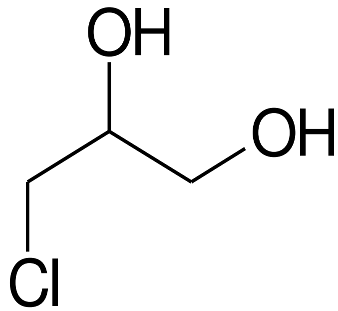
# Fatty acid esters of chloropropanols and glycidol in foods – analysis and exposure

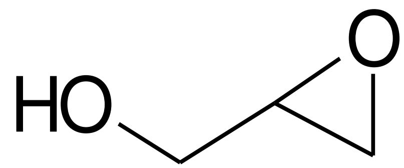
International Association for Food Protection European Symposium on Food Safety

Warsaw 21-23 May 2012

**Colin Crews**

# MCPD and Glycidol




- MCPD discovered in acid-hydrolysed vegetable protein (acid-HVP) by Jan Velisek 1979
- Formed from action of HCl on residual glycerides
- Soon after found in foods not treated with HCl :– heated cereals, salami and others

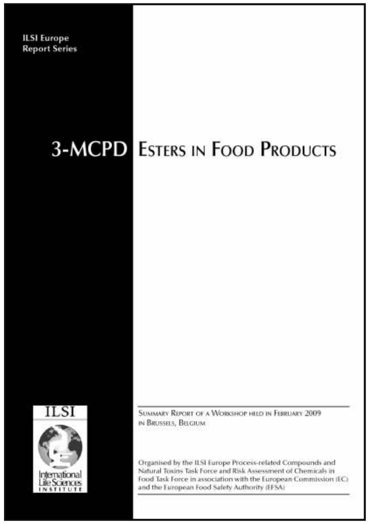
**Forms: dichloropropanols, chloropropanediols, chloro-propanediol mono- and di- fatty acid esters, and glycidyl fatty acid esters**

# ILSI Europe activity

- MCPD fatty acid esters reported at high levels in refined oils (Zelinkovä *et al.* 2006)

- ILSI Europe Risk Assessment of Chemicals in Food Task Force Workshop held in February 2009

- Joint ILSI Europe Process-related and Natural Toxins and the Risk Assessment of Chemicals in Food Task Forces shared a Second Workshop held Brussels November 2011



