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Recent advances in toxicity and analytical methods of monochloropropanediols and glycidyl fatty acid esters in foods

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An emerging group of process-induced food contaminants including esterified forms of monochloropropanediols (MCPDE) and glycidol (GE) has attracted significant attention of fats and oils producers in the past few years. These substances are mainly formed during the deodorization step of the refining processing of vegetable oils. Literature indicates different precursors, mechanisms and process conditions for the formation of these contaminants. Nephrotoxicity, developmental and reproduction toxicity, and carcinogenicity have been described as the most important adverse effects of MCPDE and GE. Analytical methods for the determination of these contaminants include direct and indirect approaches, and some of them are fully validated for different matrices. However, important gaps still exist, which motivates many research opportunities on this topic.

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Introduction

Edible oils are generally refined in order to remove impurities and other compounds that can affect quality (smell, appearance, and taste) and storability [1]. However, undesirable chemical changes may also occur during the refining process. Monochloropropanediol esters (MCPDE), which comprises fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPDE) and 2-monochloropropane-1,3-diol (2-MCPDE), and glycidyl (3-hydroxy-1,2-epoxypropane) esters (GE) are process contaminants formed at the high temperatures employed during the deodorization step of the refining process [2,3].

The presence of 3-MCPDE in a large variety of processed foods was first described in 2004 [4], followed by the discovery of high concentrations in refined, bleached and deodorized (RDB) vegetable oils in 2006 [5]. The occurrence of GE in refined edible oils was first reported in 2008 [6]. Preliminary risk assessments raised an immediate health concern due to human exposure to these substances, which has stimulated intensive research on this topic [7–9,10••,11].

The list of food and ingredients in which 3-MCPDE have been detected includes refined vegetable oils, fried foods, infant formula, meat products, dairy products, cereal and bakery products, soups, sauces, and roasted coffee [4,5,7–9,12•,13]. The higher amounts found in vegetable oils, especially in refined palm oil and derived products, have attracted considerable attention and challenged industries from this segment. So far, no or only traces of 3-MCPDE have been found in unrefined vegetable oils. GE is mainly found in refined palm oil as well, while the database related to 2-MCPDE is still limited [10••]. Table 1 shows the concentrations of 3-MCPDE, 2-MCPDE and GE in foods and food ingredients recently reported in literature.

The formation of these substances in edible oils seems to be directly associated to the refining process, and it is favored at temperatures above 140 °C [10••]. Literature indicates that the precursors involved in the formation of MCPDE can be acylglycerols (triacylglycerols, diacylglycerols, and monoacylglycerols) in the presence of a chlorine source [22]. Palm fruits have a significant content of chlorides which come from the endogenous metabolism of the plant as well as the application of fertilizers containing chloride salts in palm cultivation [3]. Formation mechanisms based on nucleophilic substitution SN2, in which chlorine acts as a nucleophile, were proposed by some authors [22], while others described a reaction mediated by free radicals to generate MCPDE [23]. For GE, the main precursors have been identified as diacylglycerols and monoacylglycerols, and the formation of the contaminants seems to occur by an internal nucleophilic attack of diacylglycerols or from the acyloxonium ion generated by the displacement of the hydroxyl group [10••].

Table 1

Occurrence levels of 3-MCPDE, 2-MCPDE and GE in foods and food ingredients.

