MCPD AND GLYCIDYL ESTERS IN FOOD PRODUCTS

SUMMARY REPORT OF A WORKSHOP HELD IN NOVEMBER 2011

Organised by the ILSI Europe Risk Assessment of Chemicals in Food Task Force and Process-related Compounds & Natural Toxins Task Force
About ILSI / ILSI Europe

Founded in 1978, the International Life Sciences Institute (ILSI) is a nonprofit, worldwide foundation that seeks to improve the well-being of the general public through the advancement of science. Its goal is to further the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. ILSI is recognised around the world for the quality of the research it supports, the global conferences and workshops it sponsors, the educational projects it initiates, and the publications it produces. ILSI is affiliated with the World Health Organization (WHO) as a non-governmental organisation and has special consultative status with the Food and Agricultural Organization (FAO) of the United Nations. By bringing together scientists from academia, government, industry, and the public sector, ILSI fosters a balanced approach to solving health and environmental problems of common global concern. Headquartered in Washington, DC, ILSI accomplishes this work through its worldwide network of branches, the ILSI Health and Environmental Sciences Institute (HESI) and its Research Foundation. Branches currently operate within Argentina, Brazil, Europe, India, Japan, Korea, Mexico, North Africa & Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia Region, as well as a Focal Point in China.

ILSI Europe was established in 1986 to identify and evaluate scientific issues related to the above topics through symposia, workshops, expert groups, and resulting publications. The aim is to advance the understanding and resolution of scientific issues in these areas. ILSI Europe is funded primarily by its industry members.

This publication is made possible by support of the ILSI Europe Risk Assessment of Chemicals in Food Task Force and the Process-related Compounds & Natural Toxins Task Force, which are under the umbrella of the Board of Directors of ILSI Europe. ILSI policy mandates that the ILSI and ILSI branch Boards of Directors must be composed of at least 50% public sector scientists; the remaining directors represent ILSI’s member companies. Listed hereunder are the ILSI Europe Board of Directors and the industry members of the ILSI Europe Risk Assessment of Chemicals in Food Task Force and Process-related Compounds & Natural Toxins Task Force.

ILSI Europe Board of Directors

Non-industry members

Prof. A. Boobis, Imperial College London (UK)
Prof. P. Calder, University of Southampton (UK)
Prof. G. Eisenbrand, University of Kaiserslautern (DE)
Prof. C.L. Galli, University of Milan (IT)
Prof. A. Grynberg, INRA – University Paris 13 (FR)
Prof. R. Hurrell, Federal Institute of Technology – ETH (CH)
Prof. G. Rechkemmer, Max Rubner-Institut – Federal Research Institute of Nutrition and Food (DE)
Prof. V. Tutelyan, National Nutrition Institute (RU)
Prof. G. Varela-Moreiras, University San Pablo-CEU of Madrid (ES)

Industry members

Mr. R. Fletcher, Kellogg Europe (IE)
Dr. M. Knowles, The Coca-Cola Company (BE)
Dr. G. Kozianowski, Südzucker/BENEO Group (DE)
Dr. G. Meijer, Unilever (NL)
Dr. M. Michalik, PepsiCo International (PL)
Prof. J. O’Brien, Nestlé (CH)
Prof. C. Shortt, McNeil Nutritional (UK)
Dr. J. Stowell, DuPont Nutrition & Health (UK)
Dr. G. Thompson, Danone (FR)
Dr. P. Weber, DSM (CH)

ILSI Europe Risk Assessment of Chemicals in Food Task Force and Process-related Compounds & Natural Toxins Task Force industry members

Ajinomoto Europe
Barilla G. & R. Fratelli
Bunge Europe
Cargill
DSM
Danone
Kikkoman Foods Europe
Kraft Foods Europe
Luigi Lavazza
Mars
Nestlé
PepsiCo International
Premier Foods
Procter & Gamble
Soremartec Italia – Ferrero Group
MCPD AND GLYCIDYL ESTERS
IN FOOD PRODUCTS

By Colin Crews

SUMMARY REPORT OF A WORKSHOP HELD IN NOVEMBER 2011 IN BRUSSELS
ORGANISED BY THE ILSI EUROPE RISK ASSESSMENT OF CHEMICALS IN FOOD TASK FORCE AND
PROCESS-RELATED COMPOUNDS & NATURAL TOXINS TASK FORCE

MAY 2012
CONTENTS

FOREWORD 4
Session 1 “Introduction and background” 5
Session 2 “Analysis of MCPD- and glycidyl-esters” 7
Session 3 “Mechanisms of formation and mitigation” 10
Session 4 “Working groups” 14
PUBLICATIONS 17
CONCLUSIONS 17
LIST OF PARTICIPANTS 18
ABBREVIATIONS AND GLOSSARY 20
REFERENCES 23
FOREWORD

Following the discovery of fatty acid esters of the carcinogens 3-monochloropropanediol (3-MCPD) and glycidol in refined vegetable oils, the European branch of the International Life Sciences Institute (ILSI) held a workshop on “MCPD and Glycidyl Esters in Food Products” in Brussels, Belgium on 9 and 10 November 2011. Its purpose was to give an update on progress since the previous workshop on “3-MCPD Esters in Food Products” held in 2009. The event was attended by 67 participants from industry, research and government organisations. The meeting was chaired by Alfonso Lampen and co-chaired by Gerhard Eisenbrand.
Session 1 was an introduction to the European branch of the ILSI by Alessandro Chiodini, and to the background and purpose of the current meeting. Gerhard Eisenbrand offered feedback from the previous workshop and reviewed the objectives of the present workshop. Following the discovery and confirmation of relatively large amounts of 3-MCPD esters in foods and food ingredients, the first workshop was organised by ILSI Europe in February 2009 in order to review all the available data relevant for risk assessment (Larsen, 2009). Significant knowledge gaps and research needs were identified. Recommendations on how best to address issues related to 3-MCPD esters and related compounds, i.e., 2-MCPD esters and glycidyl esters, in foods were formulated. That workshop triggered widespread activities on analytical challenges including formation, occurrence, mitigation and toxicology (sub-chronic toxicity and biokinetics of 3-MCPD esters). It was decided to follow these scientific developments through a shared activity between the ILSI Europe Risk Assessment of Chemicals in Food and the Process-Related Compounds and Natural Toxins Task Forces.

Preliminary contacts with stakeholders and investigators indicated that a workshop in 2011 would have been too early to expect new breakthrough information on toxicology to be available. At the same time, significant progress had been made in the development of analytical methods, including the direct analysis of glycidol and its esters in products similar to those containing MCPDs and their esters. An ILSI Europe expert group initiated a critical review of these methods in the context of their performance and applicability to address key issues such as occurrence in food, mechanisms of formation and mitigation. It is now considered that several important analytical challenges encountered earlier can be resolved. In addition, new information on the mechanisms of formation together with evidence of possible application in mitigation approaches was emerging. In this context, it was decided to focus the second workshop mainly on analytical methods and mitigation. As a first action the expert group prepared a draft working manuscript targeting the occurrence and exposure (including an inventory and review of analytical methods), the available analytical tools to study precursors, chemical formation and toxicology, and mitigation approaches. The first draft manuscript was presented during the Workshop.

Objectives of the workshop

The objectives of the 2011 workshop were to review the draft manuscript developed by the ILSI Europe expert group on analytical methods (with a particular emphasis on whether they were valid methods for addressing key issues such as risk assessment and verification of mitigation), to clarify conclusions, identify remaining issues and define further research needs in dedicated working groups, and to communicate the outcome to the relevant stakeholders.

