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Occurrence of chloropropanols and glycidol esters in foods – A literature review

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1. Summary

Chloropropanols and glycidol are transformation products that form during food production, essentially contaminants that form during food processing. Since their production conditions are similar, they are usually mentioned together in the relevant literature, as chloropropanols and related substances. The presence of chloropropanols was first observed in connection with acid-hydrolyzed vegetable proteins, however, they also occur in other foods. They have been detected in fried potatoes, cooking oils, but they also occur in roasted and cooked meats, snacks, biscuits, and practically all thermal-treated products that contain fat.

Based on the toxicological results so far, free glycidol was classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (2A), and also as genotoxic; free 3-MCPD (3-chloro-1,2-propanediol) was classified as possibly carcinogenic to humans (2B). Currently, regulation in the European Union exists only for free 3-MCPD in soy sauces and acid-hydrolyzed vegetable protein (EC 1881/2006), but many literature sources address the analysis of these compounds in other foods.

Fatty acid esters of chloropropanols form in the high temperature reaction of glycerides (fats, oils) in the presence of chlorine-containing compound – either organic or inorganic. Glycidol esters also form at high temperatures, for their formation diglycerides or monoglycerides need to be present. The formation of the above transformation products in fats and vegetable oils, as well as the reduction of their amounts are important areas of current research.

In this review, I would like to present literature results related to chloropropanols and glycidol, which deal with their occurrence, with special emphasis on cooking oils.

2. Introduction

Chloropropanols are compounds derived from glycerol, in which one or two of the hydroxyl groups are replaced by chlorine atoms (***Figures 1-3***). They can form in the reaction of glycerol and chlorine under acidic conditions **[41]**. Their main representatives are 3-chloro-1,2-propanediol (3-MCPD), 2-chloro-1,3-propanediol (2-MCPD), and 1,3-dichloro-2-propanol (DCP), containing two chlorine atoms. They occur both as free diols or in the form of fatty acid esters **[91]**. The physical properties of 3-MCPD esters are similar to those of acylglycerols, at room temperature they are usually solids, with their melting points depending on the carbon number of the fatty acid part **[45]**.

The presence of chloropropanols in foods was initially investigated in connection with soy sauces and acid-hydrolyzed vegetable proteins (AHP) **[93]**. AHP is produced at high temperature by acidic hydrolysis. The esters of chloropropanols are produced not from

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the protein, but from the residual fat in the starting material protein, during the reaction with hydrochloric acid. AHP is mostly used for non-fermented soy sauces, but it can also be added to fermented sauces to improve taste. The European Commission's Scientific Committee on Food (SCF) stated already in 1997 that AHP with a 3-MCPD content of more than 10 mg/kg is not acceptable. It was also recommended by the Commission that producers try to control the amount of this compound in the products, and preferably develop technologies that eliminate the presence of chloropropanols in the products **[80]**. According to the US Food and Drug Administration (FDA), soy sauces and acid-hydrolyzed vegetable proteins containing more than 1 mg/kg 3-MCPD are not safe food additives, and cannot obtain GRAS (Generally Recognized As Safe) status **[37]**. In 2001, the Tolerable Daily Intake (TDI) value for 3-MCPD was also determined by the SCF, at 2 μg/kg body weight/day **[81]**. Following this, in 2002, a Provisional Maximum Tolerable Daily Intake (PMTDI) value was published by JECFA (the Joint FAO/WHO Expert Committee on Food Additives) for 3-MCPD, which was also 2 μg/kg body weight/day, and this value was maintained in 2007 **[56], [57]**. The tolerable daily intake value was reduced to 0.8 μg/kg body weight/day by EFSA (the European Food Safety Authority) in 2016 **[32]**.

Glycidol forms under similar conditions, and so it is commonly mentioned in the literature together with chloropropanols. Glycidol contains two functional group, an alcoholic hydroxyl group and an epoxy group (***Figure 4***). It is a transparent liquid, slightly viscous at room temperature, miscible with water at any ratio, and soluble in most polar solvents **[32], [64]**. It also occurs in the free form or as esters of fatty acids. Glycidol esters can form from mono- or diglycerides at high temperatures, they are not produced directly from triglycerides **[26]**. A positive correlation between the diglyceride content of the oil and the amount of glycidol esters formed during deodorization has been reported by several studies **[22], [49] [96]**.