Food	N	3-MCPDE (μg/kg)		2-MCPDE (μg/kg)		GE (μg/kg)		Reference
		Mean	Range	Mean	Range	Mean	Range	
Extra virgin olive oil	20	0.1	ND–0.2	_	_	_	_	[14]
Olive oil	15	4.0	1.0–7.6	_	_	_	_	[14]
Olive pomace oil	7	12.3	7.4–20.53	_	_	_	_	[14]
Sunflower oil	11	230	80–960	110	20–520	230	20–900	[15]
Refined palm oil	6	1330	180–2480	650	80–1650	1870	100–3550	[15]
Refined rape seed oil	5	440	30–510	210	10–310	310	10–1100	[15]
Fish oil	5	_	1500–5500	_	100–230	_	_	[16]
Margarine	5	_	1300–7300	_	630–1700	_	_	[16]
Corn oil	38	503	502–505	233	–	650	647–654	[12•]
Olive oil	9	48	48–49	86	85–88	15	0–31	[12•]
Palm kernel oil	97	624	–	270	249–291	421	415–428	[12•]
Peanut oil	8	229	–	102	90–115	148	133–162	[12•]
Rapeseed oil	294	232	224-239	109	78–140	166	144–188	[12•]
Soybean oil	191	394	392–396	167	159–175	171	157–186	[12•]
Sunflower oil	596	521	517–524	218	207–229	269	259–279	[12•]
Coconut oil/fat	204	608	608	169	143–194	476	472–479	[12•]
Palm oil/fat	501	2912	2912	1565	1563–1566	3955	3954–3955	[12•]
Margarine and similar products	170	408	406–409	159	152–166	361	358-364	[12•]
Extra virgin olive oil	46	133	ND–116	61	ND–580	323	ND–198	[17]
Olive oil	13	855	280–3777	420	170–1910	643	ND–1880	[17]
Oil blends	17	304	180–610	120	ND–250	825	310–1840	[17]
Infant formula	40	150	ND–630	_	_	220	ND–750	[7]
Infant formula	98	370	24–920	_	_	84.4	< LOQ–400	[18]
Infant formula	88	185	0–316	41	0–52	_	_	[19]
Beef flavoring products	6	256.3	30.6–501.7	NR	NR	NR	NR	[20]
Potato Chips	5	431.4	< LOQ–604	NR	NR	1.9	ND–9.5	[21]
Corn puffs	4	195	45–267	NR	NR	ND	_	[21]
Sticks	5	318.5	25–257	NR	NR	2.3	< LOQ–11.6	[21]
Crackers	5	449.4	112–748	NR	NR	ND	_	[21]
Peanuts	3	475.3	251–753	NR	NR	ND	_	[21]
Granola	3	375.0	206–513	NR	NR	9.6	ND–28.8	[21]
Muesli	3	353.6	86–585	NR	NR	ND	_	[21]
Flakes	3	50.3	26–78	NR	NR	ND	_	[21]
Sugar free Biscuits	5	599.2	133–1501	NR	NR	ND	_	[21]
Organic farming biscuits	5	237.0	59–495	NR	NR	ND	_	[21]
Gluten free biscuits	4	326.3	91–571	NR	NR	ND	_	[21]
Baby Biscuits	6	283.5	88–443	NR	NR	ND	_	[21]
Classic biscuits	5	590	363–870	NR	NR	ND	_	[21]
Bread and bread rolls	75	29	23–36	14	9.8-19	8	7.8–8.3	[12•]
Breakfast cereals	66	26	19–33	15	10-20	17	16–18	[12•]
Fine bakery wares	88	172	167–178	87	82-92	112	112–113	[12•]

N: number of samples; NR: Not reported in the study; ND: Not detected; LOQ: Limit of quantification.