The current situation was that 3-MCPD was known to be genotoxic in several assays in vitro but not genotoxic or mutagenic to rats or mice in vivo; however, it did cause tumours in the kidney, testes and mammary glands. The occurrence of 3-MCPD in processed foods, e.g., cereals, coffee, fish, meat products, dairy products and soy sauce based on acid hydrolysed vegetable protein has been well-documented, and the exposure calculated using food occurrence and consumption data from surveys. From this the Joint Expert Committee on Food Additives (JECFA) was able to set a Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg bodyweight per day (JECFA, 2002).
The presence of fatty acid esters of 3-MCPD in goat’s milk had been reported in 1984 (Cerbulis et al., 1984), but details of its detection in refined vegetable oils sparked the current interest (Zelinková et al., 2006). Initial information was that the esters could be detected in refined vegetable oils and consequently in products containing these oils, and also at lower levels in coffee and in heated cereal foods such as malts.

From a limited database of occurrence, the adult exposure to 3-MCPD, assuming complete cleavage of the esters in the gut, could be estimated as 1–9.8 µg/kg bodyweight 3-MCPD/day, which is between 0.5 and 5 times the PMTDI of 3-MCPD. For infants on a diet of infant formula the estimated exposure was 7.3–25 µg/kg bodyweight 3-MCPD/day which is 3.6–7.7 times the PMTDI (Lampen, 2009).

Contrary to 3-MCPD, no health-based guidance values have been established for glycidol due to the presumed genotoxic mode of action of its carcinogenicity. The release of glycidol by gastrointestinal lipases in a fashion similar to MCPD esters can also be assumed. Occurrence data available to date are insufficient to estimate what degree of exposure (dietary intake) would be needed to provide a dose, estimated from animal studies, as likely to cause cancer.

These findings indicated the need for research on several aspects of MCPD- and glycidyl- esters, such as on mechanisms of formation, development of validated analytical methods (separate for 3-MCPD monoesters, diesters and glycidyl esters) and a comprehensive database on occurrence in food. There was also a need for feeding studies in animals to compare the effects of 3-MCPD diesters and monoesters with free 3-MCPD and to examine the liberation, adsorption, distribution, metabolism and excretion of 3-MCPD derived from ingested esters. Related to this, the stability and hydrolysis of esters under simulated gut conditions required study. Similar research needs existed for glycidyl esters.
SESSION 2
“ANALYSIS OF MCPD- AND GLYCIDYL-ESTERS”

Karel Hrnčířík of Unilever R&D (Vlaardingen, The Netherlands)
“Indirect methods”.

In this session Karel Hrnčířík gave an overview of indirect methods, in which individual esters are converted into single analytes (2-MCPD and 3-MCPD), which are quantified using established methods to give a sum of the “bound forms”. Indirect methods have the advantages that synthesis of a series of standards is unnecessary and the methods have good sensitivity with limits of detection (LOD) of less than 0.1 mg/kg. They are undemanding in terms of equipment and easy to perform.

The basic analytical protocol consists of the addition of an internal standard, transesterification, salting out and extraction, derivatisation and gas chromatography-mass spectrometry (GC-MS). The most common variations are the use of free or bound forms of deuterium-labelled 3-MCPD (3-MCPD-d5 or dipalmitoyl-3-MCPD-d5), transesterification under acidic conditions (sulphuric acid/methanol), rapid alkaline conditions (sodium methoxide/methanol), or slow alkaline conditions. The salting out stage can be non-specific for bound 3-MCPD (including glycidyl esters) using NaCl or can avoid including glycidyl esters by substituting non-chloride salts for NaCl. The derivatising agents used for GC-MS are phenylboronic acid (PBA) or heptafluorobutyrylimidazole (HFBI).

Points of concern regarding indirect methods are that they give just a sum, i.e., the total content of MCPD and glycidyl esters, and that sample preparation involves chemical transformations of the analytes, leading to uncertainty as to the true result.

The quality of results of indirect methods varies, possibly on account of the different performance of individual indirect methods and their modifications, and a lack of understanding of what is being measured. The acid hydrolysis methods are lengthy but are reliable, robust and suitable for routine work. The rapid alkaline methods require care, especially in the timing of the transmethylation step, and are best suited to rapid (semi-quantitative) screening (Kuhlmann, 2011a,b; Hrnčířík et al., 2011; Ermacora and Hrnčířík, 2012). The slow alkaline method avoids complex side reactions encountered in the fast method, and gives simultaneous determination of MCPD esters and glycidyl esters, but it has so far had little evaluation. Proficiency test results have confirmed that a good understanding of the methodology and careful application are required, as non-specific methods had been used previously.

The current status is that enormous progress has been made in analytical methodology, and a better understanding of the chemistry of the methods has been achieved. Nevertheless, too many methods are available, leading to persistent confusion. Harmonisation of current analytical methodology for MCPD and glycidyl esters is highly desirable. The way forward is to review the methodology and identify methods that are “fit for purpose”, to run more comparative studies and to evaluate selected methods by ring-test, prior to validation and adoption as official methods.

Thomas Wenzl of the European Commission Institute for Reference Materials & Measurements (Geel, Belgium) “Direct methods”

Direct methods determine individual species of MCPD and glycidyl esters directly without any chemical transformation. This provides information useful in studying their formation, fate and toxicity, and thus complements the simpler indirect methods.

There is great structural diversity in the case of MCPD esters on account of the numerous fatty acids, the occurrence of mono- and di- esters and the positional isomerism along the 3-carbon backbone, as well as isomerisation of the MCPD moiety. Only a core of six or seven of the most common number of...
fatty acids needs to be considered for analysis; nevertheless, about 100 potential MCPD esters could be derived from even that small number of acids. Positional isomers of MCPD mono- and di- esters cannot be distinguished by LC-MS when they have the same mass. The situation with glycidol is much simpler as it forms only one ester with each fatty acid (Dubois, 2011).

Direct analysis strategies include “Dilute and Shoot”, in which solutions of the oil or lipid extracts from food are injected directly into the LC-MS. Other methods involve prior isolation or clean-up. The “Dilute and Shoot” approach has proved to be quite challenging on account of the poor solubility of the MCPD esters in the LC solvent, rapid deterioration of sensitivity, and inconsistent behaviour of different types of instrument (Collison and Blumhorst, 2011; Haines et al., 2011; Pinkston and Stoffolano, 2011).

The different clean-up strategies used have been based on dual-stage solid-phase extraction (SPE), fractionation by column chromatography on silica SPE, or on gel permeation chromatography (GPC) for glycidyl esters alone. Dual-stage SPE uses a C_8 sorbent and acetonitrile eluent followed by a silica column with dichloromethane, chloroform or hexane/ethyl acetate eluents. It is applicable to glycidyl esters and MCPD monoesters but MCPD diesters co-elute with triacylglycerols (TAG). Reported recoveries have varied from 50% to 110% (Zelinková et al., 2009; Dubois et al., 2011; Granvogl and Schieberle, 2011; MacMahon et al., 2011; Masukawa et al., 2010, 2011; Shiro et al., 2011a,b).

The second method, fractionation by column chromatography, is also applicable to 3-MCPD esters and GC. Large silica gel 60 columns have been used and the analytes eluted with petroleum ether/diethyl ether or with pentane/diethyl ether mixtures. Recoveries are lower than with the dual column SPE, and solvent consumption is very high.

For GPC Bio-Beads® S-X3 have been used, with elution by a mixture of cyclohexane and ethyl acetate. Additional SPE on silica may be required where the monoacylglycerol (MAG) and diacylglycerol (DAG) contents are high. Limits of quantification (LOQ) are below 0.1 mg/kg and recoveries high for most glycidyl esters (Weisshaar and Perz, 2010; Dubois, 2011).

