Snapshot survey for 2-MCPD, 3-MCPD, glycidol and their esters in selected vegetable oils and infant formulas in Australia and New Zealand

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Prepared for New Zealand Food Safety
by Darren A. Saunders (ESR), Sarah Molyneux (ESR), Panan Sitthirit (ESR) & Jeane Nicolas (NZFS)

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Scientific Interpretative Summary

This SIS is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for MPI risk managers and external readers.

FW18005 Snapshot survey for 2-MCPD, 3-MCPD, glycidol, and their esters in selected vegetable oils and infant formulas in Australia and New Zealand.

3-Monochloropropanediol (3-MCPD), 2-Monochloropropanediol (2-MCPD) and their esters and glycidyl esters (GE) occur in food as a result of high temperature refining of vegetable oils that are ingredients of many foods including infant formulas. There is evidence that 3-MCPD and GE can cause toxic effects and cancer in laboratory animals, there is however currently no evidence that these substances cause harm to humans in the normal diet. Nevertheless, international efforts continue to reduce exposure to any potential harm. New Zealand sources its vegetable oils from international markets, and the survey described in this report was designed to benchmark New Zealand levels of 3-MCPD, 2-MCPD and their esters and GE in oils and infant formula against international ranges.

Analytical methods for these contaminants were previously not available in New Zealand. This study, carried out by New Zealand Food Safety (NZFS) with input from Food Standards Australia New Zealand (FSANZ), validates an indirect method implemented at New Zealand's Institute of Environmental Science and Research (ESR) as well as providing comparability and further validation for a direct method used by the United States Food and Drug Administration (USFDA). This provides a high level of confidence in the method implemented by ESR.

As expected, 3-MCPD esters, GE were detected in a range of oils including rice bran, grapeseed and palm oil at concentration ranges consistent with those reported overseas. Concentrations in infant formulas of 3-MCPD esters and GE were generally very low and within the range of those found internationally. These data provide confidence that the New Zealand diet does not constitute undue exposure and are consistent with international studies.

New Zealand and Australia are actively involved in the Codex Committee on Contaminants in Foods. The 40th Session (2017) of the Codex Alimentarius Commission approved work aimed at reducing 3-MCPD esters and GE in refined oils and products made with refined oils. A new code of practice has been adopted and will provide guidance for producers and users to reduce the presence of 3-MCPD and GE in refined oils (e.g. canola, soybean, sunflower, safflower, walnut and palm oils). This will continue to ensure the production of a safe product to protect consumer health and ensure trade flow of refined oils by producing countries.

Due to the adoption of the code of practice by supplier countries, NZFS will identify opportunities to monitor uptake of the guidance and consequential change in concentrations of 3- and 2-MCPD and their esters, and GE in vegetable oils and foods including infant formula.

Science for Communities

SNAPSHOT SURVEY FOR 2-MCPD, 3-MCPD, GLYCIDOL AND THEIR ESTERS IN SELECTED VEGETABLE OILS AND INFANT FORMULAS IN AUSTRALIA AND NEW ZEALAND

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Prepared by/Author(s):

Darren A. Saunders, Sarah Molyneux, Panan Sitthirit

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REVIEWED BY: **Andrew Chappell & Sarah Molyneux**

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Manager	Peer reviewer	Author
Dr Rob Lake	Andrew Chappell	Darren A. Saunders
Manager, Risk, Response and Social Systems	Chemistry Group	Environmental Science Group

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EXECUTIVE SUMMARY

The aim of this project, coordinated by New Zealand Food Safety (NZFS) and Food Standards Australia New Zealand (FSANZ), was to conduct a 'snapshot' of total 3-monochloropropanediol (3-MCPD) ester, and glycidyl ester (GE) levels in vegetable oils and infant formulas available for sale in Australia and New Zealand. Although not part of the original project aim, an analysis of 2-monochloropropanediol (2-MCPD) esters was also conducted.

A total of 100 samples were assayed, with 44 samples of vegetable oils and 56 samples of infant formulas. Approximately 50% of each product was sourced from Australia and New Zealand. New Zealand samples were purchased in Christchurch with some vegetable oil samples being acquired from online suppliers based in New Zealand during August, September and October 2017. Several samples were also obtained directly from infant formula manufacturers. Australian samples were purchased primarily in Canberra during August and September 2017. The Australian samples were sent by FSANZ to the ESR Food Chemistry Laboratory, Christchurch (New Zealand) for analysis. ESR also dispatched sub-samples to the United States Food and Drug Administration (USFDA) for analysis by an alternative method, as a means of validating the method used by ESR.

The levels of 2-MCPD, 3-MCPD and their esters and GEs in vegetable oils were found to be generally consistent with previous international surveys conducted over the last 10 years. Methods used by ESR and the USFDA gave similar results. Rice bran, grapeseed and palm oils had higher levels of 3-MCPD esters and GEs compared with other oils (see Table 3 for ranges found in different oil varieties). The highest concentration of 3-MCPD esters and GEs was found in a rice bran sample, at 10.914 mg/kg and 7.110 mg/kg respectively (ESR result).

Some previous surveys of 3-MCPD ester and GE concentrations in various edible oils found levels ~30 mg/kg. However the range of concentations found in any given study varied considerably (see Table 7). The concentrations in this snapshot survey, bearing in mind the small number of samples (sometimes *n* = 1) of any given oil variety examined, can be said to be very broadly consistent with previous findings.

The average levels of 3-MCPD esters and GEs found in infant formulas, as reported by the USFDA (which had a lower limit of detection (LOD) and limit of quantitation (LOQ) for this product), were 0.092 mg/kg (range 0.005 to 0.669 mg/kg) and 0.026 mg/kg (range <LOD to 0.484 mg/kg) respectively. The concentrations found in this study were relatively low compared with most previous surveys.

1. INTRODUCTION

1.1 PROJECT AIM

Free chlorinated propanediols, 3-monochloropropanediol (3-MCPD) and 2-monochloropropanediol (2-MCPD), have been known as food toxicants for more than thirty years. 3-MCPD was discovered in acid-hydrolysed vegetable protein used for soy sauce production in 1978.

3-MPCD, 2-MCPD, glycidol and their esters are processing contaminants that are formed in vegetable oils when they are being decolourised and deodorised prior to their sale or use as an ingredient in foods. Vegetable oils are commonly used as an ingredient in infant formulas as well as many other foods such as margarines and cooking oils etc. Research has shown that the esters are metabolised back to the parent compounds 3-MCPD and glycidol after ingestion, and are considered to be potential human carcinogens. Less is known about the toxic effects of 2-MCPD although similar in structure to 3-MCPD, studies suggest that the position of the chlorine has a significant impact on toxicity.

The aim of this project, coordinated by New Zealand Food Safety (NZFS) and Food Standards Australia New Zealand (FSANZ), was to conduct a ‘snapshot’ survey of total 3-MCPD and its esters, and glycidol esters (GEs) levels in vegetable oils and infant formulas available for sale in Australia and New Zealand. Although not part of the original project aim, an analysis of 2-MCPD and its esters was also conducted. It included establishing confidence in the selected method; the determination of the concentration; and assessment of whether these compounds in these matrices differ significantly from those found in overseas markets, especially those of the European Union and the United States. For information regarding the formation of these processing contaminants and potential mitigation, see Appendix A.

2. MATERIALS AND METHODS

The samples were analysed by the ESR Food Chemistry Laboratory, Christchurch (New Zealand) employing an indirect Gas Chromatographic Mass Spectrometric method (GC-MS, see MPI Client Report FW17079, December 2017, for validation details). ESR employed an indirect method of analysis based upon that of American Oil Chemists' Society (AOCS) method (Cd 29a-13) validated for the determination of 2-MCPD, 3-MCPD esters and GEs in edible oils and fats by Gas Chromatography Mass Spectrometry (AOCS, 2013). This method is indirect because it quantitates various 2-MCPD esters, 3-MCPD esters and GEs (see Figure 4, Appendix A for structures) as total 2-MCPD, 3-MCPD and glycidol equivalents i.e. as three analytes.

Briefly, GEs are converted to 3-monobromopropanediol (3-MBPD) in an acid solution containing a bromide salt. 3-MBPD esters, together with 2- and 3-MCPD esters are then converted into the free (non-esterified) form in an acid methanolic solution and quantitated.

The method incorporated a preliminary clean-up step to remove monoacylglycerides (MAGs) from vegetable oils and infant formula lipid fractions. MAGs can react in a similar way to GEs when exposed to bromine during the indirect analysis and their presence can give a false positive result for GEs.

As a component of this survey, the Centre for Food Safety and Applied Nutrition, United States Food and Drug Administration (USFDA), Maryland, United States analysed the same samples using a direct, LC-MS based method for each ester individually (Leigh and MacMahon, 2016; MacMahon *et al.*, 2013a; MacMahon *et al.*, 2013b).

A similar method was employed for the extraction of the lipid fraction from infant formulas by both the USFDA and ESR. ESR used a method adapted from that of Leigh and MacMahon (2016) using ethyl acetate.

The development of a direct LC-MS method similar to that employed by the USFDA was considered by ESR and NZFS. Such a method would have quantitated numerous individual 3-MCPD esters and GEs potentially producing more refined toxicity data and lower LODs and LOQs. However, the direct method would have required validation for approximately forty individual analytes (compared to the indirect method’s three) at a cost which was outside the scope of this project.

The LODs and LOQs were different for both methods and are tabulated below (Table 1). ESR LODs and LOQs were determined using signal to noise (S/N) ratios for each analyte at concentrations corresponding to the lowest concentration on the standard curve in each instance. USFDA LODs and LOQs were given by S. MacMahon in personal correspondence.

While the ESR method had an LOD of total GEs in infant formula of 0.075 mg/kg, the USFDA method had a lower LOD and their results have also been employed in reference to other researchers for comparative purposes (see Table 1 below for ESR and USFDA LODs and LOQs).

Table 1: Limits of quantitation and detection for USFDA and ESR methods

Matrix	LOD and LOQ values ESR			LOD and LOQ values USFDA		
	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
Vegetable oils						
LOD	0.156	0.325	0.126	0.008	N/A	0.005
LOQ	0.521	1.083	0.421	0.027	N/A	0.017
Infant formula(1)						
LOD	0.071	0.225	0.075	0.0026	N/A	0.0017
LOQ	0.236	0.750	0.251	0.009	N/A	0.0056
Infant formula (oil/fat component)						
LOD	0.272	0.866	0.290	0.010	N/A	0.0065
LOQ	0.906	2.885	0.967	0.035	N/A	0.022

(1) ESR LOD/LOQ assumes a fat content in infant formula of 26% (w/w) i.e. the average fat content in infant formulas assayed by ESR.

