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Occurrence of 3-chloro-propane-1,2-diol (3-MCPD) and related compounds in foods: a review

C. G. Hamlet†*, P. A. Sadd†, C. Crews‡, J. Velíšek§ and D. E. Baxter¶

† *RHM Technology Ltd, The Lord Rank Centre, Lincoln Road, High Wycombe HP12 3QR, UK*

‡ *Central Science Laboratory, Sand Hutton, York Y041 1LZ, UK*

§ *Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 1905, 166 28 Prague 6, Czech Republic*

¶ *Brewing Research International, Lyttel Hall, Nutfield RH1 4HY, UK*

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A critical review of the occurrence of 3-chloro-propane-1,2-diol (3-MCPD) in foods not known to contain hydrolysed vegetable proteins is presented. The review covers the properties and chemistry of 3-MCPD and the current methods of analysis in foodstuffs. The results of UK surveys of 3-MCPD occurrence in both retail foods and commercial food ingredients are discussed with particular reference to cereal, meat and dairy products. The possible mechanisms for the formation and decay of 3-MCPD in foods are suggested. The review does not cover the detailed toxicology of 3-MCPD and its occurrence in hydrolysed vegetable proteins, which have been considered elsewhere, nor possible issues such as in-vivo formation.

Keywords: 3-MCPD, 3-chloro-propane-1,2-diol, 3-monochloropropanediol, analysis, food, formation, occurrence, mechanisms, stability

Introduction

3-Chloro-propane-1,2-diol (3-MCPD) is a member of a group of chemical contaminants known as chloropropanols that also includes known genotoxic animal carcinogens such as 1,3-dichloro-propan-2-ol(1,3-DCP) (Joint FAO/WHO Expert Committee on Food Additives 2001). Toxicological studies have shown that 3-MCPD is carcinogenic in rat (Sunhara *et al*. 1993) and has genotoxic activity *in vitro*. These studies have been adequately reviewed by Olsen (1993) and Lynch *et al*. (1998). New studies of *in-vivo* genotoxic potential (Fellows 2000, Marshall 2000) have since been reviewed by the UK Committees on Mutagenicity (COM 2000) and Carcinogenicity (COC 2000), the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2001) and the EC Scientific Committee on Food (EC 2001). The consensus of the expert committees was that the genotoxic activity seen *in vitro* was not expressed *in vivo*. Based on these evaluations, a provisional maximum tolerable daily intake of $2\,\mu g\,kg^{-1}$ body weight (bw) has been established (EC 2001, JECFA 2001). The EC has recently set a regulatory limit of $0.02\,mg\,kg^{-1}$ for 3-MCPD in HVP and soy sauce (Commission Regulation 2001). The UK Food Advisory Committee (FAC) has advised industry that they should 'continue to take all steps necessary to reduce concentrations of 3-MCPD in foods and food ingredients to the lowest technologically achievable level' (FAC 2000).

It is known that 3-MCPD and 1,3-DCP can be formed as by-products of the manufacture of hydrolysed vegetable proteins (HVP) and soy sauces made by acid hydrolysis (MAFF 1999, Wu and Zhang 1999, Macarthur *et al*. 2000, FSA 2001a, b, Jin *et al*. 2001). The precursors of 3-MCPD and 1,3-DCP formed in HVPs and soy sauces have been shown to be hydrochloric acid and residual lipids from the raw materials used (Velíšek *et al*. 1978). Mechanisms of formation (Collier *et al*. 1991) and degradation (Dolešal and Velíšek 1992, 1995) in HVPs and model systems have been studied. Contamination of HVPs by chloropropanols has since been adequately resolved by the manufacturing industry and several patented methods exist for their effective removal (Faesi *et al*. 1986, Brown *et al*. 1989, De Rooij 1989, Hirsbrunner and Weymuth 1989, Payne 1989).

* To whom correspondence should be addressed. e-mail: cghamlet@rhmtech.co.uk

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Studies carried out by laboratories on behalf of the UK food industry have shown that 3-MCPD may also be formed in other processed foods, notably a range of cereal products that have been subjected to heat treatments such as baking, roasting or toasting. These findings, passed to the UK regulatory authorities, have recently been confirmed by surveys of foodstuffs undertaken by the UK Food Standards Agency (Crews *et al.* 2001a, Hamlet *et al.* 2001) and an investigation into the effects of domestic preparation (Crews *et al.* 2001b). These foods do not use HVP or soy sauce as an ingredient and the mechanism of formation is not therefore fully understood.

The present paper considers the occurrence of 3-MCPD in these foods in terms of the chemistry of 3-MCPD, the relevant methods of analysis, the potential mechanisms of formation and the known precursors. The objective is to provide a foundation for further research into the origin and formation of 3-MCPD in foods that do not use HVP as an ingredient.

Chemistry of 3-MCPD: structures, properties, reactions

Structures

3-MCPD is a glycerol chlorohydrin so named when one hydroxyl group of the parent molecule glycerol is replaced with a chlorine atom. The single positional isomer, 2-chloro-propane-1,3-diol (2-MCPD), and the two enantiomers of 3-MCPD arising from the substitution of –OH by –Cl at the stereospecifically numbered carbons atoms (*sn*) 1 or 3 on the prochiral glycerol molecule are given in figure 1.

It has been reported that each enantiomer of 3-MCPD exhibits different biological activity: the (*R*)-isomer has a detrimental effect on the kidneys (Porter and Jones 1982), whereas the (*S*)-isomer has antifertility activity (Ford and Waites 1982, Jones and Ford 1984).

The current opinions of the EU expert committees and the EC regulatory limit for 3-MCPD in soy sauces and HVPs apply to the racemate.

