





Toolbox for the Mitigation of 3-MCPD Esters and Glycidyl Esters in Food



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Preface

Food must be safe – this is in everybody's interest: the food business operators, the consumers and the authorities. Nevertheless, nobody is immune to surprises. Method development continuously leads to new and improved analytical techniques which allow to detect new substances in food, in particular when the substance is only present in traces (milligram or microgram per kilogram food or levels even below that). In 2002, with the detection of acrylamide in food, a completely new class of undesired substances in food was found: the process contaminants. These substances are formed from natural food constituents when heat is applied in the production process or the treatment of food. In 2006, the process contaminant "3-MCPD ester" was described in various vegetable edible oils and fats for the first time. Later on, "related compounds" to the 3-MCPD esters were detected: Glycidyl esters and 2-MCPD esters.

Within the European Union, the minimization principle applies to undesired substances (contaminants) in food. This as well as principles for consumer health protection have been laid down in basic Regulation (EEC) No 315/1993 of the European legislation on contaminants. Immediately after the detection of 3-MCPD esters and based on health concerns expressed by the Federal Institute for Risk Assessment (BfR), research in the building process and the development of measures were started which aimed at reducing the levels of 3-MCPD esters in food and in particular in refined vegetable oils and fats. For this purpose, the food sector in Germany initiated two comprehensive research projects via the Research Association of the German Food Industry (FEI) which led to a number of findings. The producers of edible oils and fats evaluated the results, tested them in practice and developed them further. Parallel to this, the food processing industry worked on reducing the levels of 3-MCPD esters and Glycidyl esters in the final products. During this work it was also shown that under certain conditions 3-MCPD esters can be newly formed in some processing and preparation processes.

Due to the positive experiences made with the "Acrylamide toolbox" which has been developed by the European food industry under the leadership of FoodDrinkEurope to mitigate the acrylamide levels in food, the development of a toolbox for 3-MCPD esters and Glycidyl esters was proposed by interested associations and companies of the German food sector. The toolbox presented here was developed by a group of representatives from the German food sector, research institutes and private laboratories under coordination of the BLL. It contains tested "tools" for the entire food chain. It will enable the individual user to profit from the knowledge and experience available in research and practice in order to reduce the levels of 3-MCPD esters and Glycidyl esters in his products accordingly. The toolbox is therefore to be understood as practice-related guidance to help ensuring the protection of consumer health.

BLL, February 2016

Introduction and Background Information

Depending on the process conditions, so-called heat-induced contaminants (process contaminants) can be formed at various stages of food production and food processing. These contaminants are either formed – such as acrylamide or furan – together with the flavoring substances which are of the great importance for food quality and food taste or they are built during the refining process of food raw materials. In practice it is difficult to completely avoid the formation of heat-induced contaminants while at the same time still ensuring the product properties in such a way so that the food continues to meet consumer expectations and quality and safety of the product are still guaranteed.

The presence of 3-monochloropropane-1,2-diol esters (3-MCPDE) in edible oils and fats is one example of the occurrence of such process contaminants. These were described by Zelinková et al. [1] in 2006 for the first time. While no 3-MCPDE could be detected in native olive oil, increased levels were found in refined oils and oils made from roasted seeds. In December 2007, the Chemical and Veterinary Investigatory Office (CVUA) of Stuttgart and the Max Rubner-Institut (MRI) published the results of 3-MCPDE analysis of various edible oils and fats [2] and thus initiated the discussion on the new process contaminant 3-MCPD ester in Germany. Due to the progress of further method development for 3-MCPDE, later on also high levels of Glycidyl esters (GE) were found, mainly in refined palm oil.

3-MCPD esters and fat molecules (triglycerides) have a strong resemblance in their chemical structure. Triglycerides have a glycerol structure that is esterified with three fatty acids. 3-MCPD diesters have a glycerol structure that is esterified with two fatty acids while the third fatty acid is replaced by a chlorine atom. In 3-MCPD monoesters the glycerol structure is esterified with only one fatty acid. The term “3-MCPD ester” is derived from the assumption that “chlorinated glycerol” (3-MCPD) forms the basic structure instead of glycerol. Nowadays, 3-MCPDE are often called “bound 3-MCPD” in order to differentiate the esters from “free 3-MCPD”.

Unlike 3-MCPD esters, free 3-MCPD, which chemically belongs to the group of chloropropanols, has been known since the end of the 1970s as a reaction product from the production process of acid-hydrolyzed vegetable protein [3]. The detection of 3-MCPDE in edible oils and fat was significant from the health point of view because long-term studies with rats showed that the intake of 3-MCPD can cause renal damage. There have also been reports on benign tumors after the intake of higher doses of 3-MCPD [4]. Onami et al. [5] showed that free 3-MCPD and 3-MCPD esters are not genotoxic in vivo but possibly nephrotoxic. The Scientific Committee on Food (SCF) of the European Commission and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the later one also being responsible for contaminants, derived a Tolerable Daily Intake (TDI) of 2 µg free 3-MCPD/kg body weight based on available evidence and under consideration of a safety factor of 500. The International Agency for Research on Cancer (IARC) has classified free 3-MCPD as “possibly carcinogenic to humans” (Group 2B) [6]. For free 3-MCPD in hydrolyzed vegetable protein and soy sauce, maximum levels of 0.02 mg/kg, referring to the liquid product with a dry matter of 40 percent which corresponds to a maximum level of 0.05 mg/kg for the dry product, were laid down in the European contaminants legislation already several years ago [7].

