis of 318 samples of different food items: 101 types of food contained 3-MCPD at the concentration range between 3 and 66 μg/kg.

Edible Oils and Fats

This group of foodstuffs is presented separately although it is processed at high temperatures during both industrial processing (refining process including seed roasting or deodorization) and domestic cooking, e.g. frying. In most cases, oils and fats, including lipid fractions of some foodstuffs, e.g. goat's milk (Rahn and Yaylayan, 2011), potato fries, doughnuts, or salty crackers (Hamlet et al., 2011), contain fatty acid esters of 3-MCPD. The bound form of 3-MCPD was determined for the first time in this group of foods by Gardner et al. (1983) in rapeseed oil (3800 μg/kg) in the presence of hydrochloric acid. Further investigations of edible oils were carried out by Zelinkova et al. (Zelinková et al., 2006) after analyzing 45 samples of crude and refined oils. Oils containing free 3-MCPD at the concentration range between <3 and 24 μg/kg exhibited much higher levels of the esterified 3-MCPD, which varied from <100 μg/kg (virgin oils) to 2462 μg/kg (refined olive oils). This finding resulted in an intensive investigation of the oil and fat sector of food industry. In the years 2007 and 2008, over 400 samples of different fats and oils were analyzed by the Chemical and Veterinary Test Agency in Stuttgart (German Food Control Agency) (ILSI, 2009), and significant amounts of bound 3-MCPD were found in almost all refined fats and oils. The highest levels (>4000 μg/kg) were determined in palm oils, whereas frying fat and margarine

contained relatively high concentrations of 3-MCPD esters. Large amounts of 3-MCPD esters (540–4840 μg/kg) were also determined in the fat fraction of coffee creamers, cream aerosols, and bouillon cubes (Karsulinova et al., 2007). Foodstuffs prepared by frying in palm oil, such as potato fries and potato chips, also contained significant amounts of 3-MCPD esters (Zelinková et al., 2009a). An overview on bound 3-MCPD determined in fat, oil, and lipid fractions was presented by Weisshaar (2011). Undoubtedly, this group of foodstuffs needs to be investigated further with regard to the precursors and formative mechanism of 3-MCPD because it contributes to the overall food consumption and, consequently, to the daily intake of 3-MCPD by humans in a significant way.

Infant Foods and Human Breast Milk

This group encompasses foods meant for consumption by infants. It is presented separately because of the small body weight of the consumers, which contributes significantly to risk assessment in relation to dietary exposure. Human breast milk can be used as a toxicity indicator of various compounds with regard to human tissues and biological fluids. Velisek et al. (2007) analyzed 12 samples of human breast milk. None of the samples contained free 3-MCPD at concentrations higher than the LOD (3 μg/kg); however, all samples contained significant levels of esterified 3-MCPD. The average concentration of 3-MCPD was 1014 μg/kg isolated fat, which corresponds to 35.5 μg/kg milk. For comparison, samples of human breast milk collected after 14–76 days after childbirth contained 930 μg/kg isolated fat, which corresponds to 12 μg/kg milk. These results indicate that, at the onset of lactation, lipids are secreted into the breast milk together with toxicants in women who have digested contaminated food. Zelinková et al. (2009b) analyzed 14 samples of infant foods that had a composition similar to that of human breast milk. None of the samples contained significant amounts of free 3-MCPD; however, high levels of bound 3-MCPD were detected in all of them. The concentration level of bound 3-MCPD was 62–588 μg/kg isolated fat, which corresponds to <300–2060 μg/kg milk. The determined amounts were proportional to the fat content in each infant food. As previously mentioned, the tolerable daily intake can be easily exceeded in this case because of the fact that infants have small body weight and only consume the above-described foods. It goes without saying that this topic needs to be investigated further.

ABSORPTION, METABOLISM, AND TOXICITY OF 3-MCPD

Absorption, metabolism, and short- and long-term toxicity of chloropropanols have been investigated since the 70s. In the studies conducted on rodents, 3-MCPD exhibited a damaging effect on the urinary tract and decreased fertility (Schilter et al., 2011). In addition, mutagenic activity was observed in vitro; however, in vivo studies did not confirm this finding. Clinical studies in human beings have not been reported (JECFA, 2002). A summary of both early and more recent publications on the toxicological aspects of 3-MCPD is presented below.