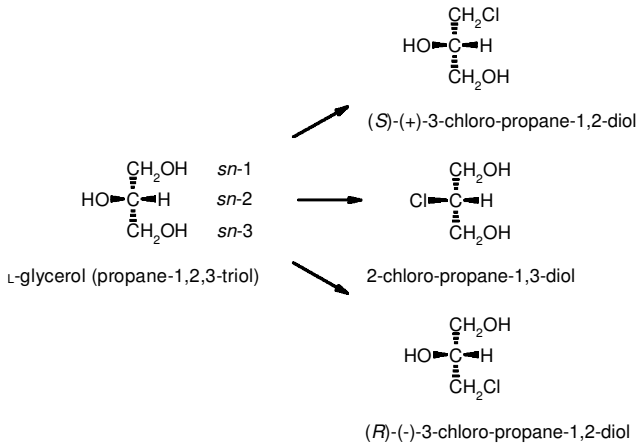


Figure 1. Monochlropropanediol isomers and their relationship to the prochiral molecule L-glycerol shown in Fischer projection (stereospecific numbering). Optical isomers (enantiomers) of 3-MCPD are formed when -OH is replaced by -Cl at the sn-*1 or* sn-*3 positions on the glycerol backbone*.

Physical properties

3-MCPD (CAS Registry No. 96-24-2) is a colourless, slightly oily liquid with a faint and pleasant odour. 3-MCPD boils at 213°C, has a density of 1.3204 g cm^{-3} (20°C), and is readily soluble in water and ethanol. Full details of all physical characteristics are available from the Royal Society of Chemistry (1999).

Stability/degradation

3-MCPD and 2-MCPD are relatively unstable in aqueous alkaline media and are decomposed to glycerol via the intermediate epoxide glycidol according to the reaction given in figure 2. This reaction is commonly used to reduce the level of 3-MCPD in commercial HVPs (Brown *et al.* 1989)

The degradation reactions of monochloropropanediols have been studied in aqueous model systems at pH range 5–9 and temperatures from 25 to 85°C. Dolešal and Velíšek (1992) found that 3-MCPD decayed according to first-order kinetics and that the stability of 3-MCPD was very sensitive to both pH and temperature. The degradation reaction obeyed an Arrhenius temperature dependence with a constant activation energy (E) of 119.2 kJ mol^{-1} over the pH range 5–9.

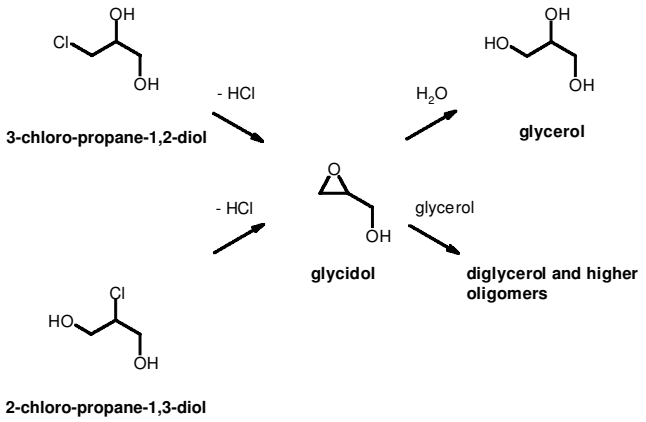


Figure 2. Decomposition reactions of 3-MCPD and 2-MCPD in aqueous alkaline media (adapted from Doležal and Velíšek 1992, 1995).

The stability of the two chloropropanediols is not equivalent. For example, the rate constants for the degradation of 3-MCPD and 2-MCPD at pH 9 and 85°C have been reported as 9.79×10^{-3} (s^{-1}) and 7.60×10^{-4} (s^{-1}) respectively (Doležal and Velíšek 1995).

The stabilities of 3-MCPD and 2-MCPD have not yet been fully investigated over the pH and temperature ranges of interest in food systems.

Reactions

3-MCPD undergoes reactions characteristic of both alcohols and alkyl chlorides, and reacts readily with acids, alcohols, aldehydes, ammonia, amino compounds, ketones and thiols (Velíšek *et al.* 1991a). Some of these are summarized in figure 3. For example, 3-MCPD derived amino alcohols and amino acids have been identified in HVPs (Velíšek *et al.* 1991b, 1992). It is not known if these compounds are formed in other foodstuffs.

Methods to determine 3-MCPD and related compounds

Despite the apparent simple chemical structure of 3-MCPD, analysis at the sub-mg kg^{-1} level is complex. The three main physical characteristics that contribute to this difficulty are the absence of a suitable chromophore, a high boiling point and a low molecular weight. The former characteristic has made approaches based on high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection unfavourable. The latter characteristics also complicate direct analysis by gas chromatography (GC). The low volatility and high polarity of 3-MCPD give rise to unfavourable interactions with components of the GC system that result in a poor peak shape and a low sensitivity. The low molecular weight of 3-MCPD makes mass detection difficult since diagnostic ions cannot be reliably distinguished from background chemical noise. Despite these apparent limitations, methods based on GC predominate. Earlier methods and their limitations have been reviewed by Hamlet and Sutton (1997) and Hamlet (1998). Recent methods and their adaptations rely on the formation of a stable GC volatile derivative so that 3-MCPD may be readily characterized by selective detection.

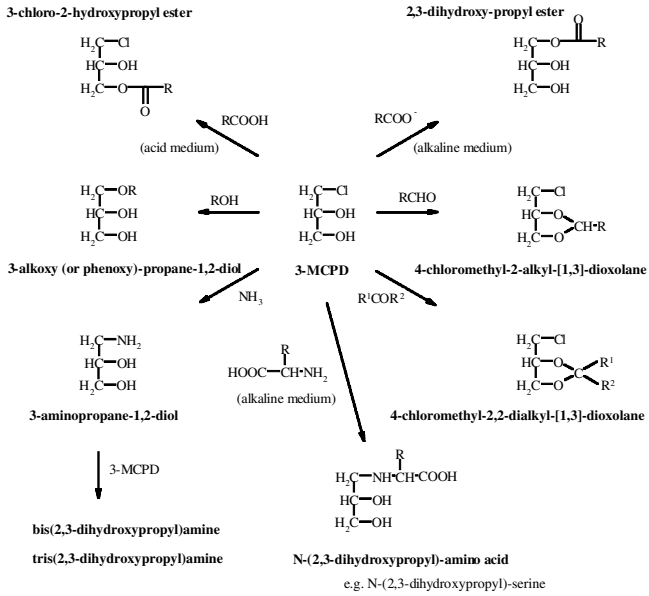


Figure 3. Typical reactions of 3-MCPD.