Glycidyl esters are also estimated as harmful to human health [8], [9]. Free Glycidol has mutagenic and carcinogenic properties and was classified by the IARC as “probably carcinogenic to humans” (Group 2A) [10]. Therefore, the levels of this compound in food should be as low as reasonably achievable (ALARA).

In December 2007, the Federal Institute for Risk Assessment (BfR) presented a preliminary toxicological assessment of 3-MCPDE in which it is assumed that there a complete cleavage of the esters by enzymes occur in the human body releasing free 3-MCPD [11]. Therefore, it was recommended to apply the available toxicological assessment for the free compound to the assessment of the esters. The European Food Safety Authority (EFSA) issued a statement in which it agreed with this assumption [12].

Newer investigations of Buhrke et al. [13] and Abraham et al. [14] prove that 3-MCPD is almost completely released from 3-MCPDE in the body. Similar results were found by Appel et al. [15] with respect to the release of Glycidol from GE. According to a study published by Barocelli et al. [16] (Parma study) the main target organs for free 3-MCPD are the kidneys and the testicles. The fatty acid ester 3-MCPD

dipalmitate showed a similar effect as the free compound; approximately 70 percent of the ester was excreted in the urine as free 3-MCPD. These results generally confirm the assessment done by the BfR in 2007.

In its statement of December 2007, the BfR requested the oil producing industry to develop "alternative methods for the production of refined fats and oils" [11]; recommendations of the EFSA [12] pointed in the same direction. During the investigations into the reasons of the presence of 3-MCPDE and GE in vegetable fats and oils it quickly became evident that in particular the deodorization step applied in the oil refining process has a significant impact on the formation of 3-MCPDE and GE.

The formation of 3-MCPD esters and Glycidyl esters follows complex mechanisms in which not only the process conditions (temperature, time, pH) but also the presence of suitable precursors play an important role. In the case of 3-MCPDE, these are the presence of a chlorine source in an organic or inorganic form and the presence of fats (lipids) such as mono, di and triglycerides or phospholipids. Chlorides and other chlorinated compounds are taken up by the plant via water, fertilizers or pesticides and are stored in the seeds. During vegetable oil production they are transferred from the seeds into the oil. Nagy et al. [17] identified a number of organic chlorinated compounds in palm oil, as well as inorganic substances such as iron(III)chloride, iron(II)chloride, magnesium chloride and calcium chloride which can be converted during processing into more or less lipophilic chlorinated compounds ("chloride cascade"). With regard to the building mechanisms of 3-MCPDE, Destailats et al. [18] proposed that organic chlorine compounds break down thermocatalytically at high temperatures during the deodorization step into reactive chlorinated substances such as hydrochloric acid which then reacts with glycerides to form 3-MCPDE. The break down process of organic chlorinated compounds starts already at temperatures above 120°C. At temperatures of more than 150°C the formation of 3-MCPDE starts with a substitution of a fatty acid at the glycerol structure by a chlorine atom. A second reaction path for the formation of 3-MCPDE includes the intermediate step of an acyloxonium ion and a nucleophilic substitution.

In case of the building process of Glycidyl esters, a strong correlation with the diglyceride level in the fat or oil was found. Several studies [19], [20], [21] showed that the formation of GE increases exponentially from a temperature of 230-240°C and at a diglyceride level of more than 4 percent. The reaction mechanism proposed by Craft et al. [22] includes an intramolecular rearrangement with subsequent release of a fatty acid and formation of an epoxide bond as a result of the thermal treatment. Especially in fruit pulp oils such as palm oil or olive oil, higher levels of partial glycerides (diglycerides, monoglycerides) were found as a result of the enzymatic degradation process that occurs during the storage of the fruits prior to processing.

Different types of oil show a different potential for the formation of 3-MCPD esters and Glycidyl esters. For seed oils such as canola oil or sunflower oil 3-MCPDE levels of less than 1 mg/kg – calculated as free 3-MCPD – are typical. The 3-MCPDE levels in palm oil are usually between 2 and 4 mg/kg, and depending on the process conditions, GE levels of between 4 and more than 10 mg/kg – calculated as free Glycidol – can be found in addition. Due to the building pathways described, the quality of the raw material, in particular in case of fruit pulp oils, has an important impact on the potential to form 3-MCPDE and GE.