Absorption, excretion, and biotransformation of 3-MCPD and its metabolites were studied by Edwards et. al. (1975) and Jones et al. (1978), but the data obtained were conflicting. Edwards et al. (1975) claimed that 3-MCPD is absorbed and distributed in the body fluids, whereas a study by Jones et al. (1978) did not confirm the accumulation of either of 3-MCPD or its metabolites in tissues. However, the latter reported that approximately 30% of the initial dose of [^{36}Cl]3-chloro-1,2-propanediol was recovered as β-chlorolactate (3-MCPD metabolite).

Jones (1978) explained the metabolic pathways of 3-MCPD via two different routes (Figure 2). The bacterial pathway (tested on *Pseudomonas* and *Arthrobacter* [Slater, 1994]) develops through glycidol (genotoxic compound) to glycerol or mercapturic acid. Metabolites found in vivo in rats, mice, and other mammals differ from those identified in bacteria. Toxic effects on rats are associated with β-chlorolactaaldehyde and β-chlorolactic acid causing testicular and renal toxicities (Olsen, 1993). These effects derive from the inhibition of enzymes involved in glycolysis by β-chlorolactaaldehyde (Jones and Porter, 1995). Moreover, oxalic acid, a metabolite of β-chlorolactic acid, has an influence on the renal system (Bakhiya et al., 2011). Nephrophathy and spermatocele formation in rats is associated with a similar mechanism because both kidneys and the epididymis have the same embryonic origin (Jones, 1983).

The short-term toxicity of 3-MCPD was examined in rodents. The kidney was the organ most affected after a short exposure (four weeks). A single intraperitoneal dosage of 3-MCPD caused acute glomerular nephritis in Sprague–Dawley rats (Jones et al., 1978) and severe proteinuria in Wistar rats (Morris and Williams, 1980). Moreover, an increase in kidney weight was observed (Cho et al., 2008a). Apart from rodents, some research on the short-term toxicity of 3-MCPD was also carried out in primates (Kirton et al., 1970). After a six-week study, a damaging effect on bone marrow was observed as manifested by anemia, leucopenia, and thrombocytopenia. However, these toxicologic effects have not been investigated further and in more detail.

Long-term toxicity (carcinogenicity) was studied in mice and rats. Research carried out on mice did not demonstrate the presence of tumors after 3-MCPD intake (Van Duuren et al., 1974). Several bioassays regarding 3-MCPD carcinogenic effect were conducted. The reported lesions associated with carcinogenic activity are summarized in Table 1. Conflicting data were reported by Jayoung et al. (2010); dosing 3-MCPD

