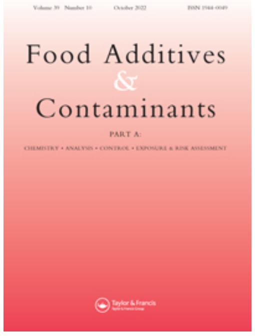

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Occurrence of 2- and 3-monochloropropanediol esters (MCPDE) in infant formula products on the Canadian market between 2015 and 2019

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ABSTRACT

2- and 3-monochloropropanediol esters (MCPDEs) are most commonly formed as process-induced contaminants during the refinement of vegetable oils used for food production. 'In vivo' hydrolysis of 3-MCPDEs releases the potential carcinogen 3-monochloropropanediol (3-MCPD). Levels of MCPDEs in infant formula are of particular concern, as refined oils are commonly used as main fat ingredients. For this study, infant formula samples (powders, liquid concentrates and ready-to-feed infant formula samples) from the Canadian market were purchased and analysed in 2015 (35 samples) and 2019 (33 samples). MCPDE concentrations (expressed as free MCPD equivalents) were examined through an indirect analytical approach, applying acid-catalysed ester cleavage and using cyclohexanone as derivatising agent. Labelled diesters were used as internal standards. 2015 Survey data were analysed by gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring mode (SIM). 2019 Survey data were analysed with an updated method using GC-MS/MS in multiple reaction monitoring modes (MRM). In 2015, levels in reconstituted formula ranging from 3.7 ng/g to 111 ng/g for 3-MCPD and 2.2 ng/g to 56.2 ng/g for 2-MCPD were found. In 2019, levels ranging from 3.9 ng/g to 74.8 ng/g for 3-MCPD and 1.0 ng/g to 33.9 ng/g for 2-MCPD were found. A significantly reduced mean of combined MCPDEs was observed between 2015 and 2019 data (64.5 ng/g, standard deviation (SD) 8.6 ng/g in 2015 to 31.8 ng/g, SD 5.6 ng/g in 2019, *p*-value = 0.024). For the majority of manufacturers, the data comparison among brand products over time shows decreased levels of MCPDEs. Occurrence data of MCPDEs, including data from previously published surveys (2012/2013), were also compared and a temporal trend was established.

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Introduction

2-Monochloropropane-1,2-diol (2-MCPD) and 3-monochloropropane-1,3-diol (3-MCPD, racemic) can occur in food products either as fatty ester derivatives or in their free forms. Esters of MCPD include monoesters of 2- and 3-MCPD or diesters with identical or different fatty acid moieties. Monochloropropane-diol esters (MCPDEs) are most commonly formed as process-induced contaminants during the refinement of vegetable oils used for food production, whereas free MCPDs can occur in various foods. MCPDEs are formed predominantly during the deodorization step of vegetable oil manufacturing, to remove undesirable compounds such as tocopherols, sterols or free fatty acids (Tiong et al. 2021). The process of steam distillation under vacuum requires temperatures of up to 260 °C (Eskin and Przybylski 2003) and fosters the formation of MCPDEs. MCPDEs are therefore present in significant quantities in refined oils and fats, primarily but not exclusively in palm oil or palm olein (Kuhlmann 2019; Matthäus and Pudel 2022). Various other vegetable oils, such as sunflower, safflower, corn or coconut oils also contribute to MCPDE quantities. Under these conditions and in the presence of organic or inorganic chloride sources, MCPDEs can form through the reaction of acylglycerols,

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predominantly by direct and indirect nucleophilic substitution (Yao et al. 2019).

MCPDEs are potentially toxic, as they release free MCPDs from the parent esters after ingestion. Free 3-MCPD is known to be entirely liberated from its ester derivatives during digestion (Abraham et al. 2013; Knutsen et al. 2018 (EFSA); JECFA 2016). 3-MCPD is a known rodent kidney carcinogen and is classified by the International Agency for Research on Cancer as possibly carcinogenic to humans (Group 2B) (International Agency for Research on Cancer 2012). The adverse human health effects and toxicological concerns of 3-MCPD are well characterized, with kidneys and testis identified as the main target organs for 3-MCPD-induced toxicity (EFSA 2016).