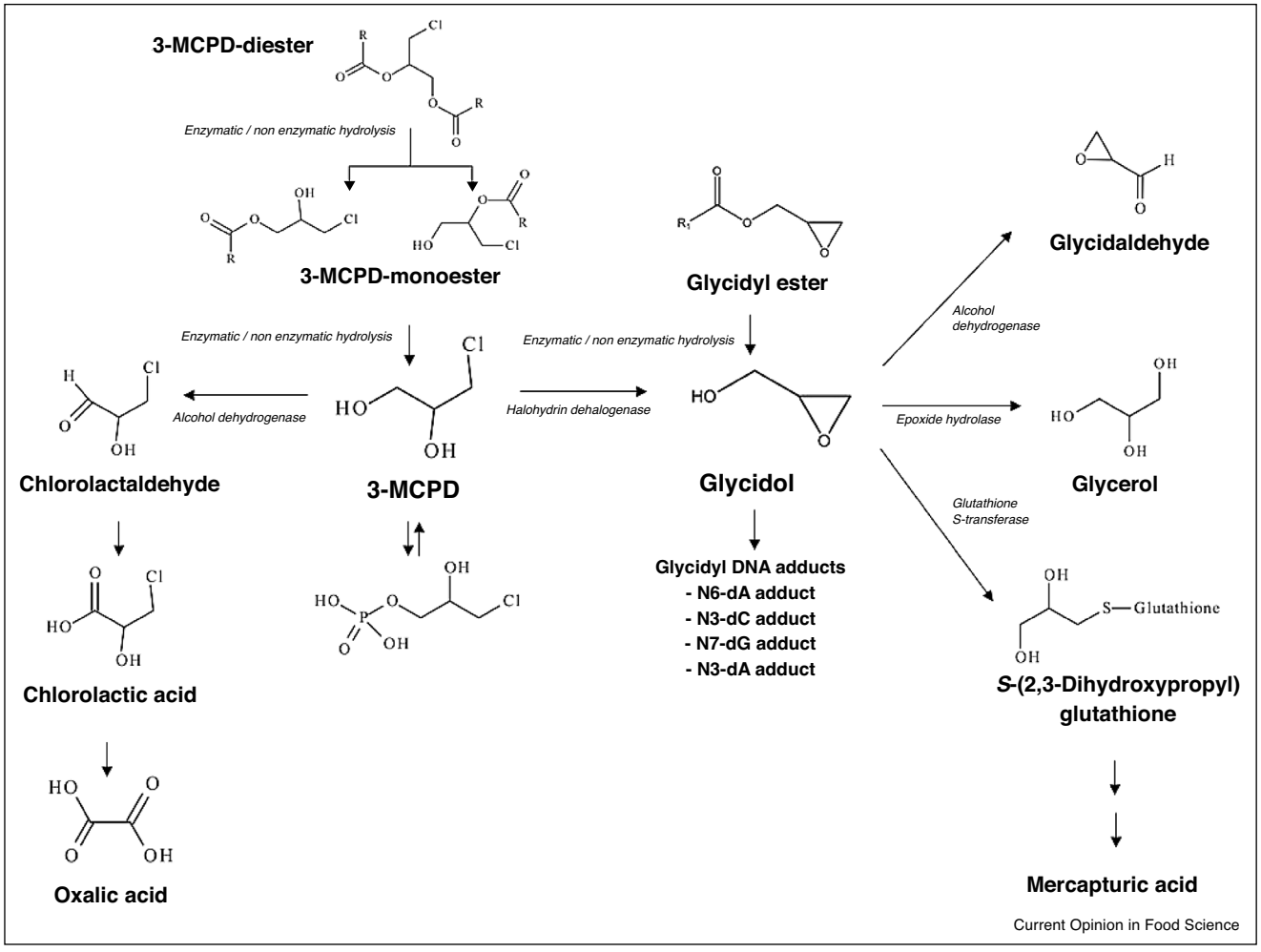
Recently, various strategies have been proposed focusing on the reduction of these contaminants. In general, three approaches have been investigated: removal of precursors, such as chlorinated compounds present in the crude oil; modifications of the processing parameters, in order to reduce the drastic conditions applied in the refining process; and degradation or removal of the contaminants formed in the final product using adsorbent agents [24]. Toxicity and analytical methods used to determine MCPDE and GE in food products have also presented important advances, which will be discussed in the following sections.

Toxicological aspects

Experimental evidence suggests that both 3-MCPDE and GE are substantially hydrolysed to their free forms in the gastrointestinal tract and elicit toxicity as free 3-MCPD and glycidol, respectively [25,26]. The hypothesized metabolic pathways for MCPDE and GE are illustrated in Figure 1.

Toxicological assessments were already performed for free 3-MCPD by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the forty-first, fifty-seventh and sixty-seventh meetings while the esterified form (3-MCPDE) and GE have been evaluated at the eighty-third meeting of the Committee in 2016 [27•]. The contaminants were also assessed by the European Food Safety Authority (EFSA) in 2016 and 2018 [12•,28•]. Because of currently limited food occurrence data and insufficient toxicological database, 2-MCPD and its esters have not been evaluated so far.

Figure 1



Hypothesized metabolic pathways for 3-MCPDE and GE (adapted from IARC [30] and Rietjens *et al.* [49]).

Regarding free 3-MCPD and 3-MCPDE, studies conducted with rodents have demonstrated that kidneys and testes are the main target organs for toxicity. Short-term oral exposure to 3-MCPDE resulted in increased relative kidney weight and, at higher doses, tubular epithelial hyperplasia, glomerular lesions and accumulation of hyaline casts. Increased testis weight and histopathological findings in testes and epididymis were generally observed at doses equal to and above 30 mg/kg body weight (bw) per day expressed as 3-MCPD. Renal tubular hyperplasia was identified as the most sensitive toxicological endpoint [29].