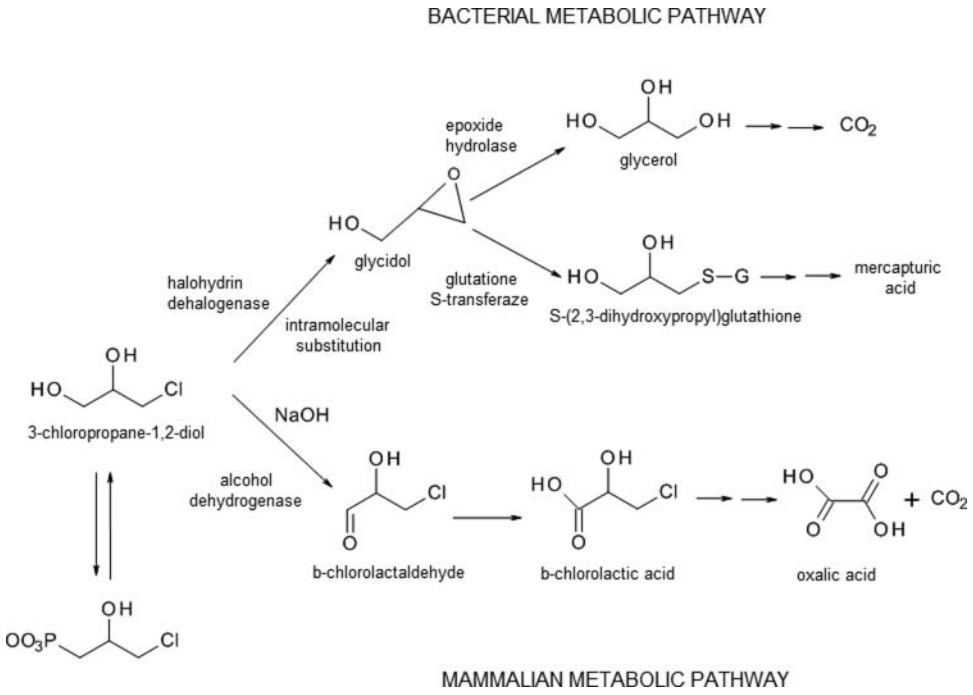

Figure 2 Bacterial and mammalian metabolic pathway of 3-MCPD.

in the drinking water to B6C3F1 rats had no carcinogenic effect. Further, Ramya et al. (2006) did not observe any negative effect on rat testicular organogenesis after exposure to 3-MCPD.

The potential genotoxicity of 3-MCPD raises a lot of questions. In bacteria, the compound exhibits a positive mutagenic effect (Silhankova, 1982). Generally, the tests conducted on mammalian cells, including gene mutation test in mouse, were also positive (May, 1991). However, it is commonly believed that 3-MCPD causes tumors through nongenotoxic mechanisms (Jones, 1978).

ANALYTICAL PROCEDURES USED FOR DETERMINATION OF 3-MCPD IN FOOD SAMPLES

Determination of trace or even ultra-trace levels of 3-MCPD in food samples characterized by complex matrix composition and the presence of compounds displaying similar physicochemical properties as 3-MCPD poses a great challenge for analytical chemists. In addition, the lack of chromophore eliminates the application of high performance liquid chromatography with ultraviolet or fluorescence detection, while both a high boiling point and low molecular weight

Table 1 Data on carcinogenicity studies of 3-MCPD in rats

Organ	Lesion	Rat species	Gender	3-MCPD* [ppm]	Ref.
Kidney	Nephropathy	Fisher 344	Male	20	Sunhara et al., 1993
Kidney	Urothelial hyperplasia	Fisher 344	Male	20	Sunhara et al., 1993
Kidney	Tubular hyperplasia	Fisher 344	Male	20	Sunhara et al., 1993
Kidney	Tubular adenoma	Fisher 344	Male	20	Sunhara et al., 1993
Kidney	Tubular carcinoma	Sprague–Dawley	Male	400	Cho et al., 2008b
Testis	Leydig cell hyperplasia	Fisher 344	Male	20	Sunhara et al., 1993
Testis	Leydig cell adenoma	Fisher 344	Male	20	Sunhara et al., 1993
Testis	Leydig cell carcinoma	Fisher 344	Male	500	Sunhara et al., 1993
Testis	Atrophy	Sprague–Dawley	Male	25	Cho et al., 2008b
Testis	Arteritis/periarteritis	Sprague–Dawley	Male	25	Cho et al., 2008b
Kidney	Nephropathy	Fisher 344	Female	20	Sunhara et al., 1993
Kidney	Urothelial hyperplasia	Fisher 344	Female	100	Sunhara et al., 1993
Kidney	Tubular hyperplasia	Fisher 344	Female	20	Sunhara et al., 1993
Kidney	Tubular adenoma	Fisher 344	Female	20	Sunhara et al., 1993
Kidney	Tubular carcinoma	Sprague–Dawley	Female	100	Cho et al., 2008b

*Lowest significant dose.