ILSI Europe
Report Series

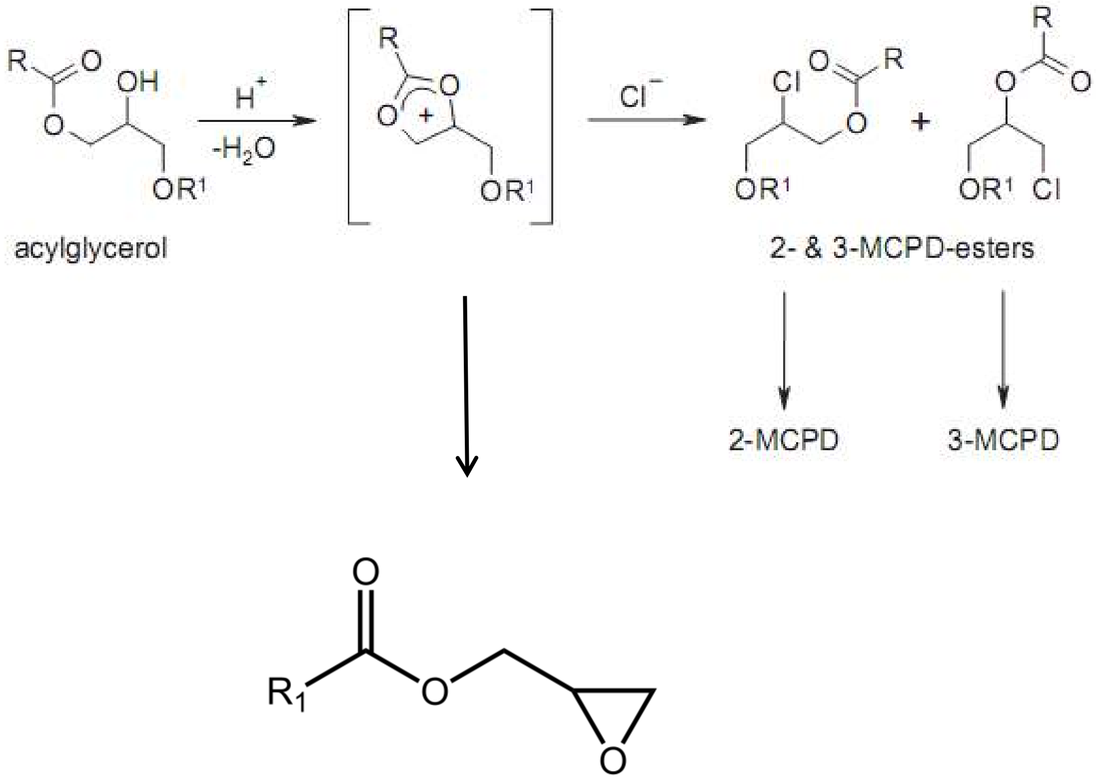
3-MCPD Esters in Food Products

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)

ILSI
International Life Sciences Institute

# 3-MCPD formation

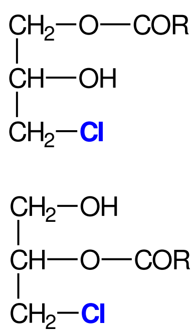


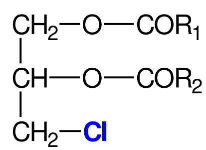

glycidyl ester

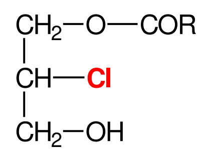
*[Hamlet & Sadd 2009]*

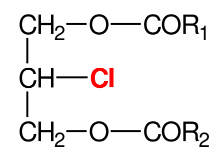
# MCPD and glycidol esters

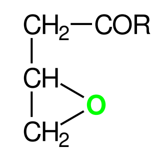
| 3-MCPD | | 2-MCPD | | Glycidyl esters |
|---|---|---|---|---|
| monoesters | diesters | monoesters | diesters | |




**For 7 fatty acids (C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3)**

**- number of individual esters is:-**

| | | | | |
|---|---|---|---|---|
| **14** | **49** | **7** | **28** | **7** |

$\underbrace{\qquad 14 \qquad 49 \qquad}_{\sum 63}$ $\underbrace{\qquad 7 \qquad 28 \qquad}_{\sum 35}$

*Karel Hrncirik ILSI Workshop 2011*

# Formation in oils

- Crude oil
- ↓
- degumming
- ↓
- bleaching
- ↓
- deodorizing

**Formed during refining processes (deodorisation) at 240-270°C**

**MCPD di-esters are formed mainly from triacylglycerols (TAG), less from DAG, and need a chlorine source**

**Glycidyl esters are formed mainly from DAG not TAG, no chlorine needed**

**MCPD esters and glycidyl esters typically formed at up to ~ 20 mg/kg**

# Occurrence - MCPD esters in oils

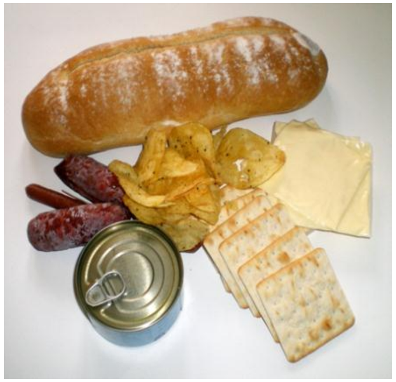
**2- MCPD and 3-MCPD linked in esters mg/kg**

| | 3-MCPD | 2-MCPD |
|---|---|---|
| Unrefined vegetable oils | < 0.1 - < 0.3 | < 0.1 |
| Refined vegetable oils | < 0.2 - 18.8 | < 0.1 – 2.4 |
| Frying oils (fresh/used) | < 0.2 - 16.2 | |
| Refined palm oil | 1.1 - 10.0 | 0.2 - 5.9 |
| Refined coconut oil | 1.4 - 1.7 | |
| Refined hazelnut/walnut oil | 1.2 - 19.0 | 0.5 - 11 |
| Refined olive oil | < 0.3 - 2.5 | |