3. Analytical possibilities

When talking about the quantities of chloropropanols and glycidol, it is important to define exactly in what chemical bonds we would like to determine the concentrations of the compounds. Methods used for the quantitative determination of compounds in ester bonds are basically divided into two groups: during the indirect method, the compounds released from all of its esters is detected, while in the case of direct methods, esters with different fatty acids are detected **[34]**. Given that esters of 3-MCPD, 2-MCPD and DCP, as well as those of glycidol are high molecular weight compounds, liquid chromatography can be used for their direct detection **[11], [12], [21], [26], [30], [43]**. Currently, direct methods cannot be used for routine quality control, these are not user-friendly procedures, requiring several standards. For the time being. Experiments are also underway using other methods, such as LC-MS combined with gel permeation extraction **[29]**, SFC–QqQMS **[48]**, as well as ^{1}H NMR spectroscopy **[88]**.

For the indirect determination, i.e., the measurement of the 3-MCPD, 2-MCPD, DCP or glycidol released by the esters, sample preparation usually involves derivatization, to form volatile MCPD, DCP or glycidol derivatives for gas chromatography. Ester bonds can be broken several ways. Acidic transesterification with sulfuric acid can be used **[28], [50], [107]**, as well as alkaline transesterification **[50], [62],** or enzymatic transesterification **[72]**. During sample preparation, hydrolysis is one of the most critical steps, because glycidol esters can be transformed into 3-MCPD under alkaline conditions **[60]**. In the case of 3-MCPD and glycidol, the most often used derivatizing agents include phenylboric acid/PBA **[28], [97], [107],** and heptafluorobutyrylimidazole/HFBI **[46]**. In the case of DCP, also HFBI **[92]**, heptafluorobutyric anhydride/HFBA **[2]**, or N,O-bis(trimethylsilyl)trifluoroacetamide/BSTFA **[16]** can be used. However, there is a method already that could detect chloropropanols without derivatization, using gas chromatography **[102]**. According to Ermacora & Hrncirik **[34]**, for indirect determination in the case of vegetable oils, the three most accepted methods are the combined method of Ermacora & Hrncirik **[33]**, the procedure of DGF (Deutsche Gesellschaft für Fettwissenschaft) **[27]**, and the SGS „3 in 1" method **[62], [63]**, which was also accepted as an official method by the AOCS (American Oil Chemists' Society) **[3], [4], [5]**.

4. Toxicological aspects

Free glycidol was classified by IARC into category 2A, because the compound is probably carcinogenic to humans and genotoxic **[51]**. In contrast, the toxicity of glycidol esters is low, according to toxicological results obtained so far, in fact it is the hydrolyzed, free glycidol that poses a food safety risk **[82]**. In two-year long experiments in rodents – mice ($B6C3F_1$) and rats (F344) – glycidol has caused tumors in several organs, including the liver, the forestomach, in mammary glands, in the thyroid and the lungs **[54], [55]**. During digestion, the majority of the glycidol is released from glycidol esters, therefore, foods containing glycidol esters can be considered glycidol sources. In a static digestion model, in the presence of lipase at pH 4.8, rapid hydrolysis took place, according to the study of Frank et al. **[38]**, while inhibition of the lipase was observed at pH 1.7. Without lipase, during a period of 2 hours, esters proved to be stable. Based on the processes that took place in the dynamic model it was concluded that esters are rapidly broken down by the lipases of the digestive system **[38]**. According to *in vivo* studies in rats, the hydrolysis of glycidol esters comes to nearly completion during digestion **[6]**.

Based on toxicological data, 3-MCPD was classified into category 2B by the International Agency for Re-

search on Cancer, i.e., it is possibly carcinogenic to humans **[52]**. Different MCPD esters behave similarly to glycidol esters in the digestive system. In an *in vitro* experiment carried out by Seefelder et al., more than 95% of monoesters were hydrolyzed after 1 minute in the presence of lipase, while 45, 65 and 95% of diesters hydrolyzed after incubation periods of 1, 5 and 90 minutes, respectively. In the same study, it was found that 3-MCPD released from 3-MCPD esters only contributed dietary 3-MCPD exposure to a small extent **[83]**. On the one hand, this conclusion is based on the calculation that the proportion of monoesters, according to their measurements, was no more than 15% of all esters. On the other hand, it was assumed that the metabolism of 3-MCPD esters in the body is similar to that of acylglycerides, i.e., fatty acids in positions 1 and 3 are favored by pancreatic lipases during hydrolysis, and so, in the diesters present in larger amount, the ester bond on carbon 2 is not broken **[83]**.