Today, there are several validated indirect methods available for the determination of 3-MCPDE and GE in vegetable oils and fats which cover the esters as groups and express the results as their sums [23], [24], [25], [26]. These methods are based on the cleavage of the esters into the free form, their isolation and derivatization with subsequent gas chromatographic separation and mass-selective detection (GC/MS). The indirect methods differ in the way the esters are cleaved, either acid-catalyzed or base-catalyzed. For the mitigation of 3-MCPDE and GE during the production and processing of fats and oils, it has shown to be sufficient to determine the levels of the individual ester groups as a sum. Direct methods which are able to determine individual 3-MCPDE or GE are based on liquid chromatographic methods with mass spectrometry detection (e.g. LC-MS/MS) after enrichment. However, they have not been able so far to be established in routine analysis. In March 2015, the European Reference Laboratory Joint Research Centre - Institute for Reference Materials and Measurements (JRC-IRMM, Geel, Belgium) published in-house validated indirect methods both for the esters (3-MCPDE, 2-MCPDE and GE) and the free

compounds (3-MCPD and 2-MCPD) in different kind of food [27]. With that, for the first time analytical methods have become available for fat-containing foods like bakery ware, cereal and potato-based snacks or fish and meat products that can be used consistently throughout the EU.

When considering the entire production chain of vegetable fats and oils, there are three general approaches available for the reduction of 3-MCPD esters and Glycidyl esters:

- (1) Selection of suitable oil or fat raw materials and reduction or prevention of precursors in the raw materials prior to processing
- (2) Modification of oil refining conditions and the introduction of new oil refining steps
- (3) Reduction of esters in the refined oil by use of suitable adsorbents (post refining step).

Since the request of the BfR in 2007 to develop "alternative methods for the production of refined fats and oils" [11], a number of proposals for the reduction of 3-MCPDE and GE in the production process of edible fats and oils have been published. Today, there are different tools available that can be used for their mitigation.

Structure and Use of the Toolbox

The toolbox contains tools that can be used to reduce the levels of 3-MCPD esters (3-MCPDE) and Glycidyl esters (GE) in refined vegetable fats and oils and in products containing these fats and oils. It can be assumed that the tools that are suitable for 3-MCPDE are also suitable for reducing the formation of 2-MCPD esters (2-MCPDE); however this has not been explicitly verified during the development of this toolbox. Furthermore, the toolbox also covers the aspect of new formation of 3-MCPDE during industrial processing and preparation of certain animal foods that are roasted, fried or grilled. The toolbox presented here does not contain measures for the mitigation of free 3-MCPD which has been known as a process contaminant for quite some time. The reason for this is that the formation of free 3-MCPD is traditionally linked to "other kind of food" like soy sauce and hydrolyzed vegetable protein and that free 3-MCPD has not yet been detected in fats and oils. Moreover, the mitigation strategies for 3-MCPDE and free 3-MCPD are different to current knowledge.

The toolbox for 3-MCPDE and GE presented here is based on the four pillars which represent the different stages of the food chain.

- Agriculture
- Oil Mill and Refinery
- Industrial Use of Oils and Processing
- Preparation (e. g. in the household or in restaurants/catering).

The individual main groups are divided into subgroups, e.g. the main group Agriculture consists of the subgroups Breeding, Cultivation and Harvest as well as Storage and Transport. The individual tools are allocated to the respective subgroup of a main group of the toolbox.

The tools provide the following information:

- name and/or short description of the tool
- mode of action/mitigation effect of the tool
- suitability of the tool for the following oils (assumption)
- the tool reduces the following process contaminant: 3-MCPDE, GE
- conditions and limitations of use of the tool
- consequences of use of the tool
- test level
- reference

Some of the information is explained here in more detail.

The information given for the „suitability of the tool for the following oils“ in the toolbox main groups Agriculture and Oil Mill/Refinery is based on assumptions made by the Working Group that developed the toolbox. This is due to the fact that most of the research on 3-MCPDE and GE has been carried out on palm oil; however, it was important for the Working Group to be able to offer tools ideally for the mitigation of esters in all vegetable oils. Therefore, the column „suitability of the tool for the following oils“ contains the following possible entries: "all oils", "fruit pulp oils (only)" or "palm oil (only)".

With regard to the information provided under „the tool reduces the following process contaminant“, the Working Group aimed at providing as much precise information as possible. This is why a differentiation was made between 3-MCPDE, GE and 3-MCPD plus GE. If it was not possible to clarify beyond doubt whether the tool covers both kind of esters or only one, the entry is specified as „sum, not specified“. This

information goes back to the beginnings of the analytical methods on 3-MCPD esters when it was not possible to distinguish between 3-MCPDE and GE.

The information provided under „test level“ shall help to evaluate whether and to what extent the tool has already been tested under practical conditions. Possible entries in this column include: industrial scale, pilot scale, laboratory scale, model experiment, single result and theoretical consideration. The test level „industrial scale“ covers tools that have already been established in practice. Those tools should be given preference in the consideration of the order of tools to be tested. Because the development of mitigation measures for 3-MCPDE and GE is still on-going, also tools belonging to lower test levels and even tools at the test level „theoretical consideration“ are mentioned in this toolbox as far as they seem to be plausible and are worth to be examined somewhat closer.