Single stage SPE is applicable to 3-MCPD esters and glycidyl esters. The sample is applied to a silica cartridge in 2-methoxy-2-methylpropane (also known as MTBME)/diethyl ether and eluted with n-hexane/diethyl ether. Recoveries of 3-MCPD esters of 85–111% and of glycidyl esters at 79–114% have been reported (MacMahon et al., 2011).

Extracts for MCPD ester determination can be analysed by GC-MS using non-polar high temperature columns, but less harsh conditions are required for glycidyl esters. LODs are in the order of 0.2 mg/kg for 3-MCPD mono- and di- esters (Zelinková et al., 2009; Weisshaar and Perz, 2010).
fractionated forms that are almost always refined, and they have a wide range of uses. The high content of 3-MCPD esters and glycidyl esters in palm oils is considered to be related to the relatively high DAG content. Exposure from refined oils is rather higher than that from other food sources, but very little information is available concerning formation from processing of foods other than oils.

Determination of 3-MCPD esters has presented challenges that have required various changes to the analytical methods. Unfortunately, indirect methods with acid methanolysis do not always give the same result as alkali methanolysis, and direct methods with and without clean-up also often disagree with each other and with indirect methods. This means that the validity of some older reported data is questionable, and true differences between samples are obscured. Human exposure assessment requires that information on the dietary intake of possibly contaminated food be correlated with the measured contamination levels. The effect of measurement errors and changes in manufacturing processes and consumer behaviour on exposure assessment is therefore very important.

Consumers, regulators and the food industry all need far more information on the contents of 3-MCPD esters and glycidyl esters in foods, their toxicity and fate, the effects of processing and cooking and the potential for mitigation of formation and exposure. On the positive side, we now have good platforms for the exchange of information between industry and researchers, increasing availability of standard compounds and the means to prepare reference materials and organise collaborative trials and ring tests. Correlation between some methods also appears to be improving.

Future requirements are for data on the effects of oil-processing on formation, detailed information on the metabolism of MCPD and glycidyl esters and knowledge of the effects of food-processing on their formation and fate. There is much too little information on the occurrence of 2-MCPD esters and glycidyl esters, and a lack of validated methods for separate mono- and di- esters.

Elisabeth Apel of the Fraunhofer Institute for Toxicology and Experimental Medicine ITEM (Hanover, Germany) “Analytics in biological samples”

There is a need for quantification of 3-MCPD esters in biological samples with high sensitivity to enable biomonitoring. Methods for free 3-MCPD are available, but at the moment tracer experiments with labelled esters of MCPD and glycidol are more reliable.

Investigations on the bioavailability of 3-MCPD fatty acid esters and glycidyl esters have been carried out. A method for the quantification of free 3-MCPD in rat blood, urine and organs was devised based on silica column extraction, derivatisation and GC-MS. The method had high recovery and LODs of 2 ng/ml blood and 10–12 ng/ml urine, and 10–12 ng/g of liver and kidney. 3-MCPD in rat organs and intestine was measured by extraction, ester cleavage with sodium methoxide, derivatisation with phenylboronic acid and GC-MS.

Investigations on the bioavailability of glycidyl fatty acid esters were carried out after oral administration of glycidol and its esters. The conjugated metabolite N-acetyl-S-(2,3-dihydroxypropyl)cysteine was measured in rat urine by extraction, dilution and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) using a hydrophobic interaction liquid chromatography (HILIC) column. The haemoglobin adduct of glycidol, N-(2,3-dihydroxypropyl) valine, was measured in rat globin by a modified Edman procedure, acetylation and gas chromatography-negative chemical ionisation-mass spectrometry (GC-NCI-MS).

Knowledge gaps and research needs were identified as a direct method for quantification of 3-MCPD esters and glycidyl esters in biological samples with high sensitivity, studies of oral administration of radioactive 3-MCPD ester to rats with radio-HPLC of biological samples (e.g., urine) and structural elucidation via MS² and nuclear magnetic resonance (NMR) spectroscopy.
SESSION 3
“MECHANISMS OF FORMATION AND MITIGATION”

Bertrand Matthäus of the Max Rubner-Institute (Detmold, Germany)
“Mitigation: the FEI project”

Minimisation strategies require clarification of the relationship between the formation of 3-MCPD esters and related compounds, process conditions and the composition of the components involved. The Research Association of the German Food Industry (FEI) organised a project under the title “Investigations on the formation of 3-monochloropropane-1,2-diol fatty acid esters (3-MCPD-FE) in vegetable oils and development of strategies on their avoidance”. Its aims were the clarification of the relationship between formation of 3-MCPD esters and related compounds, process conditions and composition of the components involved, and to make recommendations for the definition of processes which would result in the mitigation of 3-MCPD esters and related compounds, without impairing product quality, and in the removal of 3-MCPD esters and related compounds from the refined product.

The mitigation methods highlighted were removal of precursors from the raw material, changing the refining process and removal of the esters from the product. After heating at 240°C for 2 hours, crude palm, corn and coconut oils all produced larger quantities of 3-MCPD esters and related compounds than did olive oil, palm kernel and some other oils. Palm oil from different locations of Malaysia showed remarkable differences in their abilities to form 3-MCPD and glycidyl esters. The reason is not clear yet, but it can be supposed that soil, fertiliser, genotype or harvest technique is responsible for these differences. The project showed that deodorisation is the most important step for the formation of the esters. Deodorisation at high temperatures, especially those higher than 240°C, produced large increases in glycidyl ester formation, while the amounts of 3-MCPD esters formed were relatively independent of the deodorisation temperature. This shows that deodorisation causes more of a glycidyl ester problem than a 3-MCPD ester problem at higher temperatures. Therefore, it is recommended that chemical rather than physical refining be used, so that lower temperatures can be used during deodorisation. The other refining steps reduce the ability of oils to form 3-MCPD and glycidyl esters during deodorisation.

There was some correlation between the formation of 3-MCPD and glycidyl esters and the content of DAG and polar compounds in the oil; washing before deodorisation reduced MCPD ester formation in model studies. It is becoming clear that the potential for the formation of the esters increases significantly when the content of DAG is greater than 4% of the oil. Therefore, it is recommended that refining should start with raw material containing less than 4% DAG. Organic chlorine compounds are probably one important chlorine source for the formation of 3-MCPD esters. Since the DAG content increases with increasing time between ripening and processing, optimising the speed of palm fruit processing is absolutely necessary for the mitigation of the esters in the oil. It is recommended that raw material with a low content of precursors such as DAG and organic or inorganic chlorine compounds be chosen. In a novel approach, the addition of diacetin as a competitor for chlorination was effective as the product was relatively volatile and could be removed. Use of zeolite during deodorisation removed MCPD esters and varying proportions of glycidyl esters; however, quite large proportions of zeolite were required. Two-step deodorisations and short-path distillations also partially reduced MCPD esters and related compounds. Treatment of the refined oil with various adsorbent zeolites or synthetic magnesium silicate was tested in model systems and found to provide further reductions.
Effective mitigation of MCPD and glycidyl esters has not been realised because their exact precursors, chemical pathways for their formation and the source of chlorine responsible have not been identified. It is known that MCPD and glycidyl esters are formed during edible oil refining, with especially high levels observed in palm oil and olein fractions. The critical step is deodorisation, which is performed at 240–270°C by injected stripping steam under vacuum (3–7 mbar).