There are two possible metabolisms for diesters in the body, one is hydrolysis by the lipase of the pancreas, and the other is intracellular degradation **[14]**. Based on an *in vivo* experiment in rats, biological utilization of 3-MCPD diesters was 86% of that of free 3-MCPD, therefore, in terms of exposure, 3-MCPD released from the esters should be evaluated in the same way as free 3-MCPD **[1]**. 3-MCPD was found to be genotoxic in *in vitro* experiments, however, there is no proof of its *in vivo* genotoxicity **[68]**. In two-year-long *in vitro* experiments carried out on mice, no clear evidence of carcinogenicity was found **[58]**, however, a correlation between the development of Leydig cell tumors and kidney tubule carcinoma, and the consumption of drinking water containing 3-MCPD was discovered in experiments in male rats **[18]**.

Currently, there is a limited amount of data available on the toxicity of 2-MCPD and of 2-MCPD esters, they are not yet classified in terms of carcinogenicity. More information is available on DCP and its esters, having been classified into category 2B by IARC **[52]**. Their genotoxicity has been shown to be negative in *in vivo* experiments, positive in *in vitro* experiments. In large doses, in a two-year-long test on rodents, the rates of certain cancerous diseases, such as kidney tubule carcinoma or liver cell adenoma were increased significantly **[99]**.

4.1. Human exposure

TDI and PMTDI values for free 3-MCPD have been calculated by JECFA based on the LOAEL (Lowest Observed Adverse Effect Level), which is the smallest dose with observable health effects. This value is 1.1 mg/kg body weight, which was divided by a safety factor (500), resulting in the 2 µg/kg body weight/day value **[56]**. In 2016, in its scientific opinion on MCPD and glycidol esters, the TDI value was reduced to 0.8 µg/kg body weight/day by EFSA **[32]**. JECFA found **[56]** that regulation was unnecessary to be introduced for DCP, containing two chlorine atoms, on the one hand, because the dose causing tumor in animal experiments was 20,000 times higher than the average amount introduced into the body by consuming soy sauce. On the other hand, there are always large amounts of 3-MCPD alongside DCP, so regulation of the former is the important thing **[56]**. No tolerable daily intake levels were determined for glycidol and its esters. Since the compounds are not only carcinogenic, but genotoxic as well, therefore, the ALARA principle (As Low As Reasonably Achievable) should be followed.

Concerning the topic of chloropropanols and glycidol, studies on human exposure have also been produced in recent years. Per capita daily intakes of 3-MCPD esters and glycidol esters have been calculated for the German population by Weißhaar **[98]**, which was divided by the average body weight (60 kg), and so the daily intake of 3-MCPD esters was estimated at 1.5 µg/kg body weight/day, while that of glycidol esters was estimated at 0.9 µg/kg body weight/day this way. In the calculation, only contaminants coming from vegetable oils were included **[98]**. The exposure of Hong Kong high school students was investigated by Yau et al. **[103]**. According to their results, the amount of DCP ingested is 0.003-0.019 µg/kg body weight/day by an average student, 0.009-0.040 µg/kg body weight/day by "large consumers" (5%), while the amounts for 3-MCPD are 0.063–0.150 µg/kg body weight/day for the average student, and 0.152-0.300 µg/kg body weight/day for "large consumers" **[103]**. According to another study, the average amount of 3-MCPD ingested with bakery products on the Polish market, in the case of adults, is 0.008-0.013 µg/kg body weight/day, which is 0.4-0.65% of the TDI, while it is 0.022-0.036 µg/kg body weight/day in the case of children, which is 1.1-1.8% of the TDI. These values are for free 3-MCPD **[89]**.

Summarizing the results of previous exposure studies it can be concluded that the tolerable daily intake value of 2 µg/kg body weight determined by JECFA and SCF, and even the reduced value of 0.8 µg/kg body weight of EFSA are above the consumption of the average consumer, but there may be special cases where the amount digested can approach these values, for example, in the case of brand loyalty.