Compared to 3-MCPD, data suggests that 2-MCPD bears a different toxicity profile, with significant changes observed in the heart tissue (Schultrich et al. 2017; Frenzel et al. 2018). Subacute and -chronic dietary exposure animal studies, conducted at Health Canada, demonstrate significant incidences of non-neoplastic lesions in the heart tissues of rats of both sexes exposed to dietary 2-MCPD (50 or 40 mg/kg BW, respectively), together with significantly higher wet weights (Raju 2022). A provisional maximum tolerable daily intake (pMTDI) for 3-MCPD of 4 µg/kg body weight was established by JECFA (2016). A recent addendum (European Union 2020) to European Union regulation 2018/290 (European Union 2018) for 3-MCPD furthermore sets to limit the sum of 3-MCPD and 3-MCPDEs (expressed as 3-MCPD) in infant formula to 125 ng/g for powders and 15 ng/g for liquids. Additionally, a 750 ng/g limit in vegetable oils and fats dedicated to the manufacturing of infant formula was adopted. Infant formula is the sole source of nutrition for many young infants (0–6 months) and a significant source of nutrition for older infants (6–12 months), which warrants a specific focus on MCPD and MCPDE levels therein. Refined vegetable oils are commonly used by manufacturers in the fat blends of infant formula, which provide more than 50% of total energy in infant diets. Following an exposure assessment in 2020, the German Federal Institute for Risk Assessment (BfR) observed no exceedance of the tolerable daily intake (TDI) for 3-MCPD and 3-MCPDEs for adult consumers (BFR 2020). However, the same study found that for children and infants the TDI was exceeded in some cases. An exceedance (by a factor of 1.5–3) of the TDI was found for infants (BFR 2020).

MCPD and MCPDE occurrence data in infant formula have been researched globally (Zelinková et al. 2009; Weißhaar 2011; Wöhrlin et al. 2015; Becalski et al. 2015a; Jędrkiewicz et al. 2016; Wang et al. 2016; Arisseto et al. 2017; Beekman et al. 2020; Nguyen and Fromberg 2020; Azmi et al. 2021; Beekman et al. 2021). Several direct and indirect analytical methods for the quantitative analysis of MCPDEs have evolved. Direct analytical approaches quantify MCPDEs without further chemical transformation, whereas indirect methods cleave MCPDEs to liberate 2- and 3-MCPD, which is further derivatised for GC-MS analysis. The latter type of methods can be distinguished largely between conditions for transesterification as well as derivatising agents. Several indirect methods of analysis for MCPDE analysis have recently been established. Two indirect methods by Dubois et al. (2019) and Kuhlmann (2019) that both have a scope specifically dedicated to infant formula, were adopted by the Association of Official Analytical Chemists (AOAC International) as First Action official methods (AOAC 2018.03 and 2018.12 respectively). Other indirect methods with an analytical scope on fats and oils have been adopted by the American Oil Chemist's Society (AOCS) and the International Organization for Standardization (ISO). (Fiebig 2011; Ermacora and Hrncirik 2013; Koyama et al. 2016; Zwagerman and Overman 2019).

Regulatory restrictions such as those stated above show a recent awareness of exposure of infants to MCPDEs. The occurrence of 2- and 3-MCPDEs in infant formula products on the Canadian market were first investigated with a pilot survey in 2012/2013 (Becalski et al. 2015a). This study was undertaken to monitor temporal trends and to verify if recently-developed mitigation techniques to lower MCPDE concentrations in refined oils were adopted in the industry and reflected in baseline levels in infant formula (Matthäus and Pudel 2022).

Infant formula products collected in 2015 and 2019 were analysed for MCPDE (reported as free MCPD equivalents) by gas chromatography-mass spectrometry (GC-MS) (2015) and GC-MS/MS (2019) following an indirect analytical approach using acidic hydrolysis (Divinová et al. 2011) and subsequent derivatisation with cyclohexanone and a fluorinated sulfonic acid resin (Nafion) as catalyst (Becalski et al. 2013).

Materials and methods

Chemicals and standards

3-Monochloro-1,2-propanediol 98% was obtained from AlfaAesar (WardHill, MA, USA). 2-monochloro-1,3-propanediol 98%, 2-monochloro-1,3-propanediol-d5 98%, isotopic purity >99 atom% D, 3-monochloro-1,2-propanediol-d5 dipalmitate 97%, isotopic purity 99 atom% D, and 2-monochloro-1,3-propanediol-d5 distearate 98%, isotopic purity 98 atom% D were supplied by Toronto Research Chemicals Inc. (Toronto, ON, Canada). 3-Monochloro-1,2-propanediol-d5 97%, isotopic purity 98 atom% D was obtained from CDN Isotopes (Pointe-Claire, QC, Canada). Nafion^{TM} NR50 (super acidic ion exchange resin catalyst) was purchased from Alfa Aesar, and cyclohexanone 99.8% was obtained from MilliporeSigma (St.Louis, MO, USA). Sulfuric acid 98%+ (trace metal use, A-510-500) was from Fisher Scientific while anhydrous sodium sulfate was obtained from EMD (Gibbstown, NJ, USA). Sodium sulfate was muffled at 650 °C for 16 h before use. All other reagents were of analytical grade. A Millipore Milli-Q water purification system was used.

