ILSI Europe
Report Series

3-MCPD Esters in Food Products

Summary Report of a Workshop held in February 2009
in Brussels, Belgium

Organised by the ILSI Europe Process-related Compounds and Natural Toxins Task Force and Risk Assessment of Chemicals in Food Task Force in association with the European Commission (EC) and the European Food Safety Authority (EFSA)

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3-MCPD ESTERS IN FOOD PRODUCTS

By John Christian Larsen

SUMMARY REPORT OF A WORKSHOP HELD IN FEBRUARY 2009 IN BRUSSELS, BELGIUM
ORGANISED BY THE ILSI EUROPE PROCESS-RELATED COMPOUNDS AND NATURAL TOXINS TASK FORCE AND RISK ASSESSMENT OF CHEMICALS IN FOOD TASK FORCE IN ASSOCIATION WITH THE EUROPEAN COMMISSION (EC) AND THE EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

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SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Recent studies have identified high levels of 3-MCPD esters in refined edible fats, such as margarine and oils, and in fat-containing foods including infant formula (both starter and follow-on) and human milk. Other related ester compounds such as 2-MCPD esters and glycidol esters are also expected to occur.

No toxicological data are available on 3-MCPD esters. The full hydrolysis of these esters in the gastrointestinal tract would result in significant exposures to free 3-MCPD. Considering the highest levels of 3-MCPD esters found in oils and fats, and assuming 100% hydrolysis, exposures of 10- to 20-fold the 3-MCPD tolerable daily intake (TDI) could be calculated for infants fed on formulae, and fivefold for adult men on a fat-rich diet.

However, structural information and preliminary digestion data suggest that 3-MCPD esters behave like triacyl-*sn*-glycerols (TAGs) in the gastro-intestinal tract, where gut lipases with higher affinity for positions *sn*1 and *sn*3 release 2-monoacylglycerol (*sn*2-MAG) from the dietary TAGs. *sn*2-MAGs are readily taken up by enterocytes, re-esterified by acyltransferases and then incorporated into lipoprotein particles. Assuming a similar metabolism for 3-MCPD esters raises the hypothesis that ingestion of (*sn*1) monoesters would result in release of free 3-MCPD, while (*sn2*) monoesters would be absorbed as such. 2-MCPD esters are also expected to be good substrates for lipases (AOCS, 2009).

Therefore, assuming a complete hydrolysis for only (*sn1*) monoesters of 3-MCPD and considering that only 15% of the total 3-MCPD esters consist of (*sn1*) monoesters, the calculated excursion above the TDI for 3-MCPD would be about one sixth of that mentioned above and most adult consumers would probably be exposed to 3-MCPD levels within the TDI.

The workshop therefore agreed that the toxicological profile of 3-MCPD esters will strongly depend on the rate of lipase-mediated hydrolysis in the gut. Therefore, for the risk assessment it is necessary to clarify whether the esters might simply be considered as "prodrugs" for MCPD and/or glycidol or whether they need to be toxicologically evaluated individually.

3-MCPD esters have been found in all refined vegetable oils. The lowest levels were observed in refined rapeseed oil (0.3–1.5 mg/kg) and the highest levels in refined palm oil (4.5–13 mg/kg). 3-MCPD esters are now also 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 and 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.

In the EU, maximum levels of 0.02 mg/kg for free 3-MCPD in hydrolysed vegetable proteins and soy sauce were established in 2001. The maximum levels have applied since April 2002 and are integrated into the Commission Regulation (EC) 1881/2006. These limits were not designed to account for 3-MCPD esters.

3-MCPD esters are formed at high temperatures during the refining of edible fats and oils, mainly during the deodorisation step. The proposed mechanism for the formation of 3-MCPD esters involves the formation of 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. There are no toxicological data on glycidol esters but glycidol is a known genotoxic and carcinogenic compound. 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. It has been observed that the presence of glycidol esters could be the source of significant overestimation of 3-MCPD bound as esters, but this will depend on the analytical method applied. Standardised and agreed analytical methods are therefore necessary.

Looking at the possibilities for reducing the exposure to 3-MCPD esters makes it clear that the consumer has only limited options. Therefore, the producers have a duty to develop strategies for minimising the amount of these compounds in fats and oils by optimising the processing, removing 3-MCPD esters from the product, or by avoiding relevant reactants in the raw material.

The information obtained so far is insufficient for optimising the refining process for 3-MCPD ester mitigation, while at the same time maintaining the quality of the refined product. It was concluded that there are no easy solutions to this problem. 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.

The workshop agreed that, in order to develop effective mitigation measures, the database on occurrence of 3-MCPD esters in food and on the monoester/diester ratio needs to be improved and substantiated by research on the mechanisms of 3-MCPD/glycidol-ester formation.

The workshop considered that collaboration among different research institutes, industry and authorities is key to resolving this potential public health problem and gave the following recommendations for future work in order to address the gaps in knowledge that at the present time prohibit both an adequate risk assessment and effective mitigation procedures.

- The database on occurrence of 3-MCPD esters in food and on the monoester/diester ratio needs to be improved.
- Research on the mechanisms of 3-MCPD/glycidol ester formation is required in order to develop effective mitigation measures. Studies aimed at understanding the pathways of 3-MCPD/glycidol ester formation in refined oils and fats and during the processing of foods are needed in order to design the most appropriate strategies for reducing the levels of these compounds.
- There is a need for agreement on and development of analytical methods that are validated separately for each component (free and bound 3-MCPD monoesters and diesters and other related compounds such as free and bound 2-MCPD and glycidol).

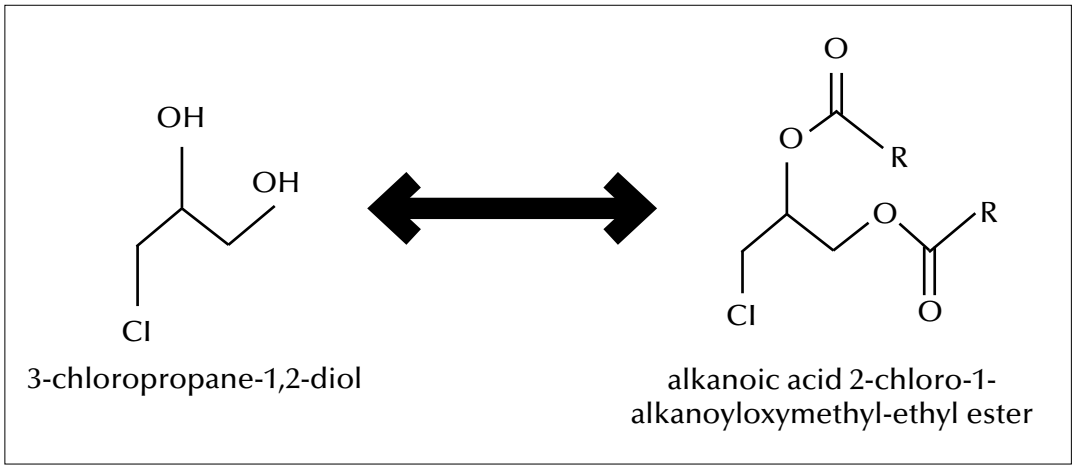
- As glycidol esters are an emerging issue, a critical appraisal of existing research priorities and ongoing actions should be undertaken to determine whether further research is warranted to study in detail the mechanisms of formation of 3-MCPD/glycidol esters.
- As a first priority it was recommended that a subacute (28 days) or subchronic (90 days) feeding study be conducted in rats. The study should be designed to determine the effects and toxicity of pure 3-MCPD diester, (*sn*1) 3-MCPD monoester and (*sn*2) 3-MCPD monoester, in comparison with the effects of free 3-MCPD. The doses of the esters should contain the same molar amount of free 3-MCPD as reference. Palmitic acid would be the fatty acid of choice since it is the major fatty acid in palm oil.
- Experiments addressing absorption, distribution, metabolism and excretion were considered important for supporting the toxicological studies. Levels of 3-MCPD esters in blood (in lipoprotein-rich particles) and selected tissues are also required. This will require development of analytical methods to measure 3-MCPD esters in blood and tissues.
- Studies addressing the rate of hydrolysis of 3-MCPD esters in the human gut, taking into account the impact of gut development (i.e. accounting for lower lipase activity in the infant gut) and the effects of food matrices on the hydrolysis rate of 3-MCPD esters in the gut should be performed using the TNO gastrointestinal model (TIM). TIM reproduces very closely the dynamic *in vivo* conditions of the human gastrointestinal tract.