# Occurrence- 3-MCPD esters in foods

**3-MCPD linked in esters**

| Class | Food | Range (mg/kg) |
|---|---|---|
| Cereals | Biscuits | 0.25 - 0.70 |
| | Bread | < 0.01 - 0.04 |
| | Bread toasted | 0.06 - 0.16 |
| | Breakfast cereals | 0.04 - 0.89 |
| | Crackers | 0.10 - 1.14 |
| | Crispbread | 0.42 - 0.58 |
| | Doughnuts | 0.42 - 1.21 |
| | Cereals | < 0.005 - 1.02 |
| | Malt and beer | 0.004 - 0.65 |
| Infant/baby | Cereal | < 0.01 - 0.23 |
| | Infant biscuits | 0.11 - 0.31 |
| | Jarred food | < 0.011 |
| | Human breast milk | < 0.01 - 0.08 |
| | Infant formula | < 0.08 - 0.59 |
| Meat | Chicken grilled | 0.26 - 0.74 |
| | Ham | n.d. - 2.64 |
| | Salami | 0.88 - 6.40 |
| | Fish incl. Smoked | 0.28 - 1.08 |
| Potato | Potato crisps | 0.05 - 1.19 |
| | French fries | 0.04 - 0.40 |
| Coffee | Coffee | < 0.10 - 0.39 |
| Dairy | Cheese | n.d. - 1.28 |



# Occurrence- glycidyl esters in oils

**Glycidol linked in esters**

| Food | Range (mg/kg) |
|---|---|
| Unrefined vegetable oils | < 0.10 |
| Refined vegetable oils | < 0.1 - 4.1 |
| Cooking oils | 3 - 28 |
| Refined palm oil | 0.30 - 10 |
| Refined hazelnut/walnut oil | 0.5 - 1.4 |
| | |
| Margarine (fat portion | < 0.15 - 5.0 |
| Infant formula (fat portion) | < 0.15 - 3.0 |

**There is no relationship between the occurrence of 3-MCPD esters and glycidyl esters beyond a common occurrence in refined oils**

# Toxicity

3-MCPD causes infertility in rats and suppression of the immune function

Genotoxic in several *in-vitro* assays, not genotoxic/mutagenic *in-vivo* (mice & rats), induced tumours in kidney, testes, mammary glands (rats)

Provisional maximum tolerable daily intake PMTDI: 2 µg/kg bw/d

Data for 2-MCPD lacking

Glycidol - IARC probably carcinogenic to humans

EC Scientific Committee on Food established a Tolerable Daily Intake for 3-MCPD of 2 µg/kg body weight – but:

It is important to assess exposure to 3-MCPD esters,2-MCPD esters and glycidyl esters separately because of their differing toxicology

# Exposure

**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.**

# Exposure

**Risk assessment : 3-MCPD from esters - BfR 2007 and 2009**

| | | | **TDI: 2 µg/kg bw/d exceeded by factor** |
|---|---|---|---|
| **Formula-fed Infants** | Maximum | 25.0 µg/kg bw/d | **12.5** |
| | Median | 15.4 µg/kg bw/d | **7.7** |
| **Adult men** | Maximum | 9.8 µg/kg bw/d | **4.9** |
| | Median | 1.0 µg/kg bw/d | 0.5 |

*Prof. Alfonso Lampen (BfR) - ILSI Europe Workshop, 9-10 November 2011*

# Bioavailability

**Feeding rats with free MCPD:**
Maximum 3-MCPD concentration reached after 0.17 hr; none detectable at 24 hr

**Feeding rats with esterified MCPD:**
Maximum 3-MCPD concentration reached after within 4 hrs; none detectable at 24 hrs

The amount of 3-MCPD absorbed is only slightly lower in case of the diester

Therefore, intestinal hydrolysis is almost complete, but it needs more time

**Feeding rats with free glycidyl palmitate, analysis of blood and urinary biomarkers:**
Haemoglobin adduct formation for the ester is comparable to that of glycidol

This supports the hypothesis that glycidyl palmitate ester is quickly hydrolysed to glycidol and palmitic acid

Toxicological and kinetic studies confirm that the esters act just as an additional source of exposure to MCPD and glycidol

# Bioavailability

## EFSA 90-DAY TOXICOLOGICAL STUDY

Repeated Dose 90-Day Oral Toxicity Study in Rodents:

Exposure to 3-MCPD dipalmitate compared to equimolar doses of 3-MCPD

Female rats were very sensitive to 3-MCPD, high mortality at the highest doses, no benchmark dose could be calculated due to major effects at the lowest dose

Unexplained sensitivity of female rats to acute kidney injury

At low (9.8 mg/kg/d) and intermediate (39 mg/kg/d) doses all dipalmitate was converted to free 3-MCPD

At high doses urinary excretion of 3-MCPD was lower by one third compared to equimolar doses of 3-MCPD

The BMD for 3-MCPD was 40 x background or 2x the lowest dose (1.84 mg/kg/d)