Sample collection

Samples of 33 different infant formula products from seven different manufacturers were collected in 2019 from grocery stores in Ottawa, Toronto, and Montreal, Canada. Samples of 35 different infant formula products were also collected in the same geographical area in 2015. Foods were collected following an internal sampling plan, developed to cover the products available and based on Canadian market share data. In both survey samplings, 17 products show identical universal product codes (UPC) for the two-time points. Infant formula products are divided into three categories: powder formula, concentrated liquid formula, and ready-to-feed liquid formula. Samples were divided into these categories to reflect potential variations in their MCPDE contamination level. All samples were furthermore distinguished between either milk- or soy-based formula products. All milk-based products in surveys presented herein refer to cow's milk. No further distinction of products according to age was done. All samples were stored to ensure stability: solid samples were stored in refrigerators at 4 °C, and liquid samples were stored in freezers at −15 °C.

Sample preparation

The procedure for sample preparation and the analytical method to determine MCPDE concentrations was generally followed as depicted previously (Becalski et al. 2015a; 2018). A sample weight of 3.0 g of reconstituted formula was used for all samples. Samples were reconstituted prior to analysis when required. A uniform dilution factor of 8.065 was applied to the powdered formula, and a factor of 2 was applied to concentrates, to allow direct comparison between different types of formula and with previous results. The ready-to-feed formula was used as is. For powdered samples, 0.37 g were taken up in 3.00 mL of Milli-Q water. For concentrated liquid formula, samples of 1.50 g were taken up in 1.50 mL of Milli-Q water. For ready-to-feed formula, samples of 3.00 g were weighed. All samples were spiked with deuterated (d_5) analogs of 2- and 3-MCPD diesters as internal standards. 5 µL of 102.5 µg/mL *rac*-bispalmitate-3-MCPD-d_5 and 5 µL of 112.2 µg/mL 1,3-distearate-2-MCPD-$d_{5,}$ equivalent to 100 ng of free 2- and 3-MCPD-d5 in 100 mg of sample lipids were added. 100 mg of sample lipids represent the fat content for the chosen sample size of reconstituted formula products (mean of 3.07 weight % in 2019 samples). The infant formula samples were extracted with 25 mL of 1/1 (v/v) dichloromethane/methanol, and the organic fraction was washed with water, dried over anhydrous sodium sulphate and concentrated. The lipids (∼100 mg) were taken up in tetrahydrofuran (THF) and were subjected to sulphuric acid (methanolic solution) transesterification to release free 2- and 3-MCPD. Hydrolysis was performed at 40 °C over 16 h. The reaction mixtures were neutralized with aqueous sodium bicarbonate and the organic layer was evaporated. The aqueous layer was washed with hexanes. The free 2- and 3-MCPD fractions (aqueous layer) were purified by solid-supported liquid-liquid extraction over diatomaceous earth (Extrelut® NT), using 10% (v/v) diethyl ether/hexanes, followed by 100% diethyl ether as eluents. The concentrated eluates were subject to derivatisation with cyclohexanone, forming acetals suitable for GC-MS/MS (GC-MS for 2015 data) analysis as follows. The solvent was removed to near dryness with nitrogen. In the presence of Nafion NR 50 acidic ion exchange catalyst (one pellet, 0.05 g) and sodium sulfate (~0.2 g), a 10% (v/v) cyclohexanone/iso-octane solution was applied as derivatisation mixture at 45 °C for 1 hour. The sample was allowed to cool to ambient temperature and the supernatant was transferred to a 2 mL GC vial. For each sample batch, two reference materials, FAPAS© 2628 (3-MCPD esters in refined palm oil) as well as Institute for Reference Materials and Measurements (IRMM) palm oil (2012 spiked cocoa oil 980), were analysed as quality control samples and ensured appropriate method stability. 6-Point calibration curves were analysed prior to each sample run ($R^2 \geq 0.999$).

Instrumental analysis

For 2019 data, GC-MS/MS analysis was performed on an Agilent GC 7890B with 7010 MS/MS triple quad (Agilent, Mississauga, ON, Canada). GC separation was performed using a VF-17ms column (30 m × 0.25 mm × 0.25 µm, Agilent, Mississauga, ON, Canada). The MS/MS was operated with electron impact ionization (EI) in Multiple Reaction Monitoring (MRM) mode with four ions for native analytes and three ions for labelled internal standards. Samples (1 µL) were injected in pulsed splitless mode using helium as carrier gas under the following parameters: injector temperature 300 °C, GC oven temperature program: initial temperature of 60 °C, hold for 1 min, increase the temperature to 120 °C at 25 °C min^{-1}, increase temperature to 150 °C at 4 °C $min^{-1,}$ increase temperature to 300 °C at 50 °C min-1 and hold for 6.1 min. The MS/MS parameters were: MS/MS source temperature 230 °C, mass spectrometer (MS) quadrupole temperature 150 °C, MS transfer line temperature 300 °C. Precursor ions 190.2 (2- and 3-MCPD) and 195.2 m/z (2- and 3-MCPD-d_5). Product ions 69, 74.6, 147.1, 161.1 m/z for 2- and 3-MCPD and 69, 152.1, 166.1 m/z for 2- and 3-MCPD-d_5. Data analysis was performed using MassHunter software.