The different test levels in the following chapters are color coded as follows.

1. industrial scale	2. pilot scale	3. laboratory scale	4. model experiment	5. single result	6. theoretical consideration
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It should be pointed out that not every tool will be suitable for every product and every process but that the selection of tools needs to be adapted to the individual product and production process. The columns „conditions and limitations of use“ and „consequences of use“ may help in the decision process because they already show some restrictions of use of the respective tool. It should also be taken into consideration that it might be reasonable to use a combination of several tools in order to achieve an effective reduction in the 3-MCPDE and GE levels (e. g. in the refinement process of edible oils). For tools which are within the responsibility of an upstream stage of the production chain (e. g. agricultural conditions in the plantations for the downstream stages of the production chain), the toolbox can be used as guidance for the communication between customer and supplier.

In general, it is recommended to carry out a risk-benefit assessment prior to introducing mitigation measures. In this assessment the sensory properties, other quality parameters and the possible formation of other process contaminants should be considered as well. For tools which are used in the oil refining process and here in particular in the deodorization step, it needs further to be considered that various residues and contaminants such as polycyclic aromatic hydrocarbons (PAHs), pesticide residues and mycotoxins must be removed from the oil by refinement in order to ensure the safety of the products. Furthermore, the „technical feasibility“ of the tool for the individual product has to be taken into consideration as well; aspects such as extra efforts should be considered too. It is recommended that the results of the tests and their assessment are documented in an appropriate way.

Apart from the toolbox presented here, FEDIOL, the federation representing the vegetable oil industry in Europe, has also developed a „Toolbox Document“. The FEDIOL Toolbox Document is based on an assessment of references and patents and is coupled with a ranking of the most relevant tools. The ranking was made available to the Working Group and was taken into consideration in the development of the toolbox presented here.

This toolbox shall be updated regularly in order to take into account new findings and developments and to enable a dynamic process in the reduction of 3-MCPDE and GE levels (and maybe other related compounds).

1

Toolbox Main Group Agriculture





1. Toolbox Main Group Agriculture

The tools in the Toolbox main group Agriculture are divided into the four subgroups: "Breeding", "Cultivation", "Harvest" and "Storage and Transport (up to the oil mill)". Each subgroup contains a tool that considers more than one aspect. These combinations have been deliberately chosen because the toolbox Working Group believes that they are only effective or reasonable when combined (e.g. the transportation of the fruit bundles to the oil mill as quickly as possible with immediate sterilization of the fruits, see subgroup "Storage and Transport").

The test levels for the tools in the main group Agriculture are currently within the range from „theoretical consideration“ to „model experiment“. This is due to the fact that up to now it was difficult to influence the agricultural conditions in the oil palm plantations externally, and the cultivating countries had to be sensitized to this issue first. Nevertheless, according to the assessment of the Working Group, the main group Agriculture has a large impact on the mitigation of 3-MCPD esters because at this stage there exists several possible entries for inorganic or organic chlorine compounds that may act as precursors for the chlorine-containing 3-MCPDE. Moreover, at this stage, the enzymatic reactions take place which lead to a cleavage of the fat molecules (triglycerides) forming diglycerides, monoglycerides and free fatty acids which are considered to be precursors for the formation of 3-MCPD and GE too.

The tool „minimization of the entry of chlorine-containing substances“ in the subgroup Cultivation is based on theoretical considerations only; however, it does contain a number of plausible suggestions for chlorine-containing substances whose use in cultivation should be checked and which might/should possibly be replaced or reduced (e. g. chlorine-containing fertilizers, chlorinated water for irrigation of the plantations).

Also, the aspect of propagation of error costs should not be neglected. For example, measures in the oil refinery which prevent or reduce the formation of the esters from precursors can lead to significantly higher costs and efforts compared to measures which mitigate the root cause – in this case the formation and use of precursors- at the source.



1.1 Breeding

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
model experiment	breeding or selection of oil plant varieties with low lipase content	minimizes the enzymatic cleavage of triglycerides	all oils	GE	breeding is a lengthy process; new varieties are only available after several years	higher oil yields are possible due to lower levels of free fatty acids (FFA) and diacylglycerides (DAG) in the oil	[28]

1.2 Cultivation

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
theoretical consideration	minimization of the entry of chlorine-containing substances during cultivation	minimizes the availability of chlorine atoms	all oils	3-MCPDE		e.g. check the use of chlorine- containing fertilizers and pesticides; avoid the use of chlorinated water or iron chloride when irrigating the plantations; avoid the cultivation on saline soils	[18], [29], [30]