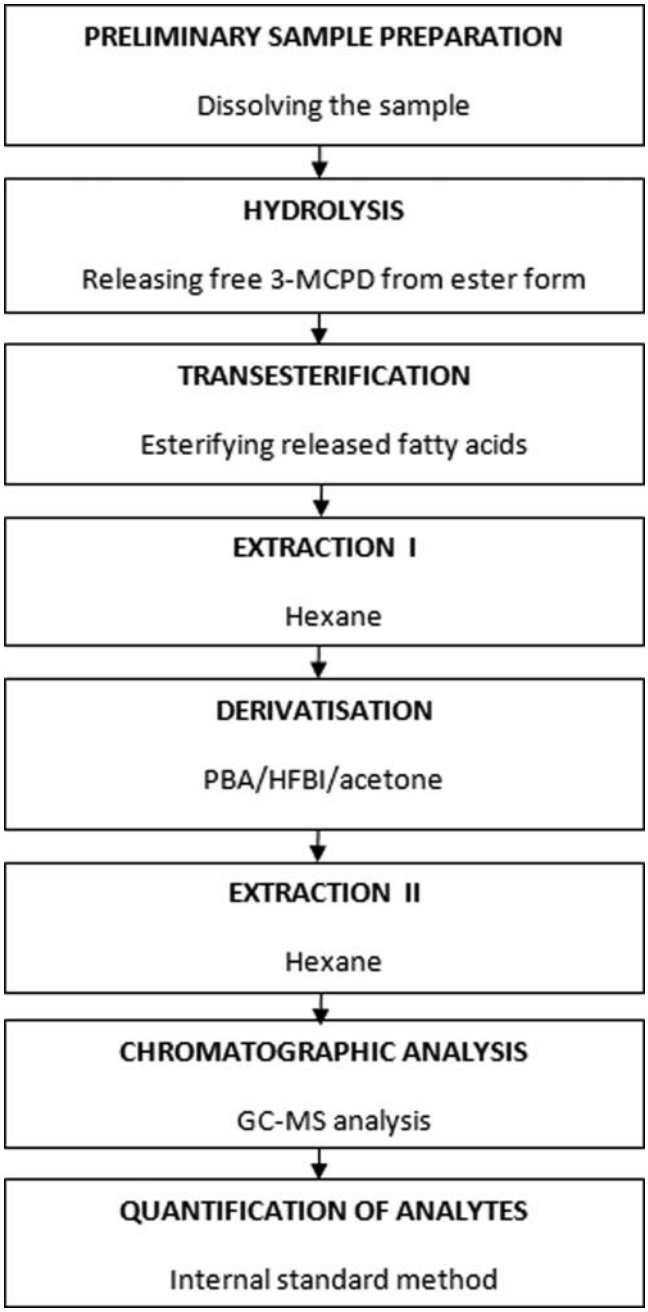

Figure 3 Analytical approach used with GC-MS technique.

affect gas chromatography and mass spectrometry (GC-MS) analysis (Baer et al., 2010).

In 1978, Velisek et al. for the first time determined 3-MCPD by using silica gel column separation and nuclear magnetic resonance. Later, GC-MS methods became widely applied in research laboratories. However, due to sample matrix composition, sample preparation and derivatization was needed to avoid interactions between 3-MCPD and GC components or compounds in the matrix. Currently, there is no analytical procedure approved by the EC, but experts have set the criteria to provide acceptable

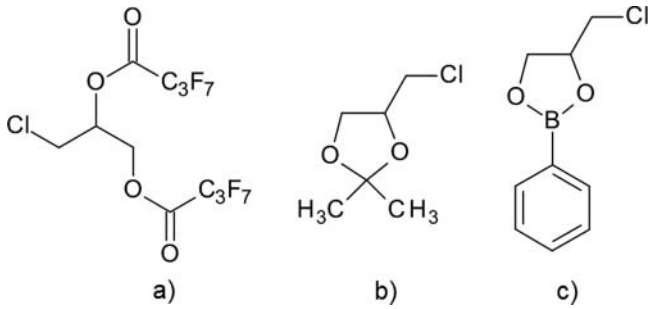

Figure 4 Derivatization reaction products of 3-MCPD with: (a) HFBI; (b) PBA; and (c) acetone.

and repeatable results, e.g. field blanks, recovery, LOD, LOQ, and precision (European Commission Regulation, 2007).