Samples from the 2015 survey were analysed by GC-MS analysis performed on an Agilent 6890 GC with 5973 N MSD (mass selective detector) system (Agilent, Mississauga, ON, Canada). GC was performed using a DB-5ms column (30 m × 0.25 mm × 0.25 µm, Agilent, Mississauga, ON, Canada). MCPD derivatives were detected in Selected Ion Monitoring (SIM) mode with four ions for native analytes and two ions for internal standards (147, 149, 161,190 m/z for 2- and 3-MCPD, 152 and 195 m/z for 2- and 3-MCPD-d_5).

QC/QA

For the 2019 survey data analysis, the LOD for 3-MCPD (0.8 ng/g) was calculated as blank average (0.6 ng/g) + 3 × standard deviation (0.07 ng/g) of the blank. For 2-MCPD, the limit of detection (LOD) of 0.2 ng/g was based on a signal-to-noise ratio (S/N) of 9 for spiked samples. The limit of quantification (LOQ) for 3-MCPD was determined as three times the LOD, 2.5 ng/g for 3-MCPD and 0.6 ng/g for 2-MCPD. Due to instrument upgrades, the LOD for MCPDs was improved during the 2019 survey of infant formula products, as compared to previous surveys. These improvements allowed obtaining lower LOD/LOQ values in 2019. To compare, the analytical method applied for 2015 survey data afforded a LOD for 3-MCPD of 1 ng/g and for 2-MCPD of 0.6 ng/g, using the same calculations as described above. The LOQ was defined as 3 ng/g for 3-MCPD and 2 ng/g for 2-MCPD. The LOQ for data reported in a previous survey in 2012/2013 was 6 ng/g for 3-MCPD and 2 ng/g for 2-MPCD (Becalski et al. 2015a).

For 2019 survey data, mean relative standard deviations (RSD) of 1.3% for 3-MCPD and 1.8%

Table 1. The concentration of 2- and 3-MCPDEs (expressed in ng/g as free MCPD equivalents) in 35 infant formula samples from the Canadian market sampled in 2015.

			3-MCPD (ng/g)		2-MCPD (ng/g)		Total bound MCPD (ng/g)*
Sample type	Base	*n*	Mean	Range	Mean	Range	Mean
Powder	Milk	15	48.6	3.7–91.9	21.3	2.4–42.6	69.9
Powder	Soy	3	62.1	7.4–111	29.0	2.2–56.2	91.1
Concentrate	Milk	7	42.2	5.1–107	19.1	2.2–47.5	61.3
Concentrate	Soy	1	12.5		6.2		18.7
Ready-to-feed	Milk	9	37.7	7.6–101	16.7	4.0–46.7	54.4
All		35	44.6	3.7–111	19.9	2.2–56.2	64.5

LOQ: 3 ng/g for 3-MCPD and 2 ng/g for 2-MCPD. For 3-MCPD the LOD (1 ng/g) was calculated as blank average (0.5 ng/g) + 3 × standard deviation (0.18 ng/g) of the blank. For 2-MCPD no contamination of the blank was observed, and the LOD of 0.6 ng/g was based on an S/N ratio of 9 for spiked samples. * Sum of bound 2- and 3-MCPD.

for 2-MCPD were obtained for duplicate analyses ($n = 8$). The average of duplicates is reported for these samples. To compare, a mean RSD of 4.2% for 3-MCPD was found for duplicate analysis of samples ($n = 17$) in 2015. A comparison with Food Analysis Performance Assessment Scheme (FAPAS, Sand Hutton, York, UK) #2646 was carried out as quality control ($n = 5$) for 3-MCPD. All samples were within two standard deviations (SD) of 17 ng/g from the assigned value (586 ng/g) and within a range for $|z| \leq 2$. Additionally, an Institute for Reference Materials and Measurements (IRMM) sample was used as a comparison. All samples ($n = 6$) were within 1.3 SD for 3-MCPD (assigned value 3261 ng/g) and within 1.3 SD for 2-MCPD (assigned value 972 ng/g). Intra-lab quality control (QC) comparison in 2019 with one sample from the 2015 survey gave results for 3-MCPD within three SD (4.6 ng/g) and within 1.7 SD (1.6 ng/g) for 2-MCPD ($n = 5$). Spike experiments were carried out with virgin olive oil samples, tested to be free of 2- and 3-MCPD. Native 2-MCPD distearate and 3-MCPD dipalmitate were added (equivalent of 100 ng 2- and 3-MCPD equivalent per sample) and the sample preparation process was carried out. Spiked samples ($n = 8$) showed mean recoveries of 96.3% (SD 3.2) for 3-MCPD and 106.1% (SD 2.8) for 2-MCPD. Mean internal standard recoveries of 97.6% for 3-MCPD and 109.3% for 2-MCPD were obtained.