1.3 Harvest

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
single result	quick harvest at the optimal time; careful separation of loose/damaged fruits	minimizes the enzymatic cleavage of triglycerides	fruit pulp oils	3-MCPDE and GE	not always possible in case of remote/hard to reach plantations; directly available as so-called special quality (SQ) oils in certain growing countries (e.g. Malaysia); currently not available on the European market	more work during harvest, but possibly lower follow-up costs	[31], [32]

1.4 Storage and Transport

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
single result	transportation of the fruit bundles (FFB) to the oil mill as quickly as possible and immediate sterilization of the fruits	minimizes the enzymatic cleavage of triglycerides	palm oil	3-MCPDE and GE	not always possible in case of remote/hard to reach plantations; directly available as so-called special quality (SQ) oils in certain growing countries (e.g. Malaysia); currently not available on the European market	more work during harvest, but possibly lower follow-up costs	[31]

2

Toolbox Main Group Oil Mill/Refinery





Toolbox Main Group Oil Mill/Refinery

The Toolbox main group Oil Mill/Refinery which comprises the vegetable oil production process contains by far the highest number of tools. The tools are divided into the following five subgroups: "Production of crude oils", "Refining of vegetable oils", "Post-treatment of refined vegetable oils", "Modification of vegetable oils" and "Storage and Transport (up to the customer)". There are two tools available for the production of crude oils, thirteen for the refining of vegetable oils, four for the post-treatment of refined oils and two tools for the modification of vegetable oils. Currently there is no tool for the subgroup "Storage and Transport (up to the customer)". Four tools from the subgroup "Refining of vegetable oils" have been tested on "industrial scale"; the same applies for the two tools from the "Modification of vegetable oils" subgroup.

The Working Group for the toolbox points out that it may be reasonable to apply several tools from the subgroup "Refining of vegetable oils" in combination in order to achieve a more effective reduction of the esters; this is especially true when the tools belong to different steps of the refinement process. In general, the refinement process consists of the following steps: de-gumming, de-acidification, bleaching and deodorization. The deodorization step is considered to be the most relevant for the formation of 3-MCPDE and GE. Because 3-MCPDE are already formed at temperatures of around 180°C and the formation of GE rises exponentially at temperatures above 230°C, both the temperature and temperature profile in the refinement process have a large impact on the reduction of the esters.



2.1 Production of crude oils

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
model experiment	extraction of palm fruit pulp with hexane: water (2:1, v/v)	reduces the entry of reactive chlorine- containing compounds into the crude oil	palm oil	3-MCPDE		changes in the process required, this results in expenses for investments and additional costs for solvents; more work involved; environmental aspects (use and disposal of organic solvents)	[29]
	extraction of palm fruit pulp with isopropanol	reduces the entry of reactive chlorine- containing compounds into the crude oil	palm oil	3-MCPDE		changes in the process required, this results in expenses for investments and additional costs for solvents; more work involved; environmental aspects (use and disposal of organic solvents)	[29]



2.2 Refining of vegetable oils

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
Industrial scale	limiting of DAG and FFA levels in the crude oil (a) <4 % DAG and <2.5 % FFA; b) <5.5 % DAG and <1.5 % FFA by selection of suitable raw materials	reduction of the potential of GE formation	palm oil	GE	only high quality oils can be used for refinement; directly available in the form of so-called special quality (SQ) oils in certain growing countries (e.g. Malaysia); currently not available on the European market; only available as smaller batches	use of loose fruits not possible, this results in yield losses; extra efforts in logistics; higher prices for the crude oils	[29], [33], [34]
	replace physical refining by chemical refining	lower temperatures during the deodorization step possible; removes possible precursors by washing them out	all oils	3-MCPDE and GE		chemical refining has a higher environmental impact and results in higher oil losses	[33], [35]



	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
Industrial scale	use of significant higher amounts of bleaching earth	adsorption of precursors	all oils	3-MCPDE and GE		higher costs due to higher amounts of bleaching earth; higher oil losses	[36], [37]
	the first bleaching and deodorization process is followed by a second bleaching and deodorization process at lower temperatures	reduction of GE and prevention of new formation of GE	all oils	GE		extra efforts due to double bleaching and deodorization; higher oil losses	[38], [39]
pilot scale	replacement of deodorization by short path distillation	reduces temperature stress on the oil	all oils	3-MCPDE and GE	not suitable for food in which the red color of the palm oil is undesired; up to now no standard process in oil refineries	results in red-colored palm oils; quality problems with those treated oils possible; extra efforts	[40]



	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
laboratory scale	washing of the crude oil with water (*)	removes reactive chlorine-containing compounds from the oil	all oils	3-MCPDE		more work involved; separation of the water phase may be difficult	[17], [36]
	de-gumming with water at 70°C or de-gumming with 5% water and 0.2% phosphoric acid with subsequent bleaching with more than 1.5% bleaching earth at 80-100°C	prevents a too low pH level in the oil	all oils	3-MCPDE		may result in higher phosphorus levels in the oil	[17], [33], [35]
	reduced addition of acid during de-gumming	prevents a too low pH level in the oil	all oils	3-MCPDE and GE			[37]

(*) According to information provided by the research institutes who developed the tool, the tool showed good results on a laboratory scale but not on a pilot scale. A reduction of 3-MCPDE was also observed in the tests on pilot scale, but at the same time the GE levels strongly increased. Therefore, the research institutes are no longer recommending this tool. However, the Toolbox Working Group believes that the tool should be tested again under different conditions. Therefore, the tool is still listed in the toolbox, but at test level "laboratory scale".



