Research Activities at the Center for Food Safety and Applied Nutrition (CFSAN) Related to the Analysis and Occurrence of MCPD Esters and Glycidyl Esters in Edible Oils, Infant Formula, and Other Processed Foods

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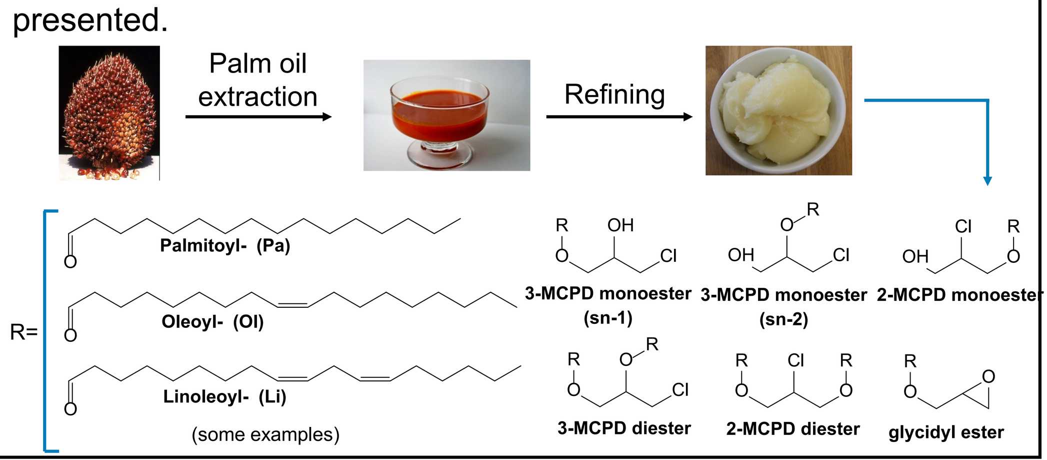
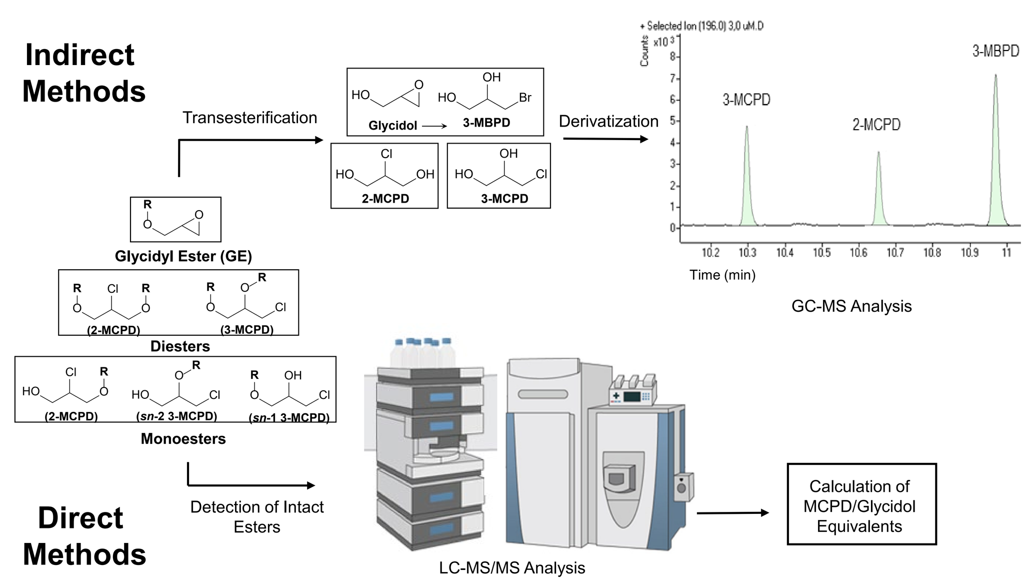
Introduction