Statistical comparisons for observed MCPD levels between surveys from different years were performed, including previously published data (Becalski et al. 2015a). Analyses were done by SAS Enterprise Guide version 9.4, and the comparisons included: 1). Pairwise comparison of four different survey years (2012, 2013, 2015 and 2019), and 2). 2012–2015 combined years vs. 2019. Further, MCPD levels of products from individual manufacturers between different years were compared. Non-parametric testing methods (Kruskal-Wallis test and Wilcoxon two-sample test) were employed for the statistical comparisons of non-normally distributed data, and p-values of pairwise comparisons for the four different years were adjusted applying the Bonferroni adjustment method.

Results and discussion

Occurrence

Table 1 depicts 2- and 3-MCPDE concentrations (expressed as free MCPD equivalents, assuming complete '*in vivo*' hydrolysis of MCPDEs to free MCPDs) in 35 infant formula products sampled in 2015 in the Canadian market. The analytical methods used for surveys presented here allow the determination of MCPDEs (expressed as 'bound' MCPD equivalents), excluding the determination of free 2- and 3-MCPD if present in food samples. Summarized MCPD contents refer to the sum of bound 2- and 3-MCPD stemming from their respective MCPDEs. All results are reported "as consumed" to allow comparison between different types of formula and with previous results. Powders and concentrates were therefore reconstituted prior to analysis. A uniform dilution factor of 8.065 was determined empirically and applied to all powdered samples. The dilution factor of 8.065 differs marginally from the value of 7.7 used by the European Food Safety Authority (EFSA) for its calculations to convert occurrence data for powdered products

Table 2. The concentration of 2- and 3-MCPDEs (expressed in ng/g as free MCPD equivalents) in 33 infant formula samples from the Canadian market sampled in 2019.

			3-MCPD (ng/g)		2-MCPD (ng/g)		Total bound MCPD (ng/g)*
Sample type	Base	*n*	Mean	Range	Mean	Range	Mean
Powder	Milk	15	28.6	3.9–74.8	12.2	1.0–33.9	40.8
Powder	Soy	4	12.3	4.6–29.2	4.9	1.6–12.4	18.2
Concentrate	Milk	6	12.2	5.9–24.2	4.6	2.6–8.6	16.7
Ready-to-feed	Milk	8	22.7	6.2–63.8	11.0	2.6–32.8	33.8
All		33	22.2	3.9–74.8	9.7	1.0–33.9	31.8

LOQ: 2.5 ng/g for 3-MCPD and 0.6 ng/g for 2-MCPD. For 3-MCPD the LOD (0.8 ng/g) was calculated as blank average (0.6 ng/g) + 3 × standard deviation (0.07 ng/g) of the blank. For 2-MCPD no contamination of the blank was observed, and the LOD of 0.2 ng/g was based on an S/N ratio of 9 for spiked samples. * Sum of 2- and 3-MCPD.

to ready-to-feed liquid formula (EFSA 2016). A factor of 2 was applied to concentrate formula products, and the ready-to-feed formula was used as is. These factors must be applied to obtain actual concentrations of MCPDs in concentrates and powders (if expressed on an "as is" basis). Levels ranging from 3.7 ng/g to 111 ng/g for 3-MCPD and from 2.2 ng/g to 56.2 ng/g for 2-MCPD were observed among all products. Mean values of all samples of 44.6 ng/g for 3-MCPD and 19.9 ng/g for 2-MCPD were found. Comparing the mean of the sum of 2- and 3-MCPDs in the different categories of infant formula samples, the indication of a lower occurrence was observed in concentrates and ready-to-feed formula compared to powdered formula (56.0 ng/g mean concentration in concentrates and 54.4 ng/g mean concentration in ready-to-feed formula vs 73.4 ng/g mean in the powdered formula). A slightly higher total bound MCPD content was observed in soy-based formula compared to milk-based formula (means of 73.0 ng/g for soy- and 63.4 ng/g for milk-based formula products).

In the 2019 survey, 33 infant formula samples were procured. Table 2 shows the concentrations of 2-MCPDE and 3-MCPDE (expressed as free MCPD equivalents) observed in the samples obtained in 2019. Levels ranging from 3.9 ng/g to 74.8 ng/g for 3-MCPD and from 1.0 ng/g to 33.9 ng/g for 2-MCPD were observed. A mean value over all samples of 22.2 ng/g for 3-MCPD and 9.7 ng/g for 2-MCPD was found.