MCPD diesters are predominantly formed from triacylglycerols (TAG), which represent 88–96% of palm oil. There is a correlation between the DAG content and the formation of 3-MCPD and glycidyl esters in oils. Obviously, chlorine-donor molecules are required and these need to be identified in non-deodorised palm oil. MCPD formation from TAG may be initiated by hydrochloric acid that is itself released by thermal degradation of other chlorine-donor molecules. Formation of MCPD diesters is a regio-selective reaction and 1(3)-MCPD diesters are favoured. MCPD diesters can also be formed from DAG, but in lower quantities with slower kinetics. Glycidyl esters are mainly formed from DAG by elimination of fatty acids, whereas direct formation from TAG is not significant. The reaction proceeds at relatively high temperatures (>230°C), when oxopropyl-esters are also formed (Craft et al., 2011, 2012; Destaillats et al., 2012a,b).

Recommendations were made that oil suppliers produce palm oil and fractions with low levels of MCPD esters and glycidyl esters. At the plantation and mill, palm fruits should be harvested, transported, sterilised and pressed within the shortest acceptable time (< 3 days) when they are at optimum ripeness, and only high quality fruit should be used. At the refinery, segregated crude palm oil based on quality – judged by the free fatty acid (FFA) content – should be used for food applications as it has lower DAG and lower potential for MCPD ester and glycidyl ester formation. Lowering deodorisation temperatures (max 230 °C) can be used to avoid glycidyl ester formation.

A significant feature of the presentation was the report of the discovery of chlorine sources in palm oil. Mass Defect Filtering was applied to LC-MS data to indicate chlorinated compounds in palm oil, of which over 300 were observed (Nagy et al., 2011). One group was identified as having a structure resembling phytosphingosines, which were possible plant metabolites. Inorganic chlorine sources such as salts of iron, magnesium and calcium were also found in palm oil possibly derived from fertilisers. These salts could lead to the biosynthesis of water-soluble organochlorines in the palm fruits, and further transformation to lipid-soluble organochlorines. Reaction of lipid-soluble organochlorines with palm oil TAG could give MCPD esters during deodorisation.

Iekje Berg of Sime Darby Unimills (Rotterdam, The Netherlands)
“The impact of agricultural practices”

Palm oil has a very high yield per hectare compared to other vegetable oil sources providing 38% of world vegetable oil output from 6% of the area. The harvesting, milling and refining processes were described. Special quality palm oil products were available, made from fruits just before full ripeness and processed less than 24 hours after harvesting to minimise MCPD ester formation. The fruit bunches were selected to be fresh, with no loose fruits. The free fatty acid content can be used as an indicator of the quality of the oil and therefore its DAG content. Mild refining of the oil with low contents of fatty acids and DAG gave a red palm product with natural colour (carotenoids) and tocopherols. MCPD esters were reduced from 6.5 mg/kg to 0.2–0.6 mg/kg in the refined olein (Nagy et al., 2011).
Nils Hinrichsen of ADM Research (Hamburg, Germany)

“Mitigation”

The edible oil processing industry is making progress in producing 3-MCPD- and glycidyl ester-reduced products based on blends of palm and other oils. Glycidyl esters have been reduced considerably more (~90%) than MCPD esters (65%).

Alfonso Lampen of the Federal Institute for Risk Assessment – BfR (Berlin, Germany)

“Biokinetics study”

Studies initiated by the German Federal Institute for Risk Assessment (BfR) on the bioavailability and metabolism of 3-MCPD esters in rats were described. Hydrolysis in vitro using Caco-2 cells in the absence of pancreatin showed that MCPD fatty acid esters were hydrolysed (Buhrke et al., 2011). After oral administration of 3-MCPD diesters in rats, free 3-MCPD and 3-MCPD esters were determined in faeces, blood and various tissues and organs. Glycidyl ester metabolism was similarly studied in rats after oral administration of palmitoyl glycidol and the corresponding radiolabelled compound. Adducts and metabolites were determined in blood, urine and various tissues and organs. Rats given single oral doses of 3-MCPD-1,2-dipalmitate in a corn oil gavage at 53.2 mg/kg bodyweight (equivalent to 10 mg 3-MCPD/kg bodyweight) had free 3-MCPD in their blood over a 10 hour period. Preliminary results show that after feeding 3-MCPD ester there was an increase of free 3-MCPD in blood to maximum concentration level within 4 hrs and no free 3-MCPD was detectable after 24 hrs. After feeding free 3-MCPD the maximum concentration was reached after 10 mins. No free 3-MCPD was detectable after 24 hrs.

The peak concentration (Cmax) and the time to reach Cmax (tmax) values were significantly different for 3-MCPD ester and 3-MCPD, whereas the area-under-the-blood-decay-curve (AUC) values in blood were similar. The amount of 3-MCPD absorbed was only slightly lower in the case of the diester compared to the free compound. Therefore, intestinal hydrolysis may be more or less complete but it takes several hours, leading to delayed absorption and lower peak levels in blood.

For glycidol studies small amounts of [2-14C] glycidyl palmitate and glycidyl [9,10-3H] palmitate were added to non-labelled glycidyl palmitate and administered by gavage in corn oil. The labelling showed that 14C (on the glycidol part of the molecule) in erythrocytes and plasma decreased over 24 hours while tritium (fatty acid part) increased. The entire 14C radioactivity was accounted for after 7 days. Determination of the haemoglobin N-terminal bound N-(2,3-dihydroxy-propyl) valine adducts in blood and urine conjugate N-acetyl-S-(2,3-dihydroxy-propyl) cysteine (DHPMA) in rats showed that maximum DHPMA values persisted from 0–8 hrs, followed by a continuous decrease. DHPMA concentrations over the time courses were equal after the administration of either glycidol or glycidyl fatty acid ester. Glycidyl fatty acid esters show a different kinetic behaviour for the glycidol (14C) and the fatty acid (3H) parts of the molecule. Maximum levels of haemoglobin adducts were observed from 24 up to 48 hrs and were also similar for both glycidol and its fatty acid ester. The data support the hypothesis that glycidyl palmitate ester hydrolysis to glycidol and palmitic acid proceeds at a very high rate.

Antonio Mutti of the University of Parma (Parma, Italy)

“Results of the EFSA 90-day toxicity study”

The 90-day study organised by the European Food Safety Authority (EFSA) involved dosing with 3-MCPD ~2–30 mg/kg bodyweight per day) or 3-MCPD dipalmitate (~10–150 mg/kg bodyweight per day). The results showed that urinary excretion of 3-MCPD and 3-MCPD mercapturate can be used to monitor exposure to both 3-MCPD and its dipalmitate, whereas only trace amounts of α-chlorolactic acid were recovered in urine. Urinary excretion rates of both 3-MCPD and 3-MCPD mercapturate were about 30% lower from the esters than after exposure to equimolar doses of 3-MCPD. Female rats were
extremely sensitive to 3-MCPD, having a high mortality rate at the highest doses. Surviving females showed changes in nephrotoxicity biomarkers and mild and dose-related normochromic anaemia was common to all experimental groups. Histopathological examination confirmed that, in male rats, extensive testicular toxicity was related to high doses of both 3-MCPD and 3-MCPD dipalmitate.

Moderate testicular damage was observed with the intermediate dose. Biological effects of dipalmitate were similar to those of free 3-MCPD, but milder, in proportion to the urinary excretion of metabolites. Benchmark doses (BMD10) for severe damage to renal and testicular structures in male rats were respectively 5.6 and 8.4 mg/kg body weight per day for 3-MCPD. The corresponding BMDL10 (a dose corresponding to the lower limit of the 95% confidence interval for 10% tumour incidence) were 2.5 and 6.0 mg/kg bodyweight per day, respectively. The BMD10 and BMDL10 for mortality in female rats were 7.4 and 2.3 mg/kg bodyweight per day, respectively. Different BMDs were obtained for 3-MCPD dipalmitate, depending on the contribution of the 3-MCPD moiety to the molecule and probably a slower excretion rate. In male rats, the BMD10s for severe renal and testicular damage induced by 3 MCPD dipalmitate were 41.1 and 64.4 mg/kg bodyweight per day, respectively (equivalent to 7.7 and 12.2 mg/kg bodyweight per day 3-MCPD). The corresponding BMDL10s were 17.4 and 44.3 mg/kg bodyweight per day (equivalent to 3.3 and 8.4 mg/kg bodyweight per day 3-MCPD), respectively.
SESSION 4
“WORKING GROUPS”

Participants were invited to join one of three Working Groups, which discussed the major topics – Analysis, Exposure and Mitigation Measures in parallel sessions.