The workshop considered it unnecessary to generate any genotoxicity data on 3-MCPD esters. Furthermore, the toxicological database on free 3-MCPD esters was considered adequate and did not require any additional studies. The toxicological data from the studies recommended above should be used to decide on the need for additional information on the potential effects of 3-MCPD esters on development.

BACKGROUND

3-Monochloropropane-1, 2-diol (3-MCPD) and other chloropropanols 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. 3-MCPD is formed when fat- and salt-containing foods are processed at high temperatures during production. The EC Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Group on Food Additives (JECFA) have evaluated 3-MCPD in food. In 2001, the SCF derived a tolerable daily intake (TDI) for 3-MCPD at 2 µg/kg body weight (SCF, 2001) and in 2002 the JECFA established a provisional maximal tolerable daily intake (PMTDI) for 3-MCPD, also at 2 µg/kg body weight (JECFA, 2002).

Figure 1. Chemical structures of 3-MCPD and its corresponding fatty acid diester



The presence of fatty acid esters (free or bound 3-MCPD esters) has recently been reported in various types of foods (including human milk) and food ingredients, especially in vegetable oils. Although there is no evidence to suggest any adverse health effects from 3-MCPD esters in food, an indirect toxicological concern is raised by the potential release of free 3-MCPD through the action of gut lipases.

Due to the fragmentary data available and the uncertainties involved, it is currently difficult to obtain an accurate exposure assessment for 3-MCPD esters in the general population. Recently, it was reported that the potential exposures to 3-MCPD if it were efficiently released from 3-MCPD esters would be significantly higher than the previously observed background exposure to free 3-MCPD. Exposures to 3-MCPD esters in infants resulting from consumption of products containing palm-oil ingredients can be better estimated. Applying a scenario considering the maximum levels of 3-MCPD esters measured in oils, and a high food intake, the hydrolysis of 100% of 3-MCPD esters would result in calculated exposures corresponding to about 10- to 20-fold the TDI of 3-MCPD.

The assessment of the health significance of dietary exposure to MCPD esters is complex and will require in-depth scientific investigations, which need to be defined. In order to ensure both the application of the best science and the optimisation of resources, it is essential to facilitate a thorough review of the situation. Therefore, two ILSI Europe Task Forces (Risk Assessment of Chemicals in Food, and Process-related Compounds and Natural Toxins) organised a workshop on 5-6 February 2009 in Brussels in collaboration with the European Commission (EC) and the European Food Safety Authority (EFSA) with the following objectives:

- Review all available data required for risk assessment
- Identify the key data gaps for risk assessment
- Define experimental research strategies to fill data gaps
- Propose an action plan

More than 70 participants from 20 countries, including experts from regulatory bodies (DG SANCO, EU Member States and US FDA), risk assessment organisations (EFSA, UK FSA and BfR), academia and industry participated in the workshop. The topics addressed were: the assessment of risks posed by 3-MPCD esters in food; analysis and method validation; occurrence, exposure and toxicology; formation routes; and mitigation options, followed by a round-table discussion.

The workshop was a two-day event with three sessions that included 11 presentations. The first session addressed the state of the art in relation to the evaluation of 3-MCPD esters, highlighting the positions/ statements of BfR, EFSA and DG SANCO. The presentations in the second session focussed on the mechanism of formation, occurrence and biological significance of various MCPD esters in foods and discussed potential ways of reducing levels of 3-MCPD esters in vegetable oils. The third session on the second day of the workshop involved three working groups on:

1. Analytical and exposure assessment
2. Technologies and reduction strategies
3. Toxicology and toxicokinetics

The participants were equally distributed in the three working groups on the basis of their background and professional interest.

The workshop was chaired by **Dr. Ib Knudsen**, ILSI Europe President who welcomed the participants and introduced the workshop by highlighting its objectives and pointing out the uncertainties related to the chemical structures of the 3-MCPD esters present in food, the difficulties in performing reliable exposure assessments, and the lack of knowledge about the actual fate and metabolism of the MCPD esters in the animal and human gastrointestinal tract as well as the potential role of the food matrix.

The overall rapporteur was **Dr. John Christian Larsen**, (DTU National Food Institute, Denmark) and co-rapporteurs were **Prof. Varoujan Yaylayan** (McGill University, Canada), **Dr. Claire-Lise Bechert** (The EU Oil and Protein Meal Industry, Belgium) and **Dr. Benoît Schilter** (Nestlé, Switzerland) who served as rapporteurs of working groups 1, 2, and 3, respectively.

SESSION 1: STATE OF THE ART

Risk assessment of 3-MCPD esters in food: position of the German Bundesinstitut für Risikobewertung (BfR)

Prof. Dr. A. Lampen from the Federal Institute for Risk Assessment, (BfR, Germany) gave an introduction to the potential toxicological properties of 3-MCPD and reported on the BfR assessment of 3-MCPD esters in vegetable oils and in infant formulae and follow-on formulae (BfR, 2007).

In animal experiments, 3-MCPD administration led to an increased incidence of renal tubule hyperplasia and, at higher dose levels, it induced kidneys tumours, Leydig cell tumours and mammary fibroadenomas. As 3-MPCD did not show genotoxicity *in vivo* (negative micronucleus test in mouse bone marrow and unscheduled DNA synthesis test in rat liver), it has been assumed that the tumours observed in the long-term animal study (mainly benign) only occur above a threshold value. According to the SCF and the JECFA, hyperplasia occurred in a dose-dependent manner in the kidneys of male rats (SCF, 2001; JECFA, 2002). The fact that the renal effects, although not statistically significant, occurred even at the lowest dose of 1.1 mg/kg body weight per day was taken into account by applying an uncertainty factor of 500. A TDI of 2 µg 3-MCPD/kg body weight was established by the SCF and a provisional maximum tolerable daily intake (PMTDI) of 2 µg 3-MCPD/kg body weight was established by the JECFA. Recent data obtained in an *in vivo* rat study applying the comet assay in kidney and testes of male rats confirmed the absence of genotoxicity. These data further support the opinion that the kidney tumours are induced by a threshold mechanism.