Extensive surveys have recently been conducted in other studies. Results presented herein for the dry powdered formula in the 2019 survey showed levels between 31.5 and 603 ng/g of bound 3-MCPD ('as is', recalculated from diluted sample values reported throughout this study). In comparison, values ranging from 13 to 950 ng/g of bound 3-MCPD were reported in a survey of infant formula in the US market (Beekman et al. 2020). Surveys in the markets of New Zealand and Australia showed levels of bound 3-MCPD from 14 to 164 ng/g (New Zealand) and from 5 to 669 ng/g (Australia) (New Zealand Food Safety 2020). In a survey carried out in China in 2016, a maximum level of 316 ng/g of 3-MCPD was found (Wang et al. 2016). Lower values of 24–99 ng/g of bound 3-MCPD were obtained in a survey in the German market (Beekman et al. 2021), and 3-MCPD values ranging from 5.3 to 102.8 ng/g in a survey of regular and specialised infant formula in Denmark (Nguyen and Fromberg 2020).

As was observed in our 2015 data, samples in the 2019 concentrate infant formula category showed a lower mean of the sum of MCPDs, as compared to powders and ready-to-feed formula (16.7 ng/g mean concentration in concentrates vs mean concentrations of 35.8 ng/g and 33.8 ng/g in powder and ready-to-feed formula, respectively) (Table 2). Comparing milk- and soy-based products, the data indicated a reverse in the trend observed in 2015, with a lower total bound MCPD content in soy-based formula (means of 17.2 ng/g for soy- and 33.9 ng/g for milk-based formula products). A mean ratio of 3- to 2-MCPD in all samples surveyed in 2015 and 2019 of 2.46 was observed (median 2.34), within the common range of values for refined vegetable oils (Becalski et al. 2015b; Kuhlmann 2011).

Comparison to previous surveys of infant formula procured in the Canadian market

Significant efforts have been made by manufacturers of refined oils to reduce the formation of MCPDEs during oil processing in recent years,

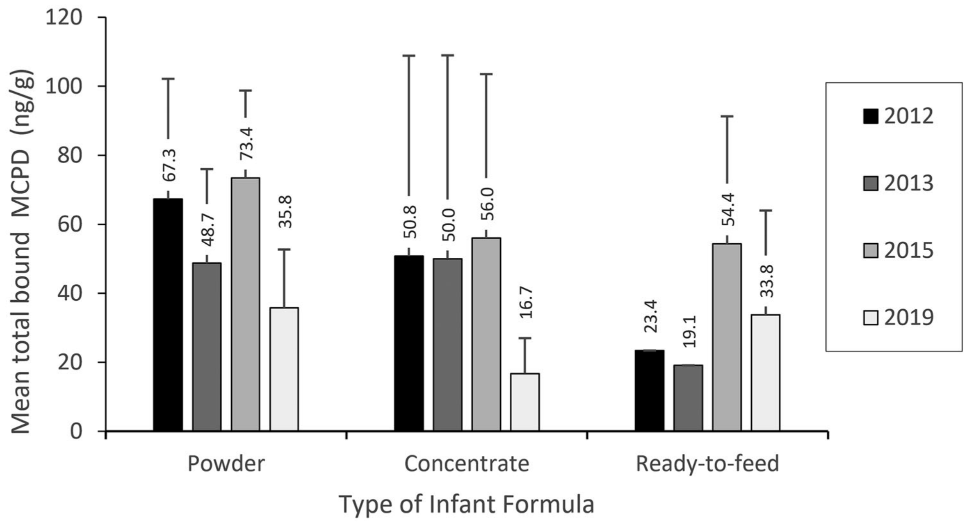

Figure 1. Comparison of concentrations of MCPDs (sum of 2- and 3-MCPD) by type of infant formula in surveys from 2012, 2013, 2015 and 2019, expressed in ng/g (means and 95% confidence interval (CI)). Sample size: powders: 2012 ($n = 10$), 2013 ($n = 10$), 2015 ($n = 18$), 2019 ($n = 19$); concentrates: 2012 ($n = 5$), 2013 ($n = 5$), 2015 ($n = 8$), 2019 ($n = 6$); ready-to-feed formula products: 2012 ($n = 1$), 2013 ($n = 1$), 2015 ($n = 9$), 2019 ($n = 8$). When averages of sample ranges for MCPD concentrations were calculated including samples with 2- or 3-MCPD concentrations below the LOQ, a concentration of zero was used.

focussing on palm oil and palm olein (Matthäus et al. 2011; Strijowski et al. 2011; Shimizu et al. 2013; Oey et al. 2020). Palm oils are used as a fat source in some infant formulas, and contain the highest MCPDE content among vegetable oils (JECFA 2016). Toolboxes exist to address each step of oil manufacturing that may contribute to the formation and presence of MCPDEs (Bund für Lebensmittelrecht und Lebensmittelkunde (BLL) 2016). Especially conditions for high-temperature oil deodorization, a crucial step in the formation of MCPDEs, were optimized to limit MCPD occurrence.