2.1 SAMPLE PLAN

A total of 100 samples were acquired in New Zealand and Australia, one half from each country. Of these, a total of 44 were vegetable oil samples (*n* = 22 from each country) while the remainder (*n* = 56) were powdered infant formulas (*n* = 28 from each country).

New Zealand samples were purchased from retail outlets in Christchurch during August, September and October 2017. Retail outlets included supermarkets and specialist retailers as well as online stores (located in New Zealand). In addition, three samples of vegetable oils used in the manufacture of infant formulas, and the two formulas they were used in, were provided directly to ESR by the industry for analysis. New Zealand obtained samples were given unique identifiers 17FC0239-001 to 17FC0239-050.

Australian samples were purchased from retail outlets in the Australian Capital Territory (ACT), during August and September 2017, with several samples also purchased in New South Wales (NSW) and Victoria. Retail outlets included supermarkets, pharmacists, health food shops and other specialist retailers. Australian purchased samples were given unique identifiers 17FC0239-051 to 17FC0239-100.

As a snap-shot survey, the intent was to sample as broad a range of available products and brands and, as such, no duplicate samples were purchased by either country. Oil samples were selected based on market share data and also taking into account those types of oils reported in the literature to have particularly high levels. Infant formula samples were also selected based on market share data, but also ensuring that a wide variety of different brands and types of infant formula (e.g. standard, premium and specialised) were included.

2.2 SAMPLE PREPARATION

ESR took sub-samples from each sample. One complete set of sub-samples was dispatched to the USFDA for analysis using the direct method. Selected sub-samples of infant formulas were also dispatched to Callaghan Innovation (69 Gracefield Road, Lower Hutt 5010) for indirect GC-MS analysis. A further complete set of sub-samples of infant formula was retained by ESR (frozen at -30°C) for analysis by industry should this be desired.

The sub-samples submitted to the USFDA and Callaghan Innovation were anonymised in such a manner that no details about the sample could be determined. The USFDA results were then compared with those obtained by ESR. Results from Callaghan Innovation were not available for comparison at the time of finalising this report.

The method employed to extract fat from infant formula samples was that of Leigh and MacMahon (2016). In brief, 2 g infant formula was added to 12 ml deionised water (a ratio similar to that recommended by most infant formula manufacturers) before extraction into an equal volume of ethyl acetate. The solvent and aqueous phases were separated by centrifugation and the solvent layer removed. The extraction was repeated two more times and the solvent layers combined and evaporated under a stream of nitrogen.

2.3 SAMPLING CHARACTERISTICS

Table 2 provides a summary of the number of each type of vegetable oil sampled, by country of purchase.

Table 2: Vegetable oils by type

OIL TYPES (Percent total vegetable oil samples)	Number of samples (percent total vegetable oil samples, n = 44)	
	Australian set (n = 22)	New Zealand set (n = 22)
Almond (2)	1 (2)	0 (0)
Canola (5)	1 (2)	1 (2)
Coconut (5)	1 (2)	1 (2)
Corn (5)	1 (2)	1 (2)
Grapeseed (5)	1 (2)	1 (2)
Olive (11)	3 (7)	2 (5)
Palm (9)	2 (5)(1)	2 (5)
Peanut (5)	1 (2)	1 (2)
Rice bran (9)	2 (5)	2 (5)
Safflower (5)	1 (2)	1 (2)
Sesame (9)	2 (5)	2 (5)
Soybean (11)	2 (5)	3 (7)(2)
Sunflower (9)	2 (5)	2 (5)
Walnut (2)	1 (2)	0 (0)
Submitted by industry as an infant formula ingredient or labelled as a "vegetable oil" with no further details (9)	1 (2)	3 (7)

(1) One Australian palm oil sample was a mixture of palm and canola oils.

(2) One New Zealand soybean oil sample was a mixture comprising 90% soybean oil and 10%canola oil.

Vegetable oil and infant formula samples are also listed in Appendix D: Vegetable oil and infant formula samples according to criteria including:

Table 13. Vegetable oils by country of manufacture
Table 14. Infant formulas by country of manufacture
Table 15. Infant formulas by oil type

3. RESULTS AND DISCUSSION

3.1 ESR RESULTS

A summary of the results in vegetable oils is given in Table 3 (ESR). For full results see Appendix B, Table 9. All three samples submitted by industry as an infant formula ingredient had levels of 3-MCPD, 2-MCPD and glycidol equivalents under their respective LODs.

Table 3: Summary of vegetable oil results (ESR)

OIL TYPES (TOTAL No)	Range of values (% samples >LOD)		
	3-MCPD (mg/kg)	**2-MCPD (mg/kg)**	**Glycidol (mg/kg)**
Almond (1)	0.838 (100%)	<0.325 (0%)	<0.126 (0%)
Canola (2)	<0.156 (0%)	<0.325 (0%)	<0.126 (0%)
Coconut (2)	<0.156-1.681 (50%)	<0.325-0.908 (50%)	<0.126 (0%)
Corn (2)	<0.156-0.880 (50%)	<0.325 (0%)	<0.126-0.770 (50%)
Grapeseed (2)	3.203-5.326 (100%)	2.453-2.924 (100%)	0.903-2.927 (100%)
Olive (5)	<0.156-0.529 (60%)	<0.325-0.426 (40%)	<0.126-1.574 (20%)
Palm (4)(1)	<0.156-5.080 (50%)	<0.325-2.144 (50%)	<0.126-3.147 (75%)
Peanut (2)	<0.156 (0%)	<0.325 (0%)	<0.126-1.450 (50%)
Rice bran (4)	0.945-10.914 (100%)	0.560-4.518 (100%)	2.401-7.110 (100%)
Safflower (2)	<0.156 (0%)	<0.325 (0%)	<0.126 (0%)
Sesame (4)	<0.156-0.892 (50%)	<0.325 (0%)	<0.126 (0%)
Soybean (5)(2)	<0.156-0.755 (60%)	<0.325 (0%)	<0.126-0.732 (20%)
Sunflower (4)	<0.156 (0%)	<0.325 (0%)	<0.126-0.408 (50%)
Walnut (1)	2.087 (100%)	1.403 (100%)	0.471 (100%)
Submitted by industry as an infant formula ingredient or labelled as a "vegetable oil" with no further details (4)	<0.156 (0%)	<0.325 (0%)	<0.126 (0%)

(1) One Australian palm oil sample was a mixture of palm and canola oils.

(2) One New Zealand soybean oil sample was a mixture comprised of 90% soybean oil and 10% canola oil.

Infant formula results above the LOD (ESR) for 3-MCPD esters are given in Table 4. For full results see Appendix B, Table 10, which shows results in the oil/fat component, as well as the results for 2-MCPD esters.

Table 4: 3-MCPD esters (as free 3-MCPD) in infant formulas, ordered from highest to lowest in the fat component (>LOD of 0.272 mg/kg) (ESR)

SAMPLE No	Fat %(w/w)	3-MCPD (mg/kg) in oil/fat component	3-MCPD (mg/kg) infant formula
17FC0239-062	27.3	2.164	0.591
17FC0239-077	24.0	1.585	0.380
17FC0239-078	27.6	1.013	0.280
17FC0239-069	26.6	0.999	0.266
17FC0239-075	26.7	0.865	0.231
17FC0239-073	27.3	0.664	0.181
17FC0239-064	26.8	0.571	0.153
17FC0239-074(1)	26.5	0.571	0.151
17FC0239-022	28.1	0.540	0.152
17FC0239-001	25.1	0.502	0.126
17FC0239-068	27.8	0.482	0.134
17FC0239-076	27.5	0.426	0.117
17FC0239-023	25.3	0.354	0.090
17FC0239-024	28.0	0.348	0.097
17FC0239-072	27.9	0.345	0.096
17FC0239-071	28.1	0.295	0.083
17FC0239-060	26.7	0.286	0.076
17FC0239-061	27.7	0.284	0.079

(1) Sample had reached best before date prior to purchase

Infant formula results for GEs in powdered infant formula are given in Table 5. For full results see Appendix B Table 10 (ESR), which shows results in the oil/fat component, and Appendix C Table 11 (USFDA), which shows results for both the oil/fat component and infant formula.

Table 5: Glycidyl esters (as free glycidol) in infant formulas, ordered from highest to lowest (>LOD (ESR))

SAMPLE No	Fat %(w/w)	Glycidol (mg/kg) in oil/fat component	Glycidol (mg/kg) infant formula
17FC0239-062	27.3 28.3 (USFDA)	2.232 (ESR) 1.709 (USFDA)	0.609 (ESR) 0.484 (USFDA)
17FC0239-077	24.0 24.9 (USFDA)	0.573 (ESR) 0.538 (USFDA)	0.138 (ESR) 0.134 (USFDA)
17FC0239-027 (1)	25.4	0.333 (USFDA)	0.085 (USFDA)

(1) Analytes not detected in ESR analysis so USFDA results used.

3.2 FDA RESULTS

The USFDA results for vegetable oil and infant formula samples are given in Appendix C Table 11.

3.3 COMPARISON OF ESR AND USFDA RESULTS

The results for total fat, 3-MCPD esters and GEs (2-MCPD esters were not included in the USFDA analysis) as determined by both laboratories are discussed in sections 3.3.1-3.3.3.

3.3.1 TOTAL FAT

The inter-laboratory reproducibility for total fat determinations was estimated by calculating the precision of the measurements regarding the USFDA total fat determination as one duplicate and the ESR total fat determination on the same sample as the other for each infant formula sample (*n* = 56).

The precision of the total fat results expressed as a standard deviation was 0.73 %(w/w) fat/oil. Using a coverage factor of k = 2 this gives a 95% confidence interval of ± 1.5 %(w/w) fat/oil.

3.3.2 3-MCPD ESTERS (as free 3-MCPD)

Ideally, two different methods of quantitating the same analytes should give precisely the same results. If one set of results for the analysis of the same samples, determined by method A were plotted on a Cartesian axis against the results as determined by method B a linear regression analysis would give a regression line whose slope was 1.000, had a coefficient of determination (r^2) of 1.000 and pass precisely through the origin.