In male rats the NOAEL was about 20 times the background

# Analysis

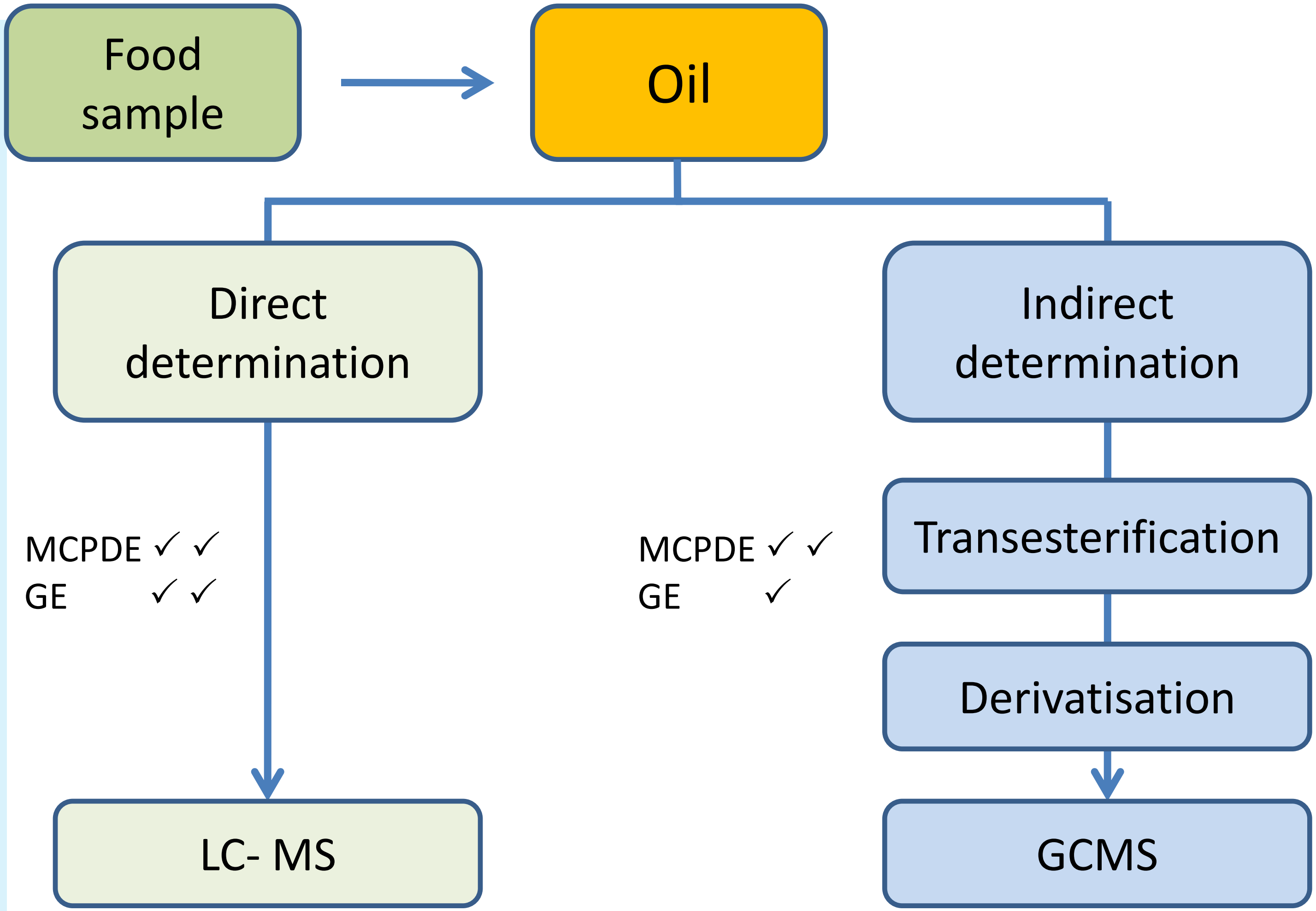
**To provide estimates of consumer exposure analytical methods are required that give a true level of the potential (free + esterified) quantity of 2-MCPD, 3-MCPD and glycidol in food**

**To provide information on metabolism, bioavailability and toxicology analytical methods are required that can separately quantify indidiual esters of 2-MCPD, 3-MCPD and glycidol, and their isomers in food and body tissues.**

