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In

SCIENZA E TECNOLOGIA DELL'ALIMENTAZIONE E NUTRIZIONE UMANA

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Corso di

Caratterizzazione chimico-fisica, microbiologica e sensoriale degli alimenti

Modulo

Determinazione di contaminanti negli alimenti

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3-monochloropropane-1,2-diol (3-MCPD)

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1. Introduction

1.1. Chemical Structure

Figure 1: Chemical structure

Synonims:

3-chloro-1,2-dihydroxypropane,alpha-Chlorohydrin,3-Chloro-1,2-propanediol, Chlorodeoxyglycerol, Monochlorohydrin, 3-Chloropropane-1,2-diol, 1,2-Propanediol 3-chloro-, Glycerol chlorohydrin, Glycerol alpha-monochlorohydrin.

Chemical type: Organochlorine, chiral compound.

1.2. Properties Computed from Structure:

Table 1: Properties computed from structure

Molecular Weight	110.53948 [g/mol]
Molecular Formula	$C_3H_7ClO_2$
Linear Formula	$ClCH_2CH(OH)CH_2OH$
XLogP3-AA	-0.5
H-Bond Donor	2
H-Bond Acceptor	2
Rotatable Bond Count	2
Exact Mass	110.013457
MonoIsotopic Mass	110.013457
Topological Polar Surface Area	40.5
Heavy Atom Count	6
Formal Charge	0

Complexity	32
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	1
Defined Bond StereoCenter Count	0
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	1
Feature 3D Acceptor Count	2
Feature 3D Donor Count	2
Effective Rotor Count	2
Conformer Sampling RMSD	0.4

1.3. Physical and chemical properties

Table 2: Property

Property		Value	Interpretation
Solubility - In water at 20^{o}C (mg l^{-1})		100000	High
Solubility - In organic solvents at 20^{o}C (mg l^{-1})			Soluble in ethanol, and organic solvents
Melting Point (^{o}C)		-40	
Boiling Point (^{o}C)		Decomposes before boiling	
Degradation point (^{o}C)		213	
Flashpoint (^{o}C)		58	
Octanol-water partition coefficient at pH 7, 20^{o}C	P	1.41 X 10^{-01}	Low
	Log P	-0.85	
Bulk density (g ml^{-1})/Specific gravity		1.322	
Vapour pressure at 25^{o}C (mPa)		13.0 X 10^{-05}	Volatile
Henry's law constant at 25^{o}C (Pa m^{3} mol^{-1})		6.18 X 10^{-03}	Non-volatile
Henry's law constant at 20^{o}C (dimensionless)		5.90 X 10^{-14}	Non-volatile
Refractive index:		*n*20/D 1.480(lit.)	
Optical activity		[α]20/D −0.9±0.2°, neat	
Storage temperature		2-8^{o}C	

Chemical Properties

Clear pale yellow liquid.

General Description

Denser than water. Contact may irritate skin, eyes and mucous membranes. May be toxic by ingestion. Used to make other chemicals.

Air and Water Reactions

Soluble in water. Hygroscopic.

Reactivity Profile

3-Chloro-1,2-propanediol is hygroscopic and may be sensitive to prolonged exposure to air. Glycols and their ethers undergo violent decomposition in contact with approximately 70% perchloric acid.

Health Hazard

TOXIC; inhalation, ingestion or skin contact with material may cause severe injury or death. Contact with molten substance may cause severe burns to skin and eyes. Avoid any skin contact. Effects of contact or inhalation may be delayed. Fire may produce irritating, corrosive and/or toxic gases. Runoff from fire control or dilution water may be corrosive and/or toxic and cause pollution.

Personal Protective Equipment to wear: eyeshield, faceshields, full-face respirator (US), gloves, multi-purpose combination respiratory cartridge (US), type ABEK (EN14387) respirator filter.

Fire Hazard

Combustible material: may burn but does not ignite readily. When heated, vapors may form explosive mixtures with air: indoors, outdoors and sewers explosion hazards. Contact with metals may evolve flammable hydrogen gas. Containers may explode when heated. Runoff may pollute waterways. Substance may be transported in a molten form.

1.4.3-monochloro-1,2-propanediol esters

The largest amount of 3-MCPD esters occurring as food contaminants are formed with long-chain (C14-C18) fatty acids, mainly palmitic and oleic acids.

Palmitic acid, C16H32O2

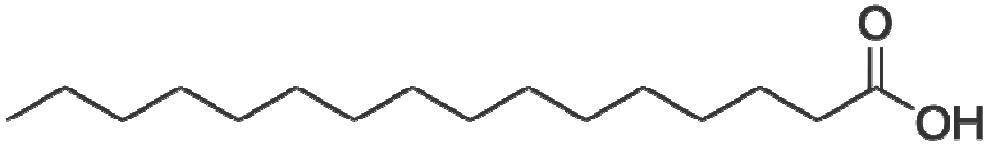


Figure 2: Palmitic acid

Oleic acid, $C_{18}H_{34}O_2$

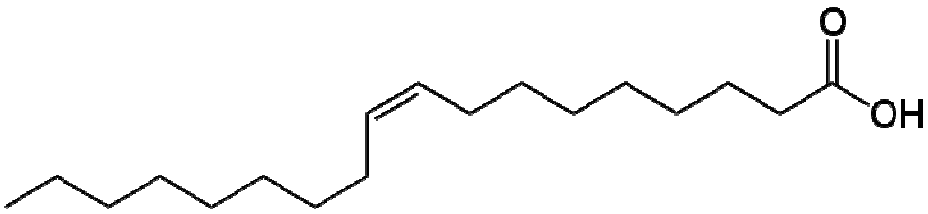


Figure 3: Oleic acid

3-MCPD esters can occur as monoesters, where only a single acyl chain (R) is present, either at position sn-1 or at position sn-2 of 3-MCPD molecule, or as diesters.

Diesters can be symmetric when both acyl residues are of the same kind (i.e. 1,2-dypalmitoyl ester), or asymmetric when acyl groups are different (i.e. 1-palmitoyl, 2-oleoyl ester).

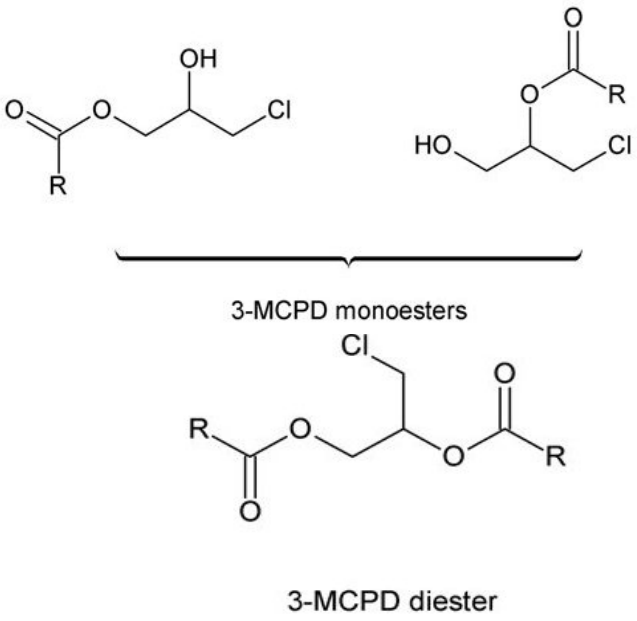


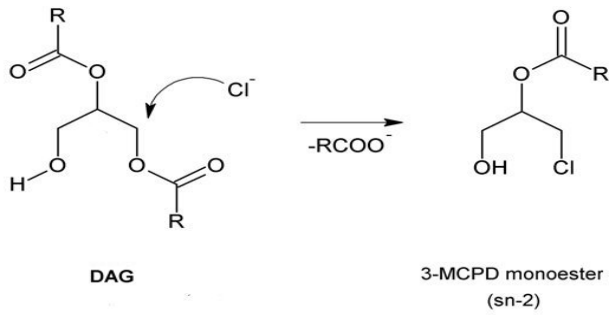
Figure 4: 3-MCPD mono-diester

Potential molecular mechanisms of 3-MCPD ester formation

Recently different pathways of 3-MCPD esters formation have been proposed.

a) Direct nucleophilic attack

Direct nucleophilic substitution by the chloride ion through S_N2 pathway on diacylglycerol molecule (DAG) in DAG/hydrochloric acid mixtures, either at carbon carrying an ester group or a hydroxyl group, resulting in the formation of 3-MCPD monoester and 3-MCPD diester.



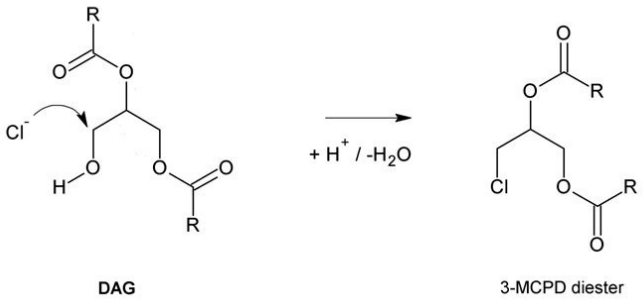
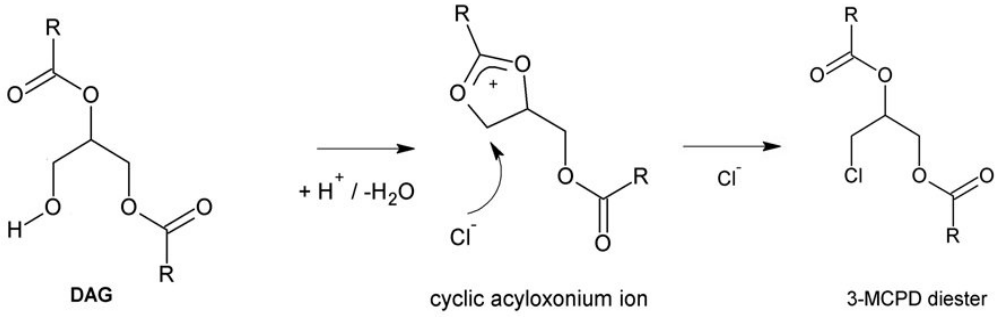


Figure 5: Direct nucleophilic attack

b) Formation through acyloxonium ion intermediate

Acylxonium ions can be formed through internal nucleophilic attack of the ester carbonyls in triacylglycerol (TAG), diacylglycerol (DAG) or monoacylglycerol (MAG) with the simultaneous separation of the leaving group from the glycerol backbone catalyzed by an acid. The leaving group could be either a protonated hydroxyl group as in the case of MAG and DAG or carboxylic acid groups as in the case of TAG or DAG.

In the following figure is depicted 3-MCPD diester formation through acyloxonium for DAG only.



6: Formation through acyloxonium ion intermediate

c) Formation through glycidol ester (GE)

There is convincing evidence that glycidol can be converted into 3-MCPD through ring opening of the epoxide in the presense of chloride containing reagents. Diacylglycerol (DAG) is considered as probable precursor of glycidol through intramolecular S_N2 reaction.