Analysis of foodstuffs

Hamlet and Sutton (1997) reported a procedure for the determination of 3-MCPD at the low μg kg^{-1} level in HVPs and seasonings. 3-MCPD was extracted into

saline solution and then partitioned into diethyl ether using a solid-phase extraction technique based on diatomateous earth. Concentrated extracts were then reacted with heptafluorobutyrylimidazole (HFBI) to give the corresponding 3-MCPD di-esters, which were then analysed by combined gas chromatography-mass spectrometry (GC-MS). Figure 4a shows the full-scan electron impact (EI) mass spectrum of the HFBI derivative of 3-MCPD. Although dominated by ions derived from charge retention on the heptafluorobutyryl moiety (*m/z* 69, 100, 119, 169), there are sufficient diagnostic ions for confirmation purposes. These ions together with their proposed fragmentation pathways are given in figure 4b. Quantification was by the stable isotope internal standard method using deuterium-labelled 3-MCPD added to the sample before extraction. The three main advantages of the approach were: high sensitivity resulting from the formation of a GC volatile 3-MCPD derivative; high specificity associated with mass spectrometric detection at higher mass; and accurate quantification from the use of a stable isotope internal standard. The procedure has been extended to cover other food matrices (Hamlet 1998) and has recently been validated by collaborative trial (Brereton *et al.* 2001). The limit of quantification has been established as being close to 0.01 mg kg^{-1}. The method is also suitable for the determination of 2-MCPD and other chloropropanols including 1,3-DCP. Commercial preparations of 2-MCPD are not available, necessitating a custom synthesis. The compound may be quantified to a first approximation by using the response factors from 3-MCPD calibration standards.

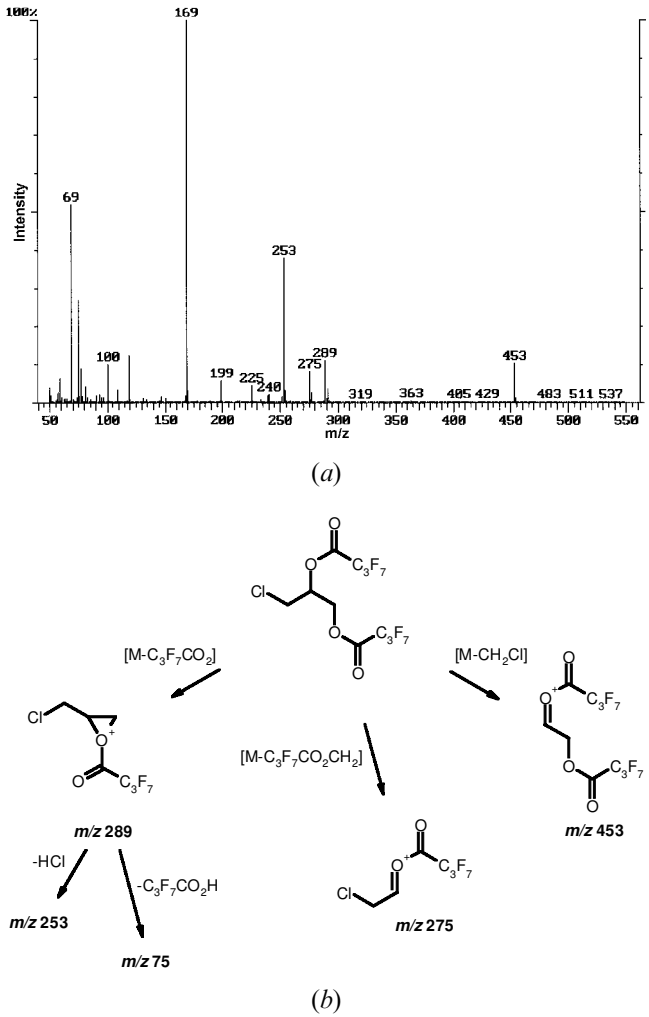
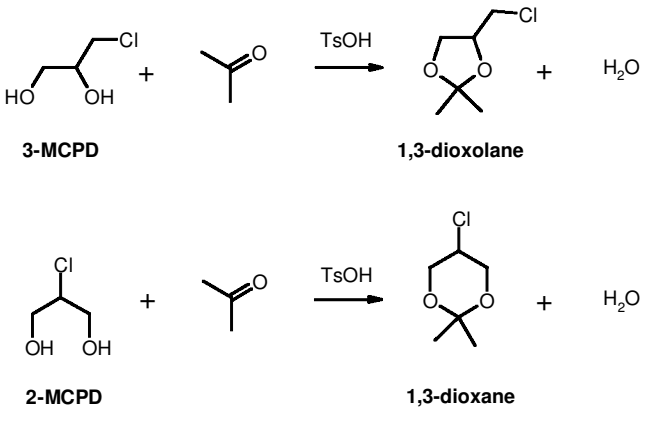


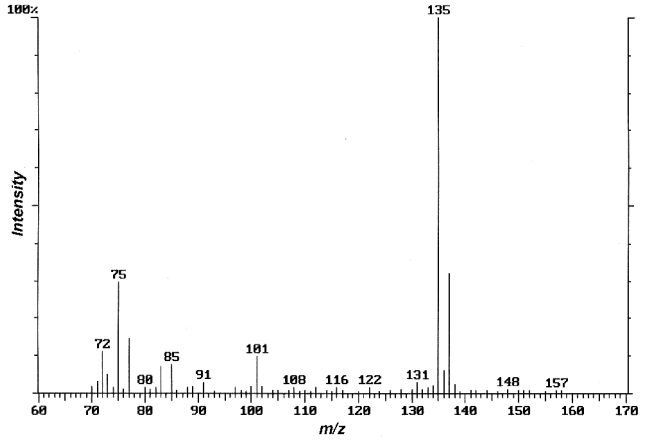
Figure 4. Mass spectrometry of HFBI derivatives of 3-MCPD: (a) EI mass spectrum of HFBI derivative of 3-MCPD; and (b) diagnostic ions and possible fragmentation pathways (adapted from Hamlet and Sutton 1997).