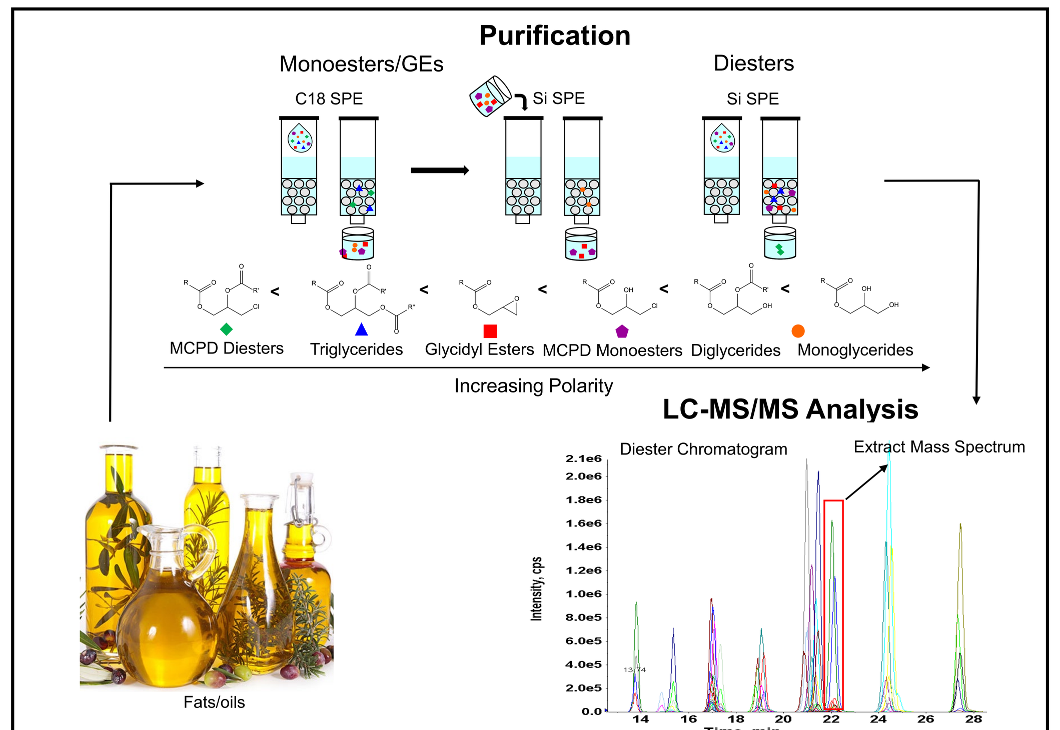
3-Monochloro-1,2-propanediol (3-MCPD) esters, 2-monochloro-1,3-propanediol (2-MCPD) esters, and glycidyl esters are process-induced chemical contaminants formed during the deodorization step of the refining of edible oils. These compounds are considered potentially carcinogenic and/or genotoxic, making their presence in refined oils and other food products containing these oils a potential health concern. Over the last 10 years, researchers at the U.S. Food and Drug Administration (FDA) have developed methods for the analysis of these contaminants in refined vegetable oils, infant formula, and other complex food matrices in an effort to determine their occurrence in many food products on the U.S. market and abroad. This poster will summarize the extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies developed at the U.S. FDA. In addition, the occurrence of these compounds in a variety of foods (including infant formula) and the impact of food processing on the concentrations of these compounds in the final products will be presented.