	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
laboratory scale	introduce neutralization with calcium oxide prior to deodorization	prevents a too low pH level in the oil	all oils	3-MCPDE and GE		neutralization results in higher waste water quantities and in oil losses	[35]
	washing with ethanol/water (1:1, v/v) prior to deodorization	removes reactive chlorine-containing compounds from the oil	all oils	3-MCPDE		higher costs for solvents, more work involved; because of the use of ethanol the product is no longer suitable for Halal and Kosher production	[29]
	use of neutral bleaching earths	prevents a too low pH level in the oil	all oils	3-MCPDE and GE	only applicable for oils with low chlorophyll levels; more bleaching earth required	higher costs	[35]
	deodorization at temperatures between 190 and 230°C	reduces temperature stress on the oil and thus the reaction speed for the formation of the esters	all oils	GE		possible problems with the quality and product safety of the refined oil	[33], [41]
	deodorization using a two-step temperature profile (2-step-deodorization)	reduces temperature stress on the oil	all oils	3-MCPDE and GE		extra efforts	[36]



2.3 Post-treatment of refined vegetable oils

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
laboratory scale	post-treatment of the refined palm oil with calcinated zeolite	removes GE from the oil	all oils	GE	zeolite must be of food-grade quality	extra efforts; high oil losses (approx. 5% of the oil); separate plant unit required; zeolite must be disposed after use	[42]
	post-treatment of the refined palm oil with synthetic magnesium silicate	removes GE from the oil	all oils	GE		minor losses in oil quality; extra efforts; high oil losses (more than 5% of the oil); separate plant unit required; magnesium silicate must be disposed after use	[42]
	post-treatment of the oil with carboxymethyl-cellulose in combination with a nitrogen treatment	precursors of 3-MCDP esters or the esters themselves are bound to the carboxymethyl-cellulose and removed in this way	all oils	3-MCPDE		extra efforts, additional costs for nitrogen; oil losses; carboxymethylcellulose must be removed after the treatment and disposed afterwards	[43]



	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
laboratory scale	post-treatment of the oil with cation-exchange resin in combination with a nitrogen treatment	precursors of 3-MCDP esters or the esters themselves are bound to the exchange resin and removed in this way	all oils	3-MCPDE		extra efforts; additional costs for nitrogen; the exchange resin must be separated from the oil after use	[43]

2.4 Modification of vegetable oils

	tool	mode of action/ mitigation effect	tool most probably suitable for	tool reduces substance class	conditions/limitations of use	consequences of use	reference
industrial scale	carry out the deodorization step after hardening and bleaching at lower temperatures (< 220°C)	reduction of GE and prevention of new formation of GE	all oils	GE			[38], [39], [44]
	carry out the deodorization step after bleaching and esterification at lower temperatures (< 220°C)	reduction of 3-MCPDE and GE and prevention of new formation of 3-MCPDE and GE	all oils	sum, not specified			[44]



3

Toolbox Main Group Industrial Use/Processing





3. Toolbox Main Group Industrial Use/Processing

The Toolbox main group Industrial Use/Processing distinguishes two subgroups: the recipe (“exogenous source”) and further processing of the food to a finished product (“endogenous formation”). The term “exogenous source” refers to the use of a refined vegetable oil or fat as an ingredient in food stuffs while the “endogenous formation” deals with possible new formation of 3-MCPDE and GE during processing.

For the subgroup “exogenous source”, only two tools could be identified: the selection of a suitable vegetable oil and the reduction of the fat content of the individual food product. Both tools belong to the test level “industrial scale”, thus they are already applied in practice. The mitigation effect of the tool “selection of the vegetable oil” is described as follows: “the use of a suitable oil with lower levels of 3-MCPDE and GE results in lower levels (of 3-MCPDE and GE) in the final product”. This wording has been deliberately chosen by the Working Group that developed the toolbox in order to focus on the suitability of an individual oil for both the product and the production process instead of favoring certain types of oil. On one hand, initially increased levels of 3-MCPDE and GE were especially found in palm oil; on the other hand, most research for a reduction of the esters was carried out on palm oil so that palm oil may be the “suitable oil” today. Moreover, it is not only the levels of 3-MCPDE and GE that are important to decide if an oil is suitable for an individual product and process but all oil properties in total (e.g. range of fatty acids, heat stability). However, the wording of this tool as chosen also requires that the levels of 3-MCPDE and GE in the oil are known. That means that an analysis of the oil on 3-MCPDE and GE may be necessary which up to now can only be carried out with elaborate instrumental analytical methods.