Solid-phase extraction on diatomaceous earth has also been utilized for the determination of 3-MCPD and 2-MCPD as acetonide derivatives in soy sauces, seasonings and bouillon (Meirerhans *et al.* 1998, Jin *et al.* 2001). The reaction of chloropropanediols with acetone was catalysed with toluene-4-sulphonic acid and the resulting 1,3-dioxolane (3-MCPD) and 1,3-dioxane (2-MCPD) derivatives were characterized by GC-MS. The reaction schemes and accompanying EI mass spectrum of 3-MCPD showing the intense and diagnostic molecular ion isotope pattern (^{35}Cl/^{37}Cl) at *m/z* 135 and 137 are given in figure 5. Quantification was by the external standard method with detection limits in the low μg kg^{-1} range. Although the authors claim good precision and accuracy for the method, a more robust quantification might result from the use of a stable isotope internal standard. Furthermore, the requirement for a custom synthesis of the acetonide standards from both 3-MCPD and 2-MCPD may place restrictions on the general applicability of this method. The method is not suitable for the determination of chloropropanols such as 1,3-DCP since these compounds do not form cyclic acetonide derivatives.

3-MCPD has also been determined in HVP and in soy sauce using phenylboronic acid (PBA) as a derivatization reagent. PBA reacts specifically with diol groups to form cyclic derivatives that may be characterized by GC-MS. Wu and Zhang (1999) carried out derivatization and extraction simultaneously. Since there is no sample clean up stage and non-diol



(*a*)



(*b*)

Figure 5. Characterization of 3-MCPD and 2-MCPD as acetonides: (a) reactions of 3-MCPD and 2-MCPD with acetone; and (b) full-scan EI mass spectrum of acetone derivative of 3-MCPD [4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane].

compounds are not derivatized this method may not be applicable to complex and fatty foods. Ushijima *et al.* (1995) employed the solid-phase extraction and clean-up stage described previously with derivatization before GC-MS. This latter adaptation may be more applicable for processed foods. Quantification was by the internal standard method using hexane diol added before derivatization. Both methods offer good sensitivity but suffer from poor selectivity since there are relatively few diagnostic ions of sufficient intensity. Since PBA reacts specifically with diol compounds, other chloropropanols such as 1,3-DCP can not be determined by this method.

A method to separate the (*R*)- and (*S*)-enantiomers of 3-MCPD as PBA derivatives on a Chiraldex G-TA-fused silica capillary column (Alltec, USA) and GC-MS has also been developed (J. Velíšek, personal communication, 2001). As far as the authors are aware, chiral separation of 3-MCPD enantiomers as HFBI or acetonide derivatives has not been attempted.

Occurrence in foods

Published information on levels of 3-MCPD in foodstuffs, other than HVPs and soy sauces, is scarce. There are currently two food survey publications (Crews *et al.* 2001a, Hamlet *et al.* 2002) and a publication on the effects of domestic preparation (Crews *et al.* 2001b).

UK survey of commercial food ingredients

Levels of 3-MCPD $>0.01\,mg\,kg^{-1}$ were found in 14 (22%) of 63 food ingredients available in the UK (Hamlet *et al.* 2001). Samples taken during 1999–2000 included breadcrumbs, caramels, enzyme-hydrolysed vegetable proteins, gelatines, malt products (malt extracts, malt flours and other malt-based ingredients), meat extracts, modified starches, and yeast extracts. Nine malt samples found to contain 3-MCPD were all without exception products used for colouring and flavouring applications. The additional heat treatments to which these malts had been subjected to produce the desired colours and flavours would appear to be a significant factor in the formation of 3-MCPD from endogenous precursors.

Modified starches found to contain 3-MCPD were a potato and a maize yellow dextrin. These samples were believed to have been produced by heat treatment in the presence of a mineral acid. The authors offered the suggestion that 3-MCPD was formed in these products by the reaction of chloride anion with residual starch lipid in a mechanism analogous to that of acid-HVP (Collier *et al.* 1991). Since the level of starch lipid was greater in the maize product this accounted for the higher level of 3-MCPD found.

UK survey of retail food products

A survey of 3-MCPD levels in approximately 300 retail foods purchased between 1999 and 2000 has been reported (Crews *et al.* 2001a). 3-MCPD was detected at levels $>0.01\,mg\,kg^{-1}$ in 30% of the samples tested. Food groups were selected to focus on foods that might contain 3-MCPD resulting from a high salt content (soups and sauces), a high fat content (meats and dairy products), heat processing (cereal products) and other factors such as malt-based ingredients or combinations of factors (miscellaneous foods). Where necessary, foods had been prepared and cooked according to the manufacturers' instructions.

The foods specified in the original report (Food Standards Agency 2001c) have been reclassified to reflect foods containing principally cereal, meat or dairy produce in their make-up. The summary given in table 1 shows that the highest incidence of 3-MCPD was found in meat and cereal commodities.

Beefburgers and bacon were found to contain 3-MCPD in the uncooked state and this level did not change after cooking. It might be significant that all of the beefburger samples analysed were char grilled, i.e. they might have been subjected to local heating to a high temperature. Also all of the bacon samples were smoked and the 3-MCPD detected might have originated in the smoking process. Artificial flavours that contain acid-HVP could also be a source of 3-MCPD in these and other products.