Long-term carcinogenicity studies with rodents are only available for free 3-MCPD and, according to the International Agency for Research on Cancer (IARC), this compound is classified as a possible human carcinogen (group 2B) [30]. Carcinogenic effects were observed in a 2-year oral (drinking-water) study in rats [29]. At the highest dose (29.5 mg/kg bw per day), the authors verified increased incidences of renal cell tumours (adenoma or carcinoma) in both sexes and of Leydig cell tumours in males when compared to controls. No positive results were found in *in vivo* genotoxicity experiments with both 3-MCPD and 3-MCPDE [31,32].

At its eighty-third meeting, the JECFA considered the lowest $BMDL_{10}$ (Benchmark dose lower confidence limit for 10% increase in the response) of 0.87 mg/kg bw per day of 3-MCPD for renal tubular hyperplasia in male rats [27•]. This value was obtained from the study published by Cho *et al.* [29], using the restricted log-logistic model, and applied to derive a group Provisional Maximum Tolerable Daily Intake (PMTDI) after the application of a 200-fold uncertainty factor. The established group PMTDI of 4 μg/kg bw for 3-MCPD and 3-MCPDE singly or in combination (expressed as 3-MCPD equivalents) replaced the previous PMTDI of 2 μg/kg bw for 3-

MCPD, established at the fifty-seventh meeting and retained at the sixty-seventh meeting [27•]. Using the same toxicological data, but different BMD modelling techniques, a group Tolerable Daily Intake (TDI) of 2 μg/kg bw for 3-MCPD was recently established by the EFSA [28•], which replaced the TDI of 0.8 μg/kg bw previously derived in 2016 by the authority [12•]. Despite these differences, the outcome of the evaluations was consistent and indicates that extrapolation of the TDI/PMTDI could be observed only for specific exposure scenarios, especially for children.

Glycidol is considered a genotoxic carcinogen and is classified by the IARC as probably carcinogenic to humans [33]. In the evaluations performed by JECFA and EFSA, carcinogenicity was chosen as the most sensitive toxicological end-point [12•,27•]. As it is not appropriate to establish a health-based guidance value for substances that are genotoxic and carcinogenic, both scientific bodies applied the margin of exposure (MOE) approach using the results from the National Toxicology Program (NTP) chronic bioassay for carcinogenicity [34]. However, different reference points were selected and some divergence was also observed.

Considering the limitations in the design of the NTP carcinogenicity study, and in view of the high uncertainties associated with the BMD analysis of the chosen dataset, EFSA derived a T25 of 10.2 mg/kg bw per day as reference point for the application of the MOE approach [12•]. On the other hand, JECFA considered the lowest $BMDL_{10}$ of 2.4 mg/kg bw per day for mesotheliomas in the tunica vaginalis/peritoneum in male rats [27•]. Both JECFA and EFSA concluded that glycidol represents a human health concern for some exposure scenarios.

Analytical methods

Analytical methods for the determination of 3-MCPDE, 2-MCPDE and GE can be grouped into direct and indirect approaches. An overview of their application is presented in Table 2.

Indirect methods, which require the conversion of esterified forms of the compounds into their free forms before chromatographic analysis, were firstly used followed by gas chromatography coupled to mass spectrometry (GC–MS). These methods present many advantages for routine purposes, such as the lowest number of analytical standards and a simpler interpretation of results, but generally involve several steps such as transesterification, neutralization, salting out and derivatization [11,13,43,44••].

Transesterification is a critical step for the cleavage of 3-MCPDE, 2-MCPDE and GE in their respective free forms. Acid, alkali or enzymes may be applied to catalyze the reaction. The low stability and degradation of 3-MCPD in alkali solution has already been demonstrated by some authors [43]. Furthermore, under alkaline conditions, the presence of GE and chlorinated compounds can produce an overestimation of 3-MCPDE levels [45]. In contrast to the alkali-catalyzed transesterification, the use of acid does not promote degradation of 3-MCPDE [43]. The enzymatically catalyzed transesterification involves the use of lipases from some biologic systems such as *Candida rugosa* and can be chosen as an alternative strategy [46].

Indirect methods also need derivatization before GC–MS analysis. The free forms of MCPDE and GE exhibit high polarity and low volatility, resulting in analyses with low sensitivity [43]. Generally, phenylboronic acid (PBA) is

Table 2

Analytical methods for the determination of 3-MCPDE, 2-MCPDE and GE in foods and food ingredients.

Matrix	Method	Hydrolysis	Compounds	LOD (μg/kg)	LOQ (μg/kg)	Reference
Fish oil	Indirect	Alkali-catalyzed	3-MCPDE	50	200	[35]
			GE	20	70	
Edible oils, fish oil, lipid fraction of margarine	Indirect	Alkali-catalyzed	3-MCPDE, 2-MCPDE	10	30	[16]
Oils, chips and crisps, infant formula	Indirect	Alkali-catalyzed	3-MCPDE, 2-MCPDE, GE	0.8–30	2.5–100	[36]
Edible oils	Indirect	Alkali-catalyzed	3-MCPDE	25	50	[37]
Edible oils and fats	Indirect	Alkali-catalyzed	3-MCPDE	14	43	[38]
			2-MCPDE	17	52	
Several foodstuffs	Indirect	Acid-catalyzed	3-MCPDE	7	13	[39]
			2-MCPDE	8	15	
			GE	17	31	
Fish oil	Indirect	Enzymatic	3-MCPDE, GE	NR	NR	[40]
Vegetable oils	Direct	Not applied	3-MCPD monoesters	0.08–12.7	0.98–38.0	[9]
			3-MCPD diesters	0.033–18.61	0.1–55	
Edible oils	Direct	Not applied	3-MCPD diesters	10–25	25–50	[41]
Fishery products	Indirect	Alkali-catalyzed	3-MCPDE, 2-MCPDE, GE	NR	20	[42]

NR: Not reported in the study.

used to convert the compounds into more volatile derivatives for instrumental analysis [44••].

On the other hand, direct methods allow the analysis of MCPDE and GE as they are found in foods and require simple extraction procedures, without the need of transesterification and derivatization steps. High performance liquid chromatography–mass spectrometry (LC–MS/MS) has been employed for the identification and quantification of the different compounds. The main drawback is the need of a large number of analytical standards, since each species of MCPDE and GE is identified and quantified individually [13].

The American Oil Chemists' Society (AOCS) published three official methods (Cd 29a 13, Cd 29b 13, and Cd 29c 13) for the analysis of 3-MCPDE, 2-MCPDE and GE in vegetable oils and fats [47]. All protocols recommend indirect approaches and the main difference among them is that Cd 29a uses a solution of acidic methanol while Cd 29b and Cd 29c employs an alkaline alcoholic solution. In the method Cd 29a, GE is converted to 3-bromo-1,2-propanediol fatty acid ester (3-MBPDE) before transesterification by a reaction with acid solution of sodium bromide. In the method Cd 29b, this reaction is carried out after the transesterification step. The method Cd 29c involves differential measurement of GE by the application of two protocols and does not quantify 2-MCPDE. The AOCS Official Methods recently included the new protocol Cd 30-15 for determination of the contaminants in emulsions [48].

Recent publications have described analytical methods for the determination of 3-MCPDE, 2-MCPDE and GE in several food products [38,42]. Jedrkiewicz *et al.* [38] used alkali-catalyzed transesterification and derivatization with PBA to analyse cookies, salty deep-fried snacks and instant products. Karl *et al.* [42] developed an indirect method to analyze fish products whereas Miyazaki and Koyama [40] optimized an enzymatic indirect method for fish oils. The use of high-resolution mass spectrometry (HRMS-Orbitrap) and a modified QuEChERS protocol for sample preparation have already been cited in the recent literature [9,41].

Conclusions

Intensive research concerning MCPDE and GE has been conducted in the past years. Despite this, foods with high concentrations of these contaminants are still widely available for consumers. Toxicological assessments conducted by different committees have drawn similar conclusions, suggesting a potential health concern, especially for infants. The application of strategies to mitigate the formation of the contaminants has been recommended, but their effectiveness at an industrial level requires efforts of all those involved in the production chain, thus fulfilling the present gaps. Advances in analytical methods were noted, but fully validated procedures were only available for oils, fats and margarine. Future challenges also include increasing the database of 2-MCPDE.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sampaio KA, Ayala JV, Van Hoed V, Silva SM, Ceriani R, Verhé R, Meirelles AJA: **Impact of crude oil quality on the refining conditions and composition of nutraceuticals in refined palm oil**. *J Food Sci* 2017, **82**:1842-1850.

2. Pudel F, Benecke P, Fehling P, Freudenstein A, Matthäus B, Schwaf A: **On the necessity of edible oil refining and possible sources of 3-MCPD and glycidyl esters**. *Eur J Lipid Sci Technol* 2011, **113**:368-373.

3. Craft BD, Chiodini A, Garst J, Granvogl M: **Fatty acid esters of monochloropropanediol (MCPD) and glycidol in refined edible oils**. *Food Addit Contam Part A* 2013, **30**:46-51.

4. Svejkovska B, Novotny O, Divinova V, Reblova Z, Dolezal M, Velisek J: **Esters of 3-chloropropane-1,2-diol in foodstuffs**. *Czech J Food Sci* 2004, **22**:190-196.

5. Zelinková Z, Svejkovská B, Velísek J, Dolezal M: **Fatty acid esters of 3-chloropropane-1,2-diol in edible oils**. *Food Addit Contam* 2006, **23**:1290-1291298.

6. Weißhaar R, Perz R: **Fatty acid esters of glycidol in refined fats and oils**. *Eur J Lipid Sci Technol* 2010, **112**:158-165.

7. Arisseto AP, Silva WC, Scaranelo GR, Vicente E: **3-MCPD and glycidyl esters in infant formulas from the Brazilian market: occurrence and risk assessment**. *Food Control* 2017, **77**:76-81.

8. Kyselka J, Matějková K, midrkal J, Berčíková M, Peek E, Bělková B, Ilko V, Doleal M, Filip V: **Elimination of 3-MCPD fatty acid esters and glycidyl esters during palm oil hydrogenation and wet fractionation**. *Eur Food Res Technol* 2018 http://dx.doi.org/10.1007/s00217-018-3101-9.

9. Graziani G, Gaspari A, Chianese D, Conte L, Ritieni A: **Direct determination of 3-chloropropanol esters in edible vegetable oils using high resolution mass spectrometry (HRMS-Orbitrap)**. *Food Addit Contam Part A* 2017, **34**:1893-1903.

10. •• Cheng WW, Liu GQ, Wang LQ, Liu ZS: **Glycidyl fatty acid esters in refined edible oils: a review on formation, occurrence, analysis, and elimination methods**. *Compr Rev Food Sci Food Saf* 2017, **16**:263-281.
This recent review aims to deliver a detailed summary of studies on the occurrence of GEs in different refined edible oils, in combination with mechanisms and factors responsible for the formation of GEs and proposed measures for reducing the GE levels in refined edible oils.

11. MacMahon S: **Direct detection techniques for MCPD esters**. In *Processing Contaminants in Edible Oils MCPD and Glycidyl Esters.* Edited by MacMahon S. AOCS Press; 2014:121-167.

12. • European Food Safety Authority (EFSA): **Risks for human health related to the presence of 3- and 2-monochloropropanediol**

(MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA J* 2016, **14**:4426.
This document reports the complete risk assessments of MCPDE and GE, including exposure data from several countries.

13. MacMahon S, Begley TH, Diachenko GW: **Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States**. *Food Addit Contam Part A* 2013, **30**:2081-2092.

14. Hung WC, Peng GJ, Tsai WJ, Chang MH, Liao CD, Tseng SH, Kao YM, Wang DY, Cheng HF: **Identification of 3-MCPD esters to verify the adulteration of extra virgin olive oil**. *Food Addit Contam Part B Surveill* 2017, **10**:233-239.

15. Kuhlmann J: **Analysis and occurrence of dichloropropanol fatty acid esters and related process-induced contaminants in edible oils and fats**. *Eur J Lipid Sci Technol* 2016, **118**:382-395.

16. Je?drkiewicz R, Głowacz A, Gromadzka J, Namieśnik J: **Determination of 3-MCPD and 2-MCPD esters in edible oils, fish oils and lipid fractions of margarines available on Polish market**. *Food Control* 2016, **59**:487-492.

17. Kamikata K, Vicente E, Arisseto-Bragotto AP, de O. Miguel AMR, Milani RF, Tfouni SAV: **Occurrence of 3-MCPD, 2-MCPD and glycidyl esters in extra virgin olive oils, olive oils and oil blends and correlation with identity and quality parameters**. *Food Control* 2019, **95**:135-141.