5. Occurrence of chloropropanols and glycidol esters in foods

Since their first detection, there have been several studies on chloropropanols and glycidol. In 1980, 3-MCPD was present in acid-hydrolyzed protein, in the neutralized hydrolyzate in a concentration of 47 mg/kg, while a value of 305 mg/kg was measured in the filter cake, which is an extremely high concentration compared to today's limit value of 20 µg/kg (EC 1881/2006) **[20, 94]**. Today, the amount of chloropropanols in protein hydrolyzate can be controlled by good manufacturing practice (GMP), however, their

presence have been observed recently in other areas of the food industry as well **[45]**. The hydrolysis of winged beans and soy beans was optimized for time and temperature by Sim et al., during which the minimum 3-MCPD concentration that could be achieved was 25 mg/kg, but by applying an additional alkaline treatment, the amount of the compound in the products could be reduced to value below the limit of detection (<0.002 mg/kg) **[86]**. In the 2000s, monitoring programs for the 3-MCPD content of soy sauces have been carried out in several countries. In China, in 2001, five of the 30 soy sauces examined contained more than 1 mg/kg 3-MCPD **[59]**, while in 2007, the value was above the nationally recommended maximum level (1.0 mg/kg) in 12.2% of retail samples **[42]**. According to a study in the United Kingdom, 35% of products contained more than 0.02 mg/kg 3-MCPD **[69]**, while in a study carried out in the United States, concentrations in 60% of retail samples exceeded 0.025 mg/kg (LOQ), the highest value being 876 mg/kg **[75]**. Nearly 90% of the samples examined in the Singapore National Monitoring Program met the national limit value (0.02 mg/kg), which is the same as the European Union limit value (EC 1881/2006) **[20, 101]**. Summarizing the results, there are still soy sauce products with free 3-MCPD concentrations exceeding national or international limit values.

In EFSA's 2013 report, 3-MCPD data for 1235 foods, collected from European member states between 2009 and 2011, are published. Of the 11 food categories, the average value was highest in the case of animal and vegetable fats and oils (1020 µg/kg), and within the category, the highest values were measured for margarines and similar products (average: 1500 µg/kg) **[31]**. Since chloropropanols and glycidol esters can be present in fats and oils in the mg/kg order of magnitude, a number of related studies can be found in the literature. Industry, responding to newer and newer scientific results, tries to reduce the amounts of these contaminants in the products, by modifying technology. Results and studies on fats and oils are presented in the next chapter.

5.1. Occurrence of chloropropanols and glycidol esters in fats and oils

According to literature data, high levels of MCPD and glycidol esters are primarily measured in refined palm oil and its fractions. In a 2012 study, different oils of plant origin were analyzed. Based on the results, they occurred in largest amounts in refined palm oils (1.01-13.59 mg/kg). No 3-MCPD esters were detected in the seed oils (soy, rapeseed, maize) tested, however, certain glycidol fatty acid esters were present above the detection or quantification limits **[47]**. On the other hand, in another study, 3-MCPD esters could also be detected, in concentrations of 0.21 mg/kg in rapeseed oil, 0.54 mg/kg in sunflower and coconut oils, and 0.68 mg/kg in corn oil **[25]**. 3-MCPD esters were found by Berg et al. also in rapeseed and sunflower oils in amounts of 0.4 and 0.5 mg/kg, respectively **[10]**. Based on the results of Kuhlmann, walnut, peanut, grape seed and fish oils (salmon oil capsule) also showed a exceptionally high 3-MCPD and glycidol ester concentrations above 10 mg/kg. It was also observed by him that evening primrose oil contained a lot of 3-MCPD esters, and coconut oil, palm kernel oil and shea butter contained a lot of glycidol esters, while the concentration of 2-MCPD esters was exceptionally high in walnuts and peanuts **[62]**.