Table 1. UK survey of 3-MCPD levels ($mg\,kg^{-1}$) in retail foods.[a]

Food group	Incidence		
	Number	Mean	Range
Cereal products[b]	54/110	0.016	<0.010–0.134
Meat products	(26/63)	(0.012)	(<0.010–0.081)
Beefburgers	5/7	0.024	<0.010–0.071
Cured fish	4/6	0.022	<0.010–0.081
Salami	9/20	0.012	<0.010–0.069
Other[c]	6/15	<0.010	<0.010–0.042
Bacon	2/10	<0.010	<0.010–0.047
Ham	0/5	<0.010	<0.010
Dairy[d]	4/35	<0.010	<0.010–0.031
Miscellaneous[e]	5/55	<0.010	<0.010–0.016
Soups and sauces	0/34	<0.010	<0.010[1]

[a] Adapted from Food Standards Agency (2001); all data are reported as on a consumed basis (made up/cooked).
[b] Breakdown of the results for cereal products is given in table 2.
[c] Mainly flavoured chicken products, kebabs, cocktail sausage.
[d] Cheese and cream.
[e] Beer, cakes, coated meat and fish products, confectionery, malted drinks, pastries, pizza, pork pies, tea (bags), vegetable burgers.

The presence of 3-MCPD in the salami products could be due to migration from continental sausage casings treated with wet-strength resins based on epichlorohydrin. These resins have been shown to contain residual 3-MCPD (Bodén *et al.* 1997).

The highest level of 3-MCPD found in meat products was in a sample of canned anchovies in olive oil. The equivalent sample of canned salted anchovies did not contain 3-MCPD. This observation is difficult to explain, though it is possible that differences in water activity, pH and salt concentration (ionic strength) could be factors in formation. Equally, the nature of the can coating if any, and potential migration from epoxy materials has not been considered.

A summary of the levels of 3-MCPD for various cereal commodity subgroups is given in table 2. Table 2 has been ordered to reflect the decreasing means of 3-MCPD: savoury biscuits > doughnuts > bread > sweet biscuits. No 3-MCPD was found in the breakfast cereals, corn snacks, crumpets (cooked) or Yorkshire puddings (cooked).

Savoury biscuits accounted for the greatest incidence and highest levels of 3-MCPD reported. These products have some of the lowest moisture levels and highest baking temperatures. The combination of these conditions would appear to favour formation of 3-MCPD. Low-fat cream crackers had the highest mean 3-MCPD of $0.099\,mg\,kg^{-1}$. These were distinct as a subclass from all other crackers, which had mean 3-MCPD ranging from 0.015 to $0.037\,mg\,kg^{-1}$. Comparison of the ingredient and nutritional declarations for standard cream crackers (mean 3-MCPD of $0.037\,mg\,kg^{-1}$) with those of the low-fat variety showed these to differ only by the declared fat levels. Though the declared salt levels of both products were similar, the relative amounts of other key ingredients such as flour could not be determined. The latter may have been higher in the low-fat product. Although it is possible to speculate that the lipid may have an inhibitory effect, it is equally possible that differences in pH and temperature between product types could have a bearing on the levels of 3-MCPD found.

3-MCPD was widely distributed in the non-white breads. The highest levels, however, were found in

Table 2. 3-MCPD (mg kg^{-1}) levels in retail cereal products: UK survey.[a]

Commodity	Incidence			
	Number	Median	Mean	Range
Biscuits, savoury[b]	(30/34)	(0.026)	(0.038)	(<0.010–0.134)
Cream crackers (low fat)	4/4	0.111	0.099	0.040–0.134
Cream crackers	4/4	0.041	0.037	0.019–0.046
Toasts	12/13	0.026	0.036	<0.010–0.088
Twiglets	1/1	0.034	0.034	0.034
Crispbreads	2/4	0.019	0.031	<0.010–0.087
Matzos	1/1	0.026	0.026	0.026
Wafers	2/3	0.018	0.017	<0.010–0.030
Savoury crackers	4/4	0.015	0.015	<0.010–0.018
Doughnuts	5/5	0.019	0.018	0.011–0.024
Bread	(14/27)	(0.011)	(0.012)	(<0.010–0.049)
White	2/4	0.018	0.021	<0.010–0.049
Non-white	12/18	0.015	0.013	<0.010–0.029
Other[c]	0/5	<0.010	<0.010	-–
Biscuits sweet	5/19	<0.010	<0.010	<0.010–0.032
Breakfast cereals	0/10	<0.010	<0.010	—
Corn snacks	0/10	<0.010	<0.010	—
Crumpets[d]	0/3	<0.010	<0.010	—
Yorkshire pudding[d]	0/2	<0.010	<0.010	—

[a] Adapted from Food Standards Agency (2001).
[b] Classification according to Manley (2000).
[c] Fruit malt loaf, black olive ciabatta, plain naan bread, wholemeal pitta bread.
[d] Cooked product.

two of four white breads analysed at 0.035 and 0.049 mg kg^{-1}. Both of these samples were Scottish breads baked in a batch process without tins. Batch baked breads have a dark and thick crust that results from the long bake time required to cook the larger mass of dough. Since 3-MCPD is associated with the crust region (C. G. Hamlet, unpublished data, 1997) this probably accounts for the higher levels found.

The low levels of 3-MCPD found in all samples of doughnuts are consistent with formation at the dough–fat interface. The typical temperature range for a commercial fat fryer is only 170–190°C compared with baking oven temperatures of >200°C.

No 3-MCPD was found in any of the breakfast cereals or corn snacks. With the exception of corn flakes, these were all pressure-cooked products formed by expansion or extrusion. The lower temperatures and/or reduced heat treatment times do not appear to favour formation of 3-MCPD in these products.

Similarly, no 3-MCPD was found in the crumpets or the Yorkshire puddings. Although no details were given, these products were described as laboratory cooked.