In reality this never happens, however such a comparison gives a statistical and visual indication of how equivalent any given methods are. Figures 1 & 2 below are regression comparisons of USFDA and ESR results for 3-MCPD esters (as free 3-MCPD equivalents) in vegetables oils and infant formulas respectively.

NB The LOD and LOQ for the indirect ESR method are both higher than those of the USFDA method. Only samples with results for 3-MCPD esters above the ESR LODs from both laboratories (*n* = 18 vegetable oils), (*n* = 18 infant formulas [fat/oil component]) have been compared.

Figure 1: Regression comparison – USFDA/ESR 3-MCPD fatty acid esters in vegetable oils

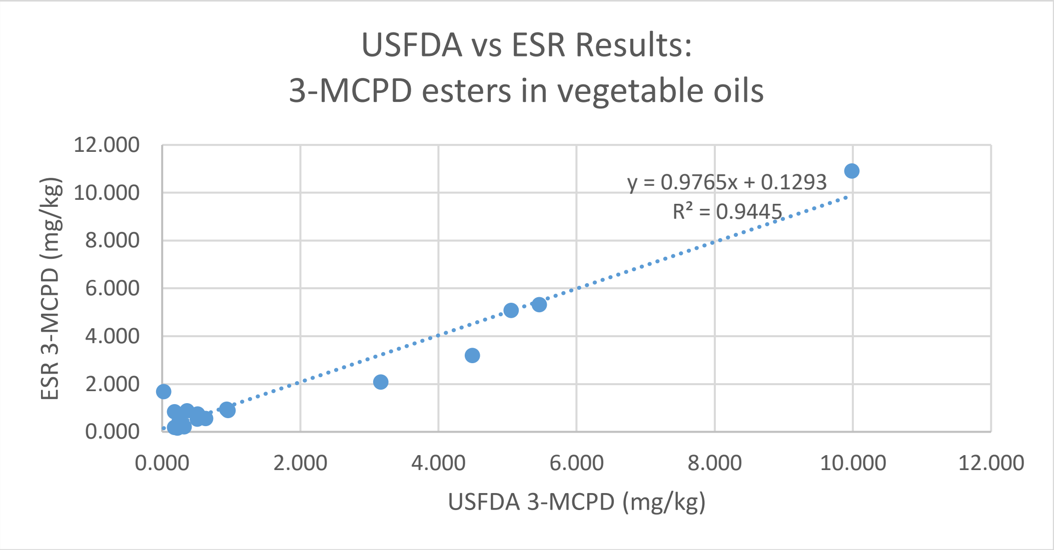
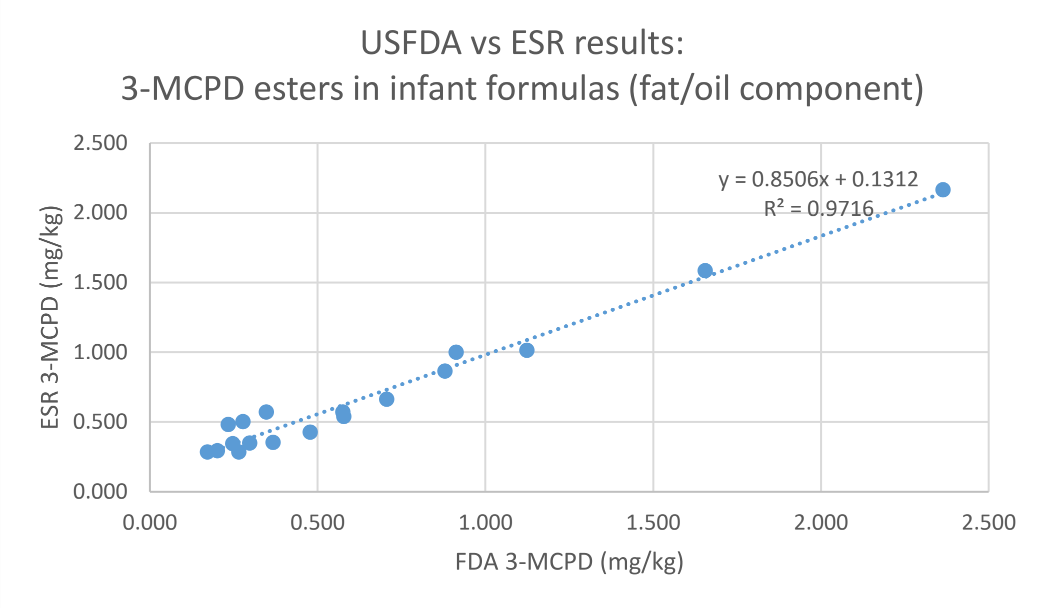


Figure 2: Regression comparison – USFDA/ESR 3-MCPD fatty acid esters in infant formulas (fat/oil component)

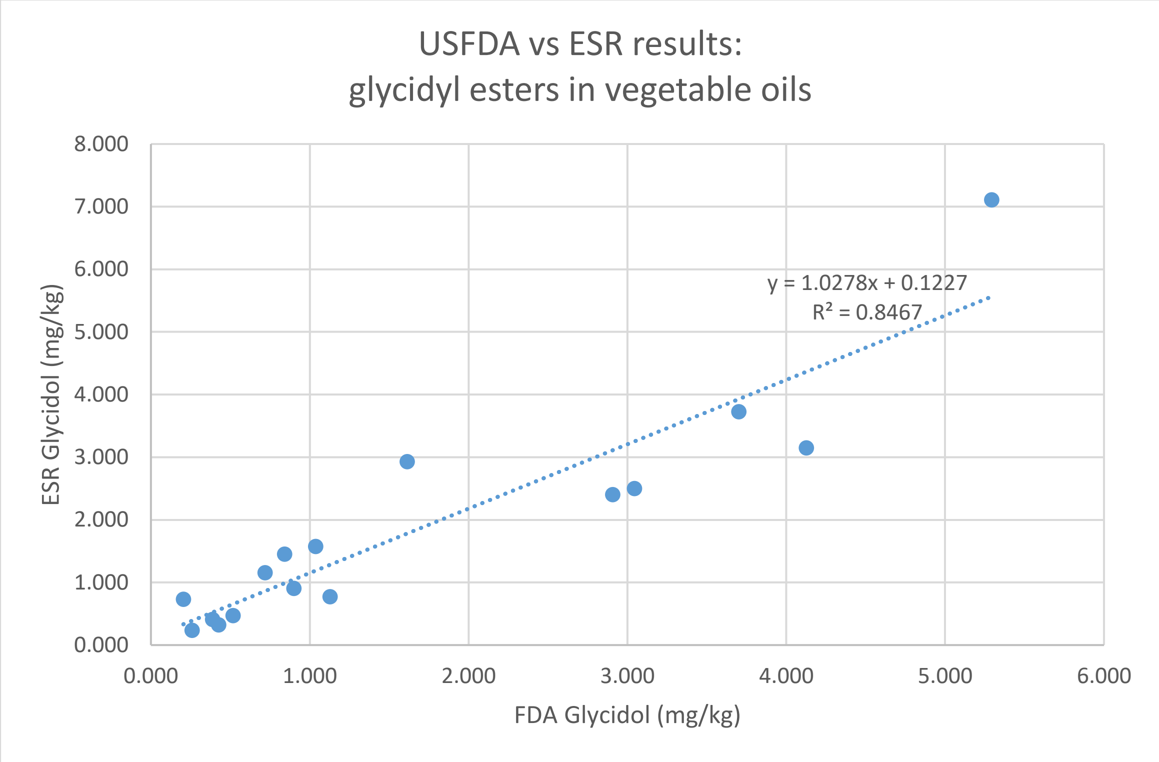


3.3.3 GLYCIDYL ESTERS (as free glycidol)

A regression comparison of USFDA and ESR results for GEs (as free glycidol equivalents) in vegetable oils was also undertaken (Figure 3). As the LOD and LOQ for the indirect ESR method are both higher than those of the USFDA direct method, only those USFDA results for which ESR results were above the ESR LOD of 0.126 mg/kg (*n* = 16 vegetable oils) have been compared with the ESR results in Figure 3.

The closeness of fit between USFDA and ESR results for total GEs in vegetable oils is (based upon the r^2 value of approximately 0.85) is not as good as that achieved for the 3-MCPD esters results in vegetable oils and infant formula (r^2 = 0.95 and 0.97 respectively). The ESR indirect method employs a procedure in the analysis that may possibly have added to the variability of the GE results. The solid phase extraction (SPE) columns used as part of the clean-up procedure are designed to remove MAGs from the samples that can give a false positive result for GE, as they can react in a similar manner to the GE epoxide ring. These steps do not affect 3-MPCD or 2-MPCD, but may add to variability in the GE results. The GE epoxide ring is also relatively unstable compared with the chlorine in 2-MCPD and 3-MCPD which may also have contributed to variability between the ESR and USFDA results for GEs.

Figure 3: Regression comparison – USFDA/ESR glycidyl fatty acid esters in vegetable oils



There were only three infant formula samples in which the GEs concentration as determined by the USFDA were above the ESR LOD of <0.290 mg/kg (for the infant formula oil/fat component). This small sample number was not considered suitable for a linear regression comparison. However, the results for the three samples from both laboratories are given below and were considered comparable within experimental variation (Table 6).

Table 6: Total glycidyl esters in the oil/fat components of infant formulas (as free glycidol), ESR results above LOD compared with USFDA results for same samples

Laboratory Sample Number	ESR result (mg/kg)	USFDA result (mg/kg)
17FC0239-027	<0.290	0.333
17FC0239-062	2.232	1.709
17FC0239-077	0.573	0.538

3.3.4 OVERALL COMPARABILITY OF ESR AND USFDA RESULTS

Based upon the high r^2 values obtained for the results shown in Section 3.3.1-3 the methods were considered highly comparable and the ESR method suitable for the determination of 3-MCPD esters and GEs.

Despite the level of comparability observed between the two methods, it should be noted that the ESR laboratory experienced difficulty with the indirect GC-MS method. The phenyl boronic acid derivatising agent was found to be highly deleterious to the equipment necessitating frequent cleaning and replacement and shortening of the GC column to maintain sensitivity.

3.4 STUDY RESULTS COMPARED WITH LITERATURE – VEGETABLE OILS

It should be noted that the number of samples assayed for each oil type in this study was not large (see Table 2 for sample numbers) and so mean concentrations should be interpreted with caution. Some broad trends could be observed from the results, however.