**Current methods for body tissues need to include better markers***

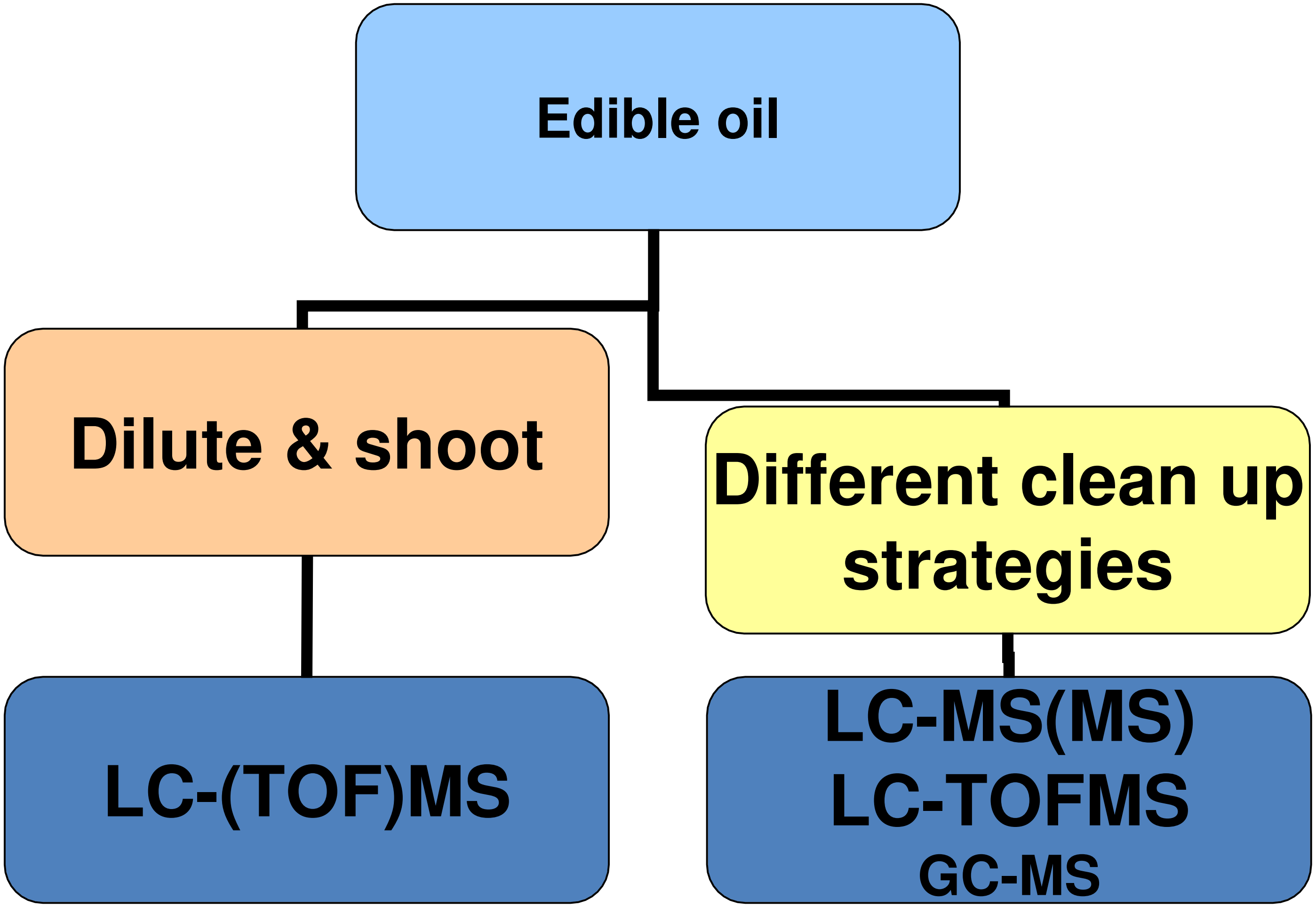
* *Ms. Elisabeth Apel (Fraunhofer ITEM) - ILSI Workshop 9-10 November 2011*

# Analysis



Food sample
Oil
Direct determination
Indirect determination
MCPDE ✓ ✓
GE ✓ ✓
MCPDE ✓ ✓
GE ✓
Transesterification
Derivatisation
LC- MS
GCMS

# Direct analytical methods 1




*Thomas Wenzl ILSI Workshop 2011*

# Direct analytical methods 2

**Dilution Solvents**

- Acetone or mixtures - Dichloromethane/Methanol/Acetonitrile

**MS systems**

- RP column, LC-MS, LC-MS/MS, LC-TOF MS
- APCI+, ESI+ generally of sodium adducts (MCPD esters)