Effects of domestic preparation

While levels of 3-MCPD in retail foods have not been found to exceed 0.1 mg kg^{-1}, the potential for additional generation of 3-MCPD resulting from domestic preparation has also been reported. Crews *et al.* (2001b) measured levels of 3-MCPD in stock cubes, gravies, cake mixes, cheeses, meats, batters and breads subjected to combinations of baking, frying, grilling, microwaving and stewing.

Significant levels of 3-MCPD were reported for all grilled cheeses, toasted breads and a laboratory-prepared batter mix fried in oil. Weight losses during cooking, although reported, were not considered. When the corresponding levels of 3-MCPD are corrected for water loss, the data given in table 3 show that four of the six cheese samples gave an increase in 3-MCPD on cooking. These were cheddar,

Table 3. Increase in 3-MCPD levels during domestic cooking.[a]

Food type	3-MCPD ($mg\,kg^{-1}$)		3-MCPD[b] ($mg\,kg^{-1}$)	
	Uncooked	Microwaved	Fried	Grilled
Cheddar cheese	< 0.005	< 0.005	—	0.017
Mozzarella cheese	< 0.005	< 0.005	—	0.061
Goat's cheese	< 0.005	0.005	—	0.035
Processed cheese	0.017	0.020	—	0.020
Cheese alternative	0.037	0.033	—	0.043
Parmesan cheese	< 0.005	0.052	—	0.099
Beef	< 0.005	0.006	0.016	< 0.005
Beefburger	< 0.005	—	< 0.005	< 0.005
Batter, laboratory	< 0.005	—	0.045	—
Batter, retail	< 0.005	—	< 0.005	—
White bread, slice	< 0.005	—	—	0.143
Brown bread, slice	< 0.005	—	—	0.205
Wholemeal bread, slice	0.010	—	—	0.275

[a] Adapted from Crews *et al.* (2001).
[b] Expressed on an uncooked weight basis (corrected for water loss).

mozzarella, goat and parmesan, which all had $< 0.005\,mg\,kg^{-1}$ 3-MCPD in the uncooked state. One possible explanation for the generation of 3-MCPD in these cheeses could be that they are formed from mono- or di-esters of 3-MCPD. These compounds have been reported in goat's, ewe's and cow's milk (Cerbulis *et al.* 1984, Kuksis *et al.* 1986, Myher *et al.* 1986), where their formation was tentatively linked to the use of chlorine-based sanitizers. These are known precursors in the formation of 3-MCPD in acid-hydrolysed vegetable proteins. The samples showing no increase in 3-MCPD during grilling were a processed cheese and a cheese alternative containing 0.017 and $0.037\,mg\,kg^{-1}$ respectively in the uncooked state. The presence of 3-MCPD could be a consequence of migration from packaging treated with epichlorohydrin-based wet-strength resins. The potential for migration in these products may be significant since processed cheese slices are often individually wrapped.

A fried batter produced from a recipe in the laboratory gave a level of $0.045\,mg\,kg^{-1}$ (corrected for water loss). It is not known how this preparation differs from a retail batter, which when fried gave $< 0.005\,mg\,kg^{-1}$ 3-MCPD. The toasting of breads gave the most significant increase in 3-MCPD levels. Highest levels were found in the non-white breads. Although the toasting times for each bread were variable, the non-white breads appear to have been toasted for slightly longer to obtain a darker colour.

Other sources of 3-MCPD in foods

Food-contact materials

Food-contact materials treated with polyamidoamine-epichlorohydrin (PAAE) wet-strength resins have been shown to contain 3-MCPD formed by side reactions from residual epichlorohydrin during production (Bodén *et al.* 1997). Some applications for these materials in the food industry include tea bags, coffee filters, meat wadding and continental sausage casings. It is possible that levels of 3-MCPD found in some salamis (Crews *et al.* 2001b) could have arisen by migration from these materials.

3-MCPD may also be present in drinking water treated with epichlorohydrin/amine co-polymers used as flocculants or coagulant aids (EC 2001).

Mechanisms of formation and potential precursors

From glycerol

Reactions of glycerol with hydrochloric acid have been used for the synthesis of 3-MCPD and 1,3-DCP. 3-MCPD can be prepared by the action of

hydrochloric acid or dry hydrogen chloride gas on glycerol alone or in the presence of an organic acid catalyst such as acetic acid (Conant and Quayle 1947a, b). The mechanism in aqueous systems has been considered by Collier *et al.* (1991) and most likely proceeds by a nucleophilic substitution reaction (SN2) of chloride anion according to the schemes given in figure 6. These reactions require prolonged heating at temperatures of about 100°C and are directly applicable to the manufacture of acid-HVP.

Glycerol and related polyols such as propylene glycol are used as humectants and flavour carriers in a wide variety of foods including confectionery products and dried fruits and vegetables. Glycerol (and free fatty acids) is also formed in processed foods by the high-temperature hydrolysis of triglycerides. This mechanism is particularly significant in the preparation of deep-fried foods of high moisture (Lawson 1995), where the frying fat reaches temperatures of 170–190°C. These conditions are also found in the crust regions of baked, grilled and roasted products. Glycerol added or formed under these conditions may be available for reaction with chloride anions present in the foodstuff.