- Rice bran oil had the highest levels of 3-MCPD esters, 2-MCPD esters and GEs of all oil varieties examined with 100% (n = 4) of samples having values above the LOD (ESR) for these analytes. The mean concentrations of 3-MCPD esters, 2-MCPD esters and GEs were respectively 5.438 mg/kg, 2.385 mg/kg (44% of 3-MCPD ester levels) and 3.934 mg/kg (ESR).
- Grapeseed oils had the next highest levels for all analytes, with both samples having values above the LOD (ESR) in each instance. The mean (n = 2) concentrations of 3-MCPD, 2-MCPD esters and GEs were respectively 4.265 mg/kg, 2.689 (mg/kg) (63% of 3-MCPD ester levels) and 1.915 mg/kg (ESR).
- Palm oils (n = 4) had maximum values slightly less than those found in grapeseed oil (except for GE with a maximum of 3.147 mg/kg compared with a grapeseed maximum of 2.927 mg/kg). It should be noted, one sample in this group was actually a blend of palm and canola oils and has been excluded from the calculation of mean levels of 3-MCPD esters, 2-MCPD esters and GEs which were respectively 1.693 mg/kg, 0.827 mg/kg (49% of 3-MCPD ester levels) and 1.434 mg/kg (ESR).
- Unrefined oils, cold pressed and virgin oils had the lowest concentration of esters in each oil type.

3-MCPD esters

The occurrence of 3-MCPD esters in edible oils and other foodstuffs was reviewed by the European Food Safety Authority (EFSA) (2013). Low levels of 3-MCPD esters were found in virgin and unrefined oils (maximum 0.3 mg/kg). Concentrations of 3-MCPD esters in palm oils varied considerably, with reported ranges from 0.11 to 10 mg/kg (n = 6 studies published between 2007 and 2012, ESR results <0.156 to 5.08 mg/kg).

The level of 3-MCPD esters found in 'refined vegetable oils' in the EFSA review (EFSA, 2013) ranged from <0.1 to 32.62 mg/kg. The maximum concentration of 3-MCPD esters found in this study was 10.914 mg/kg in rice bran oil (ranges of concentrations for 3-MCPD esters and GEs found in selected previous studies are given in Table 7).

2-MCPD esters

The vast majority of surveys have concentrated on 3-MCPD ester and/or GE concentrations. Jedrkiewicz *et al.* (2016b) determined 2-MCPD ester concentrations in select edible oils including: corn, linseed, refined and unrefined olive, refined and unrefined rapeseed, sesame, and refined and unrefined sunflower oils. These studies found 2-MCPD ester concentrations ranged from 40 to 80% of 3-MCPD ester levels. This ratio was broadly confirmed in surveys of MCPD esters in the lipid fraction of infant formulas (Jedrkiewicz *et al.*, 2016a; Wohrlin *et al.*, 2015).

A similar ratio of 2-MCPD to 3-MCPD ester concentrations was found in the current survey of vegetable oils. In the oil varieties with the highest concentration of these analytes i.e. rice bran, palm and grapeseed oils, the ratio of 2-MCPD ester levels to 3-MCPD ester levels ranged from 44 to 76.6%.

Glycidyl esters

International studies indicate that rice oil and palm oils have the highest susceptibility to the formation of GEs. These findings are broadly in line with those of the current study. The review paper by Cheng *et al.* (2017) lists GE levels found in edible oils from a variety of geographic locations in a number of studies published between 2010 and 2016. Average concentrations of GEs in palm oils range from 2.38 to 30.2 mg/kg (*n* = 4 studies, *n* = 38 samples total) and for rice bran oils 33.7 mg/kg (*n* = 1 study, *n* = 3 samples). The single study that examined grapeseed oils found a maximum concentration of 3.02 mg/kg (Cheng *et al.*, 2017).

The maximum concentration of GEs in grapeseed oil (ESR 2.927 mg/kg) found in this study is similar to that given in Cheng *et al.* (2017) (3.02 mg/kg). The average concentrations of GEs found in rice bran (3.93 mg/kg) and palm oils (1.51 mg/kg) in this study are lower than the averages for the same oil types in the review paper. There may be a number of reasons for this. Palm oil is prone to GE formation (see Appendix A) and has perhaps been the focus of mitigation procedures in oil refining which have been shown to have an impact on GE concentrations over time. Given the very small sample numbers however this assertion should be considered highly speculative. Most of the studies in the review paper were published in 2010 and 2013. Previous studies employing certain indirect GC methods have also been shown to produce artificially elevated GE results due to the reaction of bromide with MAGs. This study employed a procedure designed to remove MAGs from oil prior to reaction with bromine.

Table 7: Summary of international vegetable oil study results range of values found

Vegetable oil type	Concentrations found (mg/kg)		Reference
	3-MCPD esters (as 3-MCPD equivalents)	**Glycidyl esters (as glycidol equivalents)**	
Almond	N/A	0.03 (*n* = 1)	Cheng *et al.* (2016) (1)
Canola	<LOQ-0.33 (*n* = 7)	0.18-0.48	MacMahon *et al.* (2013a)
Coconut	N/A 0.025-0.38 (*n* = 7) 1.418-1.694 (*n* = 2)	0.50-3.00 (*n* = 2) 0.034-1.71 N/A	Cheng *et al.* (2016) MacMahon *et al.* (2013a) EFSA (2013)
Corn	ND (*n* = 3) 0.06-0.42 (*n* = 9)	0.1-0.4 0.15-1.57	Haines *et al.* (2011) MacMahon *et al.* (2013a)
Grapeseed	0.24-3.91 (*n* = 3)	0.14-3.02	MacMahon *et al.* (2013a)
Olive	<0.1-2.462 0.15-0.73 (*n* = 5) <0.3-2.462 (*n* = 5)	N/A 0.048-1.10 N/A	Zelinkova *et al.* (2006) MacMahon *et al.* (2013a) EFSA (2013)
Palm	0-1.3 (*n* = 6) N/A 1.51-7.23 (*n* = 14) <0.11-10.00 (*n* = 378)	0.1-4.2 0.30-28.0 (*n* = 40) 0.33-10.52 (*n* = 14) N/A	Haines *et al.* (2011) Cheng *et al.* (2016) MacMahon *et al.* (2013a) EFSA (2013)
Peanut	N/A 0.14-0.69 (*n* = 3)	0.40-1.10 (*n* = 7) 0.44-0.57	Cheng *et al.* (2016) MacMahon *et al.* (2013a)
Rice bran	ND (*n* = 1) N/A	9.1 27.22-28.76 (*n* = 3)	Haines *et al.* (2011) Cheng *et al.* (2016)
Safflower	0.28-1.77 (*n* = 5)	0.065-0.44	MacMahon *et al.* (2013a)
Sesame	ND (*n* = 2) <0.3-0.337 (*n* = 2) N/A	0.3-1.0 N/A 1.30-3.70 (*n* = 3)	Haines *et al.* (2011) Zelinkova *et al.* (2006) Cheng *et al.* (2016)
Soybean	ND (*n* = 2) 1.234 (*n* = 1) 0.041-0.24 (*n* = 6)	0.2-0.7 N/A 0.014-0.50	Haines *et al.* (2011) Zelinkova *et al.* (2006) MacMahon *et al.* (2013a)

Vegetable oil type	Concentrations found (mg/kg)		Reference
	3-MCPD esters (as 3-MCPD equivalents)	Glycidyl esters (as glycidol equivalents)	
Sunflower	N/A (*n* = 11) 0.19-0.93 (*n* = 4)	0.02-0.90 0.012-0.90	Cheng *et al.* (2016) MacMahon *et al.* (2013a)
Walnut	ND (*n* = 1) N/A 0.63 (*n* = 1)	0.3 0.70-1.40 (*n* = 5) 0.59	Haines *et al.* (2011) Cheng *et al.* (2016) MacMahon *et al.* (2013a)
Fish oil[(2)]	1.5-13 (*n* = 5) 0.7-13.0 (*n* = 7)	N/A N/A	Jedrkiewicz *et al.* (2016b) EFSA (2013)
Refined edible oils	0.2-14.7 (*n* = 144) 0.26-0.30 (*n* = 3) <LOD-32.62 (*n* = 7)	N/A N/A N/A	Weisshaar (2011) Jedrkiewicz *et al.* (2016b) EFSA (2013)

(1) Cheng *et al.* (2016) and EFSA (2013) reviewed a number of different studies. In each instance the range of values given may be the combined findings of a number of studies.

(2) Fish oil has been included in this list as such oils have been added to some infant formulas in this survey.

3.5 STUDY RESULTS COMPARED WITH LITERATURE – INFANT FORMULAS

3-MCPD esters

A total of 18 samples (32%) of infant formula samples had concentrations of 3-MCPD esters above the detection limit of 0.071 mg/kg in infant formula (ESR analysis). Of these the New Zealand sourced samples (*n* = 4) ranged in value from 0.089 to 0.152 mg/kg (average 0.096 mg/kg) and those sourced from Australia (*n* = 14) ranged from 0.076 to 0.591 mg/kg (average 0.201 mg/kg). The average of all USFDA results (*n* = 56) for 3-MCPD esters in infant formulas was 0.092 mg/kg (range 0.005 to 0.669 mg/kg). New Zealand sourced samples average was 0.060 mg/kg (range 0.014 to 0.164 mg/kg). Australian sourced samples average was 0.124 mg/kg (range 0.005 to 0.669 mg/kg). Infant formula products were purchased in both countries, however it should be noted that the country of purchase does not reflect the country the products were produced in.

Infant formulas International surveys

Czech Republic

Zelinkova *et al.* (2009) examined a variety of infant formulas (*n* = 14) purchased in Prague in 2006 for both free and bound 3-MCPD. Free 3-MCPD was not detected in any of the samples but bound 3-MCPD esters were detected with concentrations ranging from 0.062 to 0.558 mg/kg (mean 0.289 mg/kg) in infant formulas.

Germany

Weisshaar (2011) examined the levels of 3-MCPD esters, 2-MCPD esters and GEs in infant formulas from three separate surveys in which samples were acquired in April 2009, October 2009 and May 2010 (*n* = 40). It was noted that the levels of 3-MCPD esters changed little over this time period from an average of approximately 0.550 mg/kg in infant formula in April 2009 to 0.475 mg/kg in May 2010 (assuming a 25% fat content).