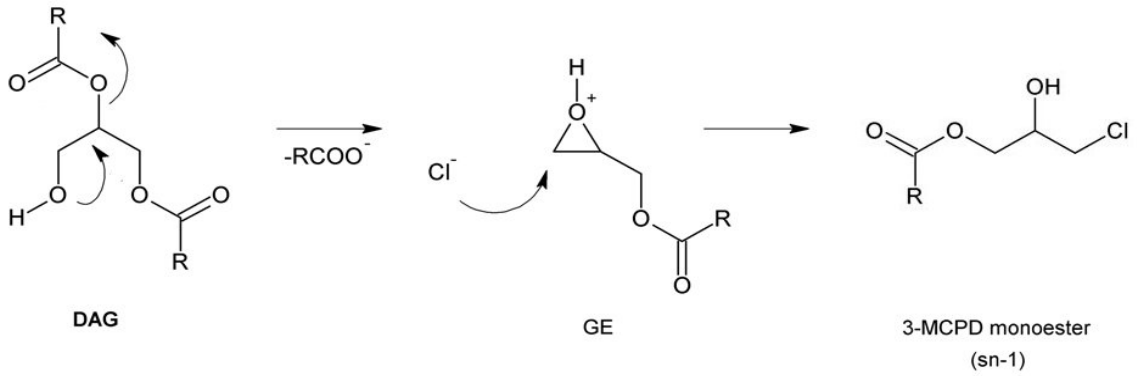


Figure 7: Formation through glycidol ester (GE)

2. Formation of 3-MCPD in food

The formation of 3-monochloropropane-1,2-diol (3-MCPD) in food is influenced by many factors, including temperature, pH, moisture and lipid content, the type of processing method employed and storage conditions (Baer *et al.*, 2010).

3-MCPD and several other chloropropanols, including 1,3-DCP (1,3-dichloropropanol), 2,3-DCP, and 2-monochloropropane-1,3-diol (2-MCPD), can be formed in foods during processing, cooking and storage as a result of the reaction of chloride ions with glycerol and other lipids present in the food (WHO, 2007). With further chlorination, 2-MCPD and 3-MCPD may form dichloropropanols. 3-MCPD and 1,3-DCP are among the major chloropropanols identified in foods (Baer *et al*., 2010).

In general, 3-MCPD can form via three pathways: acid hydrolysis, heat processing, and via 3-MCPD esters:

1) Acid hydrolysis.

Commercially, hydrolysis is carried out using 4-6 M hydrochloric acid at 100°C-130°C for 4–24 hours, which is followed by neutralisation with sodium hydroxide.

3-MCPD is formed during this process from the reaction of the acid with residual vegetable oil. Hydrochloric acid and triacylglycerols and (to a smaller extent) phospholipids and glycerol, in the raw materials are the main precursors of chloropropanols. The formation of chlorohydrins from glycerol and hydrochloric acid during HVP production was initially proposed and verified by Velíšek *et al.*, Davídek *et al.*, Collier *et al.* and Hamlet *et al.* later proposed a mechanism explaining the heat-induced formation of chloropropanols from triacylglycerols under acidic conditions. The key step involves the nucleophilic substitution of the acyl group by the chloride anion at positions activated by neighbouring ester groups. The resulting intermediate is a chloropropanediol diester that furnishes chloropropanol under hydrolytic conditions.

2) Heat processing.

3-MCPD also occurs in the absence of acid-HVP. It appears to form from lipids and sodium chloride (present naturally or added) during normal manufacturing and cooking processes, such as baking and grilling.

Free glycerol released by the high-temperature hydrolysis of triglycerides can react with the chlorides present.

3-MCPD formation also depends on other factors, as studies on model systems based on water, sodium chloride and glycerol, phospholipid or triolein have shown. With glycerol as a precursor, 3-MCPD production increases with water content up to 30% moisture, but decreases with higher moisture levels.

Furthermore, in models presenting close to no water content, glycerol still appears to be able to produce 3-MCPD. Thus, glycerol seems to be the major precursor in foods with a low water content (<15%), and other precursors like lecithin in foods with high moisture contents.

The models also showed that 3-MCPD production increased with increasing temperature above 160°C for all three precursors.

As well as temperature and moisture, 3-MCPD production is also greatly affected by the pH, and is unstable above a pH of 6.

3) Esters.

The contaminant may also be released in vivo from 3-MCPD esters by lipase-catalysed hydrolysis. Studies found that significant levels of 3-MCPD were released from bread when they were treated with bakery-grade lipase during the baking process. It also appears that hydrolytic enzymes may be directly involved in the formation of chloropropanols.

In model studies comprising lipase, vegetable oil or fat, water and sodium chloride, the generation of 3-MCPD was found to be proportional to the lipase activity in the mixture.

In dried savoury foods containing salt and fat, the residual lipase activities of certain ingredients should be monitored, as they may reflect the formation of unwanted contaminants over storage and processing time.

There is another ways of contamination, 3-MCPD can migrate from certain types of epichlorohydrin-based wet strength resins used in paper and cellulose casings, for example sausage casings, tea bags and coffee filter paper. European Commission sets a limit maximum permitted quantity of the 'residual' epichlorohydrin in the finished material, so 3-MCPD present in food from contact with casings or wrappings should be minimal in foods produced in the EU.

2.1.Occurrence in food (3-MCPD)

3-Monochloropropane-1,2-diol (3-MCPD) is one of a number of chemically related contaminants collectively known as chloropropanols.

3-MCPD was originally identified as a contaminant of the savoury food ingredient hydrolysed vegetable protein (HVP) produced by the hydrochloric acid hydrolysis of crude vegetable proteins. These protein sources contain residual lipids (triglycerides) and the reaction with hydrochloric acid to produce chloropropanols has been studied (Collier *et al.* 1991). 3-MCPD has also been reported in soy sauces (Macarthur *et al.* 2000) and some food contact materials.

3-Monochloropropane-1, 2-diol (3-MCPD) has been detected in numerous foods, amongst others in dark toast, in the crust of bread. These compounds such as 2-monochloro-propane-1, 3-diol (2-MCPD) have for a long time been known as contaminants in various foods such as liquid seasoning or bakery goods.

They also may be present as impurities in epichlorohydrin compounds.

Epichlorohydrin copolymers containing low concentrations of 3-MCPD and 1,3-DCP are also used as "wet-strength" resins for products such as tea bags, coffee filters, sausage casings, absorbents packaged with meats, and paper towels and tissues. DECs are used in flocculants and coagulants for water purification, and 3-MCPD has been detected in finished drinking water as a result of such use (EC, 2004). DECs are also used as decolorizing agents and flocculants in the clarification of refinery sugar liquors and juices, and in the production of high-fructose corn syrup (Tritscher, 2004; ILS, 2005).

3-MCPD has been detected in a variety of foods. These include:

• A wide range of prepared and processed foods to which acid-HVP is added, including many soy sauces, oyster sauces, other sauces, instant soups, bouillon cubes, gravy mixes, savory snacks, spreads, stuffings, ready-to-eat meals, instant noodles, frozen dinners, and other frozen prepared foods.

• Some processed foods that do not contain acid-HVP, such as malt products; cold-smoked meats, sausage, and fish; cooked meats (salami, bacon, hamburgers); anchovies packed in oil; melted or grilled cheese; processed cheese and cheese alternatives; roasted or toasted cereals, breads, and biscuits; instant coffee and roasted coffee beans (Hamlet *et al*., 2002; WHO, 2007; Baer *et al*., 2010).

They can be found in cereal-derived products (bread, biscuits).

In leavened dough, the main precursors are chloride ions and glycerol, which do react with each other during the baking process to form 3-MCPD. Glycerol accounts for 68% of the 3-MCPD formed upon baking proved commercial bread doughs. It is mainly generated by yeast during proving, but can also originate from flour or flour improvers.

3-MCPD is mainly found in the crust and can be present at very high levels (up to 400 µg kg−1). This is because this part of the bread undegoes the greatest exposure to high temperatures during the baking process, thus boosting the reaction. No contaminant was detected in the breadcrumbs, thus moderating the 3-MCPD content throughout the whole loaf. This is even more critical for toast, since a much higher proportion of the bread is exposed to heat, resulting in more 3-MCPD and thus an enhanced 3-MCPD intake.

Breitling-Utzmann *et al.* tested several bread ingredients for their influence on 3-MCPD formation. These included fat, baking agent, sour dough, emulsifiers, sugars, and leaven. The baking agent consisted of sugar, flour, soy flour, calcium sulfate, emulsifier (mono- and diacylglycerols of edible fatty acids, E471), enzymes and ascorbic acid. Among all these ingredients, the use of baking agent

appeared to have the greatest effect, and high levels of 3-MCPD were detected. Considering the various components of the baking agent, this effect could not be explained by the emulsifiers (which were also tested separately), but the sugar content does seem to have a strong influence on the production of 3-MCPD. However, other ingredients must have synergetic effects.

The effect of sugar was also observed in a study realized in the UK. In preparations where sugar and yeast were added to dough, the sugar content did not influence glycerol generation in the yeast, but it did appear to increase the final level of 3-MCPD produced during baking.

Another observation was that storage of the simple model dough for one week prior to cooking produced twice the amount of 3-MCPD compared to the freshly prepared doughs, and this could not be explained. It was suggested that the storage period either increased the concentrations of the precursors or removed potential inhibitors of 3-MCPD production. It was also found that the addition of yeast to the model dough significantly increased the production of the contaminant, and that pre-incubating with yeast had even more of an effect.

According to the same researchers, phospholipids, which are minor components in flour, show better reactivity during the formation of 3-MCPD than glycerol. Finally, it was also found that commercial improvers used in bakery products, which typically contain ascorbic acid, soya flours and a source of monoacylglycerols (e.g. glycerolmonostearate or modified monoacylglycerols), could promote the formation of 3-MCPD in unleavened dough.

Malt-derived products

These can be food-grade malted grains, malt flours and malt extracts, products which are used for colouring and flavouring purposes. 3-MCPD results from the dry-kilning of malted and unmalted barley at temperatures above 170°C.

Although this presence seems to be inevitable, the consequences are not that important, as levels are low and when used for brewing they will be even more diluted. It has also been shown that the endogenous components of the grain are sufficient to promote 3-MCPD synthesis. No addition of fat, acid or chloride is necessary. However, studies showed that only small amounts of 3-MCPD are detected in malt flours and pale brewing malt, whereas dark brewing malt sometimes contains very high levels (247 µg kg−1).

Although strongly diluted in the resulting product, 3-MCPD may be bound to other components contained in the beer, such as acids, aldehydes or alcohol. It appears that the level of 3-MCPD esters is indeed much higher. The bound form has been shown to exceed the free form by factors of 0.4–36.

Coffee

Coffee can contain 3-MCPD, but at low levels. It has been found in roasted coffee, but the highest levels were seen in instant coffee and in products with prolonged roasting. The final colour of the coffee beans is directly linked to 3-MCPD formation, with the darker beans having the greatest concentration.