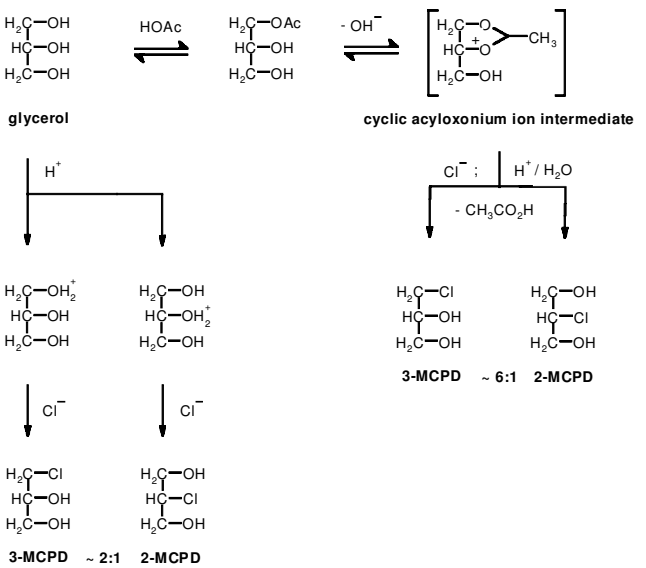


Figure 6. Formation of chloropropanediols from glycerol (adapted from Collier et al. *1991).*

From allyl alcohol

3-MCPD and 2-MCPD may be formed by the addition of hypochlorous acid (HOCl) across the double bond of allyl alcohol (prop-2-en-1-ol). The reaction of allyl alcohol with chlorine and water is reported to proceed rapidly at 50–60°C to yield 88% monochloropropanediols and 9% dichloropropanols (Myszkowski and Zielinski 1965).

Allyl alcohol is a thermal decomposition product of alliin [(*S*)-allyl-L-cystein sulphoxide], a cystein amino acid found in garlic and related species (Kubec *et al.* 1997). Formation of 3-MCPD and other chloropropanols from allyl alcohol in these compounds is therefore very probable since chlorinated water used in any process procedures will contain hypochlorous acid and chlorine (Cl_2). The addition of HO–Cl to the double bond of allyl alcohol will follow Markovnikov's rules governing carbonium ion stability to yield preferentially 3-MCPD according to the mechanism given in figure 7.

The opportunity also exists for considerable enhancement of chloropropanol levels from the dehydration of raw onion and garlic (98% water) to produce dry powdered ingredients.

From lipids and hydrochloric acid

Collier *et al.* (1991) studied the formation of chloropropanediols in acid-HVP by monitoring reactions of glycerol, triacyl glycerols and phospholipids with

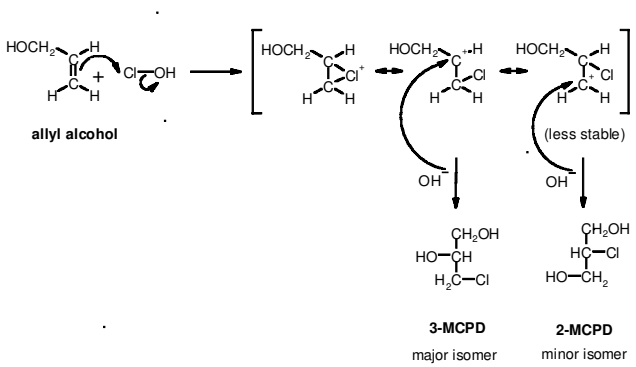


Figure 7. Reaction of allyl alcohol (prop-2-en-1-ol) and hypochlorous acid (HOCl): preferential formation of 3-MCPD from the more stable carbenium ion (adapted from Allinger et al. *1976).*

5.5 M hydrochloric acid. The yield of chloropropanediols was found to decrease as follows: triacyl glycerols > phospholipids > glycerol. The distribution of the isomers 3-MCPD/2-MCPD from both glycerol, and phosphatidylcholine were in agreement with statistical substitution at two primary methylenes (CH_2) and one secondary methine (CH), i.e. about 2:1. Although phosphate and phosphoryl choline are good leaving groups, they exhibit little regioselectivity due to facile intramolecular isomerization as shown in figure 8 (*a*). The increases in yield and isomer ratio observed from the addition of acetic acid to glycerol and from the reaction with trigyceride provided evidence for the formation of a cyclic alkyloxonium intermediate. Chloropropanediol isomer ratios have not been studied in any other foodstuffs and could therefore be an indication of potential mechanisms/precursors.

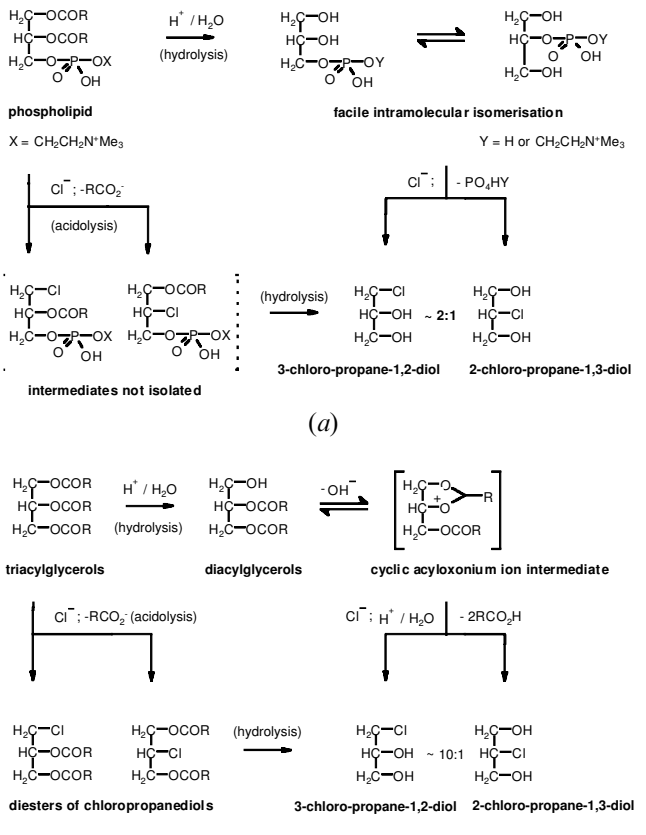


Figure 8. Formation of chloropropanediols from phospholipids and triacylglycerols (adapted from Collier et al. *1991): (a) Formation from phospholipids; and (b) formation from triacyl glycerol*

The authors concluded that the reaction involved nucleophilic substitution of an acyl group by chloride anion at positions activated by neighbouring ester groups as summarized in figure 8 (*b*).