Another German study by Wohrlin *et al.* (2015) found average concentrations of 3-MCPD esters in infant formula (*n* = 70) of 0.109 mg/kg (range of averages from different manufacturers, *n* = 5 samples per manufacturer was 0.067 to 0.177 mg/kg, ranges of concentrations 0.048 to 0.305 mg/kg).

Poland

Jedrkiewicz *et al.* (2016a) examined 24 samples of mixed infant formula varieties from seven different manufacturers obtained in the Polish market with most of the formulas obtained also available in the European market. The concentration of 3-MCPD esters in infant formula was found to range from 0.15 mg/kg to 0.95 mg/kg (2-MCPD esters ranged from 0.0125 to 0.3 mg/kg).

United States

Leigh and MacMahon (2017) examined 98 infant formula samples purchased in the United States from seven different manufacturers. Concentrations of total bound 3-MCPD esters ranged from 0.021 to 0.92 mg/kg in infant formulas. Average results for individual manufacturers ranged from 0.055 to 0.62 mg/kg with a median of averages for the seven manufacturers of 0.26 mg/kg.

Canada

Becalski *et al.* (2015) examined 20 powdered infant formula samples purchased in Canada in 2012 and 2013. The range of 3-MCPD ester concentrations found were <0.006 to 0.089 (n = 10, average = 0.051 mg/kg) in 2012 and <0.006 to 0.080 mg/kg (n = 10, average = 0.039 mg/kg) in 2013 in infant formulas.

Summary

The average concentration of 3-MCPD esters found in the current survey by both ESR and the USFDA are similar to average results found in surveys of European and American powdered infant formulas.

Surveys conducted earlier than 2009 have not been included in the comparisons above as analytical methodological issues such as the use of sodium chloride and alkaline transesterification can convert GEs to MCPD esters resulting in an overestimation of the latter.

2-MCPD esters

Some of the surveys listed above examined concentrations of 2-MCPD esters. Wohrlin *et al.* (2015) found average concentrations of 2-MCPD esters in infant formula (n = 70) of 0.044 mg/kg (range of averages from different manufacturers of 0.023 to 0.071 mg/kg with a maximum concentration of 0.58 mg/kg in the lipid fraction of one sample which equates to approximately 0.145 mg/kg in infant formula). Levels of 2-MCPD esters found in this study were approximately 45% of 3-MCPD ester concentrations. A similar ratio was also found by Jedrkiewicz *et al.* (2016a) who found 2-MCPD ester levels corresponded to 30-50% of 3-MCPD ester content.

The LOD for the ESR method for 2-MCPD esters in infant formulas was <0.225 mg/kg and <0.866 in the oil/fat component. No 2-MCPD esters were detected in the infant formula samples and only two samples had detections in the oil/fat component of the infant formula. (USFDA did not test for 2-MCPD esters).

Glycidyl esters

Of the 56 samples of infant formulas examined two were found to have GE concentrations above the ESR LOD of <0.290 mg/kg in infant formula oil/fat (or 0.075 mg/kg in infant formula). The USFDA method had a lower LOD for GE in infant formula (0.0017 mg/kg).

The USFDA found a mean level of GE in the infant formula samples (entire component, not just the fat/oil component) of 0.026 mg/kg and a median of 0.013 mg/kg (range ND – 0.484 mg/kg, n = 56). New Zealand sourced samples had an average concentration of 0.017 mg/kg in infant formula (range <LOD to 0.085 mg/kg, *n* = 28) and Australian sourced samples had an average concentration of 0.034 mg/kg (range 0.002 to 0.484 mg/kg, *n* = 28). Infant formula products were purchased in both countries, however it should be noted that the country of purchase does not reflect the country the products were produced in.

Infant formula international surveys

<u>Europe</u>

In most European studies, while samples may have been purchased in a particular European Union (EU) member state, it was noted by most authors that the products acquired were typical of those available throughout the EU.

Researchers did not report results in a uniform manner. Some reported levels of 2-MCPD esters, 3-MCPD esters and GEs in the lipid fraction for infant formulas and some in the infant formula, neither were ranges necessarily reported. Where possible the author has adjusted results reported as mg/kg in the lipid fraction to the concentration in the infant formula generally assuming a fat content of approximately 25%(w/w).

<u>Germany</u>

In the Weishaar study (2011), in which samples were acquired in April 2009, October 2009 and May 2010 (a total of 40 samples in all), between surveys the levels of GEs decreased substantially from an average of approximately 0.375 mg/kg in infant formula in April 2009 to 0.275 mg/kg in May 2010 (assuming a 25% fat content).

Another German study by Worhlin *et al.* (2015) examined 70 infant formula samples purchased in Berlin (between January and March 2013, 7 different manufacturers, two types of product from each and five lots of each product). The study found average GE levels of 0.36 mg/kg (maximum level of 1.3 mg/kg) in the lipid fraction. The average concentration of GE in infant formula was 0.088 mg/kg (range of averages from different manufacturers of 0.032 to 0.213 mg/kg as glycidol).

<u>United States</u>

Leigh and MacMahon (2017) examined 98 infant formula samples purchased in the United States from seven different manufacturers. Concentrations of GEs ranged from <LOQ to 0.40 mg/kg in infant formulas. Average results for individual manufacturers ranged from 0.005 to 0.36 mg/kg GE with a median of averages for the seven manufacturers of 0.093 mg/kg.

<u>Canada</u>

Becalski *et al.* (2015) examined 20 powdered infant formula samples purchased in Canada in 2012 and 2013. The range of GE concentrations measured was <0.010 to 0.070 (*n* = 10, average = 0.024 mg/kg) in 2012 and <0.010 to 0.040 mg/kg (*n* = 10, average = 0.020 mg/kg) in 2013 in infant formulas.

<u>Summary</u>

The concentrations of GE found in infant formulas by the USFDA in the current study are, on average, at the low end of those found in studies conducted on samples sourced in North America and Europe. Results from the current study were most similar to Becalski *et al.* (2015). USFDA results have been employed in making these comparisons as the ESR indirect method, having a higher LOD, did not have enough data points for such a comparison.

Ranges of concentrations for 3-MCPD esters and GEs found in selected previous studies are given in Table 8.

Table 8: Summary of international infant formula study results (Mean results except where otherwise indicated with ranges where available)

	ESR/USFDA	**Zelinkova *et al.* (2009)**	**Weisshaar *et al.* (2011)**	**Wohrlin *et al.* (2015)**	**Leigh and MacMahon (2017)**	**Jedrkiewicz *et al.* (2016a)**	**Becalski *et al.* (2015)**
Year of sampling	2017	2006	2009-2010	2015	2013-2016	2016	2012/2013
Sample purchase area	Australia/New Zealand	Prague	Germany	Germany	United States	Poland	Canada
Sample No:	56	14	40	70	98	24	20 (n = 10 each year)
3-MCPD (mg/kg) in infant formulas	0.117 (<0.071 to 0.152, NZ) 0.201 (<0.071 to 0.591, AU) (ESR results) All samples 0.092 (0.005 to 0.669 USFDA)	0.289 (0.062 to 0.588)	0.550 (0.15 to 0.825)	0.109 (0.048 to 0.305 assuming 25% fat in infant formula)	Means of 7 different manufacturers 0.055 to 0.62 mg/kg (range of concentrations for all samples 0.021 to 0.92)	Range of concentrations found 0.15 to 0.95 mg/kg	0.051 (<0.006 to 0.089, 2012) 0.039 (<0.006 to 0.080, 2013)
Glycidol (mg/kg) in infant formulas	0.017 (<LOD to 0.085, NZ) 0.034 (0.002 to 0.484, AU) All samples 0.026 (<LOD to 0.484 USFDA)	N/A	(Mean range 0.1 to 0.375 (<0.025 to 1.325)	0.088 (0.325 maximum)	Means of 7 different manufacturers 0.008 to 0.36 (range of concentrations over all samples 0.005 to 0.40 mg/kg)	N/A	0.024 (0.003 to 0.070, 2012) 0.020 (0.002 to 0.040, 2013)

4. DISCUSSION

The findings from this study indicate:

- The methods employed by the USFDA and ESR to determine total fat in infant formula give very similar results with a 95% confidence of ± 1.5 %(w/w) fat/oil.
- The USFDA direct method had lower LOD and LOQ concentrations than the indirect ESR method for the determination of 3-MCPD esters and GEs.
- The direct (USFDA) and indirect (ESR) methods for the determination of 3-MCPD esters and GEs give similar results for both vegetable oils and infant formulas where the results were above the ESR LOD (noting that for infant formula, this refers to the ESR LOD for the oil/fat component). Correlation coefficients (r^2) for 3-MCPD esters between method results were calculated to be 0.9445 (vegetable oils), and 0.9716 (infant formula lipid fractions). For GEs the correlation coefficient of determination was 0.8467 (vegetable oils).
- The oil types having the highest concentrations of the analytes examined were rice bran, grapeseed and palm oils. This finding is similar to that found in previous international surveys. Grapeseed oil samples (*n* = 2) were found to have concentrations similar to those of palm oil samples. The one published study of grapeseed oil found levels of GEs similar to those found in the current survey.
- Maximum concentrations of 3-MCPD esters and GEs reported in some previous edible oil surveys were substantially higher (>30 mg/kg each) than the maximum concentrations found in the current survey (10.914 mg/kg 3-MCPD esters and 7.110 mg/kg GEs in rice bran oil – ESR). The results for the current survey were generally consistent with findings of previous surveys.
- The ratio of 2-MCPD ester to 3-MCPD ester concentrations found in previous studies are consistent with the ratios observed for vegetable oils in this survey.
- Mean concentrations of GEs found in infant formulas were low compared with recent studies conducted on samples sourced from Europe and the United States and only two were found to have concentrations above the ESR LOD. The mean concentration was 0.026 mg/kg in infant formula (USFDA analysis in current study).
- Mean concentrations of 3-MCPD esters in infant formulas were low compared with those found in recent surveys conducted on samples from Europe and the United States. The USFDA LOD was lower, and only 18 samples were found to have concentrations above the ESR LOD. The mean concentration was 0.092 mg/kg in infant formula (USFDA).
- A total of 8 infant formula samples reported containing palm oils (including palm olein or palm kernel oil). Six of these also had 3-MCPD ester concentrations above the LOD for infant formula for the ESR indirect method.
- New Zealand sourced samples of infant formula had mean 3-MCPD ester levels of 0.060 mg/kg (range 0.014 to 0.164 mg/kg USFDA).
- New Zealand sourced samples of infant formula had mean GE levels of 0.017 mg/kg (range <LOD to 0.085 mg/kg USFDA).