18. Leigh J, MacMahon S: **Occurrence of 3-monochloropropanediol esters and glycidyl esters in commercial infant formulas in the United States**. *Food Addit Contam Part A* 2017, **34**:356-370.

19. Wang L, Ying Y, Zhengyan H, Tianjiao W, Xianghong S, Pinggu W: **Simultaneous determination of 2- and 3-MCPD esters in infant formula milk powder by solid-phase extraction and GC-MS analysis**. *J AOAC Int* 2016, **99**:786-791.

20. Chai Q, Zhang X, Karangwa E, Dai Q, Xia S, Jingyang Y, Gao Y: **Direct determination of 3-chloro-1,2-propanediol esters in beef flavoring products by ultra-performance liquid chromatography tandem quadrupole mass spectrometry**. *RSC Adv* 2016, **6**:113576-113582.

21. Sadowska-Rociek A, Surma M, Cieslik E: **Analysis of acrylamide, 3-monochloropropane-1,2-diol, its esters and glycidyl esters in carbohydrate-rich products available on the Polish market**. *Rocz Panstw Zakl Hig* 2018, **69**:127-137.

22. Rahn AKK, Yaylayan VA: **What do we know about the molecular mechanism of 3-MCPD ester formation?** *Eur J Lipid Sci Technol* 2011, **113**:323-329.

23. Zhang X, Gao B, Qin F, Shi H, Jiang Y, Xu X, Yu L: **Free radical mediated formation of 3-monochloropropanediol (3-MCPD) fatty acid diesters**. *J Agric Food Chem* 2013, **61**:2548-2555.

24. Matthäus B, Pudel F: **Mitigation of 3-MCPD and glycidyl esters within the production chain of vegetable oils especially palm oil**. *Lipid Technol* 2013, **25**:151-155.

25. Abraham K, Appel KE, Berger-Preiss E, Apel E, Gerling S, Mielke H, Creutzenberg O, Lampen A: **Relative oral bioavailability of 3-MCPD from 3-MCPD fatty acid esters in rats**. *Arch Toxicol* 2013, **87**:649-659.

26. Appel KE, Abraham K, Berger-Preiss E, Hansen T, Apel E, Schuchardt S, Vogt C, Bakhiya N, Creutzenberg O, Lampen A: **Relative oral bioavailability of glycidol from glycidyl fatty acid esters in rats**. *Arch Toxicol* 2013, **87**:1649-1659.

27. • Food and Agriculture Organization/World Health Organization (FAO/WHO): **Evaluation of certain contaminants in foods**. *WHO Technical Report Series 1002*. 2017:74-105.
This FAO/WHO monograph reports the complete risk assessments of MCPDE and GE, including exposure data from several countries.

28. • European Food Safety Authority (EFSA): **Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters**. *EFSA J* 2018, **16**:1-48.
This document reports the complete risk assessment of MCPDE, including exposure data from several countries.

29. Cho WS, Han BS, Nam KT, Park K, Choi M, Kim SH, Jeong J, Jang DD: **Carcinogenicity study of 3-monochloropropane-1,2-diol in Sprague Dawley rats**. *Food Chem Toxicol* 2008, **46**:3172-3177.

30. International Agency for Research on Cancer (IARC): **3-Monochloro-1,2-propanediol**. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 2012, **vol. 101**349-374.

31. Aasa J, Törnqvist M, Abramsson-Zetterberg L: **Measurement of micronuclei and internal dose in mice demonstrates that 3-monochloropropane-1,2-diol (3-MCPD) has no genotoxic potency in vivo**. *Food Chem Toxicol* 2017, **109**:414-420.

32. Onami S, Cho YM, Toyoda T, Horibata K, Ishii Y, Umemura T, Honma M, Nohmi T, Nishikawa A, Ogawa K: **Absence of in vivo genotoxicity of 3-monochloropropane1,2-diol and associated fatty acid esters in a 4-week comprehensive toxicity study using F344 gpt delta rats**. *Mutagenesis* 2014, **29**:295-302.

33. International Agency for Research on Cancer (IARC): **Glycidol**. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 2000, **vol. 77**469-486.