In 43% of the refined palm oil samples tested by Razak et al., the concentration of 3-MCPD esters exceeded 2 mg/kg. In the same study, the 3-MCPD ester contents of the different fractions were also measured, and in most of the olein samples values were between 1.4 and 3.2 mg/kg, while in the stearin fraction lower values were measured, the maximum being 1.8 mg/kg. Accordingly, it was concluded that esters prefer to be in the more liquid phase during fractionation **[79]**. In addition to the oil type, the quality of the raw material also plays a central role regarding the formation of 3-MCPD and glycidol esters. Results obtained for palm oils from different regions vary widely in the study of Matthäus et al., up to ten times more of the compounds analyzed can form in Malaysian samples than in palm oils coming from Ghana **[70]**.

In unrefined oils, typically there are no 3-MCPD and glycidol esters, in oils produced from roasted seeds 3-MCPD ester results above the detection limit (0.25 mg/kg) have also been obtained **[66]**. The same was found by Zelinkova **[107]**, refined oils and oils isolated from roasted seeds contained larger amounts of 3-MCPD esters than unroasted, virgin oils.

Currently, a limit value in regulations or laws is only set for soy sauces and acid-hydrolyzed vegetable proteins, it is 20 µg/kg, which is prescribed in Hungary by an EC regulation (EC 1881/2006) **[20]**. For fats, oils and emulsions, such as margarine, there is no regulation, however, according to the recommendation of AOCS, the properties of high quality fats and oils include the presence of minimum amounts of contaminants. Therefore, for 3-MCPD and glycidol esters, in case of general use a limit value of 2 mg/kg was defined by the American society, while in the case of use in baby foods the limit value is 0.5 mg/kg **[110]**.

5.2. Formation of chloropropanols and glycidol esters in vegetable oils and the reduction of their amounts

It has been studied thoroughly by the vegetable oil industry in recent years what the factors influencing the formation of chloropropanols and glycidol were, in order to be able to modify the technology to reduce their amounts.

The production of cooking oils is a refining process consisting of several steps. Whether chemical or physical refining is used, a deodorization step is essential. During this, odorants and residual colorants are removed, as well as free fatty acids in physical refining, with the help of steam distillation. The operation is carried out at a high temperature (above 200 °C, or even at 275 °C in the case of palm oil) and in high vacuum (1-2 mbar residual pressure) **[15], [76]**. Formation of 3-MCPD esters starts already at 140 °C, and depends only slightly on the deodorization temperature **[25]**. In the case of glycidol ester formation from diglycerides, the critical temperature is ~200 °C, above which their formation accelerates with increasing temperature **[26]**. Accordingly, chloropropanols and glycidol esters typically form during deodorization, with other steps of the refining process having no direct effect **[39], [67]**, although playing an important role in the removal of precursors. There are several approaches to the reduction of the amounts of 3-MCPD and glycidol esters:

- removal of the precursors in the refining steps before deodorization;
- optimization of the refining conditions;
- reduction of the amounts of contaminants already formed **[70]**.

Removal of the precursors is of crucial importance in the prevention of the formation of 3-MCPD esters. It is critically important to recognize the chlorine donor, in order to be able to reduce the amount of 3-MCPD in the product **[71]**. However, it must be understood that dominant precursors may differ in the different matrices, different oils **[23]**. In the work of Ermacora and Hrncirik, the main chlorine donors were polar chlorinated components, the chlorine atom of the 3-MCPD detected could also come from nonpolar compounds **[35]**. There is also a case in the literature where no correlation between the formation of 3-MCPD and the presence of precursors, i.e., chlorine containing compounds, could be detected **[49]**.

Craft et al. **[21]** experimented with the laboratory deodorization of partially refined palm oil and palm oil obtained from fresh palms. Using various methods, they tried to remove precursors, and the most effective method proved to be preliminary water washing of the palm fruit, decreasing the amount of 3-MCPD formed by 95%. Even washing raw palm oil with ethanol, water, or a mixture of them decreased the detectable amount of 3-MCPD by 25-35% **[21], [70]**. In a number of experiments, when investigating the refining steps of palm oil, water degumming proved to be the most effective **[77], [78], [108]**, while in the case of degumming using phosphoric acid, the amount of 3-MCPD esters formed during deodorization could be up to an order of magnitude higher **[78]**. When using chemical refining, sodium hydroxide neutralization can contribute significantly (35%) to the decrease in the amount of 3-MCPD. During both degumming and neutralization, the decrease might be caused by the removal of the precursors by washing **[77]**. Bleaching also slightly decreases the amount of 3-MCPD formed during deodorization, with the most effective agents proved to be magnesium silicate **[109]** and non-activated bleaching earth **[78]**. The amount of MCPD formed is also determined by the presence of precursors in the case of other foods as well. For biscuits, correlations were also found with different technological parameters, such as the temperature, longer or shorter baking times, as well as the amount of NaCl **[73]**.