Detection Methodology



FDA Direct Detection

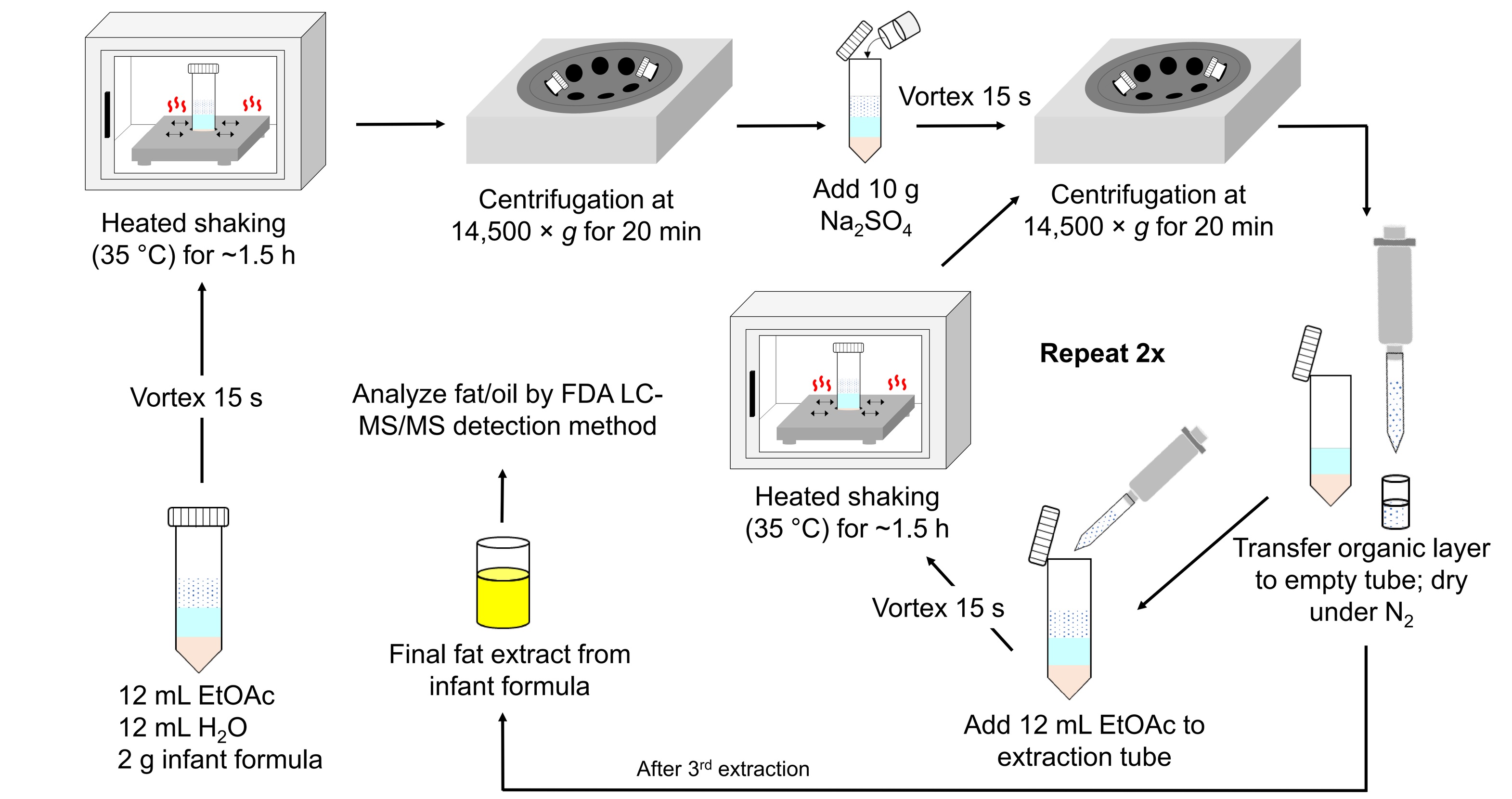


Objectives

- Because refined oils are the primary fat source in infant formula, develop a method for the extraction of MCPD and glycidyl esters from infant formula (and other complex foods)
  - Develop simple extraction procedures; ensure performance across all infant formula varieties
  - Confirm method performance beyond spiked samples
- Use established FDA direct detection method for analysis of infant formula (and other food) extracts
- Produce occurrence data for MCPD and glycidyl esters in infant formula and other processed foods to estimate levels of exposure

Methodology and Validation

This scheme illustrates the procedure developed for extracting the fat content from infant formulas (and later applied to other processed foods). The fat extract is analyzed using LC-MS/MS methodology developed at the U.S. FDA for analysis of the intact 3-MCPD and glycidyl esters.



No certified reference materials containing known levels of 3-MCPD and glycidyl esters are currently available; "homemade" reference materials were prepared and analyzed to confirm method performance (in addition to analyte recoveries from spiked samples).

Extract and analyze samples according to U.S. FDA methodology; determine % recoveries

Ester recoveries from homemade reference materials are satisfactory except for some GEs in foods. It was determined these particular GEs were already present in the ingredients of the homemade food products and not an artefact of the extraction.

Analyte	% Recovery Infant Formula	% Recovery Foods
Monoesters	89.3-114.3	89.3-114.3
Diesters	85.0-941.2	83.3-118.0
Glycidyl Esters	83.3-118.0	85.0-941.2

Glycidyl Esters	% Recovery Cookie	% Recovery Peanut Butter	% Recovery Cheese Crackers
Glycidyl Laurate	100.0	941.2	823.5
Glycidyl Stearate	93.1	276.2	101.0

Occurrence Data in Infant Formula and Processed Foods

Table 1. Bound 3-MCPD and glycidol concentrations in infant formulas purchased in the United States.