Group 1
Group 1 discussed the situation regarding analytical methods. Regarding direct analytical methods the Working Group concluded that esters of the most frequently occurring fatty acids (12:0 – 18:3) were the ones that should be determined in food as a preference. Determination of one ester for every class of contaminant (glycidyl ester, 3-MCPD monoester, 3-MCPD diester) is the minimum required. The standards to be used depend on the fat analysed (e.g., palm kernel and coconut require 12:0). For internal standardisation 13C labelled standards are preferred.

The favoured applications of direct methods are in studies of formation mechanisms. Simpler methods are usually preferred for risk assessment and legislation. Production laboratories need fast results but usually do not have LC-MS. However, direct methods are preferred for glycidyl ester determination. The most suitable methods for the determination of glycidyl esters and MCPD esters in food are LC-MS/MS and LC-TOF-MS, (TOF = time of flight) with the ionisation mode, electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI) depending on the geometry of the ion source; orthogonal sources seem to be more robust.

Current difficulties associated with direct determination are the separation of 2- and 3-MCPD esters, problems of ionisation, differences in instrumentation and the poor availability of 2-MCPD esters. Methods have to be suitable for monitoring of mitigation measures and collection of occurrence data. They therefore have to be fast, reliable and rugged, have good reproducibility and have low LOQ and LOD, especially for infant formulae.

Gaps and additional needs exist in the lack of sufficient number of standards, including isotopically labelled ones, a lack of proficiency tests and of certified reference materials.

The most urgent issues to tackle are 1) the number of method variations – we need only one or two workup procedures and clean-up procedures, 2) the absence of proficiency tests and 3) the problem of separating 2- and 3-MCPD esters.

Regarding indirect analytical methods it was agreed that they should target 3-MCPD esters, 2-MCPD esters and glycidyl esters independently where possible, but that separate detection of mono- and di- esters seemed not to be a priority, according to recent toxicological data. Extraction procedures were needed for different types of food, e.g., infant formulae, spreads and margarine.

Indirect methods need to address the scope of application. Reliable methods are needed for assembling occurrence data. The acid hydrolysis methods are suitable for 2- and 3-MCPD, and the “3 in 1” method provides complete information. The improved DGF-method (from the German Society for Fat Research) gives information quickly. For glycidyl esters direct methods are preferred. Difficulties in the indirect determination are more related to the alkaline hydrolysis methods, where artefact formation is possible and chloride must be avoided, even during fat extraction. Acid hydrolysis is considered more robust. The methods must be optimised for complex matrices.
The performance characteristics (e.g., LOD and LOQ) required for the indirect determination of glycidyl esters and MCPD esters in food should be suitable for the monitoring of mitigation measures and collecting occurrence data.

Knowledge gaps and needs in the supply of chemicals, reference materials and quality tools with respect to indirect methods are that labelled and unlabelled 2-MCPD esters are not yet available at a reasonable price, and that a certified matrix reference material is needed.

The most urgent issues are to identify the method with the best performance, to carry out comparisons of the method and to develop a robust extraction method for complex foods, e.g., infant formula.

**Group 2**

Group 2 discussed exposure. Currently the most complete set of information on occurrence in foods is available for 3-MCPD esters where there are data for several oils (the current “state of the art”). Even in this case more information is needed and more detail is needed on the various fractions of palm oil. There is an inequality of data across food groups with little information on cereals and bread, milk and milk products, frying oils and mixtures, animal fats and oils and composite processed foods. The effects of household and industrial processing are not known, neither is the proportion of food groups across diets that contain the esters.

Very few data are available for 2-MCPD esters. For glycidyl esters the apparent absence in foods other than oils needs confirmation.

The main contributor to human exposure appears to be palm oil in infant formula, but for assessment of overall exposure more data are needed on contributions from other sources. Other sources of importance are likely to be heated cereals, emulsifiers (MAGs, DAGs), milk and milk products. Causal factors, such as the influence of diet on the presence of MCPD esters in human breast milk, need study.

There seems to be no relationship between the occurrence of 3-MCPD esters and glycidyl esters beyond a common occurrence in refined oils. It is important to assess exposure to 3-MCPD esters, 2-MCPD esters and glycidyl esters separately because of their differing toxicology. Limited data suggest that 2-MCPD occurs at lower levels than 3-MCPD in oils but more data are needed, including for foods other than oils. To assess adult exposure, we need data on heat-processed foods and foods containing relevant precursors. For formula–fed infants additional data are not needed beyond monitoring what is in the component oils. Monitoring of the effects of mitigation will be necessary.

More information is required to assess the relative toxicological importance of MCPD mono- and di- esters and the different fatty acid esters; however, toxicological and kinetic studies seem to confirm that the esters act just as an additional source of exposure to MCPD and glycidol. We do not yet have enough data to update exposure estimates for 3-MCPD, and no data at all for 2-MCPD esters or glycidyl esters.

For exposure studies, additional biomarkers are required to supplement the measurement of mercapturic acid, which is non-specific.
**Group 3**

Group 3 discussed mitigation measures. TAG is the major lipid precursor of MCPD, which can be formed at temperatures as low as 150°C. It is unlikely that deodorisation can be effective at this temperature, and so reduction of chlorine sources would seem to be the most effective mitigation measure. DAGs seem to be precursors of glycidyl esters in oils, where a temperature of 220–240°C is needed for formation. MAGs are not present in refined oils, but when used in food emulsifiers can react with chloride to form MCPD esters and can also be converted to glycidyl esters to a small extent. The source for chlorine responsible for 3-MCPD ester formation is likely to be inorganic chlorine, which can be converted to organic forms in the oilseed plant. We do not yet know the status (inorganic or organic) of chloride or its levels in seeds and fruit crops. It could be added in soil fertilisers or via the processing water.

The quality of the raw materials must be defined at the plantation – the free fatty acid content could act as a marker for quality. Even when good quality oils are produced they could be refined further prior to their use in foods. It seems to be more difficult to reduce MCPD esters than glycidyl esters. Refining temperatures must be balanced with the quality of the product. A higher temperature is necessary to remove free fatty acids as well as colours and odours, but taste is affected. Chemical refining can be useful in reducing glycidyl esters – double-deodorisation has a potential to reduce both FFA and glycidyl esters.

Special quality oils can be produced, but currently only on a small scale and at a premium price. They could best be deployed for use in infant formulae. Red palm oil is lower in the contaminants but is not suitable for all food applications. Stability, colour and other potential issues will affect consumer acceptability.

Each step of the refining process can be fine-tuned to reduce these contaminants, but it is unclear if a synergy between multiple changes is achievable. However, potential reductions of 20–30% have been demonstrated. Changes may not be translated to mainstream palm oil production volumes as large economic investments are needed to make a significant impact.

Certain sorbents can be used to reduce glycidyl esters post-refining, but they have a lesser effect on MCPD, and they can affect yields and quality. Prevention earlier on is preferred.