Chloride from salt and lipids naturally present in coffee beans are responsible for 3-MCPD formation during the roasting process. However, 3-MCPD is not detected in coffee beverages because of the dilution with water.

Cheese

3-MCPD only generally occurs at low levels in cheese, but it can be found in melted or grilled cheese. Crews *et al.* found that grilling produced 3-MCPD in all studied cheese samples, whereas microwave heating significantly enhanced levels in Parmesan cheese only.

The exact mechanism of formation is not yet known, but it can be assumed that chloride ions and glycerol (or substituted glycerol) are at the heart of it, since they are both abundant in cheese.

Smoked food

The contaminant was also detected at quite high levels in a study performed in Germany on smoked meat. The level of 3-MCPD appeared to be dependent on the duration and the type of wood used for the smoking. Researchers from the same institute had the opportunity to analyse samples of one type of smoked sausage at different stages of the production process and also to analyse its ingredients.

The smoking procedure took place at a low temperature (28°C).

The smoke itself and samples scraped from the walls showed very high levels of 3-MCPD. The pellets used to generate the smoke did not initially contain any 3-MCPD.

Laboratory experiments showed that the addition of 20% calcium carbonate to the pellets prior to smoking significantly reduced the production of 3-MCPD in the smoke. Other experiments indicated

that lipids are not involved in the generation of the contaminant. The authors proposed that 3-MCPD forms via 3-hydroxyacetone following the cracking of cellulose, but agreed that more research is needed.

A similar study in the UK focussing on cooked meat, prepared cheese, smoked food, etc., also found the contaminant, but not at levels as high as in the German study. This may be explained by different preparation/smoking procedures, which would have to be verified.

The 3-MCPD content in kippers seems to increase with the salt concentration in the brine used for the process and with the smoking time.

The concentration of brine used in brine immersion curing had a significant effect on the salt content and a corresponding effect on the formation of 3-MCPD.

It should be pointed out that the smoking procedures associated with the occurrence of 3-MCPD were generally of the "cold smoking" type, meaning that they were performed at low temperatures. Nothing has been found concerning the occurrence of 3-MCPD after smoking at high temperatures.

Meat

3-MCPD can be present in cooked meats, such as salami, bacon, hamburgers, etc. However, the influence of the meat cooking process on the formation of the chloropropanediol is not understood, and there is no direct link to precursors like glycerol.

It seems that the cooking sometimes encourages 3-MCPD formation and sometimes has no effect at all. In a study by Crews *et al.*, it was found that 3-MCPD is not formed in boiled or stewed meat, suggesting that temperatures of >100°C might be necessary and that “wet” cooking may hinder its formation.

Another explanation is that the contaminant may have originated from the epichlorhydrin in the coating from salami. Furthermore, salami can contain high levels of 3-MCPD esters.

Salted fish

The contaminant was also detected in some samples of anchovy fillets in olive oil.

However, a study revealed that it was not formed during maturation with salt, but later on, during packaging and storing. The way in which 3-MCPD is formed in the anchovy fillets is not known, but it may result from enzyme action on fats. Such activity may release glycerol-related precursors which then react with chloride ions.

On the other hand, chloroesters may be formed from the interaction of fats with chloride, which then create 3-MCPD upon hydrolysis. Whatever the exact mechanism of formation is, this type of fat/enzyme-related reaction may be quite widespread. However, there does not appear to be anything in the scientific literature on this topic so far.

Soy sauce

Soy sauce can be manufactured by a range of processes, including traditional fermentation and processes which involve the use of an acid treatment or include acid-HVP as an ingredient.

Traditionally fermented soy sauces are unlikely to contain 3-MCPD, although the addition of 3-MCPD containing ingredients such as contaminated acid-HVP.

The EU has identified that there are many liquid seasoning condiments similar to soy sauce (such as fish sauce, oyster sauce, mushroom sauce, meat seasoning sauces etc.) which can contain 3-MCPD and other chloropropanols, either as a result of processing or from the use of processed ingredients.

In Canada a nationwide investigation of 3-MCPD in various soy, mushroom and oyster-flavoured sauces is being conducted**.** In 2004-2005, 45 samples of imported soy sauces were analyzed for 3-MCPD and for 1,3-DCP. The samples comprised soy sauces marketed as plain, vegetarian, seasoning, oyster, mushroom, light or dark.

The country of origin was a more consistent factor in observations of contaminated sauces than were other variables such as the type of sauce examined or whether or not the sauce was fermented.

Table 1 contains a list of products and ingredients identified as containing 3-MCPD. These products do not appear to contain acid-HVP and therefore the 3-MCPD is thought to originate from another source. Maximum levels recorded in these products have been included.

Table 3: Products that potentially contain 3-MCPD that does not originate from acid-HVP

	Codex Food Grouping	Range of 3-MCPD (quantifiable, mg/kg)	Number in Sample	Number quantifiable
1.6	Cheese	0.02 - 0.1	123	12
2.2	Fat emulsions mainly of type water in oil (comprises spreads, butters, margarine)	0.006 - 0.01	12	1
5.2	Sugar based Confectionery including hard and soft candy, nougats etc	0.020 - 0.023	15	2
6.2	Flours and Starch	0.014 - 0.029	11	4
6.3	Breakfast Cereals	0.07	45	1
7.1.1	Breads & Rolls	0.001 - 0.57	966	524
7.1.2	Crackers excluding sweet crackers	0.01 - 0.26	166	112
7.1.3	Baked Cereal Products	0.011 - 0.11	59	40
7.1.4	Bread Type Products, including Bread stuffing and bread crumbs	0.01 - 0.15	20	8
7.2.3	Other fine baked products including doughnuts, scones and muffins	0.01 - 0.11	98	44
7.2.1	Cakes, cookies and pies (eg fruit filled or custard type)	0.01 - 0.21	98	25
7.2.2	Biscuits	0.01 - 0.28	460	196
8.1	Fresh meat, in whole pieces or cuts or comminuted	0.006 – 1.9	106	19
8.2	Processed meat in whole pieces or cuts	0.005 - 0.10	109	30
8.3	Comminuted processed meat	0.007 – 1.8	158	58
21	Meat Extract	0.014 - 0.55	16	5
9.4	Fully preserved fish and fish products, including molluscs, crustaceans, and echinoderms	0.012 - 0.19	18	8
13.3, 13.4, 13.5	Dietetic Foods and Formulae	0.01 - 0.41	33	14
14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	0.01 – 0.38	58	27
14.2.1	Beer & Malt Beverages	0.003 - 0.02	104	8
15.1	Snacks including potato chips	0.01 – 0.04	60	7
22.2	Malt extract	0.005 - 0.85	31	17
23	Modified Starched Dextrins	0.012 - 0.49	9	2
26	Other ingredients	0.019 - 0.025	11	2
Includes	Flavourings	0.025	2	1
16	Composite foods	0.004 - 0.11	113	36
Includes	Pizza	0.004 - 0.09	83	31

2.2.Reduce contamination

Regarding the presence of chloropropanols in soy sauces and related foods, if these products are prepared using traditional fermentation processes (without the addition of acid-HVP), they generally do not contain 3-MCPD or other chloropropanols (Crews *et al.*, 2003; WHO, 2007).

Efforts to reduce the levels of chloropropanols in soy sauces and other foods include carefully controlling the acid hydrolysis process, following the acid hydrolysis step with an alkaline hydrolysis step, and reducing the concentrations of fats and oils in the starting materials (Crews *et al.*, 2003; Macarthur *et al.*, 2000). Baer *et al.* identified other control strategies for limiting the amount of 3-MCPD in food, including:

- Raising the pH of high moisture content food;
- Lowering the maximum processing temperature and salt content of the food;
- Avoiding low water/high temperature treatments;
- Limiting the amount of glycerol produced in the food during preparation and storage;
- Avoiding the use of partial glycerides as additives;
- Using spice extracts instead of native spices;
- Reducing microbial load via thermal treatment;
- Confirming the purity of food additives;
- Inactivating lipases and esterases;
- Screening food contact material for 3-MCPD precursors.

2.3.Formation of 3-MCPD esters in food

The mechanism for the formation of 3-MCPD esters is a cyclic acyloxonium ion from triacylglycerol, followed by reaction with chloride ions and formation of 3-MCPD esters.

The main factors for the formation of 3-MCPD esters are the presence of chloride ions, glycerol, tri-, di- or monoacylglycerides, as well as temperature and time.

In particular, increasing amounts of mono- and diacylglycerides in the oil show a linear correlation with the increased formation of 3-MCPD esters. The most prevalent isomer among the chloropropanols is 3-MCPD, but 2-MCPD might also occur in food, but at lower concentrations.

Esters of glycidol are also formed during the refining of vegetable oils. The glycidol esters seem to be precursors on the formation pathway to 3-MCPD esters. In the absence or depletion of chloride ions during the deodorisation step, the pathway is believed to end at the stage of glycidol esters (CEPA, 2010).

There are studies to investigate the formation of 2-MCPD during the deodorisation step of oils. When degummed and bleached palm oil was subjected to deodorisation at temperatures ranging from 180 to 250°C, the formation of esterified 3-MCPD and 2-MCPD was directly correlated to temperature, with the highest amounts of 3-MCPD and 2-MCPD bound in esters (approx. 4.0 and 2.5 mg/kg, respectively) being measured in samples deodorised at 250°C (for over one hour).

The ratio of 3-MCPD esters to 2-MCPD esters also seemed to be temperature-dependent, changing from approximately 4:1 at 180°C to 2:1 at 250°C. In subsequent analyses of edible oils, it was found that seed oils (sunflower, coconut and rapeseed) contained significantly lower amounts of 3-MCPD and 2-MCPD bound in esters (typically <0.3 mg/kg 3-MCPD bound in esters and <0.15 mg/kg 2-MCPD bound in esters) than the refined palm oils (1.5–5.0 mg/kg 3-MCPD bound in esters and 0.7–3.0 mg/kg 2-MCPD bound in esters) (CEPA, 2010).

In further studies it was found that roasted barley heated at increasing temperatures contained 0.6–1.9 mg/kg 3-MCPD bound in esters after a roasting period of 35 minutes.

Optimisation of the refining process is also a challenge because it is a balancing act between the necessary purification steps of the oil and the formation of other process-derived contaminants. A better understanding of the mechanisms of 3-MCPD esters formation is required to give direction to further refining trials.

Another method of formation of compounds is the molecular mechanism of 3-MCPD ester formation: **chlorinating agents and formation of cyclic acyloxonium ions.**

The proposed mechanisms behind the formation of 3-MCPD esters involve the formation of a cyclic acyloxonium ion from triacyl glycerol, followed by reaction with a chloride ion and resultant formation of an 3-MCPD ester. Cyclic acyloxonium ions are readily formed from triacyl glycerols in the presence of Lewis acids. Studies using tripalmitin, dipalmitin and ^{13}C-labeled tripalmitin (1,1,1^{-13} C_3) have indicated the formation of acyloxonium ions in tripalmitin and dipalmitin, and their subsequent chlorination to form 3-MCPD monoesters and diesters when heated at 90°C in the presence of $ZnCl_2$.