Regarding the precursors of glycidol esters, a correlation was found between the amounts of diglycerides and monoglycerides and the concentration of the glycidol esters formed, while no glycidol is formed directly from triglycerides **[22], [26], [49]**.

3-MCPD and glycidol esters form during deodorization, and this step also has many parameters influencing the properties of the final product. Treatment temperatures can vary, depending on the type of oil, temperatures as high as 260-275 °C can be used in the case of palm oil, because of the high free fatty acid and carotene contents. There are some contradictory results are presented in the literature regarding the effect of the temperature. According to several experiments, the amounts of 3-MPCD and glycidol esters increase with increasing temperature **[39], [74], [85]**, while other studies state that temperature does not have an effect on the amount of 3-MPCD esters between 180 and 265 °C **[49]**. There are also results in the literature showing that a decrease in the amount of 3-MPCD esters was observed above a certain temperature (250 °C) **[108]**. Regarding the effect of deodorization time, at a given temperature, the amounts of glycidol esters and 3-MCPD esters increases over time. However, the extent of the increase depends on the temperature applied, with a larger increase at higher temperatures **[49], [85]**. 3-MCPD esters were thermally stable over the first two hours during thermal treatment. After 24 hours, their amount decreased significantly, and the decrease (30-70%) was directly proportional to the temperature applied (180-260 °C) **[36]**. When examining the combined effect of temperature and time, Pudel et al. found that concentrations increased significantly with the length of deodorization, up to 250 °C in the case of 3-MCPD and up to 270 °C in the case of glycidol esters. However, at 290 °C, the amounts of MCPD and glycidol esters that had formed in the first two hours decreased significantly over the next four hours **[77]**.

To prevent the formation of 3-MCPD esters, Li et al. performed deodorization experiments in the presence of antioxidants. According to their results, six different antioxidants had inhibitory effects in rapeseed oil, ranging from 22 to 44%, and the formation of MCPDs was most inhibited by TBHQ (tert-butylhydroquinone) and L-ascorbyl palmitate **[65]**. The

amount of 3-MCPD esters formed can be reduced by the addition of other substances. According to the work of Velíšek et al. **[95]**, soda ash (Na_2CO_3) and baking soda ($NaHCO_3$) were the most effective, which is consistent with other literature data **[40]**, **[87],** but 3-MCPD formation was also significantly reduced by cysteine and glutathione as well **[95]**.

For the reduction of the amounts of 3-MCPD and glycidol esters, removal of the compounds formed from the refined oil can be a solution. Experiments have been performed with several adsorbents. In the research of Strijowski et al., most effective proved to be a calcined zeolite and a synthetic magnesium silicate. The effect of the applied temperature was also observed, zeolite reached its maximum binding capacity at 60 °C already, while the magnesium silicate required a temperature of 80 °C. Based on the results, the amount of the adsorbent used should be at least 5%. The other magnesium and calcium silicates did not decrease the amounts of 3-MCPD and glycidol esters, in fact, some of them even increased them **[90]**. The use of activated bleaching earth also promises to be a good method for the removal of glycidol esters. However, the mechanism is different, they are not adsorbed on the surface of the bleaching earth, but the epoxy ring is opened, and mono- and diglycerides are formed **[84]**.

Free 3-MCPD can also be degraded enzymatically **[9]**. Using enzymatic breakdown, the amount of 3-MCPD esters was reduced in cooking oils by Bornscheuer and Hesseler. To do so, first the ester bond was broken in a biphasic system using the lipase A enzyme *Candida antarctica*, and then the product was transformed into glycerol using two additional enzmyes (halohydrin dehydrogenase – *Arthrobacter sp.*; epoxide hydrolase – *Agrobacterium radiobacter*) **[13]**.