Studies by the official food control authorities (The Ministry for Nutrition and Rural Areas Baden-Württemberg) in Germany identified high levels of 3-MCPD esters in refined edible fats like margarine and oil and in fat-containing foods, including infant formula and follow-on formulae. These studies showed that only oils that had not undergone heat treatment (e.g. native olive oil) were devoid of the substances. The 3-MCPD esters are formed at high temperatures, probably during the deodorisation of edible fats and oils, which is the last stage in refinement, during which undesirable odorous and taste-bearing substances are removed.

No toxicological data were available on 3-MCPD esters. In its health-risk assessment of 3-MCPD esters, the BfR therefore assumed that 100% of the 3-MCPD is released from the 3-MCPD esters during the digestion process and subsequently used the risk assessment of 3-MCPD exposure performed by the JECFA (JECFA, 2002) and the SCF (SCF, 2001). Although the TDI value is not normally applied to infants in the first months of life, the BfR used this as an option in its risk assessment.

The BfR calculated the potential intake of 3-MCPD esters for infants fed on formula and, by assuming that the esters are fully hydrolysed, calculated that infants who are given formula with the maximum measured levels of esters (4196 µg 3-MCPD/kg fat content, corresponding to a concentration of 156 µg 3-MCPD/L in ready-to-drink milk) could have a 3-MCPD exposure of 25 µg/kg body weight per day, which is 12.5 times the TDI. The median 3-MCPD level determined in infant formula and follow-on formula (based on ten samples) was 2568 µg/kg fat in dried powder and the minimum level was 1210 µg 3-MCPD/kg fat (median and minimum levels were 96 and 45 µg 3-MCPD/L in ready-to-drink milk, respectively). This led to estimated intakes that were 7.7 and 3.6 times the TDI, respectively. The ratio between the lowest dose that led to tubular hyperplasias of the kidney in rats in the long-term study and the amounts ingested by babies fed infant formula is 44, 71 and 152. This quotient is described as the margin of exposure (MOE).

During the examination of 20 additional samples of follow-on formulae, a maximum level of 8467 µg 3-MCPD/kg fat content was found, corresponding to 250 µg 3-MCPD/kg in ready-to-drink milk. This can lead to intakes of 20 times the TDI, which corresponds to a MOE of 28. Prof. Lampen compared this value with the results of a study from the Czech Republic in which 3-MCPD esters were detected in all of the 12 samples of human milk tested. The mean and maximum concentrations found were 36 and 75 µg 3-MCPD/kg milk, respectively.

In the case of adult men, scenarios of daily consumption of vegetable fat, with either a maximum level of 3-MCPD esters identified for margarine of 7356 µg 3-MCPD/kg fat content or a median level of 3101 µg 3-MCPD/kg fat content, led the BfR to consider that the daily intake could be up to five times the TDI. The BfR concluded that there is a need to reduce the levels of 3-MCPD esters in edible fats and fat-containing foods because there is no alternative to infant formulae and follow-on formulae for infants who are not breastfed. However, because of the many unanswered questions on the toxicity of 3-MCPD esters and on the basis of a risk–benefit assessment, the BfR still advises mothers who are unable to breastfeed their infants to continue feeding them on the commercially available products.

Evaluation of 3-MCPD ester: position of EFSA

Dr. Claudia Heppner, from the European Food Safety Authority (EFSA, Italy) explained that the service within the European Commission responsible for legislation on food safety asked the members of the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM) for a statement regarding the findings in Germany of high levels of 3-MCPD esters in edible refined plant oils and fats, such as margarine, oil for frying, nougat spread and infant formula (fat component) in order to assist the European Commission in preparing its position on 3-MCPD in liquid condiments containing acid-hydrolysed vegetable protein for the meeting of the Codex Committee on Contaminants in Food in April 2008 (CAC, 2008). Maximum levels of 0.02 mg/kg 3-MCPD for hydrolysed vegetable protein and soy sauce have been laid down at a Community level; however, current discussions at the Codex Alimentarius propose higher maximum levels of 0.4 mg/kg for liquid condiments.

The CONTAM Panel was also asked to take into account the recently issued opinion from the BfR on 3-MCPD esters and recently published scientific literature. The CONTAM Panel noted that the BfR based its recent risk assessment on toxicological data on 3-MCPD, under the assumption that 100% of 3-MCPD is released from its esters since there are no toxicological data on 3-MCPD esters. The CONTAM Panel concluded that there is no scientific evidence at present to dispute this figure and agreed with the assumption that 100% of 3-MCPD is released from its esters in humans. However, the CONTAM Panel would welcome kinetic studies on 3-MCPD esters to gain further information on the time-course and site of release of 3-MCPD from its esters *in vivo*.

Regulatory issues: current and future perspectives: position of DG SANCO

Mr. Frans Verstraete (European Commission, DG SANCO, Belgium) stressed that a general objective of the EU food safety regulation is to achieve a high level of protection of human health. The "General Food Law" (Regulation (EC) 178/2002 of the European Parliament and of the Council of 28 January 2002) lays down the general principles and requirements of food law, establishes the European Food Safety Authority, and lays down procedures in matters of food safety. It provides for the general principles of food law that must be followed when measures to ensure food and feed safety are taken. The General

Food Law applies to all stages of the production, processing and distribution of food and also of feed produced for, or fed to, food-producing animals.

In order to achieve the general objective of a high level of protection of human health, the General Food Law lays down that EU food legislation shall be based on the risk analysis process, which consists of the three interconnected components: risk assessment, risk management and risk communication. The risk assessment should be based on the available scientific evidence and undertaken in an independent, objective and transparent manner.

The General Food Law also contains the general requirement that food must not be placed on the market if it is unsafe, i.e. if it is harmful to health and/or unfit for consumption. In determining whether any food is unsafe, account is taken of the normal conditions of use, the information provided to the consumer, the likely immediate or delayed effect on health, the cumulative toxic effects and, where appropriate, the particular health sensitivities of a specific category of consumers.

The basic regulation governing the measures on contaminants in food is the Council Regulation (EEC) 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. This Regulation provides that:

- Food containing a contaminant in an amount that is unacceptable from the public health viewpoint and in particular at a toxicological level shall not be placed on the market.
- Contaminant levels shall be kept as low as can reasonably be achieved following good practices at all stages of production and distribution.
- When necessary for protecting public health, maximum levels shall be established for specific contaminants. These limits may include a reference to the sampling and analysis methods to be used.
- An obligatory consultation of the EFSA Scientific Panel on Contaminants in the Food Chain be made before provisions having an effect on public health are adopted.