The subgroup “endogenous formation” includes three tools which have the status “single result” as test level. The Working Group for the toolbox considers these tools as plausible to prevent or reduce a possible new formation of 3-MCPD esters during processing. According to current knowledge a possible new formation of 3-MCPDE is limited to the food processing steps: roasting, grilling and frying. Moreover, only certain animal food such as fish and meat and products made thereof seem to be affected. When grilling, also new formation of GE is possible. During the processing of food of plant origin, new formation of 3-MCPDE and GE has not been found so far. This has been confirmed by studies carried out at the Food Chemistry Institute (LCI) in Cologne, Germany, which show that no esters are newly formed during the production process (deep-frying) of potato crisps [45]. The same applies for the baking process of e.g. cakes and cookies where also no new formation of esters was observed.



3.1 Recipe ("exogenous source")

	tool	mode of action/ mitigation effect	tool reduces substance class	conditions/limitations of use	consequences of use	reference
Industrial scale	selection of vegetable oil	the use of a suitable oil with lower levels of 3-MCPDE and GE results in lower levels in the final product	3-MCPDE and GE	limited application (depending on the individual end product, the ingredients used and the technological parameters of the production process)	additional costs; influences the technological parameters of the production process; influences the sensory properties and texture of the end product	[46]
	reduction of the fat content of the food product	by reducing the amount of fat/oil, lower levels of 3-MCPDE and GE enter into the final product	3-MCPDE and GE	limited application (depending on the individual end product, the ingredients used and the technological parameters of the production process)	changes in product properties; changes in product type; influences the technological parameters of the production process; influences the sensory properties and texture of the end product	[46]

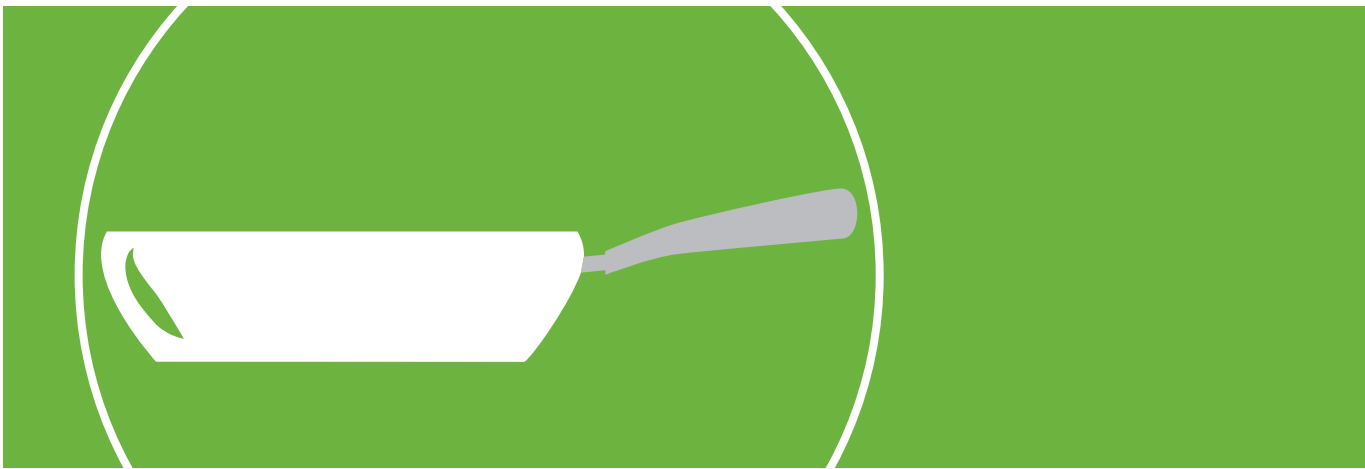


3.2 Production process (“endogenous formation”)

	tool	mode of action/ mitigation effect	tool reduces substance class	conditions/limitations of use	consequences of use	reference
single result	add only low amounts of salt; use of ingredients that are low in salt	mitigation of a possible precursor (a high salt content can promote the formation of 3-MCPD esters)	3-MCPDE	applies only to production processes which have a potential for new formation of the esters; applicability of the tool is also dependent on the individual end product, the ingredients used and the technological parameters of the production process	changes in product properties; influences the technological parameters of the production process; influences the sensory properties of the end product	[46]
	lower process temperatures	high temperatures can promote the formation of 3-MCPDE	3-MCPDE	applies only to production processes which have a potential for new formation of the esters; applicability of the tool is also dependent on the individual end product, the ingredients used and the technological parameters of the production process	changes in product properties; influence on product safety; influences the technological parameters of the production process; influences the sensory properties of the end product	[47]
	usage time of the frying fat as short as possible	thermal stress on the frying fat can promote the formation of 3-MCPDE; frying fat with low levels of 3-MCPDE results in lower ester levels in the fried product	3-MCPDE	applies only to production processes which have a potential for new formation of the esters; usage time of the frying fat can only be changed in batch processes	additional costs due to shorter usage time of the frying fat	[46]

4

Toolbox Main Group Preparation





4. Toolbox Main Group Preparation

The Toolbox main group Preparation comprises the professional preparation of food, dishes and meals within the food sector by restaurants, catering companies, food stalls etc. as well as the preparation of food and meals at home by the consumer. Most observations made for the Toolbox main group Industrial Use/Processing apply for the Toolbox main group Preparation as well.