In case of samples analyzed by GC-MS, the most important steps in sample preparation are acid or alkaline transesterification reaction (in order to release free 3-MCPD from its esterified form), derivatization reaction (with the aim of obtaining volatile derivative to be determined by GC), and the extraction of a derivative (Figure 3).

The first derivatization agent used for the determination of 3-MCPD was heptafluorobutyrylimidazole (HFBI); its reaction with diols results in di(heptafluoro)butyrate (Hamlet and Sutton, 1997) (Figure 4a). However, Retho and Blanchard (2005) suggested that the derivatization reaction with HFBI exhibits poor selectivity because HFBI may react with nucleophilic molecules. The mass spectrum of the obtained derivative displays a low-intensity signal in the region characteristic for specific ions, which may indicate insufficient (incomplete) derivatization. Instead of the imidazole form, heptafluorobutyric acid anhydride (HFBA) can be used (Xu et al., 2006). The most commonly used derivatizing agent is phenylboronic acid (PBA), whose reaction with diols results in dioxaborolane derivatives (Divinová et al., 2004) (Fig 4b). In addition, there are methods using acetone (Figure 4c) as a derivatizing agent (reaction results in dioxolanes) and reaction solvent, but they have not been widely applied because of many unwanted substances in the resulting extract (Maierhans et al., 1998). Recently, the use of N,O-bis(trismethylsilyl)trifluoroacetamide (BSTFA) as a derivatizing agent was also reported (Racamonde et al., 2011).

Analytical procedures for determination of 3-MCPD using GC-MS methods have been modified for over a decade. Nowadays, two such modified procedures are widely used in quality control laboratories. The DGF (Deutsche Gesellschaft fur Fettwissenschaft) method (2011) and the method recommended by the *Joint Research Centre Institute for Reference Materials and Measurements (JRC IRMM)* (Karasek et al., 2010) allows the indirect determination of 3-MCPD in free and bound forms. However, the aforementioned methods are questionable with regard to their reliability, as some research (Karasek et al., 2011; Kaze et al., 2011) proved that alkaline conditions cause the partial conversion of glycidol to 3-MCPD and, thus,

the final result of determination includes 3-MCPD not present in the initial sample. The solution to this problem may be the application of acid transesterification (Ermacora and Hrncirik, 2012), in which glycidyl esters do not interfere with 3-MCPD esters during analysis. However, the method is considered to be time-consuming because of the transesterification step (4–16 hours). The critical factors of the indirect determination of 3-MCPD have been summarized by Hrncirik et al. (Hrncirik et al., 2011).

Apart from GC methods, a procedure using liquid chromatography with time-of-flight mass spectrometry (LC-TOFMS) was also developed. This method seemed to be simple, not time-consuming, and without any additional steps of analyte transformation and is, thus, a direct technique. However, it has not been widely applied in routine analysis in industrial laboratories because of high costs associated with the damage to the MS system due to the presence of sodium in the mobile phase. Moreover, complex matrices such as food samples (e.g. oils) contaminate the instrument, which needs to be cleaned daily prior to use. In addition, the components of the ESI instrument, including the nebulizer needle, corrode and have to be replaced weekly and this increases the overall costs (under average conditions, the parts are replaced after a year of constant use) (Haines et al., 2011).

Basic information on analytical approaches to determine 3-MCPD are presented in Table 2. It seems that GC-MS methods, although of questionable reliability and repeatability, still dominate the present-day analytics of 3-MCPD.

There are several methods of 3-MCPD determination using sophisticated and thus expensive equipment such as dispersive microextraction followed by GC-MS (Zhao et al., 2012), supercritical fluid chromatography/tandem mass spectrometry (Hori et al., 2012), and liquid chromatography/tandem mass spectrometry (Yamazaki et al., 2013). However, they have not found wider application in laboratories so far.