Figure 1 depicts a comparison of mean concentrations of total MCPDEs by types of infant formula in surveys from the Canadian market (expressed as free MCPD equivalents). Sample data from 2015 (Table 1) to 2019 (Table 2) as discussed above, as well as data from a preliminary survey in 2012 and 2013 (Becalski et al. 2015a) were compared. To summarize previously published data, a survey of 15 infant formula products in 2012 showed a mean of 59.4 ng/g (median 43.2 ng/g) and a maximum level of 135 ng/g of total bound MCPD equivalents, whereas a survey of 15 infant formula products in 2013 showed a mean of 45.5 ng/g (median 26.4 ng/g) and a maximum level of 108 ng/g of total bound MCPD.

A reduction of total MCPDE levels in 2019 in powdered formula and concentrates was observed compared to levels observed in previous years. MCPDE levels in the ready-to-feed formula show a decrease between 2015 and 2019 but appear similar compared to 2012/2013 data.

Between 2015 and 2019, the mean concentration of 3-MCPD for all samples ($n = 35$ in 2015, $n = 33$ in 2019) was reduced by more than half, from 44.6 ng/g to 22.2 ng/g. For 2-MCPD, a comparable reduction from 19.9 ng/g to 9.7 ng/g was observed. Maximum values of 74.8 ng/g of 3-MCPD and 33.9 ng/g of 2-MCPD were found in 2019. In our previous surveys, maximum values of 111 ng/g of 3-MCPD and 56.2 ng/g of 2-MCPD in 2015, 80.0 ng/g of 3-MCPD and 32.0 ng/g of 2-MCPD in 2013, and 89.0 ng/g of MCPD and 47.0 ng/g of 2-MCPD in 2012 were observed.

We performed a statistical analysis of the observed MCPD trend between 2015 and 2019. Applying a non-parametric testing method (Wilcoxon two-sample test), we found that the difference in total bound MCPD between 2015 (mean 64.5 ng/g, SD 8.6 ng/g) and 2019 (mean 31.8 ng/g, SD 5.6 ng/g) was statistically significant (p-value = 0.024). No statistically significant difference was found between 2012 and 2015 data. However, the trend between combined 'before 2019' and 2019 data proved statistically significant (p-value = 0.004).

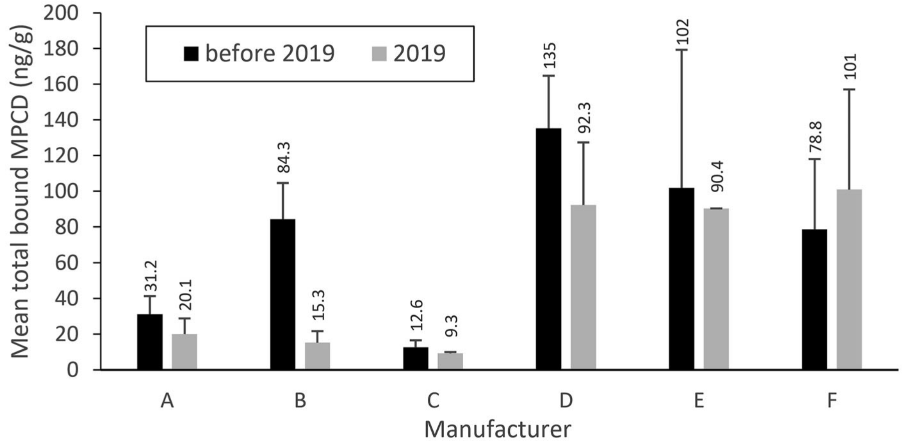

Figure 2. Comparison of MCPD concentrations (sum of 2- and 3-MCPD) by infant formula manufacturers before 2019 and in 2019 (mean and 95% CI, samples across all categories of infant formula products). [Before 2019]: Sample data from 2012, 2013, and 2015 surveys. [2019]: sample data from 2019 survey. Sample size per manufacturer: (A) before 2019 ($n = 19$), 2019 ($n = 11$); (B) before 2019 ($n = 15$), 2019 ($n = 8$); (C) before 2019 ($n = 14$), 2019 ($n = 6$). (D) before 2019 ($n = 5$), 2019 ($n = 3$); (E) before 2019 ($n = 3$), 2019 ($n = 1$); (F) before 2019 ($n = 8$), 2019 ($n = 2$).

While we observed a downward trend in the mean level of MCPDE contaminants, individual manufacturers' samples show remaining elevated levels of MCPDs. Within the sample scope, we compared MCPDE levels of infant formula products by the manufacturer between 2012 and 2019 where possible. Mean MCPD values across all brands of manufacturers were compared over time.