- Australian sourced samples of infant formula had mean 3-MCPD ester levels of 0.124 mg/kg (range 0.005 to 0.669 mg/kg USFDA).
- Australian sourced samples of infant formula had mean GE levels of 0.034 mg/kg (range 0.002 to 0.484 mg/kg USFDA).
- Infant formula products were purchased in both countries, however it should be noted that the country of purchase does not reflect the country the products were produced in.
- Two infant formula manufacturers supplied infant formula samples along with the vegetable oils added to them for the purposes of comparison (Appendix C Table 12). Unfortunately, little could be concluded from these results as one manufacturer did not submit samples of all oils added to their product (17FC0239-028). The other sample had comparable GE concentrations in the infant formula oil component and the oil added to it. The 3-MCPD ester concentration in the added oil however was substantially higher than that found in the infant formula oil component.
- Results from analyses by Callaghan Innovation or the infant formula industry were not available for comparison with the results obtained in this survey. The analyst at Callaghan Innovation indicated they were having difficulties with their GC based method and we should issue our report without their results.
- It was noted that fish-derived oils were listed as ingredients in 4 out of 9 samples of infant formula with 3-MCPD ester concentrations exceeding the ESR LOD. Previous studies (Table 7) have found concentrations of 3-MCPD esters in fish oils comparable to those found in vegetable oils.

CONCLUSIONS

In the current survey of 3-MCPD esters and GEs, concentrations (mean values and maximum concentrations found) in vegetable oils sourced in New Zealand and Australia appear lower than those found in other recent international surveys. This observation should be viewed with caution given the small sample numbers investigated for any given oil type in these surveys.

The lower concentrations measured in the current survey than recent international surveys may reflect the effect of mitigation procedures developed by industry, such as short chain distillation to avoid the formation of 3-MCPD esters and GEs, especially in palm derived oils.

Of interest were the levels of analytes found in the two grapeseed samples. These were similar to some of the palm oil and rice bran oil concentrations for 3-MCPD esters and GEs. This finding may reflect the lack of attention paid to this oil type in most studies.

The levels of 3-MCPD esters in infant formulas were, on average, comparable to other recent studies examining samples sourced in Europe and the United States. It was noted that 6 out of 8 samples reported to contain palm-derived oils had concentrations of 3-MCPD esters above the ESR LOD.

The levels of GEs in infant formulas were, on average lower than those found in recent studies examining samples sourced in Europe and the United States.

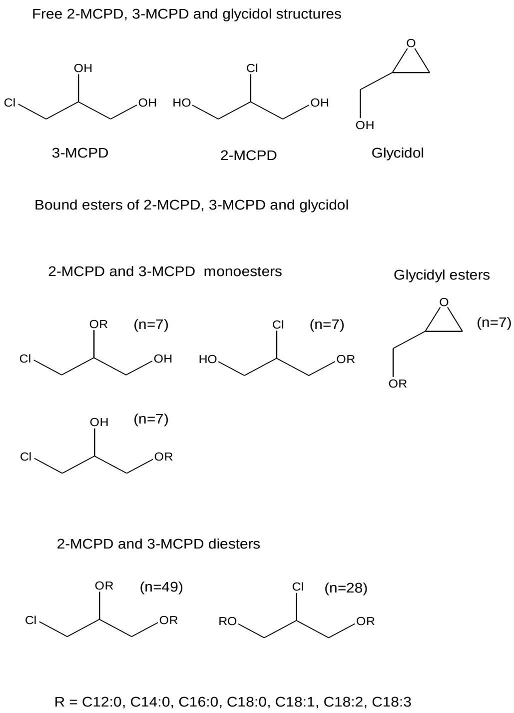
APPENDIX A: MCPD & GLYCIDYL ESTERS FORMATION AND MITIGATION

3-MCPD, 2-MCPD, glycidol and their esters are processing contaminants that are formed during decolourisation and deodorising in the manufacture of vegetable oils. Vegetable oils are commonly used as an ingredient in margarines, cooking oils and infant formulas. Research has shown that the esters are metabolised back to the parent compounds 3-MCPD and glycidol after ingestion, and these parent compounds have been shown to be potentially carcinogenic for humans.

Experiments indicate that 3-MCPD arises when either acylglycerols or glycerol react with endogenous or added sodium chloride (salt) with resultant levels depending strongly on temperature, lipid, glycerol, salt and water content (IARC, 2013; Zelinkova *et al.*, 2006). Another product of the reaction between chloride and acyl glycerols or glycerol is 2-monchloropropanediol (2-MPCD), although less is known about the toxicity of this compound. Glycidyl esters (GE) are formed from acyl glycerols during high temperature treatment of oils. 2-MPCD and 3-MPCD may inter-convert via GE as an intermediate (Cheng *et al.*, 2017).

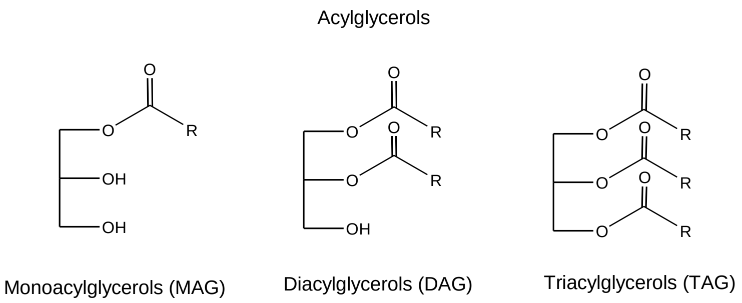
MCPD esters and GEs are formed as a result of chemical reactions between acylglycerol precursors and chloride, mainly during the deodorisation step. According to Kellen and De Greyt (2016) MCPD esters can begin to form at temperatures >140°C. GEs are also formed under similar conditions in the refining of vegetable oils but from different precursors and mechanisms. GEs can form below 200°C with levels increasing with deodorization time but the process accelerates sharply at temperatures >200°C (Arisseto *et al.*, 2017; Cheng *et al.*, 2016; Lacoste, 2014; Leigh and MacMahon, 2017).

Figure 4: Structures of free and bound 2-MCPD, 3-MCPD and glycidol



The main factors for the formation of 2-MCPD and 3-MCPD esters are the presence of chloride ions, glycerol, tri-, di- and monoacylglycerides (TAG, DAG and MAG respectively, Figure 5), as well as temperature and time. The levels of 2-MCPD esters in edible oils occur at approximately half the levels of 3-MCPD esters (Craft *et al.*, 2013). Increasing amounts of MAG and DAG in oil show a linear correlation with the increased formation of 3-MCPD esters (Haines *et al.*, 2011). In refined fats and oils, the highest concentrations of bound 3-MCPD esters are found in palm oil and palm oil-based fats. 3-MCPD esters are formed primarily from TAG and may be initiated by hydrochloric acid, released by thermal degradation of chlorine-donor molecules. 3-MCPD esters are also formed from DAG but in smaller quantities at a slower rate. GEs are formed from DAG by elimination of fatty acids at relatively high temperatures whereas formation from TAG is not significant. Palm oil is naturally rich in DAGs which make up between 4 and 12% of its composition, consequently palm fruit must be processed swiftly as DAG content further increases between ripening and processing (Crews, 2012; IARC, 2013)

Figure 5: Acylglycerol structures



After mechanical or solvent extraction most vegetable oils are not suitable for human consumption or industrial food production. This is because various associated minor compounds extracted with the oil during processing impact sensory, nutritional and technological properties (Pudel *et al.*, 2016). The major procedures are listed briefly below:

- Processing. Oilseeds are generally cleaned of foreign matter before dehulling. Kernels are ground to reduce their size and cooked with steam (inactivating lipolytic enzymes that can rapidly degrade the oil). The oil is extracted with a screw or hydraulic press and residual oil is extracted from the ‘cake’ using solvents such as food grade hexane.
- Refining. Produces an edible oil with characteristics consumers desire such as bland flavour and odour, clear appearance, light colour, stability to oxidation and suitability for frying. Classical alkaline refining method usually comprises the following steps:
    - Degumming with water to remove easily hydratable phospholipids and metals
    - Addition of small amounts of phosphoric or citric acid to convert non-hydratable phospholipids into hydratable phospholipids
    - Neutralising free fatty acids with slight excess of sodium hydroxide (NaOH) solution followed by the washing out of soaps formed by addition of NaOH and hydrated phospholipids
    - Bleaching with natural or acid-activated clay minerals to absorb colouring components (e.g. carotene, chlorophyll) and to decompose hydroperoxides

- Deodorising to remove volatile components, mainly aldehydes and ketones, with low threshold values for detection by taste or smell. Deodorisation is essentially a steam distillation process carried out at low pressure and elevated temperatures (180-220°C). In physical refining fatty acids are removed by a steam distillation process similar to the one described above. In practice a maximum temperature of 240-250°C is sufficient to reduce the free fatty acid content to levels of approximately 0.05-0.1 %(w/w) (FAO/WHO, 1993).

It has been determined which stages of edible oil refining are responsible for the formation of 3-MCPD and GEs, and methods for mitigating their formation. Intrinsic components of the oil itself could be precursors to 3-MCPD ester formation, with their production favoured in the presence of heat. When these precursors are removed or reduced the level of 3-MCPD esters formed was also reduced (Zulkurnain *et al.*, 2013).

The formation mechanisms for GEs are different from those of 3-MCPD esters. GEs are formed primarily during the deodorisation step. GE concentration in edible oils increases continuously and significantly with increasing deodorisation time below 200°C. The presence of DAGs and MAGs also contribute significantly to GE levels (Cheng *et al.*, 2016).

Mitigating the formation of 3-MCPD esters and GEs is a complex balance between the chemical and physical aspects of oil refinement and their impact on ester formation, undesirable flavours, colours and shelf-life. New processes such as ‘short path distillation’, which operate at a lower temperature than regular procedures, have resulted in levels of 3-MCPD esters and GEs in refined palm oil reduced by 30% and 50% respectively from 2010 to 2015 (Kellens and De Greyt, 2016).