The identity of the chlorinating agent during refining of oil remains elusive. In particular, the ionic nature of chloride precludes its easy access into the hydrophobic environment of the oil.

Studies have indicated that the ability of sodium chloride to chlorinate glycerol is greatly enhanced in the presence of amino acids and phosphate-containing compounds such as deoxy-guanosine monophosphate. In addition, amino acid hydrochloride salts have greater ability to chlorinate glycerol than a mixture of sodium chloride and amino acids. These studies have also indicated that covalently bound chlorine in organic compounds such as in sucralose is also able to efficiently chlorinate glycerol, supporting the hypothesis of an oil-soluble "chlorinating agent", specifically during palm oil refining, that can be formed through the reaction of carotenoid radical cations with halogens to form complexes. Such complexes can bring chloride ions into the proximity of the lipids to effect chlorination through reaction with acyloxonium ions.

2.4.Occurrence in food (3-MCPD esters)

3-MCPD esters are now found to be widespread in thermally processed foods like French fries, toasted bread, bread crust, donuts, salty crackers, roasted coffee, roasted chicory (coffee surrogate), roasted barley, roasted dark malt, coffee creamer and in fermented foods like pickled herring and sausage.

Reported levels were between 0.2 and 6.6 mg/kg in most of the analysed foodstuffs and the levels of esterified 3-MCPD were much higher than the levels of free 3-MCPD (CEPA, 2010).

Whereas untreated edible fats and oils only contain low levels of 3-MCPD, study results from the official food control authorities show that all refined, non-native edible oils and fats contain what are in

some cases substantial amounts of 3-MCPD fatty acid esters. According to the latest scientific knowledge available, they are formed during oil refining, i.e. during cleaning and processing. Crude oils also contain various accompanying substances which are removed for reasons, amongst other things, of odour and taste. In the course of refining the oils are degummed, deacidified, bleached and steamed at high temperatures. Steaming is also called "deodorisation".

Since 1980 there had been sporadic reports about 3-MCPD esters in hydrochloricacid-hydrolysed lipids and plant proteins and that fatty acid esters of 3-MCPD had been identified in,Spanish rapeseed oils treated with aniline and refined with hydrochloric acid.

No 3-MCPD esters, or only traces, were detectable in native and unrefined fats and oils, whereas significant amounts were present in nearly all refined fats and oils. Deodorisation was clearly identified as the crucial step for 3-MCPD ester formation in the refining process of fats and oils, with almost the total quantity of 3-MCPD esters being formed at this last step of the process.

Studies have classified refined vegetable oils and fats into three groups according to the level of 3-MCPD found to be ester-bound:

- Low level (0.5–1.5 mg/kg): rapeseed, soybean, coconut, sunflower oil;
- Medium level (1.5–4 mg/kg): safflower, groundnut, corn, olive, cottonseed, rice bran oil;
- High level (>4 mg/kg): hydrogenated fats, palm oil and palm oil fractions, solid frying fats.

In used frying fat, 3-MCPD levels decreased with increasing time of use. During the deep-frying process nearly no additional 3-MCPD is formed. Therefore, the level of 3-MCPD esters in French fries and other fried foodstuffs only depends on its concentration in the used frying fat.

Although there is a lack of data about 3-MCPD esters for many foodstuffs, it is obvious that thermally processed foods and refined fats and oils (as such or as a component of other foodstuffs) are the most significant sources of 3-MCPD esters for consumers. In particular, refined palm oil in different kinds of foodstuffs is responsible for a significant part of the exposure.

Recently, the presence of fatty acid esters of glycidol had also been detected in refined palm oil. Glycidol esters might be formed from an acyloxonium intermediate, which is well known from the pathway of 3-MCPD ester formation. Glycidol nearly quantitatively reacts to 3-MCPD under the conditions of analysis and there are strong indications that a significant amount (10–60%) of measured bound 3-MCPD results from fatty acid esters of glycidol.

Glycidol has been classified by the International Agency for Research on Cancer (IARC) as "probably carcinogenic to humans" (IARC Group 2A) and its formation during heat treatment of vegetable fat raises additional safety concern (IARC, 2000).

3. Toxicological studies

3.1.Acute toxicity

The LD_{50} of 3-chloro-1,2-propanediol in rats treated orally was reported to be 150 mg/kg bw (Ericsson & Baker, 1970).

3.2.Short-term studies of toxicity

Rats

Groups of eight male Fischer 344 rats were given a single subcutaneous injection of 3-chloro-1,2-propanediol at a dose of 75 mg/kg bw and killed after 24 h or 3, 8, 25, or 75 days. A slight but significant ($p < 0.05$) increase in liver weight was observed after 24 h but not at later sacrifices. Histologically, the hepatocytes showed mild-to-moderate cytoplasmatic swelling in the periportal area (Kluwe *et al.*, 1983).

Intraperitoneal injection of 3-chloro-1,2-propanediol to male Sprague-Dawley rats at a single dose of 100 mg/kg bw caused increased diuresis for up to 15 days. Higher doses (not reported) caused aneuresis and death, and histological examination of the kidney showed acute glomerular nephritis. The type of kidney lesion was characteristic of oxalic acid poisoning, and crystals of calcium oxalate were seen in urine by microscopy. Oral treatment with 3-chloro-1,2-propanediol at 10 mg/kg bw per day for 5 days did not increase diuresis (Jones *et al.*, 1978).

In another study, intraperitoneal injection of 3-chloro-1,2-propanediol at a dose of 100 or 120 mg/kg bw caused severe proteinuria and glucosuria in male Wistar rats. Oliguria and anuria were observed, and four of nine animals died. The five surviving animals had decreased appetite and body weight, proteinuria, dose-related diuresis, and increased water intake (Morris & Williams, 1980).

Testing of the (R)- and (S)-isomers of 3-chloro-1,2-propanediol, synthesized under laboratory conditions, showed that only the (R)-isomer induced diuresis and gluco-suria in rats (Porter & Jones, 1982; Dobbie *et al.*, 1988; Jones & Cooper, 1999).

Oxalic acid, a metabolite of 3-chloro-1,2-propanediol, appeared to play a important role in the development of kidney damage (Jones *et al.*, 1979). Birefringent crystals characteristic of calcium oxalate seen in tubules at the cortico-medullary junction of rats 1 day after treatment with 3-chloro-1,2-propanediol at a single subcutaneous dose of 75 mg/kg bw were considered to be early morphological changes. On day 75, focal tubule necrosis, regeneration, and tubule dilatation were observed in the kidney (Kluwe *et al.*, 1983).

Groups of 20 Sprague-Dawley rats of each sex were given 3-chloro-1,2-propanediol at a dose of 0, 30, or 60 mg/kg bw per day by gavage on 5 days per week for 4 weeks. Ten animals of each sex from each group were killed on day 2, and their blood was examined for clinical chemical parameters. On day 2, rats at the higher dose had increased serum alanine aminotransferase activity (males, $p < 0.05$; females, $p < 0.001$) and increased concentrations of creatinine (females, $p < 0.001$), urea, and glucose (females, $p < 0.05$). On day 25, the treated rats had increased alanine aminotransferase activity (males at the higher dose, $p < 0.001$; females at both doses, $p < 0.001$) and increased urea concentrations (males at the higher dose, $p < 0.001$; females at the higher dose, $p < 0.05$). Statistically significantly ($p \leq 0.05$) decreased values for haemoglobin concentration and erythrocyte volume fraction were found for both male and female treated rats. Female rats at the higher dose had a decreased erythrocyte count ($p < 0.001$). Treated rats had lower body-weight gain, which was statistically significant (statistics not reported) at termination of the study. After 2 days of treatment, the relative weight of the kidney was increased ($p < 0.001$) in males at the higher dose and in females at both doses, and on day 25, the relative weights of the kidney, liver, and testis (males at the higher dose) were significantly increased ($p < 0.01$ or 0.001) in treated rats. Histopathological examination revealed chronic progressive nephropathy in eight females at the higher dose and mild tubule dilatation in the testis of three males at the lower dose and seven at the higher dose. One male at the higher dose had severe atrophy of both testes (Marchesini & Stalder, 1983).

Groups of 20 Fischer 344 rats of each sex were given drinking-water containing 3-chloro-1,2-propanediol at a concentration of 0, 100, 300, or 500 mg/L for 90 days, corresponding to average daily intakes of 9, 27, and 43 mg/kg bw for males and 11, 31, and 46 mg/kg bw for females. Ten animals of

each sex per group were killed after 30 days of treatment. Clinical chemical and haematological parameters were determined, and histopathological examinations were carried out on controls and rats at the highest dose.

Slight anaemia ($p < 0.05$ or 0.001) was seen in females at the two higher doses after 30 days and in rats of each sex after 90 days of treatment ($p < 0.05$ or 0.01); however, there was no morphological evidence of impaired haematopoiesis or increased degradation of erythrocytes. A dose-dependent decrease ($p < 0.01$) in plasma creatinine concentration was seen in rats of each sex at the two higher doses after 30 days of treatment and in all treated groups at terminal sacrifice ($p < 0.05$ or 0.01). Serum phosphate concentrations were increased in male rats at the highest dose at the interim ($p < 0.01$) and terminal sacrifices ($p < 0.05$). A statistically significant ($p < 0.01$), dose-dependent increase in relative weights was found for the kidney and liver, and the increase in the relative kidney weight was significant at the lowest dose. Histopathological examination showed a lower incidence of crystalline precipitation in the kidneys of animals at the highest dose than in the controls. The livers of about half of the treated males were found to contain single hepatocytes with two or three nuclei after 90 days of treatment, and the epididymides of treated rats had an increased number of exfoliated spermatozoids (Marchesini *et al.*, 1989).

Primates

Three of six male macaque monkeys (*Macaca mulatta*) given 3-chloro-1,2-propanediol orally at a dose of 30 mg/kg bw per day for 6 weeks showed haematological abnormalities including anaemia, leukopenia, and severe thrombocytopenia (Kirton *et al.*, 1970). The Committee noted that two of the affected monkeys died during the study due to bone-marrow depression.

3.3.Long-term studies of toxicity and carcinogenicity

Mice

Fifty female CHR/Ha Swiss mice received 3-chloro-1,2-propanediol subcuta-neously at a dose of 1 mg/week for 580 days, and a second group of 50 mice received the compound dissolved in acetone by topical application at a dose of 2 mg three times per week. No changes were observed in the group treated by dermal application, but local sarcomas were found at the site of injection in one dosed and one control mouse treated subcutaneously (Van Duuren *et al.*, 1974).

Rats

Three groups of 26 male and female Charles River CD rats received 3-chloro-1,2-propanediol at a dose of 0, 30, or 60 mg/kg bw by gavage twice weekly. After 10 weeks, the doses were increased to 35 and 70 mg/kg bw. The animals were treated for 72 weeks, and the study was terminated after 2 years. Three parathyroid adenomas were found in male rats at the higher dose, but this finding was not statistically significant with respect to the control group. While the females showed no signs of toxicity, male rats had a higher mortality rate than controls, and all treated males had severe testicular degeneration and atrophy (Weisburger *et al.*, 1981).