The best ways forward towards mitigation consist of in-depth examination of formation mechanisms based on up-to-date findings. This is likely to be achieved by ensuring the quality of raw materials and control of the early stages of production, such as harvesting and milling. The combination of mitigation methods will require much cooperation from industry and the help of governmental organisations.
The ILSI Expert Group on 3-MCPD Esters in Food Products has prepared a review publication provisionally entitled “Analytical approaches for MCPD esters and glycidyl esters in food and biological samples – a review and future prospects for mitigation”. The document contains detailed evaluations of analytical methods in relation to monitoring, occurrence and toxicology. A review on mitigation measures will be published separately. A draft of the document was provided to all Workshop participants for discussion, and publication is expected mid-2012.

CONCLUSIONS

We now have a better understanding of the chemistry involved in indirect analysis albeit at the cost of having too many methods available, which can cause confusion. Direct methods have also improved along with the availability of reference standards. Analytical methods are still not in full agreement with each other and our goal must be the harmonisation of current analytical methodology for MCPD esters in oils, composite foods and biological tissues. The situation is somewhat better for glycidyl esters where direct methods are less challenging. There has been a gradual increase in the amount of data published on the occurrence of these esters, but caution (because of the uncertain validity of the methods) has limited large-scale surveys.

Toxicological studies have illuminated the fate and activity of both 3-MCPD esters and glycidyl esters and compared their activity with the free compounds. Thus we have an improved knowledge of metabolism and toxicology and more detailed studies of these aspects are sure to follow.

Very promising advances have been made in the mitigation of MCPD ester and glycidyl ester formation in palm oil. Studies of all stages of palm oil production have indicated where contaminant formation can be reduced. A multifaceted approach was considered to be potentially the most beneficial, beginning with a reduction of chloride application in fertiliser, continuing with the use of plant varieties low in MAG and DAG precursors, the selection of young fruit of good quality and technical changes to the refining steps, and ending with the removal of the esters using inorganic adsorbents.

Workshop presentations focused on palm oil, but all of these operations should be applicable to most commercial oils. It was hoped that the various parts of industry could work in concert on the approaches that have the highest impact, helped by trade associations.

The conclusions of the meeting were that great progress has been made towards meeting the requirements outlined in the 2009 Workshop. There has been rapid and intensive improvement of analytical methods and an improved knowledge of metabolism and toxicology. There had been only a gradual increase in the volume of occurrence data published, but this was likely to be due to caution regarding the capability of the current methods. There is now an expectation that we shall eventually see a real decrease in contamination of processed oils by these compounds and a subsequent reduction in exposure.
## LIST OF PARTICIPANTS

<table>
<thead>
<tr>
<th>Ms. Elisabeth Apel</th>
<th>Fraunhofer ITEM</th>
<th>DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Nagendran Bala Sundram</td>
<td>Embassy of Malaysia</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Marie-Héléne Bani-Estivals</td>
<td>Danone Research</td>
<td>FR</td>
</tr>
<tr>
<td>Ms. Claire-Lise Bechert</td>
<td>FEDIOL</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Iekje Berg</td>
<td>Sime Darby Unimills</td>
<td>NL</td>
</tr>
<tr>
<td>Mr. Krish Bhaggan</td>
<td>Loders Croklaan BV</td>
<td>NL</td>
</tr>
<tr>
<td>Dr. Marco Binaglia</td>
<td>EFSA</td>
<td>IT</td>
</tr>
<tr>
<td>Dr. Almut Bitterhof</td>
<td>European Commission</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Gerhard Brankatschk</td>
<td>OVID</td>
<td>DE</td>
</tr>
<tr>
<td>Mr. Paul Brereton</td>
<td>The Food and Environment Research Agency – Fera</td>
<td>UK</td>
</tr>
<tr>
<td>Dr. Falk Brüse</td>
<td>Cargill</td>
<td>NL</td>
</tr>
<tr>
<td>Ms. Ana Burgos</td>
<td>Spanish Food Safety and Nutrition Agency</td>
<td>ES</td>
</tr>
<tr>
<td>Dr. Gong Chen</td>
<td>PepsiCo International</td>
<td>UK</td>
</tr>
<tr>
<td>Dr. Alessandro Chiodini</td>
<td>ILSI Europe</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Mark Collison</td>
<td>AOCS</td>
<td>US</td>
</tr>
<tr>
<td>Dr. Brian Craft</td>
<td>Nestlé</td>
<td>CH</td>
</tr>
<tr>
<td>Dr. Otto Creutzemberg</td>
<td>Fraunhofer ITEM</td>
<td>DE</td>
</tr>
<tr>
<td>Mr. Colin Crews</td>
<td>The Food and Environment Research Agency – Fera</td>
<td>UK</td>
</tr>
<tr>
<td>Dr. Kim Dae-hun</td>
<td>Nongshim, Korea</td>
<td>KR</td>
</tr>
<tr>
<td>Ms. Isabel De Boosere</td>
<td>FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Bruno De Meulenaer</td>
<td>Ghent University</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Richard Depalma</td>
<td>Procter &amp; Gamble</td>
<td>US</td>
</tr>
<tr>
<td>Prof. Gerhard Eisenbrand</td>
<td>University of Kaiserslautern</td>
<td>DE</td>
</tr>
<tr>
<td>Ms. Alessia Ermacora</td>
<td>Unilever</td>
<td>NL</td>
</tr>
<tr>
<td>Mr. Fabian Etienne-Thewissen</td>
<td>Afscia</td>
<td>BE</td>
</tr>
<tr>
<td>Mr. Emanuele Forte</td>
<td>Soremartec Italia s.r.l. – FERRERO group</td>
<td>IT</td>
</tr>
<tr>
<td>Prof. Corrado Galli</td>
<td>University of Milan</td>
<td>IT</td>
</tr>
<tr>
<td>Ms. Jilde Garst</td>
<td>ILSI Europe</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Pierre Gondé</td>
<td>McCain Continental Europe</td>
<td>FR</td>
</tr>
<tr>
<td>Dr. Michael Granvogl</td>
<td>German Research Institute for Food Chemistry (DFA)</td>
<td>DE</td>
</tr>
<tr>
<td>Mr. Helmut Guenther</td>
<td>Kraft Foods</td>
<td>DE</td>
</tr>
<tr>
<td>Prof. Jana Hajslova</td>
<td>Institute of Chemical Technology</td>
<td>CZ</td>
</tr>
<tr>
<td>Mr. Vincent Hanot</td>
<td>Scientific Institute of Public Health</td>
<td>BE</td>
</tr>
<tr>
<td>Dr. Nils Hinrichsen</td>
<td>ADM</td>
<td>NL</td>
</tr>
<tr>
<td>Dr. Karel Hrnčířík</td>
<td>Unilever</td>
<td>NL</td>
</tr>
<tr>
<td>Dr. Jon Johansen</td>
<td>Chiron AS</td>
<td>NO</td>
</tr>
<tr>
<td>Mr. Choi Jong-hun</td>
<td>Nongshim</td>
<td>KO</td>
</tr>
<tr>
<td>Dr. Gyorgy Karlovits</td>
<td>Bunge</td>
<td>NL</td>
</tr>
<tr>
<td>Ms. Annette Klomp</td>
<td>Dutch Product Board Margarine and Oils</td>
<td>NL</td>
</tr>
<tr>
<td>Dr.</td>
<td>Jan Kuhlmann</td>
<td>SGS Germany GmbH</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Prof. Dr.</td>
<td>Dr. Alfonso Lampen</td>
<td>Federal Institute for Risk Assessment</td>
</tr>
<tr>
<td>Dr.</td>
<td>Shaun MacMahon</td>
<td>FDA</td>
</tr>
<tr>
<td>Dr.</td>
<td>Bertrand Matthäus</td>
<td>Max Rubner-Institute</td>
</tr>
<tr>
<td>Prof.</td>
<td>Antonio Mutti</td>
<td>University of Parma</td>
</tr>
<tr>
<td>Dr.</td>
<td>Sarah O’Reilly</td>
<td>Dublin Public Analyst’s Laboratory</td>
</tr>
<tr>
<td>Mr.</td>
<td>Bengt Petersson</td>
<td>Aarhuskarlshamn AB</td>
</tr>
<tr>
<td>Dr.</td>
<td>Frank Pudel</td>
<td>Pilot Pflanzenöltechnologie Magdeburg e.V.</td>
</tr>
<tr>
<td>Dr.</td>
<td>Günther Raffler</td>
<td>Danone</td>
</tr>
<tr>
<td>Dr.</td>
<td>Pratima Rao Jasti</td>
<td>ILSI Europe</td>
</tr>
<tr>
<td>Ms.</td>
<td>Katalina Recseg</td>
<td>Bunge</td>
</tr>
<tr>
<td>Dr.</td>
<td>Cécile Rétho</td>
<td>Laboratoire SCL de Massy</td>
</tr>
<tr>
<td>Dr.</td>
<td>Daniela Schachner</td>
<td>Austrian Agency for Food Safety and Health</td>
</tr>
<tr>
<td>Dr.</td>
<td>Gabriele Scholz</td>
<td>Nestlé</td>
</tr>
<tr>
<td>Dr.</td>
<td>Katrin Schütte</td>
<td>Procter &amp; Gamble</td>
</tr>
<tr>
<td>Mr.</td>
<td>Ryo Shimojo</td>
<td>Kikkoman Foods Europe</td>
</tr>
<tr>
<td>Ms.</td>
<td>Hartati Simka</td>
<td>Cargill BV</td>
</tr>
<tr>
<td>Dr.</td>
<td>Radim Stepan</td>
<td>Czech Agriculture and Food Inspection Authority (CAFIA)</td>
</tr>
<tr>
<td>Ms.</td>
<td>Jacqueline van der Wielen</td>
<td>Mars</td>
</tr>
<tr>
<td>Dr.</td>
<td>Rachel Ward</td>
<td>PepsiCo International</td>
</tr>
<tr>
<td>Dr.</td>
<td>Rüdiger Weisshaar</td>
<td>Chemisches und Veterinäruntersuchungsamt</td>
</tr>
<tr>
<td>Mr.</td>
<td>Thomas Wenzl</td>
<td>European Commission</td>
</tr>
<tr>
<td>Dr.</td>
<td>Lonneke Wilms</td>
<td>DSM</td>
</tr>
<tr>
<td>Prof.</td>
<td>Varoujan Yaylayan</td>
<td>McGill University</td>
</tr>
</tbody>
</table>
ABBREVIATIONS AND GLOSSARY