APPENDIX B: SAMPLE DETAILS AND INDIVIDUAL RESULTS (ESR)

Table 9: Sample details and results for vegetable oils (ESR)

Sample number[1]	Matrix	Oil type	Results			
			Fat (% w/w)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-029	Vegetable oil	Canola	N/A	<0.156	<0.325	<0.126
17FC0239-030	Vegetable oil	Rice bran	N/A	0.945	0.560	3.727
17FC0239-031	Vegetable oil	Sunflower	N/A	<0.156	<0.325	<0.126
17FC0239-032	Vegetable oil	Coconut	N/A	<0.156	<0.325	<0.126
17FC0239-033	Vegetable oil	Olive	N/A	0.349	<0.325	<0.126
17FC0239-034	Vegetable oil	Olive	N/A	0.529	0.413	1.574
17FC0239-035	Vegetable oil	Peanut	N/A	<0.156	<0.325	<0.126
17FC0239-036	Vegetable oil	Sunflower	N/A	<0.156	<0.325	<0.126
17FC0239-037	Vegetable oil	Soybean	N/A	<0.156	<0.325	<0.126
17FC0239-038	Vegetable oil	Unknown	N/A	<0.156	<0.325	<0.126
17FC0239-039	Vegetable oil	Soybean 90%, canola 10%	N/A	0.755	<0.325	<0.126
17FC0239-040	Vegetable oil	Grapeseed	N/A	5.326	2.924	2.927
17FC0239-041	Vegetable oil	Rice bran	N/A	10.914	4.518	7.110
17FC0239-042	Vegetable oil	Unknown	N/A	<0.156	<0.325	<0.126
17FC0239-043	Vegetable oil	Palm	N/A	<0.156	0.338	1.155
17FC0239-044	Vegetable oil	Palm	N/A	<0.156	<0.325	<0.126
17FC0239-045	Vegetable oil	Sesame	N/A	<0.156	<0.325	<0.126
17FC0239-046	Vegetable oil	Safflower	N/A	<0.156	<0.325	<0.126
17FC0239-047	Vegetable oil	Soybean	N/A	0.565	<0.325	<0.126
17FC0239-048	Vegetable oil	Sesame	N/A	<0.156	<0.325	<0.126
17FC0239-049	Vegetable oil	Corn	N/A	0.880	<0.325	0.770
17FC0239-050	Vegetable oil	Unknown	N/A	<0.156	<0.325	<0.126
17FC0239-079	Vegetable oil	Olive	N/A	<0.156	<0.325	<0.126
17FC0239-080	Vegetable oil	Olive	N/A	0.189	<0.325	<0.126
17FC0239-081	Vegetable oil	Olive	N/A	<0.156	0.426	<0.126
17FC0239-082	Vegetable oil	Canola and palm	N/A	0.162	<0.325	0.233
17FC0239-083	Vegetable oil	Palm	N/A	5.080	2.144	3.147
17FC0239-084	Vegetable oil	Rice bran	N/A	4.743	2.081	2.401
17FC0239-085	Vegetable oil	Rice bran	N/A	5.151	2.380	2.499
17FC0239-086	Vegetable oil	Walnut	N/A	2.087	1.403	0.471

Sample number[1]	Matrix	Oil type	Results			
			Fat (% w/w)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-087	Vegetable oil	Unknown	N/A	<0.156	<0.325	<0.126
17FC0239-088	Vegetable oil	Grapeseed	N/A	3.203	2.453	0.903
17FC0239-089	Vegetable oil	Peanut	N/A	<0.156	<0.325	1.450
17FC0239-090	Vegetable oil	Corn	N/A	<0.156	<0.325	<0.126
17FC0239-091	Vegetable oil	Canola	N/A	<0.156	<0.325	<0.126
17FC0239-092	Vegetable oil	Soybean	N/A	0.218	<0.325	<0.126
17FC0239-093	Vegetable oil	Soybean	N/A	<0.156	<0.325	0.732
17FC0239-094	Vegetable oil	Coconut	N/A	1.681	0.908	<0.126
17FC0239-095	Vegetable oil	Sunflower	N/A	<0.156	<0.325	0.319
17FC0239-096	Vegetable oil	Sunflower	N/A	<0.156	<0.325	0.408
17FC0239-097	Vegetable oil	Safflower	N/A	<0.156	<0.325	<0.126
17FC0239-098	Vegetable oil	Sesame	N/A	0.892	<0.325	<0.126
17FC0239-099	Vegetable oil	Sesame	N/A	0.410	<0.325	<0.126
17FC0239-100	Vegetable oil	Sweet almond	N/A	0.838	<0.325	<0.126

(1) On arrival at ESR, all samples purchased were designed unique identifiers from 17FC0239-001 to 17FC0239-100.

Table 10: Sample details and results for infant formulas (ESR)

Sample number[1]	Matrix	Results (in oil/fat component)[2]			
		Fat (% w/w)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-001	Infant formula	25.140	0.502	<0.866	<0.290
17FC0239-002	Infant formula	27.089	<0.272	<0.866	<0.290
17FC0239-003	Infant formula	27.041	<0.272	<0.866	<0.290
17FC0239-004	Infant formula	24.746	<0.272	<0.866	<0.290
17FC0239-005	Infant formula	24.888	<0.272	<0.866	<0.290
17FC0239-006	Infant formula	28.570	<0.272	<0.866	<0.290
17FC0239-007	Infant formula	25.843	<0.272	<0.866	<0.290
17FC0239-008	Infant formula	28.870	<0.272	<0.866	<0.290
17FC0239-009	Infant formula	24.896	<0.272	<0.866	<0.290
17FC0239-010	Infant formula	21.891	<0.272	<0.866	<0.290
17FC0239-011	Infant formula	27.217	<0.272	<0.866	<0.290
17FC0239-012	Infant formula	23.770	<0.272	<0.866	<0.290
17FC0239-013	Infant formula	27.869	<0.272	<0.866	<0.290
17FC0239-014	Infant formula	21.533	<0.272	<0.866	<0.290
17FC0239-015[3]	Infant formula	27.194	<0.272	<0.866	<0.290
17FC0239-016	Infant formula	25.880	<0.272	<0.866	<0.290

Sample number[1]	Matrix	Results (in oil/fat component)[2]			
		Fat (% w/w)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-017	Infant formula	25.863	<0.272	<0.866	<0.290
17FC0239-018	Infant formula	25.684	<0.272	<0.866	<0.290
17FC0239-019	Infant formula	26.966	<0.272	<0.866	<0.290
17FC0239-020	Infant formula	26.108	<0.272	<0.866	<0.290
17FC0239-021	Infant formula	24.794	<0.272	<0.866	<0.290
17FC0239-022	Infant formula	28.068	0.540	<0.866	<0.290
17FC0239-023	Infant formula	25.281	0.354	<0.866	<0.290
17FC0239-024	Infant formula	27.969	0.348	<0.866	<0.290
17FC0239-025	Infant formula	23.989	<0.272	<0.866	<0.290
17FC0239-026	Infant formula	26.863	<0.272	<0.866	<0.290
17FC0239-027	Infant formula	23.608	<0.272	<0.866	<0.290
17FC0239-028	Infant formula	23.541	<0.272	<0.866	<0.290
17FC0239-051	Infant formula	25.014	<0.272	<0.866	<0.290
17FC0239-052	Infant formula	26.096	<0.272	<0.866	<0.290
17FC0239-053	Infant formula	21.925	<0.272	<0.866	<0.290
17FC0239-054	Infant formula	24.155	<0.272	<0.866	<0.290
17FC0239-055	Infant formula	25.474	<0.272	<0.866	<0.290
17FC0239-056	Infant formula	23.374	<0.272	<0.866	<0.290
17FC0239-057	Infant formula	25.837	<0.272	<0.866	<0.290
17FC0239-058	Infant formula	27.912	<0.272	<0.866	<0.290
17FC0239-059	Infant formula	28.163	<0.272	<0.866	<0.290
17FC0239-060	Infant formula	26.655	0.286	<0.866	<0.290
17FC0239-061	Infant formula	27.674	0.284	<0.866	<0.290
17FC0239-062	Infant formula	27.264	2.164	0.698	2.232
17FC0239-063	Infant formula	26.448	<0.272	<0.866	<0.290
17FC0239-064	Infant formula	26.814	0.571	<0.866	<0.290
17FC0239-065	Infant formula	28.475	<0.272	<0.866	<0.290
17FC0239-066	Infant formula	28.552	<0.272	<0.866	<0.290
17FC0239-067	Infant formula	25.064	<0.272	<0.866	<0.290
17FC0239-068	Infant formula	27.770	0.482	<0.866	<0.290
17FC0239-069	Infant formula	26.586	0.999	<0.866	<0.290
17FC0239-070	Infant formula	27.031	<0.272	<0.866	<0.290
17FC0239-071	Infant formula	28.086	0.295	<0.866	<0.290
17FC0239-072	Infant formula	27.939	0.345	<0.866	<0.290
17FC0239-073	Infant formula	27.334	0.664	<0.866	<0.290
17FC0239-074[3]	Infant formula	26.544	0.571	<0.866	<0.290
17FC0239-075	Infant formula	26.740	0.865	<0.866	<0.290
17FC0239-076	Infant formula	27.469	0.426	<0.866	<0.290
17FC0239-077	Infant formula	24.013	1.585	0.894	0.573

Sample number[1]	Matrix	Results (in oil/fat component)[2]			
		Fat (% w/w)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-078	Infant formula	27.570	1.013	<0.866	<0.290

(1) On arrival at ESR, all samples purchased were designed unique identifiers from 17FC0239-001 to 17FC0239-100.

(2) Infant formula results are given as 3-MCPD, 2-MCPD and Glycidyl esters (as free 3-MCPD, 2-MCPD and glycidol equivalents) in the oil/fat component, not the original product.