Four groups of 50 specific pathogen-free Fischer 344 rats of each sex, 5–6 weeks old at the start of the study, underwent an 11-day acclimatization period and then received drinking-water containing 3-chloro-1,2-propanediol (purity, 98%) at a concentration of 0, 20, 100, or 500 mg/L, equivalent to mean daily intakes of 0, 1.1, 5.2, and 28 mg/kg bw per day for males and 0, 1.4, 7.0, and 35 mg/kg bw per day for females, for 104 weeks. Feed and water were provided *ad libitum*. The feed was certified laboratory chow, with contaminants within an acceptable range according to the Environmental Protection Agency of the USA. The test substance was stable in water for > 4 days, and it was prepared twice a week and tested once per group per week. The water contained a mean of 2.7 mg/L of 3-chloro-1,2-propanediol, equivalent to an intake of 0.15 mg/kg bw per day for males and 0.19 mg/kg bw per day for females, determined once per week.

The animals were examined daily for changes in health or behaviour. Food consumption was recorded weekly up to week 19 and body weight weekly up to week 20, and then both were recorded monthly; from week 88 to the end of the study, body weight was again recorded weekly. Water

consumption was recorded weekly from start to week 20 of the study and fortnightly thereafter. Ophthalmological examinations were performed regularly. Haematological parameters and blood chemistry were evaluated in blood samples taken at weeks 103–105 from all surviving animals. All animals found dead, animals killed *in extremis*, and those killed at the end of the experiment were subjected to complete necropsy and histopathological examination. The liver, kidneys, spleen, pancreas, heart, adrenals, testis, epididy-mides, and brain were weighed.

The body weights of rats at the highest dose were significantly ($p < 0.05$) reduced after the first week of treatment. At termination, the body weights of animals at the two higher doses were significantly reduced ($p \leq 0.05$), with reductions of 33% in males and 35% in females at the highest dose. However, the mortality rate was unaffected by treatment: at terminal sacrifice, more than 42% of this group were still alive. The food and water intake of rats at the highest dose were significantly ($p < 0.05$) reduced. No treatment-related clinical signs were noted. The haematological and blood clinical chemical parameters varied considerably within groups, but no consistent, significant, dose-related effects were observed. The reduced body weights of rats at the two higher doses obviated a conclusion about an effect on organ weights. However, the body weights of animals at the lowest dose were unaffected, and males showed a significant ($p < 0.05$) increase in absolute kidney weight. Treatment-related pathological, hyperplastic and neoplastic findings are listed in Table 4.

Table 4: Incidences of treatment-related pathological, hyperplastic, and neoplastic lesions in a 2-year study in rats with 3-chloro-1,2-propanediol.

Organ and lesion	Dose (mg/kg bw per day)			
Males	0[a]	1.1	5.2	28
Testis				
Leydig-cell hyperplasia	39/50	27/50*	4/50***	0/50***
Leydig-cell adenoma	38/50	43/50*	50/50***	47/50*
Leydig-cell carcinoma	0/50	0/50	0/50	3/50

Mammary gland				
Glandular hyperplasia	2/45	6/48	24/47***	43/49***
Fibroadenoma	0/45	0/48	2/47	10/49**
Adenoma	0/45	0/48	1/47	1/49
Adenocarcinoma	0/45	0/48	1/47	1/49
Kidneys				
Nephropathy	36/50	40/50	45/50*	49/50***
Tubule hyperplasia	3/50	6/50	15/50**	34/50***
Tubule adenoma	0/50	0/50	1/50	5/50
Pancreas				
Islet-cell hyperplasia	14/48	8/50	5/50*	1/48***
Islet-cell adenoma	16/48	9/50	7/50*	0/48***
Islet-cell carcinoma	8/48	0/50**	2/50*	0/48**
Mixed adenoma	0/48	1/50	0/50	1/48
Preputial glands [b]				
Adenoma	1/5	2/13	6/16	5/11
Carcinoma	0/5	0/13	1/16	2/11
Females	0[a]	1.4	7.0	35
Kidneys				
Nephropathy	24/50	23/50	42/50***	48/50***

Tubular hyperplasia	2/50	4/50	20/50***	31/50***
Tubular adenoma	0/50	1/50	0/50	9/50**

From Sunahara *et al.* (1993)

* Statistically significant at $p < 0.05$; ** statistically significant at $p < 0.01$; *** statistically significant at $p < 0.001$; pair-wise Fisher's test between treated and controls

[a] Drinking-water of the control group contained 2.7 mg/L 3-chloro-1,2-propanediol, equivalent to a daily intake of 0.15 mg/kg bw per day for males and 0.19 mg/kg bw per day for females

[b] The preputial gland was not included in the protocol but was either found incidentally on skin sections or was collected at autopsy if it contained a visible nodule. As this organ was not examined in all animals, no meaningful statistical analysis of the tumour incidence could be conducted.

Chronic progressive nephropathy was found in all groups, and the incidence increased with dose, being significant at the two higher doses ($p \leq 0.05$). Female rats were more severely affected than males. Significant correlations ($p < 0.01$) were found between the severity of nephropathy and the increase in incidence of renal tubule hyperplasia and renal adenoma (see below). Advanced chronic progressive nephropathy accounted for a significant (males) and dose-dependent (both sexes) rate of premature deaths ($p < 0.005$ for males and $p < 0.10$ for females). The treatment-related distribution of advanced chronic progressive nephropathy in rats of each sex was reflected in significant, dose-dependent increases in kidney weight ($p < 0.05$), serum creatinine concentration ($p < 0.01$), and blood urea nitrogen concentration ($p < 0.01$). Papilliform hyperplasia of the urothelium covering the renal papilla was seen almost exclusively in animals at the two higher doses. Both the incidence and the severity of the lesions increased in a dose-dependent pattern. The incidence of papillary urothelial hyperplasia correlated to the severity of chronic progressive nephropathy. A dose-dependent increase in the frequency of epithelial single-cell degeneration was observed in the epididymides, which was significant at the two higher doses ($p < 0.001$).

Dose-related alterations in the incidence of hyperplasia and/or tumours were observed in all groups, with increases in the kidney (tubule hyperplasia and adenomas), the testis (Leydig-cell hyperplasia, adenomas and adenocarcinomas), mammary gland (males: fibroadenomas, adenomas, and adenocarcinomas), and preputial gland (adenomas and carcinomas), and decreases in the pancreas (males: hyperplasia, adenomas, and carcinomas) (see Table 1). The increased incidence of tubule

hyperplasia in the kidneys of animals of each sex was the most sensitive end-point, as it was seen even at the lowest dose. Although it did not reach statistical significance at this dose (p = 0.073 for males and 0.099 for females), the Committee considered that it reflected a treatment-related, dose-dependent increase, which was highly significant ($p < 0.0001$) in a trend analysis. Nodular Leydig-cell hyperplasia was present in a high proportion of controls, and the incidence in treated animals decreased significantly in a dose-dependent pattern.

When the incidences of nodular Leydig-cell hyperplasia, adenomas, and carcino-mas were combined for statistical analysis, no significant difference was found between groups. The Committee noted that the decreased frequency of hyperplasia might be associated with the concomitant increases in the incidences of adenomas and carcinomas, so that the effect would not be significant when all three were combined. When the incidences of preputial gland adenomas and carcinomas were combined for statistical analysis, the resulting increased incidence was significant at the two higher doses. The Committee noted that the report clearly stated that the preputial gland was not included in the standard protocol and was examined only when it was removed incidentally with other tissues or organs. Thus, few were investigated. When pancreatic hyperplasia and neoplastic lesions were combined for statistical analysis, the decrease in incidence was significant at all doses ($p \leq 0.05$).

The authors concluded that treatment with 3-chloro-1,2-propanediol increased the incidences of renal and testicular Leydig-cell tumours. The renal tumours developed in a dose-dependent fashion in animals of each sex and were considered to be secondary to the treatment-related increase in the incidence of chronic progressive nephropathy. The treatment-related increase in the incidence and frequency of Leydig-cell tumours was considered to represent hormone-mediated promotion and was suggested to be associated with the treatment-related decrease in testosterone concentration and the increase in those of estradiol, prolactin, progesterone, and follicle-stimulating and luteinizing hormones. 3-Chloro-1,2-propanediol also caused a dose-related increase in the incidence of mammary tumours in males, and this effect was considered to be secondary to the hormonal activity of functionally active Leydig-cell tumours, which were suggested to produce less androgen and more estrogen or progesterone. The Committee noted that the hormones mentioned were not measured in the study. In addition, the treatment caused a dose-related increase in the incidence of preputial gland tumours, which was suggested to be secondary to the disturbed endocrine balance of treated animals

with large Leydig-cell tumours, analogous to the induction of mammary tumours (Sunahara *et al.*, 1993).

3.4.Genotoxicity

A test for micronucleus formation in bone marrow *in vivo* was performed in Crl:HanWistBR rats according to a protocol conforming to OECD 474. The highest dose was determined from a range-finding study in which single oral doses of 20–100 mg/kg bw were administered once daily for 2 days to groups of male and female rats; doses $\geq$ 60 mg/kg bw per day were severely toxic and caused some deaths. Male animals were used in the main study, as no substantial sex difference in toxicity was seen. Groups of six males were given 3-chloro-1,2-propanediol orally at a dose of 15, 30, or 60 mg/kg bw per day for 2 days. Piloerection was seen at the highest dose and was associated with a clear reduction in the ratio of polychromatic to normochromatic erythrocytes, indicating bone-marrow cytotoxicity and hence indicating that the substance and/or its metabolites had reached the bone marrow. There was no increase in the number of micronucleated polychromatic erythrocyte stem cells at any dose (2000 polychromatic erythrocytes scored per animal). Cyclophosphamide, used as the positive control, caused a clear increase in the number of micronuclei (Marshall, 2000).

A test for unscheduled DNA synthesis was performed in male Han Wistar rats according to a protocol that conformed to OECD 486. The highest dose of 100 mg/kg bw was chosen on the basis of a study that had shown severe toxicity at an oral dose of 150 mg/kg bw. In the main study, a single oral dose of 40 or 100 mg/kg bw was administered to the animals, and hepatocytes were recovered for analysis of unscheduled DNA synthesis by autoradiography after 12–24 h (four animals per dose) and 2–4 h (five animals per dose). No signs of toxicity were seen at either dose, and no increase in unscheduled DNA synthesis was seen. The two positive control compounds, *N*-2-fluorenylacetamide and *N*-nitrosodimethylamine, both gave clear positive results (Fellows, 2000).

3.5.Reproductive toxicity

3-Chloro-1,2-propanediol has been reported to inhibit male fertility (Gunn *et al.*, 1969; Helal, 1982), although the effect was reversible (Ericsson & Youngdale, 1970; Jones, 1983). The mechanism of this activity of 3-chloro-1,2-propanediol is unknown, but its metabolites inhibit enzymes involved in spermatozoan glycolysis, reducing the motility of the spermatozoa (Jones, 1983). The inhibition of

spermatozoan motility by 3-chloro-1,2-propanediol was suggested to be due in part to alkylation of cysteine (Kalla & Bansal, 1977). The compound also affects several enzymes of epithelial cells in the testis and caput epididymis, resulting in decreased glycolysis (Gill & Guraya, 1980). Only the (S)-isomer, synthesized under laboratory conditions, specifically inhibited glycolysis in boar sperm (Stevenson & Jones, 1984).

3-Chloro-1,2-propanediol has two specific effects on the reproductive tract of male rats. These effects are dose-dependent and have been termed the 'high-dose effect' and the 'low-dose effect'. The 'high-dose effect', seen after a single intra-peritoneal injection of 75 mg/kg bw 3-chloro-1,2-propanediol, consisted of bilateral retention cysts or spermatocoele of the caput epididymis 5–7 days after treatment (Cooper & Jackson, 1973). Use of electron microscopy showed that administration of 3-chloro-1,2-propanediol by gavage at a dose of 140 mg/kg bw specifically affected the epithelia in the initial segment of the epididymis 2 h later. The cellular lesions were characterized by sloughing of the epithelium, which led to obstruction of the epididymal tract (Hoffer *et al.*, 1973). The back-pressure of the testicular fluid caused oedema, inhibition of spermatogenesis, and atrophy of the testis (Jones, 1983). Histological examination of testes from rats given injections of 40 mg/kg bw per day for 20 days revealed total inhibition of spermiogenesis due to degeneration and disappearance of the spermiogonia from the tubules. Proliferation of the epithelial cells of the ducts in the cauda epididymis was observed, and several blood vessels showed thickened walls (Samojlik & Chang, 1970).

The 'low-dose effect', which was evident a few days after an oral dose of 5–10 mg/kg bw per day, was directed towards mature sperm contained in the cauda epididymis. The spermatozoa were rendered incapable of fertilization but showed no visible change in morphology (Jones, 1983). Male rats given 3-chloro-1,2-propanediol by subcutaneous injection at 15 or 40 mg/kg bw per day became infertile 6 and 3 days after commencement of treatment, respectively. Treatment with 15 mg/kg bw per day for 30 days, followed by a recovery period of 18 days, resulted in recovery of fertility (Samojlik & Chang, 1970). The lowest daily oral doses shown to cause infertility in male rats, as determined by mating studies, were 5 mg/kg bw for 14 days (Coppola, 1969), 6.5 mg/kg bw for 10 days (Gunn *et al.*, 1969), 2.5 mg/kg bw with 'continuous' treatment (Erickson & Bennett, 1971), 8 mg/kg bw for 4 days (Turner, 1971), and 8 mg/kg bw (by subcutaneous injection) for 3 days (Black *et al.*, 1975).

Groups of five albino male rats given 3-chloro-1,2-propanediol orally at a dose of 0.5, 1, 2, 4, or 6 mg/kg bw per day for 10–12 days showed 2.5%, 20%, 45%, 85%, and 100% sterility (on the basis of histological degree of spermiogenesis), respectively (Helal, 1982).

As reported in a summary, groups of five male Wistar rats were given 3-chloro-1,2-propanediol in distilled water at a dose of 0, 0.1, 0.5, 1, 2, 3, 4, 5, or 10 mg/kg bw per day by gavage for 7 days before and during mating. Each male was mated with five virgin females, which were killed on day 14 of gestation and examined for pregnancy status. 3-Chloro-1,2-propanediol had no adverse effect on male fertility at doses $\leq$ 3 mg/kg bw per day, with respect to pregnancy rate and total numbers of implantations and live embryos; however, the pre-implantation loss was significantly greater ($p = 0.05$) for female rats mated with males given 3 mg/kg bw per day than in controls. The NOEL was 2 mg/kg bw per day (Parish, 1989).

3-Chloro-1,2-propanediol has also been reported to affect the fertility of male hamsters (30–100 mg/kg bw per day orally for 7 days), gerbils (20 mg/kg bw per day orally for 50 days), guinea-pigs (50–70 mg/kg bw per day orally or subcutaneously for 45 days), dogs (8 mg/kg bw per day subcutaneously for 30 days), rams (25 mg/kg bw per day intramuscularly for 4 days), and rhesus monkeys (30 mg/kg bw per day orally for 42 days) (Jones, 1978, 1983; Jones & Cooper, 1999). The compound was reported to have no effect on fertility in mice, quail, or rabbits (Jones, 1978).

Groups of 10 female rats were given 3-chloro-1,2-propanediol subcutaneously at a dose of 0 or 10 mg (approximately 25 mg/kg bw) every second day for 30 days. Significant ($p < 0.01$) decreases were noted in the relative weights of the ovary, uterus, and vagina when compared with controls. Histological examination ahowed that the ovary was small and had widespread follicular atresia and degeneration of corpora lutea; the uterus was regressed, and the lumen was lined with columnar epithelium; atrophic changes were observed in the vaginal epithelium. The protein and RNA contents of the uterus and vagina were significantly ($p < 0.01$) reduced when compared with controls. The authors suggested a luteolytic and possibly antiestrogenic effect of 3-chloro-1,2-propanediol in female rats (Lohika & Arya, 1979).

In a computer-assisted analysis of sperm motion to determine the relationship between dose and effect on sperm glycolysis as evidenced by impaired sperm mobility in the epididymis, male Long-Evans rats were given drinking-water containing 3-chloro-1,2-propanediol at concentrations providing a dose of 5, 10, or 20 mg/kg bw per day for 8 days. The percentage of motile sperm was significantly

reduced at the two higher doses. Multivariate analysis of end-points of motion, including curvi-linear velocity, linearity of swim path, velocity, and lateral head displacement, showed significant differences from controls at the two higher doses (Toth *et al.*, 1992).

3-Chloro-1,2-propanediol was administered by gavage to groups of 10 adult male CD rats at a dose of 0, 1, 5, or 25 mg/kg bw per day for 14 days. The animals were killed on day 15 or 29. At necropsy, testis weight, distribution of DNA ploidy in testicular cell suspensions, testicular and epididymal histological appearance, and epididymal sperm concentration, motility, morphology, and breakage were determined. Before the kill at day 15, the males were cohabited with untreated females in a 1:2 ratio. The females were killed on presumed gestational day 13 and examined for pregnancy status. At the highest dose, minor decreases in body weight and relative food consumption were reported, and testicular and epididymal lesions were observed. The distribution of DNA ploidy was found to be predictive of testicular damage; however, the effects were more pronounced on day 29 (in the group allowed a 2-week recovery) than on day 15. Sperm motion was altered, and the percentage of motile sperm was reduced on day 15 at the two higher doses. At the highest dose, sperm velocity, amplitude of lateral head displacement, and epididymal sperm concentrations were reduced, and the incidence of sperm breakage was increased. The NOEL was 1 mg/kg bw per day (Hoyt *et al.*, 1994).

3-Chloro-1,2-propanediol was given orally to male Sprague-Dawley rats at a dose of 0, 2, or 8 mg/kg bw per day for 2 weeks. An additional group was given the higher dose for 4 weeks. At the end of dosing, the males were mated with untreated females. No treatment-related effects were found on body weight, food consumption, or the weights of the testis, epididymis, or prostate, and no significant effects were found on sperm number, viability, or maturation rate. At the higher dose, sperm motility was decreased after 2 h of incubation, and sperm activity was decreased both at the time of initial collection and after 2 h of incubation. At the lower dose, sperm activity was decreased only after 2 h of incubation. The group given the higher dose and allowed to recover showed no effects on sperm motility or activity. None of the females mated with males at the higher dose became pregnant. The lower dose had no effect on fertility. After recovery, males at the higher dose copulated with and successfully impregnated females (Yamada *et al.*, 1995).

3-Chloro-1,2-propanediol administered orally to male Han rats at a dose of 20 mg/kg bw per day for at least 5 days caused lesions in the testes and epididymides. At doses of 5, 10, and 20 mg/kg bw per

day, sperm motility was significantly depressed. Females mated with males at any dose failed to become pregnant. No effects of treatment were found on sperm morphology (Woods & Garside, 1996).

Male rats received 3-chloro-1,2-propanediol by oral gavage at a dose of 1, 3, or 10 mg/kg bw per day for 9 days and were then mated with untreated females. At the highest dose, no pregnancies resulted from the matings. A decreased pregnancy rate and number of implants were reported at 3 mg/kg bw per day. Analysis of sperm motility revealed treatment-related decreases in the percentage of motile sperm, sperm velocity, and amplitude of lateral head displacement at the highest dose and decreased sperm velocity and amplitude of lateral head displacement at the intermediate dose. Sperm from males treated at the highest dose did not reach the oviducts of females, and few sperm from males treated at the next lowest dose reached this location. Similarly, the percentage of fertilized eggs in the oviducts of mated females was decreased in a dose-dependent manner. The NOEL was 1 mg/kg bw per day (Ban *et al.*, 1999).

3.6.Special studies: Neurotoxicity

Groups of three male BALB/c mice were given racemic 3-chloro-1,2-propanediol intraperitoneally at a dose of 25, 50, or 100 mg/kg bw per day for up to 5 days. The mice given three daily doses of 100 mg/kg bw died and were found to have discrete widespread lesions in the gray matter, from the cortex to the spinal cord. No signs of lesion haemorrhage were observed. Administration of 50 mg/kg bw per day for 5 days did not cause deaths; however, mice killed on day 6 showed small vacuolated lesions in many brainstem centres. Another group of mice treated with an additional four daily doses developed more severe and widespread lesions. Only one spinal cord lesion developed in one mouse at the lowest dose administered on day 5. However, as with the intermediate dose, additional dosing during the second week resulted in severe lesions. The authors attributed the development of central nervous system lesions partly to inhibition of glycolysis by the (S) enantiomer of 3-chloro-1,2-propane-diol (Cavanagh & Nolan, 1993; Cavanagh *et al.*, 1993).

Single intraperitoneal injections of 3-chloro-1,2-propanediol to female Wistar rats at doses of 250–1000 mg/kg bw resulted in deaths associated with the development of neurological lesions within 24 h. Histologically, the lesions appeared as watery cytoplasmic swelling of astrocytes, mainly in the brain stem. Lower doses did not cause brain lesions. Administration of 100 mg/kg bw per day for 2 days was overtly toxic and resulted in death, with the formation of large vacuolated lesions at many sites within

the brain. Treatment at 50 mg/kg bw per day for up to 5 days resulted in cumulative central nervous system lesions. For several days after the completion of treatment, regenerative processes rather than necrosis of astrocytes predominated. As in the case of mice, the authors attributed the development of central nervous system lesions after a high dose of 3-chloro-1,2-propanediol in part to inhibition of glycolysis by the (S) enantiomer and energy deprivation of the affected brain regions (Cavanagh & Nolan, 1993; Cavanagh *et al.*, 1993).

Observations in humans

A synergistic effect of 3-chloro-1,2-propanediol and copper ions in decreasing the motility of human spermatozoa was observed *in vitro* (Kalla & Singh, 1981). When the compound was incubated with ejaculated human sperm, the motility of the spermatozoa was inhibited and their metabolic activity was reduced, as measured by glucose and oxygen uptake and lactate production (Homonnai *et al.*, 1975).

Chloropropanol esters toxicity

Currently, there is no data available on the toxicity of the fatty acid esters of chloropropanols and related compounds such as glycidyl esters.

4. Analytical methods for MCPD, DCP and their esters

During the last twenty years a great effort has been put into finding simple, rapid and affordable analytical methods to determine monochloropropanediols (MCPDs), dichloropropanols (DCPs) and their esters with long-chained fatty acids in several kinds of food, taking into consideration their toxicological aspects as a potential risk for consumers' health.

This determination requires both an efficient separation of these components from other constituents of foods and an accurate quantification in order to evaluate the compliance of a food sample with the limits established by regulatory organisms.

The main problems encountered while dealing with the development of a suitable analytical method derive first of all from these compounds' physicochemical properties, that make it particularly difficult to analyse sensitively:

- they don't have any suitable chromophore, so the application of HPLC methods with UV or fluorescence detection is quite unfavourable;
- they have a relatively high boiling point, so their volatilization and separation by gas chromatography without any manipulation may result almost impossible;
- they have a small molecular weight, so their little fragmentation affects detection by mass spectrometry;
- they are highly polar substances and as a consequence they are able to interact with components of the GC system, resulting in poor peak shape and sensitivity.

It is clear that ways to avoid these drawbacks are the most important points to be developed in a new analytical method.

Methods for the determination of MCPDs, DCP and the esters have been studied for a great variety of food matrices, such as hydrolized vegetal proteins and related foods (especially soy sauce), bakery products (bread, toasts, crackers, biscuits,…), malt-derived foodstuff, meat and fish (especially when smoked), coffee, etc…

The analysis of soy sauce for these compounds has generated a particularly great concern in the Chinese scientific community, since soy sauce is at the basis of the Eastern Asian countries culinary tradition and it is an everyday-consumed good by an extremely high number of people. For this reason a large part of the studies in this sense comes from Chinese research centres.

Neither the WHO nor the European Commission specify one standard method of analysis to apply, but rather define performance criteria that a method must fulfil in order to provide officially acceptable results, under the condition that method validation should include (if possible) the use of a certified material.

Here follows a review of the already existing validated methods for the analysis of MCPDs, DCPs and their esters, with particular focus on the comparison of different food matrices, sample preparation strategies and analytical instrumentation adopted.

4.1.Gas chromatographic methods

1) One of the oldest GC method for the quantification of 3-MCPD, 2-MCPD, 1,3-DCP and 2,3-DCP in **protein hydrolysed** (used as seasonings and savourings) was developed by Van Bergen *et al.* in 1991 and consists in an isolation of these compounds from the matrix by mixing the sample, previously spiked with a deuterated internal standard, with a salting-out NaCl solution and then extracting it through an Extrelut coloumn containing diatomaceous earth; chloropropanols are then eluted from the coloumn in two steps: first, 3-MCPD and 2-MCPD are eluted together with a mixture of hexane-diethyl ether 9:1 and secondly DCPs are eluted with diethyl ether alone.

The second action to be taken in this method is the derivatization of the extracted cholopropanols with a non-polar and high-molecular weight moiety, in order to permit their volatilization, separation in GC and detection by MS; the derivatizing agent chosen is **HFBI** (hepta-fluoro-butiryl-imidazole), which reacts with the hydroxyl functions of the analytes and generates an ester.

In this way GC separation can then be performed; for the detection of derivatized analytes an **ECD** (Electron Capture Detector) is used in this method and the quantification is carried out with reference to external standards interspersed with the experimental extracts.

The also tried to perform a GC/MS analysis of the underivatized chloropropanols, which gave an overall satisfactory result especially in order to unequivocally identify each compound by the selected ions monitoring function (SIM mode of acquisition).

2) A similar method for the quantification of 3-MCPD and 1,3-DCP was validated by Abu-El-Haj *et al.*

The main difference between this method and the previously described one consists in the food matrices considered, namely **soy sauce, cereals, bread, rice crackers, soup, soup powder and malt extract**. The first step of sample preparation differes from the previous only for bakery products, which are finely crumbled and added to the Extrelut coloumn as such without the addition of NaCl solution.

3-MCPD and 1,3-DCP are then eluted from the coloumns in one step with dichloromethane; for bakery products a previous washing of the sample with hexane is carried out to dispose of any fat.

Derivatization is performed using **HFBA** (hepta-fluoro-butiryl-anhydryde) instead of HFBI, since it is a cheaper reagent and generates a heavier diester.

GC/MS separation and detection is carried out in SIM mode and quantification is made by an external standard calibration curve.

3) One of the many methods developed by the Chinese scientific community is the one validated by Chung for 3-MCPD and 1,3-DCP in **condiments and sauces** (soy sauce and oyster sauce).

The sample preparation is the same described by Bergen *et al.* for protein hydrolysed: 3-MCPD and 1,3-DCP are separately extracted by a two step eluition on a Extrelut coloumn and then derivatized with **HBFI**. Since water is added at the end of the reaction in order to deactivate the residual imidazole, a partition with iso-octane is necessary to re-extract the chloropropanols from the aqueous phase for injection in GC.

The innovation presented in this method consists in the detection mode by **NCI/MS** (Negative Chemical Ionization/Mass Spectrometry), that was never performed before for 3-MCPD and 1,3-DCP together. The full scan spectrum revealed a number of highly characteristic ions for both compounds, excluded the ones generated by the HFB moiety, permitting a selective and accurate quantification.

4) A pioneer method, often used as a reference and cited in later works, was developed in 1991 by Plantinga *et al.* selectively for 2-MCPD and 3-MCPD in **standard acqueous solutions**.

As obvious, for standard solutions the only sample preparation steps required are the salting-out operation with a NaCl solution and the derivatization of chloropropanols, which can be performed directly in the aqueous medium using a particular kind of derivatizing agent: **phenyl-boronic acid**. As for the heptafluorobutiryl moiety, it reacts with the hydroxyl groups of chloropropanols generating a volatile cyclic boronic derivative.

The derivatized analyte is then extracted with hexane and injected in GC for separation.

According to the author the best extraction solvent eventually resulted to be hexane, even if higher recovery values for chloropropanols were obtained with toluene; the extraction with the later one, in fact, produced more peaks and this affected the resolution for the analytes.

The detection can be carried out with a simple **FID** (Flame Ionization Detector), since the precise identity of the peaks can be confirmed by retention indexes of pure standards, comparison with MS spectra and with literature spectra.

5) Another frequently cited method is the one validated in 1997 by Meierhans *et al.* for the determination of 3-MCPD and 2-MCPD in **various food matrices**.

As for many other methods, chloropropanols are extracted from the matrix on an Extrelut coloumn by eluition with diethyl-ether.

Here a new derivatization is applied and consists in the formation of a dioxolane by reaction with **acetone** under reflux in presence of toluene-4-sulfonic acid; the solution is then neutralized with aqueous NaOH and dried with an anhydrous salt.

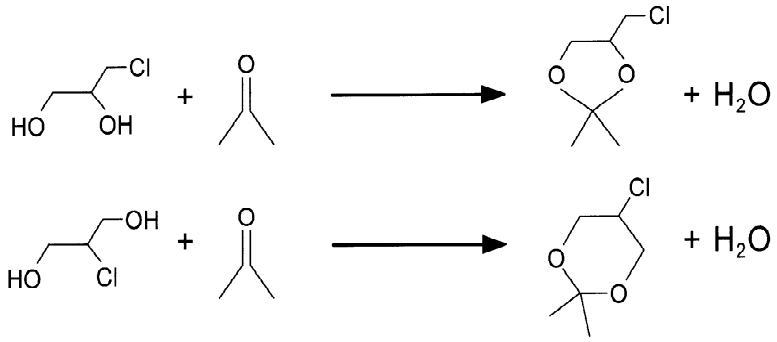


Figure 8: Formation of the dioxolane.

The analysis and quantification is performed by **GC/MS** in a SIM mode for quantification, referring to an external calibration curve in hexane.

6) All the above described methods imply a complex and time-consuming sample preparation consisting in SPE and derivatization, which can also lead to relevant mistakes in recovery of the analytes and matix effects in quantification; moreover, they adopt high amount of organic solvents and expensive reactives.

Taking all these aspect in consideration, a great effort has been put into developing faster and simpler analytical methods, without loosing sensitivity and accuracy in quantification.

An extensive interlaboratoy study (Hasnip *et al.*) managed to validate a very interesting **Headspace** (HS) method coupled with GC/MS separation and detection for the determination of 1,3-DCP in **soy sauce**.

1,3-DCP is the most volatile molecule among the chloropropanols, so it can be easily lost during solvent extraction and purification procedures; this drawback turns into a favourable characteristic when dealing with HS techniques, which can consist either in the withdrawal of a part of the vapour

phase of a sample in a trapping device or in the absorption onto a polymeric-coated fiber (Solid-Phase Microextraction device or SPME device).

In this method the HS sampling is carried out by sealing the sample as such in a screw-capped vial, leaving to equilibrate at 80°C and then removing 2 mL of the HS with an air-tight syringe; alternatively, the HS is sampled with a SPME fiber (Carboxen-Polydimethylsyloxane coating was used by authors) for 40 minutes at 80°C.

Then the content of the syringe can be injected directly in **GC/MS**, or the analyte thermally desorbed from the fiber in the injector.

For the quantification an internal standard is used and the area of the peak of 1,3-DCP is related with the one of the IS (SIM mode of acquisition on characteristic ions is adopted).

This method has been proven to have a high level of robustness and precision; also the accuracy of quantification is completely satisfactory, together with a good resolution when using a proper GC coloumn. Moreover, its application is fast, automatic and requires neither sample preparation nor the use of solvents.

4.2.Analysis of esters in gas chromatography

In some kinds of food such as refined oils chloropropanols occur in their free form only in little amounts, while the major part is esterified with long chain fatty acids; a typical example of this is represented by the low concentration of 3-MCPD as such in these oils, that can create an underestimation of its actual amount because the majority is bond to the oil's fatty acids forming 3-MCPD esters.

As a result, a proper sample preparation in these cases must include the cleavage of the bond chloropropanols and the quantification of their total free amount, assuming that the original concentration of unbond molecules was not significative.

1) The classical method to hydrolyze the ester bond is the treatment with **methanol/sulphoric acid**, hence it has a great drawback, because the treatment of the food matrix with strong acids in presence of chloride ions results in the formation of new chloropropanols; this generates an overestimation of the actual concentration of these compounds that severely affects the results.

2) In order to solve this important problem, a sample preparation strategy was developed by Weisshaar for the analysis of 3-MCPD in **safflower oil** and consists in the use of **sodium methoxide** as an hydrolytic agent.

After cleavage a salting-out step is performed with NaCl solution in presence of acetic acid and this aqueous phase is washed with iso-hexane for residuals; derivatization with **phenyl-boronic acid** is then carried out directly in the aqueous media and the analytes are extracted with hexane for GC/MS injection.

3) Very recently (2010) a **micromethod**, suitable for 3-MCPD in **different foodstuff**, was developed by Kusters *et al.* in order to reduce the amount of solvents and reagents required and also to achieve a different treatment for free and bond 3-MCPD.

In this method, after the salting-out procedure with NaCl, a small aliquot of the sample solution is taken for free 3-MCPD and another one is treated with sodium methoxide and acetic acid for the cleavage of 3-MCPD esters, with the advantage that only few hundreds microlitres of these substances are needed.

These two small aliquots are then reunited and derivatized with **phenyl-boronic acid** (also in this case a very small volume of reagent is used).

The derivatized analytes are extracted with hexane and injected in GC/MS for separation an quantification.

4.3.Other methods

Besides the majority of methods that have been developed for gas chromatography, some attempts have been made to study suitable non-chomatographic methods for chloropropanols and their esters, in spite of the big limitations that these compounds' characteristics impose.

1) Leung *et al.* carried out a very advanced study based on **molecular imprinted polymers** (MIP) as selective potentiometric chemosensors for 3-MCPD.

From an industrial applicability point of view, a robust chemosensor capable of specifically sensing the presence of chloropropanols in complex food matrices with a good level of sensitivity is a much desirable achievement.

This method includes the fabrication of a molecularly imprinted polymeric material able to selectively bind 3-MCPD and its potentiometric determination.

4-Vinylphenyl-boronic-acid is a suitable monomer for the imprinting of diols, because it forms a covalent ester bond with the template molecule, shaping a more persistent binding site after the polymerization of the substance; cross-linking of the polymer is followed by the hydrolytic removal of the template chloropropanol.

Beside acting as the recognition matrix for the diol, acid-base properties of this MIP are found to be affected by the binding of 3-MCPD; based on such phenomenon, a potentiometric approach can be used to transducer the analyte-receptor interaction into a physically measurable response for the chemosensing of 3-MCPD.

Unfortunately experimental observation have shown that the re-binding of the analyte in samples of **soy sauce** spiked at known concentrations is much lower than the original quantity of binding sites present on the polymer.

In addition, even though this MIP shows good selectivity for 3-MCPD against 1,3-DCP, its selectivity for 3-MCPD against the isomer 2-MCPD is much poorer, as it seems to bind these last two compounds equally well.

2) An **electophoresis** application for the separation and quantification of 3-MCPD in **soy sauce** was developed by Xing *et al.* and it represents a unique study in this field.

Since glycerol has been detected elettrophoretically with a copper electrode, the basis for this method is the similitude of 3-MCPD with glycerol itself, taking into consideration that a high voltage on a copper electrode can oxidize diols.

So, a suitable voltage for the electophoretic run must be evaluated, together with a proper buffer; the most effective one seems to be the borate buffer, because of its complexation properties with 3-MCPD but not with other chloropropanols, making the electrophoretic run selective for this compound only.

Quantification is performed running standard solution of known concentration in the same condition and comparing the resulting electropherogram with the one of the sample.

3) Very recently (2010) an attempt has been made by Haines *et al.* to develop a **Liquid Chromatography** (LC) method for the direct determination of MCPD fatty acid **esters** in **vegetable oils**.

Since both the acid and the basic hydrolytic procedures use harsh conditions which may alter MCPD concentration, this method is aimed to bring an alternative and more direct solution.

Indeed, samples are directly diluted in the mobile phase and separated on a C18 reversed phase coloumn by non aqueous HPLC; mobile phases are prepared with MSA (methanol/sodium acetate)/methanol/acetonitrile 1:8:1 (A) and MSA/methylene chloride/acetonitrile 1:8:1 (B), the eluition gradient is 100%A for 5 minutes then linearly to 65%B.

Detection is performed with a **TOF** (Time of Flight) analyser coupled with a ESI source and a MS detector.

The use of a TOFMS system is quite challenging in this case, especially because the sodium in the mobile phase has a detrimental effect on the MS detector and on the ESI needle. Moreover, the instrument tends to become dirty very quickly and a cleaning procedure is needed everyday.

For these reason a constant check with standard is highly advisable in order to get consistent results.

5. Legislation

Council Directive 93/5/EEC “on the assistance to the Commission and cooperation by the Member States in the scientific examination of questions relating to food” was updated on 25 February 1993. It lays down a procedure whereby Member States of the European Union can focus their scientific resources in a co-ordinated manner on problems facing the Commission in the area of food.The individual tasks to be undertaken are agreed in consultation with the Member States who also determine in which tasks they wish to participate and the extent of their participation. Directive 93/5/EEC requires that an inventory of tasks be published at least every six months. This publication, which takes the form of a Commission Decision, specifies the participating Member States that provides co-ordination and the time limit for the completion of the task.

In general terms, tasks undertaken under scientific co-operation are designed to provide a factual basis to support a Commission action in the area of food. Such support may involve the provision of information as may be required, for example, by the Scientific Committee for Food (SCF) for its

evaluation and advisory work or by the Commission's own services for the development of Community action.

In 2000, SCOOP Task 3.2.6 concerning the development of validated methods for evaluating 3-MCPD in foods was completed. This Task was successful in developing a validated method for analysing 3-MCPD and resulted in a report entitled "Provision of validated methods to support the Scientific Committee on Food's recommendations regarding 3-monochloropropane-1,2-diol in hydrolysed vegetable protein and other foods". It was noted during the Task that 3-MCPD occurrence data in foods other than soy sauce was scarce. Subsequently, Commission Decision 2001/773/EC of 26 October 2001 established Task 3.2.9 "Collection and Collation of data on levels of 3-monochloropropane-1,2-diol (3-MCPD) and related substances in foodstuffs". The UK and Sweden were designated as Member States to coordinate the Task.

All participants supplied occurrence data and all participants except Austria and Norway supplied 3-MCPD dietary intake estimates. Details of participants are provided in Annex 3. Following the appointment of the UK and Sweden as co-ordinators, the timetable of the Task was decided jointly with representatives of the Commission. For administrative reasons, the deadline for the completion of the Task was extended.

Commission Regulation (EC) No. 466/2001 sets limits for a range of contaminants in certain foodstuffs including 3-MCPD in soy sauce and HVP. The limits for 3-MCPD in soy sauce and HVP have been set at 0.02 mg/kg based on the liquid product containing 40% dry matter. The regulation was formally adopted on 8 March 2001, and applied from 5 April 2002. This SCOOP Task will inform the review of this maximum level, detailed in article 5 of Commission Regulation (EC) No. 466/2001.

The primary objective of the Task was to gather information on the levels of 3-MCPD and other chloropropanols in a range of foods. An initial meeting on 3 May 2001 introduced participants to the Task and the surveys examining 3-MCPD in selected foods6 and food ingredients9 conducted by the UK's Food Standards Agency. Participants agreed that only occurrence data obtained after

1997 should be submitted as there was concern about the robustness of data acquired prior to that date. The format for collecting and sending occurrence data was agreed in order to allow the construction of tables for data collection by the co-ordinators. An adapted version of the Codex food categorisation system7 was used to classify the food samples. The final agreed categorisation instructions, together with the list of food codes are given in Annex 2.

The second objective of this SCOOP Task was to give a best estimate of dietary exposure to 3-MCPD. As adequate national data were available, participants used their own soy sauce data to calculate exposure to 3-MCPD from soy sauce.

However, as adequate national data for other foods were unavailable, it was decided that participants would use pooled 3-MCPD occurrence data to calculate dietary exposure to 3-MCPD from all other foods.

Data provided by participants were collected and reported by the co-ordinators to provide the following:

– a description of the status of 3-MCPD and other chloropropanol levels in foodstuffs in each participating country;

– an overview of the available information on chloropropanols in individual food products and ingredients;

– best estimates of 3-MCPD dietary intake from food for each participant.

COMMISSION DIRECTIVE 2001/22/EC of 8 March 2001 laying down the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs.

COMMISSION REGULATION (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Has adopted this regulation:

(21) 3-monochloropropane-1,2-diol (3-MCPD) is created during food processing under certain conditions. In particular, it may be produced during the manufacture of the savoury food ingredient 'hydrolysed vegetable protein' that is produced through the acid hydrolysis method (acid-HVP). By adjusting production processes, a significant decrease of 3-MCPD in the abovementioned product has been achieved over the past years. Recently, several Member States have also reported high levels of 3-MCPD in certain samples of soy sauce. In order to enforce good manufacturing practice and to protect the health of consumers, maximum levels of 3-MCPD should be set. The SCF advised, in its opinion of

16 December 1994, which was confirmed on 12 June 1997, that 3- MCPD should be regarded as a genotoxic carcinogen and that residues of 3-MCPD in food products should be undetectable. Recently performed toxicological studies indicate that the substance acts as a non-genotoxic carcinogen in vivo.

(22) The maximum levels set in Annex I for 3-MCPD are based on the SCF opinion. The SCF will reevaluate the toxicity of 3-MCPD in the light of new studies. The adequacy of the maximum levels should be reconsidered as soon as the new SCF opinion is available. Member States are requested to examine other foodstuffs for the occurrence of 3-MCPD in order to consider the need to set maximum levels for additional foodstuffs.

Section 4: 3-monochloropropane-1,2-diol (3-MCPD**)**

Table 5: MAXIMUM LEVELS FOR CERTAIN CONTAMINANTS IN FOODSTUFFS

Foodstuffs		**Maximum levels (mg/kg)**	Performance criteria for sampling	Performance criteria for methods of analysis
4.1	Hydrolysed vegetable protein	0,02	Directive 2001/22/EC	Directive 2001/22/EC
4.2	Soy sauce	0,02	Directive 2001/22/EC	Directive 2001/22/EC

COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Has adopted this regulation:

46) As regards 3-monochloropropane-1,2-diol (3-MCPD) the SCF adopted on 30 May 2001 a scientific opinion as regards 3-MCPD in food , updating its opinion of 16 December 1994 on the basis of new scientific information and established a tolerable daily intake (TDI) of 2 µg/kg bw for 3-MCPD.

(47) In the framework of Directive 93/5/EEC the SCOOP-task 'Collection and collation of data on levels of 3-MCPD and related substances in foodstuffs' was performed and finalised in June 2004 . The main contributors of 3-MCPD to dietary intake were soy sauce and soy-sauce based products. Some other foods eaten in large quantities, such as bread and noodles, also contributed significantly to intake in some countries because of high consumption rather than high levels of 3-MCPD present in these foods.

(48) Accordingly maximum levels should be set for 3-MCPD in hydrolysed vegetable protein (HVP) and soy sauce taking into account the risk related to the consumption of these foods. Member States are requested to examine other foodstuffs for the occurrence of 3-MCPD in order to consider the need to set maximum levels for additional foodstuffs.

Section 4: 3-monochloropropane-1,2-diol (3-MCPD)

Table 6: MAXIMUM LEVELS FOR CERTAIN CONTAMINANTS IN FOODSTUFFS

Foodstuffs		**Maximum levels (µg/kg)**
4.1	Hydrolysed vegetable protein	20
4.2	Soy sauce	20

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