Community maximum levels for 3-MCPD have been established by Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Maximum levels for 3-MCPD in hydrolysed vegetable protein and soy sauce were established in 2001 at 0.02 mg/kg. This maximum level is given for the liquid product containing 40% dry matter, corresponding to a maximum level of 0.05 mg/kg in the dry matter. The level needs to be adjusted proportionally to the dry matter content of the products. The maximum levels have applied since April 2002 and are integrated into the Commission Regulation (EC) 1881/2006.

A task within the framework of Scientific Cooperation by Member States (SCOOP) aimed at the collection and collation of data on levels of 3-MCPD in foodstuffs was finalised in 2004 (SCOOP, 2004). It confirmed that the major contributors to human exposure to 3-MCPD are soya sauce and soya sauce products, but that other foods such as bread and noodles also contribute significantly to the exposure because of their high consumption rather than the high levels of 3-MCPD in these foodstuffs. Through the Rapid Alert System for Food and Feed (RASFF) it could be observed that since 1999 there have been regular findings of unacceptable levels of 3-MCPD, mainly in soya sauce but also in hydrolysed vegetable protein, at sometimes very high levels (>100 mg/kg and in some cases even more than 1000 mg/kg).

In the view of Mr. Verstraete, the presence of 3-MCPD esters in food is a topic of potential concern that requires close follow-up and urgent initiatives by the authorities and food business operators on the following issues:

- Availability of a validated method of analysis, including sample preparation, for the analysis of 3-MCPD esters in different foodstuffs to obtain reliable and comparable analytical results.
- Research into the release of 3-MCPD from its esters.
- Investigations into the pathways of 3-MCPD ester formation during refining of vegetable oils and fats, on risk mitigation measures to reduce the formation/presence of 3-MCPD esters in vegetable oils and fats, and on curative measures to remove 3-MCPD esters from vegetable oils and fats.
- Once risk mitigation measures have been identified, ensuring wide application of these measures by the food business operators involved in the production of vegetable oils and fats.

For the EC, the first priorities are to reduce the levels of 3-MCPD esters by risk mitigation measures to be applied by the food business operators, and to consider possible maximum levels of 3-MCPD esters in foods once more information is available on the pathways of formation and on what levels are achievable by applying appropriate risk mitigation measures.

SESSION 2: IDENTIFYING THE GAPS AND UNCERTAINTIES

Molecular mechanism of 3-MCPD ester formation: chlorinating agents and formation of cyclic acyloxonium ions

Prof. Varoujan A.Yaylayan (McGill University, Canada) outlined the molecular mechanism of 3-MCPD ester formation during the refinement of vegetable fat. 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 ($1,1,1\text{-}^{13}C_3$) 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.

Fatty acid esters of 3-MCPD: overview of occurrence in different types of foods

Dr. Rüdiger Weißhaar from the Chemisches und Veterinäruntersuchungsamt (CVUA, Stuttgart, Germany), gave an overview of the occurrence of 3-MCPD esters in different types of food. He mentioned that since 1980 there had been sporadic reports about 3-MCPD esters in hydrochloric-acid-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.

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 are between 0.2 and 6.6 mg/kg in most of the analysed foodstuffs and the levels of bound 3-MCPD are much higher than the levels of free 3-MCPD.

In 2007 and 2008 a survey of 3-MCPD esters in fats and fat-containing products was performed at CVUA Stuttgart (CVUA, 2008). More than 400 samples were analysed in the survey. It was noted that in the method used (transesterification with $NaOCH_3$ and GC-MS-detection after derivatisation with phenylboronic acid in NaCl solution), glycidol (2,3-epoxy-1-propanol) is quantitatively transformed into 3-MCPD. This method therefore detects the sum of 3-MCPD esters and glycidol esters. 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.

Dr. Weißhaar 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

Dr. Weißhaar also mentioned that levels of 0.5–10.5 mg/kg fat (median: 2.3 mg/kg fat) have been found in margarine; <0.1–16.9 mg/kg fat (median: 1.5 mg/kg) in the fillings and toppings of cookies, crackers and bars; 2.3–10.3 mg/kg fat (median: 4.9 mg/kg) in sweet spreads (hazelnut nougat spreads); and 0.5–8.5 mg/kg fat (median: 2.5 mg/kg) in infant formulae (powder).

The highest concentrations of 3-MCPD esters (up to 27 mg/kg) were found in unused frying fat. 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.

Dr. Weißhaar finally mentioned that, 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).

Structural diversity of MCPD esters in food: analytical challenges and preliminary data

The analytical and biological challenges raised by the structural diversity of chloropropanol esters were thoroughly addressed by **Dr. Walburga Seefelder** and **Dr. Benoît Schilter** (Nestlé, Switzerland). It was stressed that the most prevalent isomer among the chloropropanols is 3-MCPD, but that 2-monochloropropane-1,3-diol (2-MCPD) as well as 2,3-dichloropropan-1-ol (2,3-DCP) and 1,3-dichloropropan-2-ol (1,3-DCP) might also occur in food, but at lower concentrations. Additional diversity could result from the number, position and type of fatty acids involved as well as from potential chiral differences.

The structural diversity of chloropropanol esters in food is currently unknown, but is assumed to reflect the known complexity observed for the free chloropropanols. Very little is known about the safety significance of the different chloropropanol esters in food. In experimental test systems, the *(R-)* and *(S-)* isomers of 3-MCPD were found to produce compound-specific effects, and 2-MCPD and 3-MCPD have been shown to exhibit significantly different toxicological profiles in rats. The relevance of these differences for the safety of the esters is not known.

3-MCPD esters are accepted as substrates by lipases and it has been hypothesised that 3-MCPD esters are hydrolysed in the mammalian gut. Hydrolysis of 3-MCPD esters was demonstrated *in vitro* by incubating (*sn*1) monoesters and diesters in an intestinal model containing a large excess of pancreatic lipases. The release of free 3-MCPD from the diester was much slower than from the (*sn*1) monoester. This indicates that dietary 3-MCPD esters would not be absorbed as such and would be an additional source of exposure to free 3-MCPD. However, structural information and digestion data suggest that MCPD esters might behave as triacylglycerols (TAGs) in that gut lipases with higher affinity for positions (*sn*1) and (*sn*3) release 2-monoacylglycerol (*sn*2-MAG) from the dietary TAGs. *sn*2-MAGs are readily taken up by enterocytes, re-esterified by acyltransferases and then incorporated into lipoprotein particles. Assuming a similar metabolism for 3-MCPD esters raises the hypothesis that ingestion of (*sn*1) monoesters would result in release of free 3-MCPD, whereas (*sn2*) monoesters would be absorbed as such.

2-MCPD esters are also expected to be good substrates for lipases. Therefore, the analogy with TAG metabolism suggests that the structure of the esters will determine their metabolic fate and, possibly, toxicity. *In vitro*, 2-MCPD esters were found to be slightly more susceptible than 3-MCPD esters to the action of lipases. Other data suggested a possible significant role of the food matrix on the extent of MCPD ester hydrolysis. MCPD esters in bread were found to be more accessible (83–103% MCPD recovered after 4 hours of incubation) to gut lipases than MCPD esters in pure oils. Furthermore, 2-MCPD esters in pure palm oils (37–68% of bound 2-MCPD recovered after 4 hours of incubation) were slightly more susceptible to the hydrolytic action of lipases than the 3-MCPD esters (25–50% recovery).

Up to recently, research on MCPD esters has mainly focused on 3-MCPD esters. Current analytical methods measure the amounts of 3-MCPD released from the esters after hydrolysis. Such an approach does not differentiate between monoesters and diesters. This could constitute a significant limitation for the safety assessment because the metabolic considerations suggest that monoesters and diesters may exhibit differential sensitivities to gut lipases. A preliminary investigation of fat mixes used in food manufacture demonstrated that only a small proportion (maximum 15%) of the 3-MCPD bound in esters is in fact bound in monoesters.

Dr. Seefelder reported on 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). 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.

The application of a theoretical scenario assuming 100% MCPD ester hydrolysis in the gut would result in exposures to free MCPDs significantly higher than previously estimated. Because of the use of palm oil in infant formulae, the worst-case exposure is known to occur in infants. For 3-MCPD, exposures up to 12 times the TDI established by the JECFA (JECFA, 2002) have been anticipated in high consumers (BfR, 2007). The significance of such an excursion above the TDI during a relatively short period of time is difficult to assess. In its evaluation, JECFA emphasised the role of long-term exposure for 3-MCPD to produce significant toxicity. Therefore, to interpret the significance of excursions above the TDI, it appears to be justifiable to compare the calculated exposures with short-term animal toxicological data.

The most sensitive short-term toxicological endpoint of 3-MCPD is a reversible functional effect on mature spermatozoa causing male infertility, which might not be relevant for male human infants because the prepubertal testis only contains quiescent stem cells and no mature spermatozoa. A MOE greater than 1000 can be calculated from the LOAEL (lowest observed adverse effect level) of 30 mg 3-MCPD/kg body weight per day in a 28-day gavage study in rats and the highest anticipated 3-MCPD exposure.

In a 28-day study in rats, 2-MCPD was found to produce toxic effects qualitatively and quantitatively different from those of 3-MCPD. The main target organs were heart muscle and striated muscle. In this study, the NOAEL was 2 mg/kg body weight per day. A MOE of >1000 can be calculated from the animal NOAEL and the estimated highest exposure (same scenario as used above for 3-MCPD and applying a ratio of 2:1 for 3-MCPD to 2-MCPD).

However, Dr. Schilter concluded that preliminary *in vitro* results suggest that a 100% hydrolysis in the gut is unlikely to occur. Consequently, the MOEs calculated above for free 3-MCPD and 2-MCPD could be considered as worst-case scenarios. By assuming complete hydrolysis for only (*sn*1) monoesters of 3-MCPD and considering that only 15% of total 3-MCPD esters consist of (*sn*1) monoesters, a MOE about sixfold larger than that calculated above would be expected. With such a rate of hydrolysis, most adult consumers would be exposed to 3-MCPD levels within the TDI.

Potential ways of reducing 3-MCPD esters in vegetable oils

Dr. Gerrit van Duijn of The EU Oil and Protein Meal Industry (FEDIOL, Holland) explained that in 2007, the EU used around 23 million tonnes of vegetable oils and fats, of which more than 10 million tonnes was refined for food use.

A contaminants working group of the FEDIOL has launched a research project to investigate the possible ways to mitigate the formation of 3-MCPD esters during the oil refining process. The working group evaluated results of previous industrial trials and concluded that 3-MCPD esters were found in all refined vegetable oils. The lowest levels were observed in refined rapeseed oil (0.3–1.5 mg/kg) and the highest levels in refined palm oil (4.5–13 mg/kg). 3-MCPD esters are mainly formed during deodorisation, although some formation also occurs during bleaching. Acid pre-treatment before bleaching (as applied in physical refining) and a reduction in dosage of bleaching earth resulted in slightly higher 3-MCPD ester levels in the deodorised product. No significant effects were observed by increasing the chloride content of the bleaching earth, the use of active carbon, or the fatty acid level at the start of deodorisation.

The FEDIOL working group also initiated new pilot plant trials on various rapeseed and palm oils. In these studies, two different analytical procedures were applied for the determination of 3-MCPD esters, one using NaCl and the other $(NH_4)_2SO_4$ during the sample preparation. High levels of 3-MCPD esters were seen in the deodorised palm oils, whereas the levels of 3-MCPD in all deodorised rapeseed oil

samples were below the detection limit. When the palm oil results with the two different methods of sample preparation were compared, similar results were seen at 3-MCPD ester levels below 2 mg/kg; however, an increasing difference was distinct with increasing 3-MCPD levels. The sample with the highest level contained 10 mg/kg of 3-MCPD ester when analysed with the NaCl-based method and 4.5 mg/kg when analysed with the $(NH_4)_2SO_4$-based method. This led to a different conclusion concerning the effect of deodorisation temperature on 3-MCPD ester formation: When the NaCl-based method was used, the level more or less doubled when the deodorisation temperature increased from 180°C to 260°C, whereas with the $(NH_4)_2SO_4$-based method, the level remained more or less constant.

In conclusion, Dr. van Duijn stated that the information obtained so far is insufficient to optimise the refining process for 3-MCPD ester mitigation while maintaining the quality and food safety of the refined product. A better understanding of the formation mechanism is required to give direction to further refining trials.

Potential ways of reducing 3-MCPD esters in vegetable oils and data on mitigation

Dr. Bertrand Matthäus (The Federal Research Institute for Nutrition and Food, Münster, Germany) reported on potential ways of reducing levels of 3-MCPD esters in vegetable oils. He explained that it has become clear that deodorisation seems to be the most critical step of the refining process for the formation of 3-MCPD esters.

The main factors affecting the formation of 3-MCPD esters are the presence of chloride ions (from water and other materials used during the refining), glycerol, tri, di- and monoacylglycerides, as well as temperature and time. Increasing amounts of mono- and diacylglycerides in the oil show a linear correlation with the formation of 3-MCPD esters. This supports the assumption that higher amounts of mono- and diacylglycerides are responsible for the higher amounts of 3-MCPD esters found in palm oils and palm oil products. Therefore, the key to solving the 3-MCPD ester problem lies in the identification of the correlation between the formation of 3-MCPD esters, process conditions and the composition of the mixture of components involved.

Dr. Matthäus considered that the main problem is the dilemma between the need for reducing the content of 3-MCPD esters in fats and oils and consumer expectations regarding food quality and safety. There are several target parameters for the minimisation of the 3-MCPD ester content in fats and oils (like reaction partners, temperature and time) but the product quality given by the product properties, sensory quality and stability is also important. 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 potential formation of other process-derived contaminants. As an example of this dilemma, Dr. Matthäus mentioned the effort to reduce free 3-MCPD in pastries by increasing the pH-value of the raw material; this resulted in an increased formation of acrylamide, another undesired contaminant.