**Analytes**

- MCPD mono and diesters, glycidyl esters, mixtures

**Clean-up strategies**

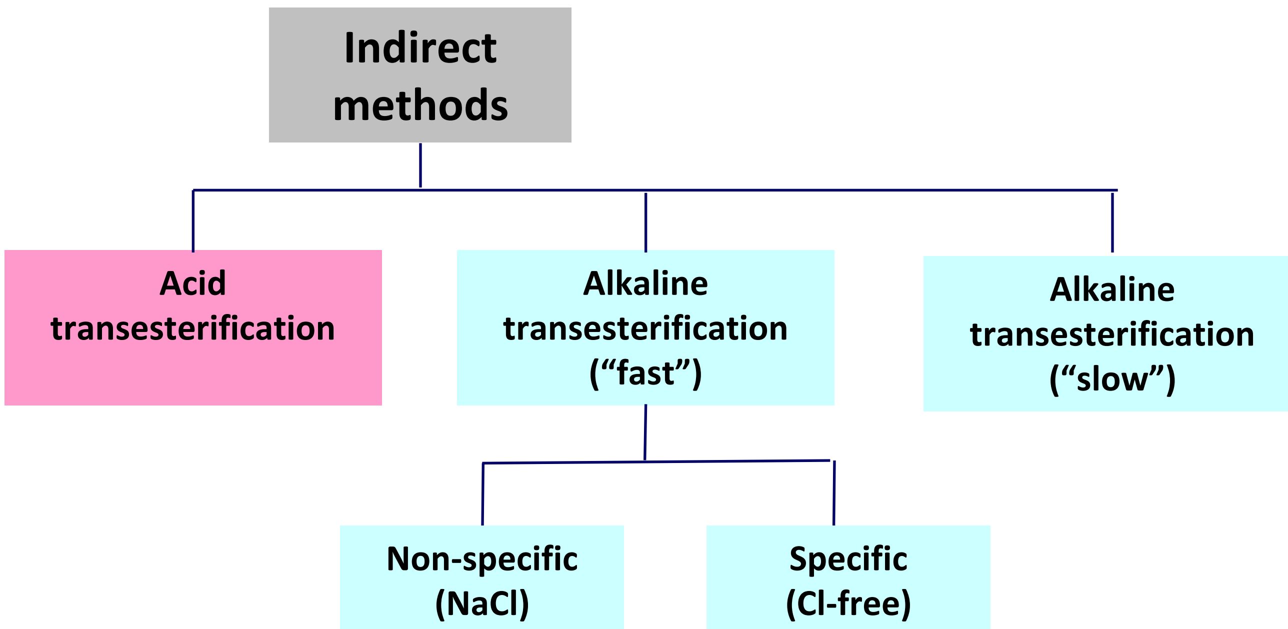
- Silica column chromatography
- Single stage SPE - silica
- Dual stage SPE - C18 then silica

**For glycidyl esters:**

Gel permeation chromatography (GPC)