Figure 2 shows the evolution of average (of all brand products, across infant formula categories) total MCPDE levels between 2012 and 2019 for products of six infant formula producers (expressed as free MCPD equivalents; only data from 2015 and 2019 was available for manufacturer D). Data from 2012, 2013, and 2015 were combined ('before 2019'), and compared to data from 2019, following the results of the statistical analysis. Five manufacturers' products show their lowest MCPD content in the 2019 survey (A-E). Manufacturer B shows the most significant difference between 2019 (mean 15.3 ng/g, SD 2.7 ng/g) and previous years (mean 84.3 ng/g, SD 9.5 ng/g), with p-value = 0.0015 by Wilcoxon two-sample test. Manufacturer C shows consistently low MCPD levels over time, between means of 9.3 ng/g in 2019 and 12.6 ng/g in previous surveys. Manufacturers D, E and F remain at levels well above the average of the 2019 survey (31.8 ng/g).

An individual analysis of total MCPDE contents per brand products and manufacturers between 2015 and 2019 is depicted in Figure 3. For 17 brand products (produced by six manufacturers), identical products were sampled in both surveys. Products with matching universal product code (UPC) were considered comparable. For all but two brand products (A4 and C1), a decrease in total bound MCPD values was observed. All 5 brands by manufacturer B see the most significant reduction (B1 to B5). Statistical analysis of the difference (mean 27.0 ng/g, SD 8.7 ng/g) between the 17 data pairs (identical UPC number) between 2015 and 2019 confirmed significance (p-value < 0.001 based on a T-test performed on Box-Cox-transformed data). These findings fit the observation by others (Beekman et al. 2020) that some manufacturers either source refined oils with low contamination content or adopt mitigation strategies during the manufacturing of infant formula products. Furthermore, 2019 survey data confirms that infant formula products that list palm oil or olein as their first fat ingredient show a higher mean sum of MCPDEs (mean of 35.7 ng/g) than those with other primary fats listed first (mean of 26.3 ng/g). Products with sunflower oil or olein listed as main ingredient show the lowest mean sum of MCPDEs (6.0 ng/g).

Conclusion

MCPDE levels in infant formula types sampled in the Canadian market were investigated. An indirect analytical method for the determination of MCPDEs, using acid-catalysed transesterification

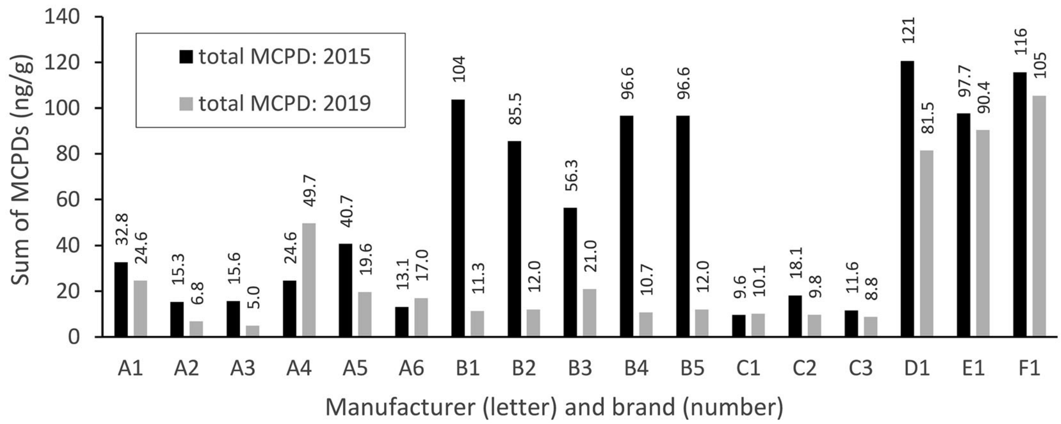

Figure 3. Comparison of MCPD concentrations (sum of 2- and 3-MCPD) by infant formula manufacturer (A–F) and brand (1–5) between 2015 and 2019.

and derivatisation with cyclohexanone was applied. Surveys were conducted in 2015 and 2019, and the data were compared, including results from a previously published, preliminary survey in 2012/2013. Samples included powdered infant formula, concentrates, and ready-to-feed formula. Most significantly, a strong reduction in mean MCPDE levels (expressed as free MCPD equivalents) for all types of infant formula between 2015 (mean of 44.6 ng/g for 3-MCPD) and 2019 (mean of 22.2 ng/g for 3-MCPD) was found. In the latest survey in 2019, 3-MCPD levels in the range of 3.9–74.8 ng/g were observed for ‘as consumed’ formula products (31.5–603 ng/g recalculated for ‘as is’ formula products). Including results from 2012 to 2013 surveys, a significant downward trend between ‘before 2019’ and 2019 data was observed. These findings may support the hypothesis that mitigation strategies for refined oil manufacturing designated for infant formula production are adopted at least partially in the industry. These observations should be substantiated in future surveys.

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Disclosure statement

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