In addition to optimisation of the refining process, it should also be possible to remove 3-MCPD esters from the product by absorption of the esters at solid surfaces, by decomposition of the formed esters and by their removal using steam distillation. A further possibility is to offer a reactant for the chloride ion and then remove the formed volatile product during deodorisation. All these possibilities will have to be assessed in the near future.

Dr. Matthäus emphasised that glycidol esters are also formed during the refining process of vegetable oils. It seems that these compounds are precursors on the pathway to 3-MCPD esters. When there is not enough chloride available in the raw oil during the deodorisation step, the reaction ends at the stage of glycidol ester formation. When sodium chloride is added during the chemical analysis, 3-MCPD esters are formed from glycidol esters and are co-analysed together with the initial 3-MCPD esters. Dr. Matthäus explained that glycidol esters might be more easily removed than 3-MCPD esters by acid treatment of the product, resulting in the formation of monoacylglycerides. The technological problem was to bring the acid and the glycidol esters in sufficient contact for the reaction.

Finally, it was mentioned that a research project with the title "Investigations on the formation of 3-monochloropropane-1,2-diol fatty acid esters (3-MCPD-FE) in vegetable oils and development of minimisation strategies" was planned to start in April or May 2009. The project is sponsored by the Research Association of the German Food Industry (FEI, 2009) and the main topic of the project is to investigate the technological possibilities for minimising 3-MCPD esters in fats and oils on the basis of knowledge gained about the technological and mechanistic aspects of 3-MCPD ester formation, the formation mechanism, the possibilities of removing 3-MCPD esters from the product and the development of a new analytical method.

Identification of gaps in knowledge concerning toxicology of 3-MCPD esters

Prof. Gerhard Eisenbrand and **Dr. Michael Habermeyer** (University of Kaiserslautern, Germany) provided an overview on the gaps in knowledge concerning the formation of 3-MCPD esters and on the open questions about their potential toxicological relevance.

3-MCPD mono- and diesters have been reported to be substrates for intestinal lipases, with diesters liberating 3-MCPD at a somewhat slower, yet efficient, rate than (*sn*1) monoesters (Hamlet and Sadd 2009). However, Prof. Eisenbrand pointed out that only a relatively small proportion of esterified 3-MCPD appears to be bound in monoesters. Diesters, accounting for the greatest share of 3-MCPD esters, appear to be hydrolysed preferentially to (*sn*2) monoesters, which are supposed to be incorporated into mixed micelles in the upper intestine and to be absorbed, together with other lipids, through the brush border into enterocytes. After potential re-esterification, the resulting diesters are expected to follow the physiological distribution pathways of lipid turnover, being potentially integrated into the various plasma lipoprotein fractions in charge of lipid transport, distribution, metabolism and storage. However, these presumptions are not yet substantiated by experimental evidence. Therefore, studies to clarify absorption, distribution, metabolism and excretion (ADME) of 3-MCPD esters are essential and should comprise representative (*sn*1) and (*sn*2) monoesters, as well as diesters. The studies might best be carried out using stable or radiolabelled isotopes and should not only address ADME, but also the distribution into plasma lipoprotein fractions as well as the potential impact on the enzymes and receptors involved.

The establishment of the TDI (e.g. JECFA, 2002) of 2 µg 3-MCPD/kg body weight was based on the induction of renal tubular hyperplasia, enhanced relative kidney weights and benign renal tumours in long-term feeding studies in F344 rats of both sexes. Very similar findings have recently been reported in male Sprague-Dawley rats, confirming that 3-MCPD induced renal tubule adenomas or carcinomas and Leydig cell tumours in a two-year feeding study (Cho *et al*. 2008). These new data thus provide support that these effects are not confined to a specific rat strain. In 2007, the JECFA estimated the exposure to free 3-MCPD for the general population to be 0.02–0.7 µg/kg body weight per day and for the high (95th) percentile consumers to be 0.06–2.3 µg/kg body weight per day (JECFA, 2007).

Using ^{36}Cl-labelled 3-MCPD, the major biotransformation route of 3-MCPD was found to follow sequential dehydrogenation and oxidation steps via ß-chlorolactaldehyde to ß-chlorolactate, both specifically affecting renal and reproductive tissues of the male rat. Whether 3-MCPD esters, if absorbed and distributed, follow metabolic pathways similar to 3-MCPD is not clear.

Recently reported findings concerning the presence of glycidol esters in refined palm oil need to be substantiated and the mechanism(s) of their formation elucidated. The toxicological relevance of glycidol esters thus needs to be addressed as well.

Prof. Eisenbrand concluded that well-designed research is needed to clarify whether the esters may just be considered as "prodrugs" for 3-MCPD and/or glycidol or whether they need to be toxicologically evaluated on their own, representing agents with discrete biological effects. Clearly, the database on occurrence of 3-MCPD esters in food and on the monoester/diester ratio needs to be improved and substantiated by research on the mechanisms of 3-MCPD/glycidol ester formation in order to develop effective mitigation measures. Potential further reaction products (e.g. those formed by reversible condensation of chloropropanols to food-borne carbonyls) need to be investigated. ADME studies need to be carried out for both chloropropanol and glycidolesters.

SESSION 3: MCPD ESTERS IN FOOD

The third session of the workshop had three working groups that discussed:

1. Analytical and exposure assessment
2. Technologies and reduction strategies
3. Toxicology and toxicokinetics

Working Group 1: Analytical and exposure assessment

The objective of working group 1 was to discuss analytical and exposure assessment of MCPD esters in food. The working group was given the following list of issues as an inspiration for the discussions:

- State of the art and literature available
- Identification of knowledge gaps
- Exposure assessment and MOEs
- Methods of determination of esters
- Other contributions from industry or organisations
- What are the ratios of mono- to diester in various foods?
- How much (*sn*2) ester is ingested preformed with foods?
- How much MCPD is expected to be liberated from diesters during digestion?
- What are the major pathways of formation?
- What are the relevant precursors?
- In addition to esters, are there other forms of MCPD-adduct formation in foods, such as 1,3 dioxolans (e.g. from food-associated carbonyls)?
- Occurrence and formation of glycidol and glycidol esters; exposure assessment
- Is artefactual formation during work-up and analysis being controlled for and/or excluded?
- Possible way forward

The working group concluded that there is not much available on the state of the art in the published literature on MCPD esters. There is therefore a need to inform the research community regarding the latest developments. This could be achieved by establishing a permanent working group dedicated to MCPD esters in food. A web page could also be useful for posting the latest publications.

In discussing what would be the major ways of formation of MCPD esters, the working group considered that the suggested mechanisms need to be verified in real food systems. In particular, all details are not known in relation to the refining of oil and some participants argued that the deodorisation step is not necessarily the major step of MCPD ester generation.

The methods of analysis of free MCPD and MCPD esters in food should be standardised and agreed upon in order to be able to compare results from different laboratories and to avoid contradictory results. Currently, there are three methods available but further investigation is needed to verify the current methods and compare their performance. Relevant questions that have to be answered are: Do the methods have biases for specific foods? Are they affected by the matrix composition? Are there interferences from the use of sodium chloride in the analytical method? Is spiking necessary?