34. National Toxicology Program (NTP): **Technical report on the toxicology and carcinogenesis studies of glycidol (CAS no. 556-52-5) in F344/N rats and B6C3F1 mice (gavage studies)**. *NTP Technical Report 374*. 1990.

35. Garballo-Rubio A, Soto-Chinchilla J, Moreno A, Zafra-Gómez A: **A novel method for the determination of glycidyl and 3-monochloropropanediol esters in fish oil by gas chromatography tandem mass spectrometry**. *Talanta* 2017, **165**:267-273.

36. Marc C, Drouard-Pascarel V, Retho C, Janvion P, Saltron F: **Determination of MCPD esters and glycidyl esters by microwave extraction in different foodstuffs**. *J Agric Food Chem* 2016, **64**:4353-4361.

37. Li C, Jia H, Wang Y, Shen M, Nie S, Xie M: **Determination of 3-monochloropropane-1,2-diol esters in edible oil method validation and estimation of measurement uncertainty**. *Food Anal Methods* 2016, **9**:845-855.

38. Jedrkiewicz R, Glowacz-Rozynska A, Gromadzka J, Konieczka P, Namiesnik J: **Novel fast analytical method for indirect determination of MCPD fatty acid esters in edible oils and fats based on simultaneous extraction and derivatization**. *Anal Bioanal Chem* 2017, **409**:4267-4278.

39. Samaras VG, Giri A, Zelinkova Z, Karasek L, Buttinger G, Wenzl T: **Analytical method for the trace determination of esterified 3- and 2-monochloropropanediol and glycidyl fatty acid esters in various food matrices**. *J Chromatogr A* 2016, **1466**:136-147.

40. Miyazaki K, Koyama K: **An improved enzymatic indirect method for simultaneous determinations of 3-MCPD esters and glycidyl esters in fish oils**. *J Oleo Sci* 2017, **66**:1085-1093.

41. Custodio Lorenzo JA, Lorenzo RA, Valente IM, Almeida PJ, Lage MA, Rodrigues JA, Carro AM: **Development of a partitioned liquid-liquid extraction-dispersive solid phase extraction procedure followed by liquid chromatography-tandem mass spectrometry for analysis of 3-monochloropropane-1,2-diol diesters in edible oils**. *J Chromatogr A* 2018, **1548**:19-26.

42. Karl H, Merkle S, Kuhlmann J, Fritsche J: **Development of analytical methods for the determination of free and ester bound 2-, 3-MCPD, and esterified glycidol in fishery products**. *Eur J Lipid Sci Technol* 2016, **118**:406-417.

43. Ermacora A, Hrncirik K: **Indirect detection techniques for MCPD esters and glycidyl esters**. In *Processing Contaminants in Edible Oils MCPD and Glycidyl Esters*. Edited by MacMahon S. AOCS Press; 2014:57-90.

44. •• Je?drkiewicz R, Kupska M, Głowacz A, Namieśnik J, Gromadzka J: **3-MCPD: a worldwide problem of food chemistry**. *Crit Rev Food Sci Nutr* 2016, **56**:2268-2277.
This review presents an overview of current knowledge about 3-MCPD, including its formation routes, occurrence in various foodstuffs, analytical approach, toxicological aspects, and future research perspectives.

45. Hrncirik K, Zelinkova Z, Ermacora A: **Critical factors of indirect determination of 3-chloropropane-1,2-diol esters**. *Eur J Lipid Sci Technol* 2011, **113**:361-367.

46. Miyazaki K, Koyama K, Sasako H, Hirao T: **Indirect method for simultaneous determinations of 3-chloro-1,2-propanediol fatty acid esters and glycidyl fatty acid esters**. *J Am Oil Chem Soc* 2012, **89**:1403-1407.

47. American Oil Chemists' Society (AOCS): *The Official Methods and Recommended Practices of the AOCS*. edn 6. AOCS Press; 2013.

48. American Oil Chemists' Society (AOCS): *The Official Methods and Recommended Practices of the AOCS*. edn 7. AOCS Press; 2017.

49. Rietjens IMCM, Dussort P, Günther H, Hanlon P, Honda H, Mally A, O'Hagan S, Scholz G, Seidel A, Swenberg J *et al.*: **Exposure assessment of process-related contaminants in food by biomarker monitoring**. *Arch Toxicol* 2018, **92**:15-40.