\(^{14}\text{C}\) carbon isotope 14. A radioactive isotope used to label compounds for detection of their metabolites by tracing the radioactivity.

\([2-^{14}\text{C}]\) glycidyl palmitate. The palmitic acid ester of glycidol, in which the 2-position carbon of the glycidol moiety is in the radioactive \(^{14}\text{C}\) form.

2-MCPD. 2-Monochloropropanediol.

3-MCPD. 3-Monochloropropanediol.

3-MCPD diesters. Esters of 3-monochloropropanediol with fatty acids in both the 1- and 2-positions.

3-MCPD esters. Mono- and di- esters of 3-monochloropropanediol with fatty acids.

3-MCPD mercapturate. A metabolite of 3-MCPD formed by binding to the amino acid cysteine.

3-MCPD monoesters. Esters of 3-monochloropropanediol with a single fatty acid in the 1- or 2-position.

3-MCPD-1,2-dipalmitate. The diester of 3-MCPD with palmitic acid in both the 1- and 2-positions.

AUC. Area-under-the-blood-decay-curve. A measure of the quantity of a compound in blood obtained by plotting its presence over time.

BMD10. A benchmark dose, a dose associated with a 10% response adjusted for background.

BMDL10. The lower bound of a 95th confidence interval on a benchmark dose corresponding to a 10% tumour incidence.

Caco-2 cells. A line of human epithelial colorectal adenocarcinoma cells cultured in vitro and used in toxicology.

Chemical and physical refining. Processes used to remove undesirable compounds such as free fatty acids from edible vegetable oils.

Cmax. The maximum concentration of a compound in a medium.

DAG. Diacylglycerol, an ester of glycerol with a fatty acid in each of the 1- and 2- or the 1- and 3-positions.

Deodorisation. A late stage of chemical and physical refining, in which oil is treated with steam under vacuum and high temperature.

Deuterium-labelled 3-MCPD (3-MCPD-d5). 3-MCPD in which the hydrogen atoms attached directly to the carbon chain have been replaced by deuterium to allow detection by mass spectrometry.

DGF-methods. Analytical methods for total 3-MCPD esters and total glycidyl esters proposed by the Deutsche Gesellschaft für Fettwissenschaft (German Society for Fat Research).

DHPMA. A urine conjugate of 3-MCPD, N-acetyl-S-(2,3-dihydroxy-propyl)cysteine.

Dilute and Shoot. A technique of LC-MS in which samples are diluted and injected with minimal clean-up.

Direct methods. Analytical methods for MCPD esters and glycidyl esters in which the individual esters are measured.

EFSA. The European Food Safety Authority.
FEI project. A German Food Industry project aimed at clarification of the relationship between 3-MCPD ester formation and process conditions.

FFA. Free fatty acids, non-esterified fatty acids in edible oils, indicators of quality.

GC. Gas chromatography, a means of separating volatile compounds.

GC-MS. GC with mass spectrometry detection. A widely used technique applied to measurement of free 3-MCPD.

GC-NCI-MS. A form of GC-MS aimed at detecting certain classes of compounds, particularly chlorinated ones.

Glycidyl [9,10-3H] palmitate. The palmitic acid ester of glycidol in which two hydrogen atoms, in the 9- and 10-positions, of palmitic acid have been replaced by radioactive tritium to allow detection of the metabolites of the palmitic acid part of the ester.

Glycidyl esters. Esters of glycidol with fatty acids.

GPC. Gel permeation chromatography. A means of separating compounds on the basis of their molecular size applied to the isolation of glycidyl esters from oil samples.

HFBI. Heptafluorobutyrylimidazole, a reagent used to make MCPD volatile for GC-MS analysis.

HILIC. Hydrophilic Interaction Liquid Chromatography, a form of HPLC in which the stationary phase is more polar than the mobile phase.

HPLC. High Performance Liquid Chromatography. A means of separating non-volatile compounds prior to mass spectrometry, in which combination the term LC-MS is used.

In vitro. Experiments modelling metabolism carried out using artificial systems simulating bodily functions.

In vivo. Studies of metabolism carried out using living tissues.

Ionisation mode (ESI or APCI). Means of producing ions in LC-MS.

JECFA. The Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives.

LC-MS. High Performance Liquid Chromatography-Mass Spectrometry.

LC-MS/MS. LC-MS in which an ion separated in a mass spectrometer is fragmented to produce a more specific signal, lowering the background signal and increasing sensitivity.

LC-TOF-MS. LC-Time of Flight-mass spectrometry, which uses an electric field to accelerate ions and measures the time they take to reach the detector. Particles with the same charge have the same kinetic energy so their velocity and the time they take to reach the detector will differ with their mass.

LOD. Limit of detection, the smallest quantity of a compound that can be distinguished from a background signal.

LOQ. Limit of quantification, the smallest quantity of a compound that can be measured repeatedly with confidence.

MAG. Monoacylglycerol, an ester of glycerol with a single fatty acid in the 1- or 2-position.

Mass Defect Filtering. A treatment of LC-MS data in which certain structural features can be obtained from overlapping information.