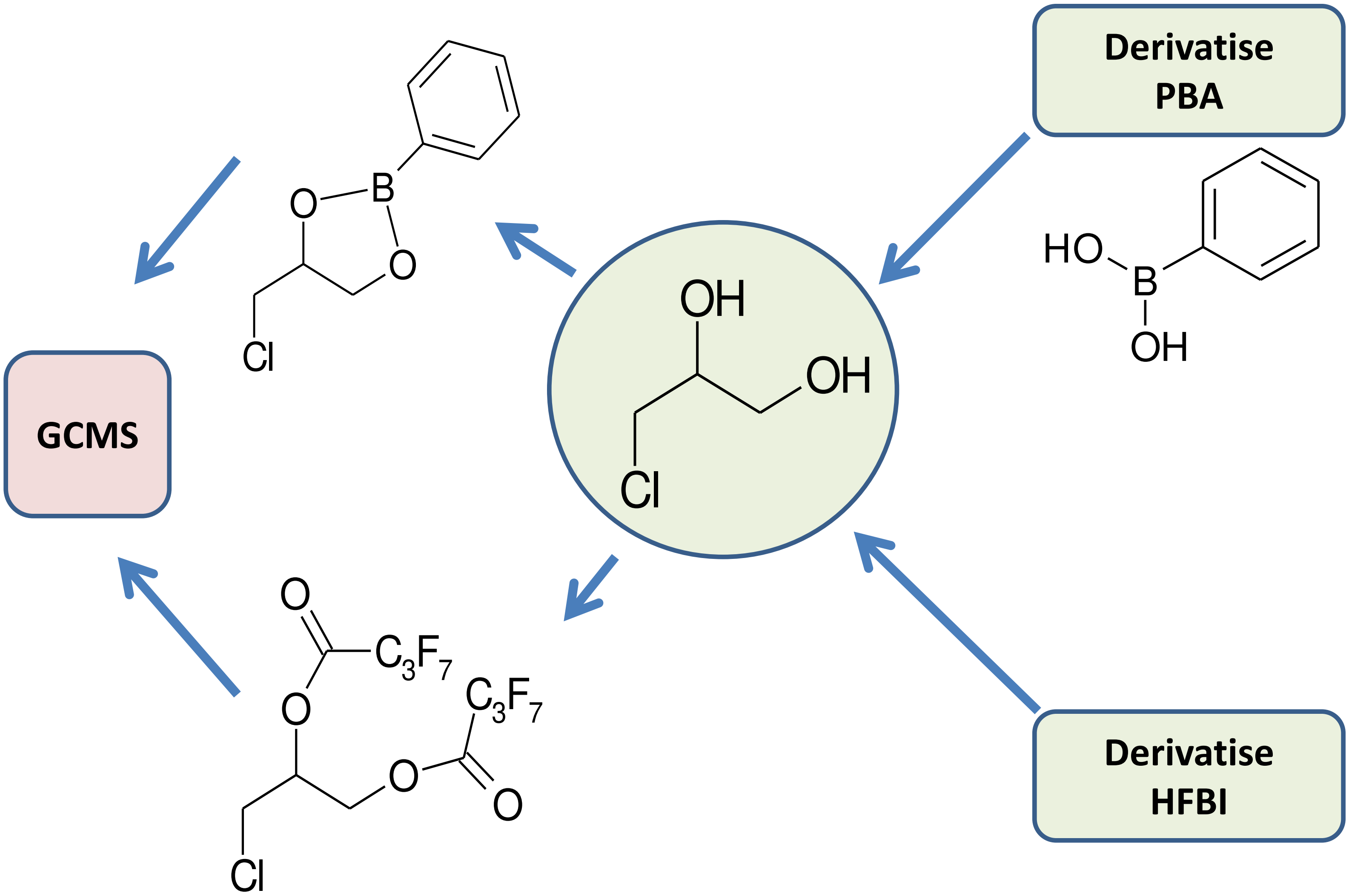
# Indirect methods 1

1. All esters converted to free 3-MCPD and 2-MCPD by methanolysis under acid or alkali conditions.
2. Free MCPD measured by GCMS



# Free MCPD analysis

**Free MCPD determined by derivatisation- GCMS**



# Indirect methods 2

**Enzyme method:** Lipase 24 hr, used less than chemical methods

**Acidic method:**

Slow reaction with methanolic sulphuric acid, glycidol esters degraded

**Alkali rapid method:**

NaOMe < 10 mins but MCPD → glycidol at varying levels depending on pH. Salt (NaCl) addition forms MCPD from glycidol

Method 1 DGF standard method C-VI 17 (10). Determines the sum of MCPD from MCPD esters + glycidyl esters

Method 2 DGF C-VI 18 (10) A. Determines MCPD from MCPD esters only, after removal of glycidyl esters
Method 2 DGF C-VI 18 (10) B. Determines MCPD from MCPD esters but no Cl used and so MCPD not produced from glycidyl esters

**Alkali slow method:**

NaOMe at – 22° C 18 hr. Prevents MCPD → glycidol
Use on bromide can allow determination of glycidyl esters

# Problems

**There are many methods available, direct and indirect causing confusion**

**Results for the same sample analysed by different methods often disagree**

**Results should be independent of the method used**

**Direct methods are very dependent on the LCMS instrument design**

**Indirect methods require understanding and care in operation**

# Ring Tests

JRC: Proficiency test on 3-MCPD esters in palm oil (2009-2010)
34 data sets for palm oil at 8.8 mg/kg

5 acid methods, range 6.7 to 9.1, compliance 5/5

27 alkali methods, range 3.0 to 16.5, of which

15 specific alkali methods (BfR/DGF B), compliance 11/15

9 non specific alkali methods  and 3 unknown, compliance 0/12

FAPAS: Proficiency test on 3-MCPD esters in palm oil (2011)