	Year Purchased: 2013-2016			Year Purchased: 2017-2019		
Manufacturer	*n*	Bound 3-MCPD (µg g^{-1} powder)	Bound Glycidol (µg g^{-1} powder)	*n*	Bound 3-MCPD (µg g^{-1} powder)	Bound Glycidol (µg g^{-1} powder)
A[a]	23	**0.13**, 0.072-0.16	**0.028**, 0.005-0.15	45	**0.12**, 0.050-0.67	**0.019**, <LOQ-0.089
B	25	**0.48**, 0.043-0.92	**0.093**, <LOQ-0.26	125	**0.070**, 0.013-0.95	**0.033**, <LOQ-0.20
C	11	**0.055**, 0.021-0.11	**0.032**, 0.011-0.057	27	**0.035**, 0.018-0.12	**0.019**, <LOQ-0.057
D	30	**0.62**, 0.31-0.89	**0.13**, 0.022-0.24	25	**0.64**, 0.36-0.81	**0.23**, 0.10-0.37

* Formulas produced by this manufacturer do not contain palm olein.

- Significant reduction in concentrations in manufacturer B products (**Table 1**); Bound 3-MCPD remains the same in manufacturer D products, while bound glycidol slightly increases (**Table 1**)
- Bound 3-MCPD in B and C formulas (containing palm) lower than A formulas (no palm); bound glycidol concentrations similar. Demonstrates effectiveness of industrial mitigation strategies for reducing contaminant concentrations (**Table 1**)

Table 2. Bound 3-MCPD and bound glycidol concentrations in U.S. and European infant formulas.

Location	Year(s) Purchased	*n*	Bound 3-MCPD (µg g^{-1} powder)	Bound Glycidol (µg g^{-1} powder)
United States	2013-2016	98	**0.38**, 0.021 – 0.92	**0.089**, <LOQ – 0.40
Europe	2015	59	**0.094**, 0.013 – 0.34	**0.010**, <LOQ – 0.081
United States	2017-2019	222	**0.14**, 0.013 – 0.95	**0.051**, <LOQ – 0.37
United States (no Man. D)	*2017-2019*	*197*	***0.077**, 0.013 – 0.95*	***0.028**, <LOQ – 0.20*
Europe	2019	45	**0.054**, 0.005 – 0.17	**0.006**, <LOQ – 0.017

- Average bound 3-MCPD and glycidol concentrations have decreased over 2-3 year period in U.S. and German formulas (**Table 2**)
- Concentrations in German formulas still lower than in U.S. formulas (**Table 2**)

Table 3. Bound 3-MCPD and glycidol concentrations in U.S. food products purchased in 2019.

Bound 3-MCPD and Glycidol Concentrations in Some Foods Purchased in the U.S. in 2019 (Average, range)

Food	*n*	Bound 3-MCPD (µg g^{-1} fat (extract))	Bound Glycidol (µg g^{-1} fat (extract))
Chips	18	**1.08**, 0.10 – 8.85	**0.60**, 0.042 – 0.88
Cookies	16	**1.40**, 0.17 – 4.39	**1.01**, 0.25 – 3.24
Snack Cakes	12	**1.41**, 0.32 – 2.47	**1.02**, 0.13 – 2.66
Spreads	14	**0.21**, 0.009 – 1.21	**0.26**, 0.026 – 0.39
Candy	18	**0.43**, <LOQ – 1.91	**0.54**, 0.25 – 1.20
Crackers	12	**0.68**, 0.015 – 3.57	**0.40**, 0.046 – 12.53
Noodles	7	**3.04**, 2.54 – 4.12	**1.67**, 0.48 – 2.50

EU Limits: 3-MCPD	Max. Level (µg g^{-1} oil/fat)
Unrefined oils, coconut, maize, rapeseed, olive, sunflower, soybean, palm kernel	1.25 2 products above level
All other refined oils	2.5 11 product above level
EU Limit: Glycidol	**Max. Level (µg g^{-1} oil/fat)**
Oils and fats placed on the market for the final consumer or used in foods	1.00 16 products above level

- Approximately 80% of products analyzed for 3-MCPD and glycidyl esters are lower than European Union (EU) limits for these contaminants (**Table 3**)

Table 4. Bound 3-MCPD and glycidol concentrations in U.S. food products from year to year (2017-2019).