Current search for new methods is focusing on eliminating (1) the complicated operations during sample preparation to avoid undesired reactions and transformations, and (2) the step of liquid extraction in accordance with the principles of green chemistry. These aims may be achieved with the use of headspace derivatization and solid-phase microextraction (HS-SPME) combined with GC analysis (Huang et al., 2005; Maw-Rong et al., 2007). The published methods were successfully applied for analyzing soy sauces and, thus, they are promising with respect to other food samples. The analytical approach is as follows: 3-MCPD was first derivatized with PBA or N-methyl-N-(trimethylsilyl)-trifluoroacetamide) (MSTFA), then extracted by HS-SPME, and finally detected with GC-MS. Moreover, a solid-

Table 2 Data on selected analytical methods for determining free and bound 3-MCPD

Sample	Analytes	Derivatization Agent	Detection	LOD for 3-MCPD (μg/kg)	Ref.
Standards	3-MCPD	PBA	GC-MI-FTIR	—	Rodman and Ross, 1986
Seasonings	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	None	GC-MS SIM	100	Wittmann, 1991
HVP	3-MCPD	PBA	GC-FID	500–1000	Plantiga et al., 1991
HVP	3-MCPD	None	GC-ECD	250	Spyres, 1993
HVP, seasonings	3-MCPD, 2-MCPD	HFBI	GC-MS/MS MRM (ion trap)	5	Hamlet and Sutton, 1997
Water	3-MCPD, 1,3-DCP (& bromo-propanediols)	HFBA	GC-ECD	0,7	Matthew and Anastasio, 2000
Soya sauce	1,3-DCP, 3-MCPD	HFBA	GC-MS SIM	5	Chung et al., 2002
Model systems	3-MCPD	None	HPLC-RI	—	Hamlet and Sadd, 2002
Various foodstuffs	3-MCPD	PBA	GC-MS SIM	3–10	Breitling-Utzmann et al., 2003
Various foodstuffs	Free and bound 3-MCPD	PBA	GC-MS SIM	3	>Divinová et al., 2004
Cereal products	Bound 3-MCPD	None	GC-MS Scan	—	Hamlet and Sadd, 2004
Oils	3-MCPD after cleavage of MCPD esters	PBA	GC-MS SIM	50–150	Cao et al., 2009
Oils	Bound 3-MCPD	PBA	GC-MS SIM	50	Kuhlmann, 2011
Oils	Bound 3-MCPD, bound glycidol	None	LC-TOFMS	—	Haines et al., 2011

phase extraction combined with GC-MS was applied to determine chloropropanols (1,3-DCP and 3-MCPD) in water samples (Gonzalez et al., 2011). Besides being environment-friendly, the above methods are simpler and faster, and thus more appropriate for routine analysis. Further development of these methods should be highly recommended.

CONCLUSIONS

3-MCPD is one of the most actively investigated chloropropanols. Because of research results indicating its carcinogenic potential, numerous analytical methods were developed in order to determine the free and bound forms of 3-MCPD in various foodstuffs. However, there is still a huge need to carry out studies on 3-MCPD as many facts still remain unknown.

Current analytical methods for 3-MCPD determination still need to be improved as regards sample preparation techniques. The methods based on liquid extraction should be replaced with those that are more efficient and environment friendly, e.g. HS or SPME. Currently, the widely applied method developed by the DGF is time-consuming and requires the use of significant amounts of solvents. Undoubtedly, the methods should be as economic and simple as possible in order to apply them in industrial laboratories for routine analyses of complex matrices described in this paper. Moreover, the formative routes and mechanisms need to be fully explained in relation to industrial processing, with the aim of mitigating the presence of 3-MCPD in foodstuffs by changing the process conditions (as in oil refining process Pudell et al., 2011; Hrncirik and van Duijn, 2011; Zulkurnain et al., 2012; Zulkurnain et al., 2013) or by treating the already processed product (as oils after refining process on adsorbent material or with enzymes Bornscheuer and Hessler, 2010; Strijowski et al., 2011). Finally, there is a growing concern associated with the release of bound 3-MCPD into its free form that results in bioavailability and absorption of 3-MCPD into human body fluids and tissues, e.g. human breast milk mentioned in this paper. For these reasons, this topic requires additional studies.

This paper provides an overview of current knowledge regarding 3-MCPD and makes a strong statement in support of further research in this area.

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