26 data sets for palm oil at 4.7 mg/kg

16 specific acid or alkaline methods, range 3.5 to 6.1, compliance 16/16

7 unspecific methods, range 9.2 to 11.5, compliance 0/0

3 failed from unknown reasons

# Mitigation

**Formation of MCPD esters is mainly from (natural) organic chlorine compounds in the oil which release chlorinating species in the deodoriser to react with TAG**

**Formation of glycidyl esters is mainly from partial glycerides in the oil**

**Reduction of MCPD esters and glycidyl esters in palm oil has been shown:**

**Use of young good quality fruit low in DAG, with rapid processing**

**Washing the oil to remove polar compound precursors**

**Addition of competitors for chlorination (diacetin)**

**Adjusting deodoriser temperatures and regimes**

**Post deodoriser treatment with zeolite**

*Dr. Brian Craft / Dr. Iekje Berg / Dr. Bertrand Matthaus (FEI Project) –*

*ILSI Workshop 9-10 November2011*

# Future needs

Harmonization of current  methods is desirable

Validation is required

Reference materials and analytical standards are required

Methods required for biomarkers in body tissues

*More information is needed  on:*

MCPD esters (2- and 3- MCPD) and glycidyl esters in cereals, bread, milk and milk products, frying oils and mixtures, animal fats and oils and composite processed foods, to update exposure estimates

The effects of household and industrial processing

Metabolic processes

# Publications

1) Analytical approaches for MCPD esters and glycidyl esters in food and biological samples – a review and future perspectives.

C Crews, A Chiodini, M Granvogl, C Hamlet, K Hrnčiřík, J Kuhlmann,

A Lampen, G Scholz, R Weisshaar, T Wenzl, P Jasti, W Seefelder

*Food Additives and Contaminants A (submitted in April 2012)*

2) Factors affecting the mitigation of MCPD esters and glycidyl esters in food products.

BD Craft, A Chiodini, J Garst, M Granvogl.

*Food Additives and Contaminants A (submitted in April 2012)*

3) MCPD and Glycidyl Esters in Food Products - Report of a Workshop held in Brussels, 9-10 November 2011

*ILSI Europe Report Series 2012*

4) MCPD and Glycidyl Esters in Food Products – Workshop Summary held in Brussels, 9-10 November 2011

*Workshop summary*

# Publications

ILSI Europe
International Life Sciences Institute

***ILSI EUROPE WORKSHOP ON MCPD AND GLYCIDYL ESTERS IN FOOD PRODUCTS***

***Summary***

**9 - 10 November 2011**

**Brussels, Belgium**

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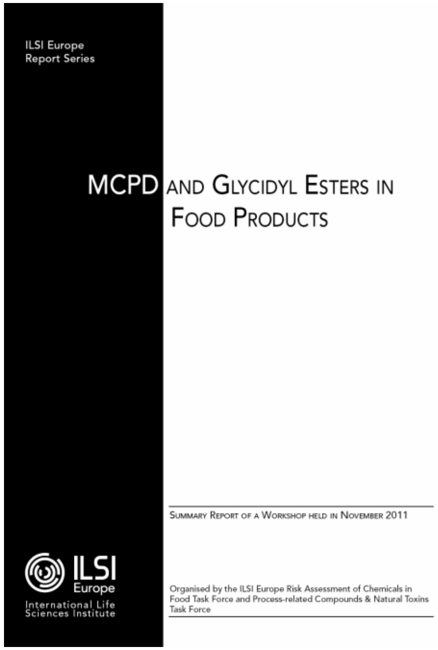

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MCPD AND GLYCIDYL ESTERS IN FOOD PRODUCTS

SUMMARY REPORT OF A WORKSHOP HELD IN NOVEMBER 2011

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

# Acknowledgements


## Expert Group Contributors

Brian Craft, Michael Granvogl, Colin Hamlet, Karel Hrncirik, Jan Kuhlmann, Alfonso Lampen, Gabriele Scholz, Rüdiger Weisshaar, Thomas Wenzl, Walburga Seefelder

## ILSI Europe Contributors

Alessandro Chiodini, Jilde Garst, Pratima Rao Jasti

# Thank you

**Colin Crews**

## Current projects on MCPD esters:

FP7: “Prometheus” http://processing-contaminants-prometheus.com/

UK Food Standards Agency ‘Formation of 3-MCPD from its esters in foods – Premier Analytical, Fera, VSCHT