The working group had the following recommendations:

- Optimise the derivatisation method
- Consider the potential analytical interference of glycidol esters and MCPD esters (in order to prevent artefact formation)
- Confirm the analytical methods based on 3-MCPD release with a method that allows the direct determination of the esters (LC- or GC-based techniques together with the application of isotopically labelled standards)
- 3-MCPD mono- and diesters should be analysed separately
- A method is needed to analyse glycidol esters
- Methods are needed to analyse free 3-MCPD in blood and urine

As this is a global issue, participants from the entire world should be involved, including oil producers. A collaborative study with agreed reference standards was considered to be helpful. As a first step, therefore, synthetic and certified standards should be made available; however, the question was raised as to whom should be responsibility for the certification.

In discussing the relevant precursors for MCPD ester formation, it was agreed that a source of chloride ions is essential. However, the origin of chlorine during the refining stage of oils is not very clear and in order to adequately study potential precursors an agreed method of analysis should be available. It was also mentioned that an unknown "component" in palm oil, such as carotenoids, might catalyse 3-MCPD formation. As regards toasting of food, the formation of MCPD esters might follow similar pathways as in the deodorisation of oils, the need for lipid and chloride being essential.

As to the question of whether, in addition to the esters, there are other forms of MCPD adducts formed in foods (such as 1,3-dioxolanes from food-associated carbonyls), the working group mentioned that reactions with thiol groups and proteins have been reported, but no other adducts confirmed.

In order to gain more knowledge about the formation, occurrence and stability of free glycidol and glycidol esters in food, an agreed-upon method is needed to quantify them. There is certainly a need to perform more studies on these issues, although there are indirect, but as yet unverified, methods under development to determine free and ester forms of glycidol. The fate of glycidol esters after consumption is not known. As the sodium chloride method of analysis of 3-MCPD converts glycidol, if present, into 3-MCPD this "artefact" can be used to measure the glycidol content. Previous studies have used the sodium chloride method for measuring 3-MCPD and this should be considered in evaluating the data generated by this method, keeping in mind that acid treatment can destroy glycidol and eliminate artefact formation.

The working group was not able to estimate how much 3-MCPD would be expected to be liberated from 3-MCPD diesters during digestion, but considered it likely to be less than 100%. It was suggested that animal feeding experiments should be carried out with foods known to contain 3-MCPD esters and then the free 3-MCPD levels measured in different tissues of the animal. It was also suggested that food might not be appropriate in the above experiments and that pure 3-MCPD esters would give more direct answers.

In order to perform better exposure assessments, the content of 3-MCPD esters in fats in different food categories should be measured or estimated from the amount of added refined oils and the results combined with the statistics on consumption of different categories of food in different countries to provide a fast first step for exposure assessment. Consumption patterns for individual consumers might not be necessary. The average consumption figures for fat-containing foods could give a good idea about the exposure to 3-MCPD esters, but not to free 3-MCPD. For free 3-MCPD, the content should be provided by the food industry in order to make an assessment of the exposure, in case it is not

already known. In addition, assumptions should be made on the type of fats being used in the food from different regions and, if the consumption of different types of oils by country is known, this data should also be used.

The working group considered the way forward to be as follows:

- Develop and confirm analytical methods before risk assessment is carried out
- Establish agreed analytical methods on free and 3-MCPD mono- and diesters before moving forward
- Adapt the current analytical methodologies and verify the details
- Obtain detailed knowledge on the mechanism of formation of MCPD esters in refined oils in order to be able to control its formation

Working group 2: Technologies and reduction strategies

The objective of working group 2 was to discuss possible strategies for reducing the presence of 3-MCPD esters in food products. The working group was given the following list of issues as an inspiration for the discussions:

- State of the art and literature available
- Identification of knowledge gaps
- Reduction strategies and their influence on other process-induced undesirable by-products
- Overview of the FEDIOL study
- Mechanisms of formation
- What are the most relevant technological operations leading to chloropropanols, chloropropanol esters, glycidol and glycidol esters?
- Possible way forward

The working group first reviewed all the current projects undertaken in the EU to deal with this newly identified process contaminant:

- The FEDIOL research project is ongoing and focuses on the refining steps and process parameters.
- The German FEI programme, which should start at the beginning of April/May 2009 for a duration of 2 years, will also deal with the refining process. In addition, this project will cover model systems to look at formation pathways, analytical methods to measure esters directly, transfer of the esters to the food products and removal of the esters from the food products.
- In the UK, the FSA will call for tender in March 2009 to work on formation pathways.

The working group then discussed where the focus of future investigations should be

Compounds related to 3-MCPD esters (such as precursors, 2-MCPD esters and others) should be looked at individually rather than as a group because there could be different mitigation strategies for each of them.

Based on the experience with acrylamide, it was highly recommended that appropriate models be used to understand the mechanisms of formation (e.g. use of radiolabelled compounds) and to ensure that research projects are structured in such a way that the potential effects arising from any particular mitigation strategy are brought to light. The use of appropriate risk–benefit models will then help in understanding all the potential effects of these mitigations.

Future research should concentrate on raw materials (e.g. endogenous chloride, carotenoids, water phase of oil fruits, etc.), particularly if the precise identification of their composition might help in understanding their potential impact on formation of the 3-MCPD esters. Particular attention should be given to palm oil.

With regards to the refining process, the presence of chloride in the processing aids (water, steam, bleaching earth, etc.) should be looked at along with the process parameters.

The possibility of reducing the concentration of esters and other compounds after refining (by using scavengers, acid treatment of refined oils, etc.) should also be an area of investigation.

In addition, data should be collected on various food products that are likely to contain 3-MCPD esters (e.g. refined oils and fats, fried foods, baby foods, etc.) to better prioritise the reductions needed, to understand 3-MCPD ester formation in food products due to the production processes and to identify the fate of 3-MCPD esters in end products.

The working group also indicated the need for legislators and authorities to undertake a risk–benefit assessment of the reduction of 3-MCPD esters in oils and fats as well as in food products. Indeed, mitigation strategies leading to the reduction of 3-MCPD esters could lead to an increase of other contaminants and residues (PAH, mycotoxins, pesticides, etc.). The sensory quality of the end product might also be affected, as well as the nutritional health benefits (e.g. the levels of trans fatty acids). It was emphasised that replacing palm oil in baby foods is not quickly and easily done because of the strict EC Regulation on fat composition and the level of palmitic acid required in these products. It was stressed that the development of new oil can take up to 20 years (for a tree oil).

As a conclusion, the working group reviewed the priorities for tackling the 3-MCPD esters issue:

- There is a clear need for analytical methods that are validated separately for each component (3-MCPD esters and others).
- There is a need for an understanding of the formation pathways for 3-MCPD esters in refined oils and fats and during the processing of foods to allow the most appropriate reduction strategies to be implemented.
- Toxicological investigations need to be undertaken in parallel with the above.
- Further investigation should focus on raw materials, particularly palm oil.

Collaboration between different research institutes, industry and authorities is key to resolving the potential public health problem posed by 3-MCPD esters in food.