Bound 3-MCPD and Glycidol Concentrations in Some Foods (Average, Range) (µg g^{-1} fat (extract))

	Year Purchased: 2017			Year Purchased: 2019		
Food Product	*n*	Bound 3-MCPD	Bound Glycidol	*n*	Bound 3-MCPD	Bound Glycidol
Chips	7	**0.88**, 0.053 – 4.68	**0.70**, 0.17– 1.23	18	**1.08**, 0.10 – 8.85	**0.60**, 0.042 – 0.88
Cookies	17	**1.18**, 0.009 – 3.93	**1.34**, 0.087 – 5.40	16	**1.40**, 0.17 – 4.39	**1.01**, 0.25 – 3.24
Snack Cakes	9	**1.07**, 0.084 – 1.93	**0.61**, 0.072 – 1.24	12	**1.41**, 0.32 – 2.47	**1.02**, 0.13 – 2.66

- Average bound 3-MCPD and glycidol concentrations in oil/fat extracts from food samples relatively similar among the products purchased in 2017 and 2019 (**Table 4**)

Impact of Infant Formula/Food Production on Contaminant Concentrations

Table 5. Relative trend in average bound 3-MCPD and bound glycidol concentrations in U.S. formulas.

	Year Purchased: 2013-2016		
Manufacturer	*n*	Bound 3-MCPD (µg g^{-1} powder)	Bound Glycidol (µg g^{-1} powder)
A	23	**0.13**	**0.028**
B	25	**0.48**	**0.093**
C	11	**0.055**	**0.032**
D	30	**0.62**	**0.13**

Sample	Bound 3-MCPD (µg g^{-1} powder)	Bound Glycidol (µg g^{-1} powder)
D1	**0.436**	0.022
D2	**0.787**	0.096
D3	**0.713**	0.112
D4	**0.542**	0.056
D5	**0.719**	0.207
D6	**0.841**	0.243
D7	**0.885**	0.181
D8	**0.711**	0.150
D9	**0.578**	0.181

- Bound glycidol concentrations in infant formulas consistently lower than bound 3-MCPD concentrations (**Table 5**)
- Production of infant formula may promote destruction/conversion of glycidyl esters

Table 6. Relative trend in average bound 3-MCPD and bound glycidol concentrations in snack foods purchased from the U.S. market.

	Year Purchased: 2017			
Snack Food	*n*	Bound 3-MCPD (µg g^{-1} powder)		Bound Glycidol (µg g^{-1} powder)
Chips	7	**0.88**	>	**0.70**
Cookies	17	**1.18**	<	**1.34**
Snack Cakes	8	**1.07**	>	**0.61**

Sample	Bound 3-MCPD (µg g^{-1} powder)	Bound Glycidol (µg g^{-1} powder)
SC1	**1.07**	0.34
SC2	0.67	**1.00**
SC3	**1.76**	0.35
SC4	0.34	**0.94**
SC5	**1.93**	1.24
SC6	**1.85**	0.30
SC7	**1.19**	0.73
SC8	**0.71**	0.54

- No trends observed in relative bound 3-MCPD and glycidol concentrations in snack foods analyzed (**Table 6**)
- Impact of processing on 3-MCPD and glycidyl ester concentrations in foods not the same as in infant formula

Conclusions

Occurrence Data

- Comparison of U.S occurrence data from 2013-2016 and 2017-2019 (**Table 1**) shows evidence of contaminant mitigation over the past 2 years (except for manufacturer D)
- Palm oils typically contain higher levels of MCPD and glycidyl esters than other refined oils - it was expected that infant formulas produced without palm oil by manufacturer A (**Table1**) would have generally lower levels of contaminants
- However, lowest levels of bound 3-MCPD were observed in formulas produced by manufacturers B and C (**Table 1**, 2017-2019), which does contain palm oil (bound glycidol concentrations were similar)
  - Strong indication that manufacturers B and C source refined oils that have undergone mitigation to reduce contaminants
  - Mitigation of contaminants in refined oils may be more effective than removing palm oil/palm olein from formulations
- Manufacturers of European formulas appear to have begun sourcing mitigated oils earlier than U.S. counterparts (**Table 2**)
- Generally, bound 3-MCPD and glycidol concentrations in fat extracts from some processed foods purchased in the U.S. below EU contaminant limits (**Table 3**)
- Bound 3-MCPD and glycidol amounts similar in food products purchased in 2017 and 2019; evidence that manufacturers have likely not changed/modified the sourcing of their refined oils (**Table 4**)

Impact of Processing

- Bound glycidol levels consistently lower than bound 3-MCPD levels in all infant formulas investigated (**Table 5**)
- No relative trends observed for bound 3-MCPD and glycidol concentrations in various snack foods (**Table 6**)
- Additional studies (not shown) indicate infant formula production may promote the reduction of glycidyl ester levels in the final product

Future Directions

- Development/analysis of certified reference materials
- Collaborative studies; acceptance of official methods
- Investigate contaminant reduction during formula production
- Expand occurrence studies in food

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