MCPD. Frequently used to mean 3-MCPD, used here to include 2-MCPD.
MS	extsuperscript{n}. A form of LC-MS/MS in which a number (n) of sequential fragmentations of an ion is induced.

MTBME. Methyl tertiary-butyl methyl ether (2-methoxy-2-methylpropane), a solvent.

NMR. Nuclear Magnetic Resonance, an instrumental means of structural elucidation of chemicals.

Olein. A liquid fraction of palm oil obtained by removal of a solid fraction by crystallisation.

PBA. Phenylboronic acid, a reagent used to make MCPD volatile for GC-MS analysis.

PMTDI. Provisional Maximum Tolerable Daily Intake, a recommended maximum tolerable level of a toxic compound.

Radio-HPLC. HPLC in which the induced radioactivity of compounds is used to monitor their metabolism.

SPE. Solid Phase Extraction, a form of chromatographic clean up of an extract prior to final analysis.

TAG. Triacylglycerol, an ester of glycerol with three fatty acids.

Tmax. The time taken for a compound to reach a maximum concentration (Cmax).

Transesterification. The breaking of the ester bonds of fatty acid esters in which the esterified alcohol is replaced, usually with methanol.

Tritium (\textsuperscript{3}H). A radioactive form of hydrogen incorporated into molecules and used to measure their metabolites through detection of radiation.

Zeolite. A mineral earth used in filtration and cleaning of edible oils.
REFERENCES


Kuhlmann, J. (2011a). Indirect determination of bound glycidol and MCPD in refined oils; Presentation at the 102nd AOCS Annual Meeting & Expo; Cincinnati, Ohio-USA, May 1-4 2011.


Other ILSI Europe Publications

Concise Monographs

• Alcohol – Health Issues Related to Alcohol Consumption
• A Simple Guide to Understanding and Applying the Hazard Analysis Critical Control Point Concept
• Calcium in Nutrition
• Carbohydrates: Nutritional and Health Aspects
• Caries Preventive Strategies
• Concepts of Functional Foods
• Dietary Fibre
• Food Allergy
• Food Biotechnology – An Introduction
• Food, Glycaemic Response and Health
• Functional Foods – From Science to Health and Claims
• Genetic Modification Technology and Food – Consumer Health and Safety
• Healthy Lifestyles – Nutrition and Physical Activity
• Healthy Lifestyles – Diet, Physical Activity and Health
• Microwave Ovens
• Nutrition and Genetics – Mapping Individual Health
• Nutrition and Immunity in Man
• Nutritional and Health Aspects of Sugars – Evaluation of New Findings
• Nutritional Epidemiology, Possibilities and Limitations
• Oral and Dental Health – Prevention of Dental Caries, Erosion, Gingivitis and Periodontitis
• Oxidants, Antioxidants, and Disease Prevention
• Principles of Risk Assessment of Food and Drinking Water Related to Human Health
• The Acceptable Daily Intake – A Tool for Ensuring Food Safety
• Threshold of Toxicological Concern (TTC)
• Type 2 Diabetes – Prevention and Management

Reports

• Addition of Nutrients to Food: Nutritional and Safety Considerations
• An Evaluation of the Budget Method for Screening Food Additive Intake
• Animal-Borne Viruses of Relevance to the Food Industry
• Antioxidants: Scientific Basis, Regulatory Aspects and Industry Perspectives
• Applicability of the ADI to Infants and Children
• Application of the Margin of Exposure Approach to Compounds in Food which are both Genotoxic and Carcinogenic
• Approach to the Control of Enterohaemorrhagic Escherichia coli (EHEC)
• Assessing and Controlling Industrial Impacts on the Aquatic Environment with Reference to Food processing
• Assessing Health Risks from Environmental Exposure to Chemicals: The Example of Drinking Water
• Beyond PASSCLAIM – Guidance to Substantiate Health Claims on Foods
• Campylobacters as Zoonotic Pathogens: A Food Production Perspective
• Considering Water Quality for Use in the Food Industry
• Consumer Understanding of Health Claims
• Detection Methods for Novel Foods Derived from Genetically Modified Organisms
• Emerging Technologies for Efficacy Demonstration
• Evaluation of Agronomic Practices for Mitigation of Natural Toxins
• Evaluation of the Risks Posed in Europe by Unintended Mixing of Food Crops and Food Crops Developed for Non-Food Uses
• Exposure from Food Contact Materials
• Foodborne Protozoan Parasites
• Foodborne Viruses: An Emerging Problem
• Food Consumption and Packaging Usage Factors
• Food Safety Management Tools
• Food Safety Objectives – Role in Microbiological Food Safety Management
• Frontiers in Food Allergen Risk Assessment
• Functional Foods in Europe – International Developments in Science and Health Claims
• Functional Foods – Scientific and Global Perspectives
• Guidance for the Safety Assessment of Botanicals and Botanical Preparations for Use in Food and Food Supplements
• Impact of Microbial Distributions on Food Safety
• Markers of Oxidative Damage and Antioxidant Protection: Current status and relevance to disease
• 3-MCPD Esters in Food Products
• Method Development in Relation to Regulatory Requirements for the Detection of GMOs in the Food Chain
• Micronutrient Landscape of Europe: Comparison of Intakes and Methodologies with Particular Regard to Higher Consumption
• Mycobacterium avium subsp. paratuberculosis (MAP) and the Food Chain
• Nutrition in Children and Adolescents in Europe: What is the Scientific Basis?
• Overview of the Health Issues Related to Alcohol Consumption
• Overweight and Obesity in European Children and Adolescents: Causes and consequences – prevention and treatment
• Packaging Materials: 1. Polyethylene Terephthalate (PET) for Food Packaging Applications
• Packaging Materials: 2. Polystyrene for Food Packaging Applications
• Packaging Materials: 3. Polypropylene as a Packaging Material for Foods and Beverages
• Packaging Materials: 4. Polyethylene for Food Packaging Applications
• Packaging Materials: 5. Polyvinyl Chloride (PVC) for Food Packaging Applications
• Packaging Materials: 6. Paper and Board for Food Packaging Applications
• Packaging Materials: 7. Metal Packaging for Foodstuffs
• Packaging Materials: 9. Multilayer Packaging for Food and Beverages
• Persistence and Survival of Pathogens in Dry Foods and Dry Food Processing Environments
• Recontamination as a Source of Pathogens in Processed Foods – A Literature Review
• Recycling of Plastics for Food Contact Use
• Safety Assessment of Viable Genetically Modified Microorganisms Used in Food
• Safety Considerations of DNA in Foods
• Salmonella Typhimurium definitive type (DT) 104: A multi-resistant Salmonella
• Significance of Excursions of Intake above the Acceptable Daily Intake (ADI)
• The Enterobacteriaceae and their Significance to the Food Industry
• The Safety Assessment of Novel Foods
• The Safety Assessment of Novel Foods and Concepts to Determine their Safety in use
• Threshold of Toxicological Concern for Chemical Substances Present in the Diet
• Tools for Microbiological Risk Assessment
• Transmissible Spongiform Encephalopathy as a Zoonotic Disease
• Trichotheccenes with a Special Focus on DON
• Using Microbiological Risk Assessment (MRA) in Food Safety Management
• Validation and Verification of HACCP
• Water Use of Oil Crops: Current Water Use and Future Outlooks

To order
ILSI Europe a.i.s.b.l.
Avenue E. Mounier, 83, Box 6
B-1200 Brussels, Belgium
Phone: (+32) 2 771 00 14 • Fax: (+32) 2 762 00 44
E-mail: publications@ilsi-europe.be
ILSI Europe’s Concise Monographs and Report Series can be downloaded from: www.ilsi.eu