Working group 3: Toxicology and toxicokinetics

The objective of working group 3 was to discuss possible strategies for improving the safety assessment of 3-MCPD esters in food products. The working group was given the following list of issues as an inspiration for the discussions:

- State of the art and literature available
- Identification of knowledge gaps
- *In vitro* model
- Physiological fat digestion and absorption pathways: To what extent are they utilised by chloropropanols/esters? What is the impact of 3-MCPD esters on the plasma lipoproteins/enzymes involved?
- Metabolism: What is the expected impact on metabolism of lipids and carbohydrates?
- Absorption, distribution, metabolism and excretion of the esters: How much do these depend on structure (monoesters versus diesters, *sn1*- versus *sn2*-substituted) and how do they compare with those of 3-MCPD?
- Toxicity: What is the expected impact on toxicity?
- Possible way forward

The working group considered the structural diversity of MCPD esters in foods to constitute the most significant challenge for the safety evaluation, impacting the hazard identification and characterisation, as well as the exposure assessment. This complexity raised the question of the value of targeting single compounds or a group of compounds as opposed to studying whole foods or ingredients. It was acknowledged that (as illustrated by the example of edible oils) addressing the toxicity of 3-MCPD esters would be highly valuable but probably insufficient to fully document the safety of oils as consumed. Indeed, the production of edible oils is a process involving relatively severe technological conditions such as high temperature, leading to the formation of a number of chemicals, all theoretically of potential concern. Amongst others, the various mono- and diesters of 3-MCPD as well as the 2-MCPD esters (together with their chiral enantiomers) were identified as potentially relevant chemicals for consideration. In addition, evidence suggesting the occurrence of glycidol esters in oils was discussed. Glycidol esters are thought to play a role as intermediates in the formation of MCPD esters. They may potentially release glycidol, a well-characterised animal carcinogen.

The currently available information on the different relevant compounds reviewed during the workshop indicated that toxicological issues were well characterised and documented for the esters of 3-MCPD, allowing the establishment of a set of questions that could be readily addressed through experimental research. This appeared less clear for the other compounds mentioned because analytical data from food materials, toxicological information and information on the technological conditions of formation are all still very limited. Therefore, it was recommended that a first step should be to focus on the toxicity and metabolism of 3-MCPD esters. It was considered important to also study 2-MCPD esters, but as a second step, with an emphasis on developing data on free 2-MCPD prior to any investigation of the corresponding esters. It was thought that additional analytical data are necessary before considering a toxicological program on other compounds such as glycidol esters.

From the thorough discussion of the information provided during the workshop regarding the metabolism and potential toxicity of 3-MCPD esters it was recognised that the toxicological profile of 3-MCPD esters will strongly depend on the rate of lipase-mediated hydrolysis in the gut. Whether 3-MCPD is the ultimate relevant toxicant was considered to be the central question to be addressed.

If only a small proportion of dietary 3-MCPD esters were hydrolysed in the gut, attention would have to focus on the toxicological uncertainties associated with exposure to the esters. However, if efficient digestion of 3-MCPD esters led to significant release of free 3-MCPD in the gut, the extensive toxicological database available for this latter compound would be applicable to assess the significance of the corresponding esters. On the basis of the information presented during the workshop, and considering the knowledge on TAG metabolism and absorption, it was recognised that a 100% hydrolysis of the 3-MCPD esters in the gut is unlikely, especially for the diesters. In addition, it was felt that some of the esters, mainly the (*sn*2) monoesters, would probably be absorbed as such. However, it was also mentioned that lipases and esterases are present in many if not all body compartments and tissues (e.g. blood) and therefore, hydrolysis of 3-MCPD esters would probably happen "somewhere" in the body. The localisation and extent of hydrolysis was thought to determine the actual toxicological significance of the esters. In this context, the following experiments were recommended, in order of priority, for the toxicological characterisation of 3-MCPD esters:

- ***Toxicological study***. Because it would indirectly cover both the metabolism and its toxicological significance, a subacute (28 days) or subchronic (90 days) feeding study in the rat was considered a first priority. The study should be designed for determining the toxicities of pure 3-MCPD diester, (*sn*1) 3-MCPD monoester and (*sn*2) 3-MCPD monoester, in comparison with the effects of free 3-MCPD. Palmitic acid would be the fatty acid of choice since it is most abundant in palm oil. The doses of the esters should be calculated using an equivalent toxic dose of free 3-MCPD as reference. Kinetics parameters, such as levels of 3-MCPD esters in blood (lipoprotein-rich particles) and selected tissues, would provide additional valuable data. This would require the adaptation of available analytical methods to cover these biological matrices.
- ***ADME studies***. To conduct additional experiments addressing absorption, distribution, metabolism and excretion was considered important to support the toxicological data obtained with doses potentially high enough to affect the metabolic capacity of the gut lipases. Such studies may apply pure labelled compounds. Double labelling using both ^{14}C and ^{36}Cl would allow the fate of the Cl to be traced.
- ***Other studies***. The TNO gastrointestinal model (TIM) consists of four serial compartments simulating the stomach, duodenum, jejunum and ileum. It reproduces very closely the dynamic *in vivo* conditions (temperature, movements, pH, enzymes) of the human gastrointestinal tract. Such a model would allow study of the rate of hydrolysis of 3-MCPD esters in the human gut and the impact of gut development (accounting for lower lipase activity in infant gut). The TIM model also appears to be a very good tool for studying the effects of food matrices on the hydrolysis rate of 3-MCPD esters in the gut.

Other toxicological issues were briefly discussed during the workshop. It was considered unnecessary to generate any genotoxicity data on 3-MCPD esters. Furthermore, the toxicological database on free 3-MCPD was considered adequate and does not require any additional studies. Toxicological data from the studies recommended above should be used to decide on the need for additional information on the potential developmental effects of 3-MCPD esters.

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ACRONYMS AND ABBREVIATIONS

ADME	Absorption, distribution, metabolism and excretion
BfR	Bundesinstitut für Risikobewertung (The Federal Institute for Risk Assessment)
CAC	Codex Alimentarius Commission
CONTAM	EFSA Scientific Panel on Contaminants in the Food Chain
CVUA	Chemisches und Veterinäruntersuchungsamt
1,3-DCP	1,3-Dichloropropan-2-ol
2,3-DCP	2,3-Dichloropropan-1-ol
DG SANCO	Directorate General for Health and Consumer Affairs
EC	European Commission
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FEDIOL	The EU Oil and Protein Meal Industry
FEI	Forschungskreis der Ernährungsindustrie (Research Association of the German Food Industry)
FSA	UK Food Standards Agency
IARC	International Agency for Research on Cancer
JECFA	FAO/WHO Expert Group on Food Additives
LOAEL	Lowest observed adverse effect level
MOE	Margin of exposure
2-MPCD	2-Monochloropropane-1,2-diol
3-MPCD	3-Monochloropropane-1,2-diol
PMTDI	Provisional maximal tolerable daily intake
RASFF	Rapid Alert System for Food and Feed
SCF	Scientific Committee on Food
SCOOP	Scientific Cooperation by Member States
TDI	Tolerable Daily Intake

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