In both Toolbox main groups Industrial Use/Processing and Preparation, there is a distinction between tools for an "exogenous source" and tools for an "endogenous formation" of the esters. If a refined vegetable oil or fat is used as an ingredient in the food preparation process (exogenous source), neither staff in restaurants, catering companies etc. nor consumers are currently able to recognize whether product A has lower levels of 3-MCPDE and GE than product B. Furthermore, as explained for Industrial Use/Processing, it is not only the levels of 3-MCPDE and GE which make an oil "suitable" but the oil has to fit with all its product properties to an individual food and its way of preparation. Such considerations are complex and thus difficult for consumers and restaurants, catering companies etc. to implement. Therefore, the Working Group for the toolbox currently does not recommend a tool for the "exogenous source" within the Toolbox main group Preparation.

For the "endogenous formation", the Working Group recommends similar tools for the preparation of food and meals as for Industrial Use/Processing. Different from tools for the "exogenous source", staff in restaurants, catering companies etc. and consumers can actively contribute to prevent or reduce possible new formation of the esters during roasting, grilling or frying. Again, it must be emphasized that according to current knowledge, new formation of esters only affects certain foods of animal origin (e.g. fish, meat). The Working Group believes that especially the reduction of added salt and salt-containing ingredients is a very suitable tool to reduce or prevent the new formation of 3-MCPDE when roasting, grilling or frying food. If the finished food is not salty enough, it can be salted after the heating process without any problems.



4.1 Production process ("endogenous formation")

	tool	mode of action./ mitigation effect	tool reduces substance class	conditions/limitations of use	consequences of use	reference
single result	reduce the addition of salt or salted ingredients when roasting, grilling or frying food	mitigation of a possible precursor (a high salt content before heating can promote the formation of 3-MCPD esters)	3-MCPDE	depending on the individual food and the type of preparation	changes in product properties; influences the sensory properties; salt can be added after preparation, if needed	[47], [48], [49], [50]
	reduce the temperature during roasting, grilling or frying food	high temperatures can promote the formation of 3-MCPDE	3-MCPDE	depending on the individual food and the type of preparation	changes in product properties; influence on product safety, influences the sensory properties	[47], [48], [49], [50]
	usage time of the frying fat as short as possible	thermal stress on the frying fat can promote the formation of 3-MCPDE; frying fat with low levels of 3-MCPDE results in lower ester levels in the fried product	3-MCPDE	depending on the individual food and the type of preparation	change oil more often; higher usage of oil	[47]

List of abbreviations

2-MCPD-FE	2-Monochloro-1,3-propanediol ester
3-MCPD-FE	3-Monochloro-1,2-propanediol ester
ALARA	as low as reasonably achievable
AOCS	American Oil Chemists Society
BfR	Bundesinstitut für Risikobewertung [Federal Institute for Risk Assessment]
CONTAM	Scientific Panel on Contaminants in the Food Chain of EFSA
CVUA	Chemisches und Veterinäruntersuchungsamt [Chemical and Veterinary Investigatory Office]
DAG	Diacylglycerides
DGF	Deutsche Gesellschaft für Fettwissenschaft [German Society for Lipid Science]
EFSA	European Food Safety Authority
FEDIOL	Federation representing the European Vegetable Oil and Protein Meal Industry in Europe
FEI	Forschungskreis der Ernährungsindustrie [Research Association of the German Food Industry]
FFA	free fatty acids
GC/MS	Gas Chromatography/Mass Spectrometry
GE	Glycidyl ester
IARC	International Agency for Research on Cancer
IRMM	Institute for Reference Materials and Measurements of JRC
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre of the European Commission
LC-MS/MS	Liquid Chromatography/Triple Quad Mass Spectrometry
LCI	Lebensmittelchemisches Institut, Köln [Food Chemistry Institute (LCI), Cologne]
MRI	Max Rubner Institut; hier: Bundesforschungsinstitut für Ernährung und Lebensmittel, Institut für Sicherheit und Qualität bei Getreide, Arbeitsgruppe für Lipidforschung, Detmold [Max Rubner-Institut, here: Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Cereals, Working Group for Lipid Research, Detmold]
PAH	Polycyclic Aromatic Hydrocarbons
SCF	Scientific Committee on Food (predecessor of EFSA)
SQ oils	Special Quality oils
TDI	Tolerable Daily Intake
v/v	concentration in volume/volume

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