From the proposed schemes given in figure 8 (*b*), it is evident that when the initial step involved the substitution of an acyl group by chloride anion, the resulting intermediate is a chloropropanediol di-ester. These compounds have been isolated in acid-HVPs (Velíšek and Davídek 1985, Collier *et al.* 1991) and adulterated cooking oils that may have been treated with hydrochloric acid (Gardner *et al.* 1983).

A chiral separation of 3-MCPD formed in acid HVP (Velíšek, personal communication, 2001) has shown this to be a racemic mixture. Both enantiomers also showed equal rates of decomposition when treated with alkali. These results are perhaps not surprising, since under the conditions employed the chloride anion is free to approach the glycerolipid at either the *sn*-1 or *sn*-3 positions. This may not necessarily be the case for formation in other foodstuffs, particularly in any mechanisms involving biosynthetic pathways and/or bound or locked conformations. Chiral separation of 3-MCPD enantiomers formed in other foods has not yet been carried out and could therefore provide useful mechanistic information.

The mechanisms outlined above apply specifically to the formation of chloropropanediols from lipids and hydrochloric acid under aqueous conditions. Although these conditions are not found during the manufacture of most foodstuffs, the underlying mechanism should not be discounted completely. Substitution reactions of small negatively charged nucleophiles, such as chloride anion, are known to proceed more rapidly in aprotic solvents (March 1985). The reason for this is that protic solvents such as water can form a shell of solvent molecules around the nucleophile, thereby constituting a barrier between it and the substrate. Under conditions of low water activity, such as the high-temperature surface regions of baked breads and grilled/roasted meats, nucleophilic substitution reactions of chloride anion could occur readily.

From carbohydrates with aqueous hydrochloric acid

Brief reference has been made by Collier *et al.* (1991) to the formation of 3-MCPD from the reaction of soluble pentosan (arabinoxylans) and pectin (mainly

methyl-esterified galacturonic acid, degree of methylation 33.7). The carbohydrates were heated with excess 5.5 M HCl for 11 h. The reduced yield of total chloropropanediols compared with that from crude soya meal (lipids) was attributed to possible formation from carbohydrate precursors. It is more probable that the chloropropanediols arose from the reaction of hydrochloric acid with residual lipids associated with the pentosan and pectin and not from the carbohydrate moiety.

Applied model systems

A brief reference to formation of 3-MCPD in a model system comprising fat, salt, glycerol and citric acid has also been reported (Gassenmeier and Goeke 1999). A level of 0.8 mg kg^{-1} 3-MCPD was formed when the combined ingredients were heated at 140°C for 30 min and that no formation occurred when either glycerol or citric acid was omitted. The authors claim that this was evidence for formation, presumably from salt and glycerol, under relatively mild conditions by catalysis with organic acids. Unfortunately no experimental details or discussion was given, making it difficult to draw any firm conclusions from this data.

The formation of 3-MCPD and 2-MCPD has recently been demonstrated in model systems from the reactions of sodium chloride with glycerol, lecithin and triolein at temperatures >160°C (Velíšek, personal communication, 2001).

Formation in polyamidoamine-epichlorohydrin resins (PAAE)

Work carried out by Bodén *et al.* (1997) on methods for the determination of 1,3-DCP and 3-MCPD has demonstrated the interchange of chloropropanol species with changing pH. Figure 9 shows the equilibrium between 2-chloromethyl-oxirane (epichlorohydrin) and 1,3-DCP in aqueous systems and the subsequent formation of 3-MCPD. Earlier studies by Collier *et al.* (1991) on HVPs have also demonstrated that 1,3-DCP may be formed from the acetic acid catalysed reaction of 3-MCPD with chloride anion. The potential for interconversion of these species may therefore exist in food systems because of pH and possibly ionic strength.

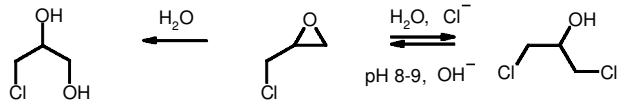


Figure 9. Equilibrium between 2-chloromethyl-oxirane and 1,3-DCP and formation of 3-MCPD.

Naturally occurring esters of 3-MCPD

Cerbulis *et al.* (1984) identified a small but significant quantity (<1% of total neutral lipids) of 3-MCPD diesters in raw milk from several herds of goats. The chloropropanediol diesters were composed of molecular species containing C_{10}–C_{18} fatty acids and corresponded closely in carbon number to glycerol moieties of goat milk triacylglycerols (their parent *sn*-1,2- but not the *sn*-2,3-diacylglycerols) (Kuksis *et al.* 1986). The carbon number of the diesters ranged from C_{26} to C_{38} and included small amounts of species with odd carbon numbers. They apparently contained two different fatty acids in the same ester molecule. The major components were dipalmitoyl, myristoylpalmitoyl, palmitoyloleoyl and palmitoylstearoyl species. Stereospecific analysis showed that the 3-MCPD diesters were racemic mixtures of both enantiomers (Myher *et al.* 1986).

These data appear equally consistent with formation from anthropogenic chlorine-containing compounds (such as chlorine-based sanitizers used in dairy operations) ex-secretion, or esterification of 3-MCPD or its monoester in the mammary gland. It is not known if the latter precursors were dietary substances or whether mono- or diesters of 3-MCPD occur in any other foods.

Conclusion

The discovery that low levels of 3-MCPD are present in a range of foods has led a renewed interest in this topic. At present, the precise mechanisms of formation have not been elucidated and it is not yet possible to explain entirely the differences in levels of 3-MCPD found in foods. However, as data become available on the stability of 3-MCPD under food processing

and storage conditions, this, together with an understanding of the mechanisms of formation, may allow food manufacturers to control levels of 3-MCPD in foods.

A task within the framework of scientific cooperation by member states (SCOOP) has been initiated to establish the dietary sources and intakes of 3-MCPD and 2-MCPD in foodstuffs (EC 2000). The UK Food Standards Agency has recently commissioned a research project to study the origin and formation of 3-MCPD in selected food products.

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