5.3. Occurrence of chloropropanols and glycidol esters in other foods

Besides fats and oils, many other foods have been studied over the last decades, assessing which food categories are characterized by the presence of chloropropanols **[7], [19], [24]**. These compounds can occur in foods either as esters or in the free form **[91]**. Generally speaking, this is true for all products that contain fats and oils which have undergone some kind of thermal treatment. However, it is not only through processing that 3-MCPD esters can be introduced into the products. Their natural occurrence was first demonstrated by Cerbulis et al. in raw goat's milk **[17]**.

There is less information available on glycidol, however, this compound is often analyzed together with chloropropanols. One of the important areas of the topic of chloropropanols and glycidol is the testing of baby foods and formulas. In baby foods analyzed in Berlin in 2013, maximum measured values of 3-MCPD, 2-MCPD and glycidol were 1.22 mg/kg, 0.58 mg/kg and 1.3 mg/kg, respectively **[100]**. MCPD and glycidol concentrations in formulas available in the Ottawa area had been measured in 2012 and 2013 by Becalski et al. Based on their results, in the 2013 samples, the total MCPD equivalent amount was somewhat lower than in the samples of the same products in 2012, in 2013 the average was 26 µg/kg and the maximum was 108 µg/kg **[8]**. A few years earlier, baby foods found on the Prague market had been analyzed by Zelinkova et al., and according to their measurements, the amount of free 3-MCPD was below the limit of detection (<3 µg/kg), while the concentration of 3-MCPD esters in the products was in the range of 62-588 µg/kg. Calculating from these results, newborns (0 to 4 months) can ingest, by consuming baby foods, up to eight times the amount of 3-MCPD than the tolerable daily intake **[105]**. 3-MCPD intake also exceeds the TDI value in the case of breast feeding, however, this value is only one half of the amount of 3-MCPD ingested when consuming formulas **[106]**.

A number of studies can be found in the literature, identifying the main sources of chloropropanols and glycidol, broken down by food category. Free chloropropanols were measured by Chung et al. in products on the Hong Kong market. In only 15 of these could DCP be detected, in sausages, grilled pork, salted, steamed fish and crabs. Only in the case of the sausages tested was it true that there was no 3-MCPD present alongside the DCP, in all other cases 3-MCPD could be detected in large amounts in the products containing DCP. 3-MCPD was detected in 32% of the products tested (3-66 µg/kg), typically in cereals, meat, fish and snack products **[19]**. In a study performed in the United Kingdom in 2002, the amount of 3-MCPD esters exceeded the limit of quantification (0.01 mg/kg) in 30% of the products tested, with the highest concentrations found in salty biscuits, ranging from 0.01 to 0.134 mg/kg **[24]**. In the same year, the amount of 3-MCPD esters was evaluated in food ingredients as well in the United Kingdom. 22% of the samples contained 3-MCPD esters in concentration ranging from 0.014 to 0.488 mg/kg, most of them being malt samples, but there were bread crumbs, modified starch, meat extract and enzymatically hydrolyzed vegetable protein samples as well **[44]**. The presence of 3-MCPD esters could be detected in the lipid fraction of coffee creamers, whipped cream and bouillon cubes. Esters are introduced into the products with the refined, possibly hydrogenated oils added during production. These compounds could not be detected in any of the samples in the free form **[61]**. Baked potatoes and potato chips were analyzed by Zelinková et al. It was found that these products belong to foods with higher MCPD concentrations. The 3-MCPD esters detected in them came primarily from the cooking oil **[104]**. In addition, in terms of 3-MCPD esters, the type of oil is of great importance as well. Only trace of 3-MCPD esters could be found in potatoes fried

in rapeseed oil, while the amount absorbed by the product increased significantly when palm oil was used **[53]**.

6. Conclusions

The investigation of chloropropanols and glycidol has been an integral part of the food industry, of food analysis and toxicological studies over the last decades. Although their toxicological assessment is not yet complete, due to their proven and supposed toxic effects these compounds deserve special attention for the safety of our foods. Following their first detection, their presence in other food groups have been noted as well. As a result, analysts have been prompted, and are still encouraged currently to develop methods that are suitable for their detection both in food raw materials and in foods themselves. Even though there are still no limit values for these compounds, in response to the challenges, great emphasis has been placed by the vegetable oil industry on reducing their amounts. To achieve this goal, continuous development of both the technological processes of food production and analytical methods is necessary.

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