(3) Sample had reached best before date prior to purchase

APPENDIX C: USFDA RESULTS

Table 11: Results (USFDA)

Sample number	Matrix	Results in oils and oil/fat extracts (in infant formula)		
		Fat (% w/w)	3-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-001	Infant formula	25.4	0.277 (0.071)	0.005 (0.001)
17FC0239-002	Infant formula	27.4	0.270 (0.062)	0.071 (0.020)
17FC0239-003	Infant formula	27.3	0.208 (0.057)	0.038 (0.010)
17FC0239-004	Infant formula	24.5	0.224 (0.055)	0.020 (0.005)
17FC0239-005	Infant formula	26.6	0.214 (0.057)	0.037 (0.010)
17FC0239-006	Infant formula	28.8	0.139 (0.040)	0.071 (0.020)
17FC0239-007	Infant formula	28.1	0.285 (0.080)	0.100 (0.028)
17FC0239-008	Infant formula	29.2	0.117 (0.034)	0.049 (0.014)
17FC0239-009	Infant formula	25.1	0.090 (0.023)	0.032 (0.008)
17FC0239-010	Infant formula	22.1	0.290 (0.064)	0.144 (0.025)
17FC0239-011	Infant formula	27.5	0.144 (0.040)	0.090 (0.025)
17FC0239-012	Infant formula	24.3	0.256 (0.062)	0.021 (0.005)
17FC0239-013	Infant formula	27.9	0.119 (0.033)	0.000 (0.000)
17FC0239-014	Infant formula	21.8	0.157 (0.034)	0.033 (0.007)
17FC0239-015	Infant formula	27.4	0.200 (0.055)	0.078 (0.021)
17FC0239-016	Infant formula	26.1	0.152 (0.040)	0.064 (0.017)
17FC0239-017	Infant formula	26.1	0.055 (0.014)	0.034 (0.009)
17FC0239-018	Infant formula	27.5	0.299 (0.082)	0.000 (0.000)
17FC0239-019	Infant formula	27.7	0.249 (0.069)	0.094 (0.026)
17FC0239-020	Infant formula	26.6	0.057 (0.015)	0.076 (0.020)
17FC0239-021	Infant formula	25.7	0.301 (0.077)	0.027 (0.007)
17FC0239-022	Infant formula	28.3	0.578 (0.164)	0.068 (0.019)
17FC0239-023	Infant formula	26.8	0.367 (0.098)	0.033 (0.009)
17FC0239-024	Infant formula	28.2	0.297 (0.084)	0.137 (0.039)
17FC0239-025	Infant formula	24.7	0.230 (0.057)	0.023 (0.006)
17FC0239-026	Infant formula	27.1	0.078 (0.021)	0.110 (0.030)
17FC0239-027	Infant formula	25.4	0.543 (0.138)	0.333 (0.085)
17FC0239-028	Infant formula	25.3	0.229 (0.058)	0.012 (0.003)
17FC0239-029	Vegetable oil	N/A	0.056	0.110
17FC0239-030	Vegetable oil	N/A	0.935	3.701
17FC0239-031	Vegetable oil	N/A	0.174	0.209
17FC0239-032	Vegetable oil	N/A	0.249	0.148
17FC0239-033	Vegetable oil	N/A	0.275	0.112
17FC0239-034	Vegetable oil	N/A	0.507	1.038

Sample number	Matrix	Results in oils and oil/fat extracts (in infant formula)		
		Fat (% w/w)	3-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-035	Vegetable oil	N/A	0.017	0.107
17FC0239-036	Vegetable oil	N/A	0.016	0.000
17FC0239-037	Vegetable oil	N/A	0.158	0.164
17FC0239-038	Vegetable oil	N/A	0.151	0.052
17FC0239-039	Vegetable oil	N/A	0.513	0.225
17FC0239-040	Vegetable oil	N/A	5.460	1.614
17FC0239-041	Vegetable oil	N/A	9.986	5.294
17FC0239-042	Vegetable oil	N/A	0.235	0.037
17FC0239-043	Vegetable oil	N/A	0.390	0.718
17FC0239-044	Vegetable oil	N/A	0.076	0.016
17FC0239-045	Vegetable oil	N/A	0.019	0.031
17FC0239-046	Vegetable oil	N/A	0.028	0.011
17FC0239-047	Vegetable oil	N/A	0.629	0.295
17FC0239-048	Vegetable oil	N/A	0.029	0.053
17FC0239-049	Vegetable oil	N/A	0.360	1.127
17FC0239-050	Vegetable oil	N/A	0.067	0.078
17FC0239-051	Infant formula	28.0	0.229 (0.064)	0.077 (0.022)
17FC0239-052	Infant formula	25.2	0.269 (0.068)	0.027 (0.007)
17FC0239-053	Infant formula	24.7	0.185 (0.046)	0.022 (0.006)
17FC0239-054	Infant formula	24.9	0.170 (0.042)	0.040 (0.010)
17FC0239-055	Infant formula	28.7	0.243 (0.070)	0.033 (0.009)
17FC0239-056	Infant formula	24.1	0.183 (0.044)	0.031 (0.007)
17FC0239-057	Infant formula	26.4	0.018 (0.005)	0.022 (0.006)
17FC0239-058	Infant formula	28.5	0.209 (0.060)	0.054 (0.015)
17FC0239-059	Infant formula	28.8	0.233 (0.067)	0.043 (0.013)
17FC0239-060	Infant formula	27.0	0.171 (0.046)	0.037 (0.010)
17FC0239-061	Infant formula	27.7	0.265 (0.073)	0.049 (0.014)
17FC0239-062	Infant formula	28.3	2.364 (0.669)	1.709 (0.484)
17FC0239-063	Infant formula	27.1	0.145 (0.039)	0.064 (0.017)
17FC0239-064	Infant formula	27.7	0.347 (0.096)	0.112 (0.031)
17FC0239-065	Infant formula	29.0	0.089 (0.026)	0.046 (0.013)
17FC0239-066	Infant formula	28.7	0.101 (0.026)	0.033 (0.010)
17FC0239-067	Infant formula	24.9	0.312 (0.077)	0.007 (0.002)
17FC0239-068	Infant formula	28.1	0.233 (0.065)	0.019 (0.005)
17FC0239-069	Infant formula	26.5	0.913 (0.242)	0.020 (0.005)
17FC0239-070	Infant formula	27.3	0.223 (0.061)	0.026 (0.007)
17FC0239-071	Infant formula	27.6	0.201 (0.055)	0.058 (0.016)
17FC0239-072	Infant formula	27.4	0.247 (0.068)	0.050 (0.014)

Sample number	Matrix	Results in oils and oil/fat extracts (in infant formula)		
		Fat (% w/w)	3-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-073	Infant formula	27.0	0.706 (0.191)	0.063 (0.017)
17FC0239-074	Infant formula	26.8	0.575 (0.154)	0.081 (0.022)
17FC0239-075	Infant formula	27.0	0.879 (0.237)	0.103 (0.028)
17FC0239-076	Infant formula	28.4	0.478 (0.136)	0.040 (0.011)
17FC0239-077	Infant formula	24.9	1.655 (0.412)	0.538 (0.134)
17FC0239-078	Infant formula	28.1	1.124 (0.316)	0.097 (0.027)
17FC0239-079	Vegetable oil	N/A	0.025	0.000
17FC0239-080	Vegetable oil	N/A	0.178	0.085
17FC0239-081	Vegetable oil	N/A	0.331	0.625
17FC0239-082	Vegetable oil	N/A	0.219	0.260
17FC0239-083	Vegetable oil	N/A	5.05	4.127
17FC0239-084	Vegetable oil	N/A	0.661	2.907
17FC0239-085	Vegetable oil	N/A	11.814	3.045
17FC0239-086	Vegetable oil	N/A	3.163	0.517
17FC0239-087	Vegetable oil	N/A	0.071	0.085
17FC0239-088	Vegetable oil	N/A	4.490	0.901
17FC0239-089	Vegetable oil	N/A	0.283	0.841
17FC0239-090	Vegetable oil	N/A	0.082	0.447
17FC0239-091	Vegetable oil	N/A	0.059	0.096
17FC0239-092	Vegetable oil	N/A	0.316	0.368
17FC0239-093	Vegetable oil	N/A	0.241	0.204
17FC0239-094	Vegetable oil	N/A	0.021	0.143
17FC0239-095	Vegetable oil	N/A	0.175	0.426
17FC0239-096	Vegetable oil	N/A	0.165	0.388
17FC0239-097	Vegetable oil	N/A	0.805	0.196
17FC0239-098	Vegetable oil	N/A	0.953	0.535
17FC0239-099	Vegetable oil	N/A	0.246	0.337
17FC0239-100	Vegetable oil	N/A	0.179	0.309

NB Results for Infant formula samples are for the fat/oil component, with results for the infant formula provided in brackets.

Table 12: 3-MCPD esters and GEs in industry supplied oils and the infant formulas made from them (USFDA results)

Sample number	Matrix	Results (in oil/fat component)			
		Fat %(w/w)	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol (mg/kg)
17FC0239-017	Infant formula	26.1	0.055	N/A	0.034
17FC0239-042	Vegetable oil	N/A	0.235	N/A	0.037
17FC0239-028(1)	Infant formula	25.3	0.229	N/A	0.012
17FC0239-038	Vegetable oil	N/A	0.151	N/A	0.052
17FC0239-050	Vegetable oil	N/A	0.067	N/A	0.078

(1) NB The oils listed below infant formula sample "17FC0239-028" were not, according to the manufacturer, the only oils added to the product and the proportion of each oil making up the total could not be obtained. Little therefore can be concluded from the 3-MCPD ester and GE results obtained for the vegetable oils compared with the concentrations found in the infant formula for this sample.

APPENDIX D: VEGETABLE OIL AND INFANT FORMULA SAMPLES BY VARIOUS CRITERIA

Table 13: Vegetable oils by country of manufacture

COUNTRY OF MANUFACTURE	Percent total vegetable oils
Australia	20
New Zealand	5
Africa	2
China	2
Ecuador	2
France	2
Italy	9
India	2
Malaysia	18
Mexico	2
Pacific Islands	2
Singapore	2
South Korea	2
Spain	7
Switzerland	2
Thailand	5
Submitted by industry or unknown country of origin	11

Table 14: Infant formulas by country of manufacture

COUNTRY OF MANUFACTURE	Percentage total infantformulas
Australia	32
New Zealand	34
Indonesia	2
France	2
Germany	5
Ireland	5
Mexico	2
Netherlands	9
Singapore	7
Unspecified	2

Table 15: Infant formulas by reported oil type

OIL/FAT TYPE	Percent total infant formulas
Vegetable oils unspecified	39
Canola	2
Coconut	14
Dairy	8
Fish	26
Palm	8
Rapeseed	2
Single-cell source long chain fats	7
Soy bean	40
Sunflower	17
Arachidonic acid (ARA)	34
Decosahexaenoic acid (DHA)	33

NB most infant formulas contained a mixture of oil/fat types

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INSTITUTE OF ENVIRONMENTAL
SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz