Occurrence of MCPD and glycidyl fatty acid esters in standard and specialized infant formula in Denmark

Nguyen, Khanh Hoang; Fromberg, Arvid

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1   **Occurrence of MCPD and glycidyl fatty acid esters in standard and specialized infant**
2   **formula in Denmark**

3

4   *Khanh Hoang NGUYEN*, Arvid FROMBERG*

5   *National Food Institute, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark*

6   **Corresponding author: khng@food.dtu.dk*

1. Introduction

Monochloropropanediol fatty acid esters (MCPDEs) and glycidyl fatty acid esters (GEs) are food processing contaminants that are formed during the thermal processing of fat via different mechanisms (Cao et al., 2019; Cheng, Liu, & Liu, 2016; Zhang et al., 2015). MCPDEs consist of two isomers depending on the position of chlorine on the propane backbone namely 2-MCPDEs and 3-MCPDEs while GEs do not have any isomers. These chemicals are well-known to present in refined vegetable oils such as palm oil or pomace olive oil due to the high temperature utilized in oil deodorization processes (EFSA CONTAM Panel, 2016; Kuhlmann, 2011). Since vegetable oils are often used in infant formula to mimic the fat composition of breast milk, infants could be exposed to MCPDEs and GEs through formula consumption. This is of high concern because 3-MCPDEs and GEs are hydrolyzed in the human gastrointestinal tract into their corresponding free form 3-MCPD and glycidol which are carcinogens according to the International Agency for Research on Cancer (IARC) (EFSA CONTAM Panel, 2016; IARC, 2000, 2013).

To date, there are still very limited studies on MCPDEs and GEs in infant formula and complex foods. However, several studies have reported the occurrence of MCPDEs and GEs in infant formula worldwide (Arisseto, Silva, Scaranelo, & Vicente, 2017; Becalski, Zhao, Feng, & Lau, 2015; Beekman, Grassi, & MacMahon, 2020; BLE, 2016; Nguyen & Fromberg, 2020; Wöhrlin, Fry, Lahrssen-Wiederholt, & Preiß-Weigert, 2015). For instance, Arisseto et al., 2017 measured concentrations of 3-MCPDEs and GEs in the range of not detected (ND) to 600 µg/kg and ND to 750 µg/kg, respectively, in powder infant formula from the Brazilian market. Our previous published study has also provided a preliminary assessment of MCPDEs and GEs in Danish liquid and powder formula (n=13) sampled in early 2019.

In this study, we aim to investigate more in-depth the degree of MCPDEs and GEs contamination in a wider range of infant formula in Denmark which covered both standard and

specialized products. Standard formula products are often readily available at supermarkets which are intended for healthy infants without any dietary or medical issues. Specialized formula is usually available at pharmacies and designed for infants with special nutritional requirements due to specific medical conditions e.g. premature newborns or infants with cow milk protein allergy (Dipasquale, Serra, Corsello, & Romano, 2020).

2. Materials and methods

2.1. Chemicals and standards

Ethyl acetate, methanol, and toluene (at least 99.8%) were purchased from Rathburn Chemicals Ltd. (Walkerburn, United Kingdom). Ammonium sulfate (99.5%), sodium sulfate anhydrous (99%), sulfuric acid (95-97%), diethyl ether (97%), isooctane (99.8%), n-hexane (99.3%), and n-heptane (99.3%) were acquired from Merck (Darmstadt, Germany). Sodium hydrogen carbonate (99.7%), sodium bromide (99.5%), phenylboronic acid (PBA, 97%), and tetrahydrofuran (THF, 99.9%) were purchased from Sigma-Aldrich Chemical (Missouri, U.S.A). Water was obtained from a Millipore Milli-Q system (Merck, Darmstadt, Germany).

Native standards (rac 1,2-bis-palmitoyl-3-chloropropanediol, 1,3-bis-palmitoyl-2-chloropropanediol (98%), and glycidyl palmitate) and internal standards (rac 1,2-bis-palmitoyl-3-chloropropanediol-d5, 1,3-bis-palmitoyl-2-chloropropanediol-d5, and glycidyl palmitate d5) were purchased from Toronto Research Chemicals (Ontario, Canada) at the highest available purity. The stock and working solutions were prepared in toluene and stored at -18°C.

2.2. Sample collection

Forty-two infant formula products from different manufacturers were purchased at physical and online supermarkets and pharmacies in Denmark during the spring of 2020. The samples covered almost all available infant formula products on the Danish market and

comprised of standard and specialized powder products and standard liquid formula. The sample details can be seen in Table 1. Upon receipt, the samples were stored at ambient conditions until analysis.

2.3. Analytical method

The sample preparation and instrument analysis were carried out according to our previously published study (Nguyen & Fromberg, 2020). The method was in-house validated and accredited according to ISO 17025:2017 by the Danish Accreditation Fund (DANAK).

2.3.1. Sample preparation

An amount of sample containing approximately 100 mg of fat was weighed into a conical centrifuge tube and spiked with 250 ng of internal standards. Twelve mL each of Milli-Q water and ethyl acetate were added and the sample was vortexed for 10 s then ultrasonicated for 15 min at 50 °C. The contaminants were extracted by high speed liquid-liquid extraction using a Geno Grinder 2100 (SPEX SamplePrep, U.S.A) at 1500 rpm for 5 min. Sodium sulfate anhydrous (10 g) was added to assist phase separation and the sample was shaken again at 1500 rpm for 3 min. After centrifugation at 4800 rpm, the upper layer was collected and the extraction process was carried out once more with 12 mL ethyl acetate. The combined upper layer containing the analytes of interest was evaporated to dryness by nitrogen stream at 40 °C then re-dissolved in 1 mL of n-hexane:ethylacetate (85:15, v/v) mixture. The sample was loaded on an aminopropyl SPE cartridge (6 cc, 500 mg, Supelclean^{TM} LC-NH₂, Sigma-Aldrich, U.S.A) and eluted with 10 mL of n-hexane:ethylacetate (85:15, v/v). The cleaned-up sample was evaporated to dryness under a nitrogen stream at 40 °C then redissolved in 2 mL of THF for bromination of GEs.

The bromination of GEs into 3-monobromopropanediol esters (3-MBPDEs) took place by incubating the mixture of the cleaned-up sample in THF and 30 µL of an acid aqueous solution of sodium bromide (sodium bromide 3 mg/mL, sulfuric acid 5% v/v) for 15 min at 50

°C. The reaction was stopped by 3 mL of sodium bicarbonate 0.6% solution and the contaminants were extracted using 2 mL of n-heptane followed by evaporation to dryness under a nitrogen stream at 40 °C. A 1 mL portion of THF was added to redissolve the sample and the sample was subjected to acid methanolysis by adding 1.8 mL of sulfuric acid in methanol solution (1.8% v/v) then incubated for 16 h at 40 °C. After the incubation period, 0.5 mL of saturated sodium bicarbonate solution was added and the remaining organic solvents were evaporated under a nitrogen stream at 40 °C. Afterward, fatty acid methyl esters were salted out from the sample by 1.3 mL of ammonium sulfate solution (40% w/v) and cleaned-up with 2 mL of n-heptane twice. Free forms of 3-MCPDEs, 2-MCPDEs, and 3-MBPDEs were extracted out of the aqueous phase twice by 2 mL of ethyl acetate. The combined extracted was derivatized by added 150 µL of PBA in diethyl ether solution (4% w/v) then ultrasonicated for 5 min at room temperature. After the derivatization process, the sample was gently evaporated to dryness under a nitrogen stream at 40 °C and reconstituted in 300 µL of isooctane. The isooctane aliquot was stored at -18 °C for 2 h and precipitated derivatization reagent was filtered out by a Mini Uni-Prep (0.45 µm, PFTE media). The filtered supernatant was transferred to a GC glass vial and analyzed by GC-MS/MS.

2.3.2. Instrument analysis

The instrument analysis was performed on a GC-MS/MS system (Thermo Fisher Scientific, U.S.A) comprised of a Trace 1300 gas chromatography, a TSQ 8000 EVO triple quadrupole mass spectrometer, and a Triplus RSH autosampler. The gas chromatography was equipped with a Supelco Equity-1 capillary column (30 m x 0.25 mm i.d. x 1.00 µm film thickness, Sigma-Aldrich, Missouri, U.S.A). Samples were injected in PTV splitless mode with the following parameters: 1.5 mL/min constant helium flow rate, 1 µL injection, 0.5 min splitless time, backflush features on at 7.5 min. The PTV temperature program was: starting at 70 °C, stayed for 0.02 min then increased to 280 °C at 8.5 °C/s, maintained for 7.5 min then

increased to 330 °C at 10 °C/s, held constant until the end of the analysis. The analytes were separated using the following oven program: starting at 60 °C for 1 min; increased to 120 °C at 30 °C/min; increased to 250 °C at 8 °C/min; then increased 30 °C/min to 260 °C, held for 3 min. The transfer line was kept constant at 300 °C. The ion source was operated at 280 °C and 70 eV.

2.4. QA/QC

The method was previously validated with excellent precision and accuracy (Nguyen & Fromberg, 2020). Several on-going QA/QC procedures were implemented to ensure the quality of obtained data. For each batch of sample, a completely new procedural calibration curve was analyzed together with the samples. All calibration curves showed r^2 greater than 0.999. Procedural blanks (5 µL of an extra virgin olive oil) showed no contamination during sample preparations. Reference material (FAPAS® 2656, powder infant formula) was also analyzed in every batch to serve as an on-going QC sample. Since this material does not contain an assigned value for GE_s, we used the in-house value for GE_s in this material instead. This in-house GE_s value was calculated by averaging the results from the analysis of 7 sub-samples of the FAPAS® 2656 material (Table S.1). On-going analysis of the reference material demonstrated a very consistent method performance (Table S.1). The concentrations of MCPDE_s and GE_s in this study were expressed as µg/kg of bound forms (meaning they were converted to free (2-)3-MCPD or glycidol concentrations, respectively) without any reconstitution correction for powder formula.

3. Results and discussion

3.1. Occurrence

The concentrations of 2-MCPDE_s, 3-MCPDE_s, and GE_s in standard and specialized infant formula available on the Danish market are described in Table 1. In standard liquid formula, 2-MCPDE_s, 3-MCPDE_s, and GE_s were measured at mean concentrations of 2.1, 5.4, and 1.7

µg/kg, respectively. These results are very similar to what we have found in liquid formula collected in 2019 (Nguyen & Fromberg, 2020). The highest concentrations of MCPDEs isomers were found in liquid products from the manufacturer C at around 3.3 µg/kg for 2-MCPDEs and 7.8 µg/kg for 3-MCPDEs. For GEs, however, the product from the manufacturer D contained the highest amount at 4.3 µg/kg. Meanwhile, the liquid formula from manufacturers A and B were roughly similar in terms of MCPDEs and GEs contamination. For normal powder formula variety, the average concentrations of 2-MCPDEs, 3-MCPDEs, and GEs were recorded at 11.8, 31.0, and 11.5 µg/kg which were also in good agreement with our preliminary study in 2019 (Nguyen & Fromberg, 2020). Among all standard powder formula, the highest concentrations of both 2- and 3-MCPDEs were found in a product from the manufacturer C at 22.7 µg/kg of 2-MCPDEs and 59.6 µg/kg of 3-MCPDEs (Item 23, Table 1). It might be plausible that these high concentrations of MCPDEs are related to the high fat content of the product. However, assessment of the MCPDEs concentrations after conversion to fat basis (data not showed) and scatter plots between revealed no such obvious trend. Indeed, the lowest concentrations of MCPDEs were found in two products from the manufacturer A with at least four times lower than other normal powder formula (Item 13 and 14, Table 1). Since items number 13, 14, and 23 were all marketed as organic products with relatively similar fat contents, such a big discrepancy in MCPDEs concentrations might be accounted for by the fact that no palm oil was used in items 13 and 14. It is well-known that palm oil is among the plant oils most affected by MCPDEs contamination due to its high contents of total chlorine and diacylglycerides (Franke, Strijowski, Fleck, & Pudiel, 2009; Oey, van der Fels-Klerx, Fogliano, & van Leeuwen, 2019). In terms of GEs contamination, normal powder products from manufacturer A generally contained slightly less amount of GEs with the lowest level found in item 13 at 3.0 µg/kg. However, the highest amounts were found in products from both manufacturers A and B and around 21.5 µg/kg.

In specialized powder formula, the GEs concentrations were quite similar to those in normal powder formula with a mean concentration of 11.2 µg/kg (ranged between 1.0 – 30.7 µg/kg). Slightly higher MCPDEs concentration in specialized products than standard ones were observed at 18.0 µg/kg for 2-MCPDEs and 49.8 µg/kg for 3-MCPDEs. In order to evaluate if the higher 2-MCPDEs and 3-MCPDEs levels in the specialized formula were significant, some statistical test was carried out at 95% level of confidence. Shapiro-Wilk test confirmed the normal distribution of both 2-MCPDEs and 3-MCPDEs in either standard or specialized powder formula. F-test also assured equal variances of the contaminant concentrations in two product types. With the satisfying conditions of normal distribution and equal variances, a t-test for mean comparison was applied. The results from the t-test revealed that both MCPDEs isomers in specialized powder formula showed significantly higher concentrations than those in standard ones ($p < 0.05$, 95% confidence level). This is the first time such a significant discrepancy was observed for MCPDEs in these two types of infant formula. Leigh & MacMahon, 2017 also assessed 3-MCPDEs and GEs in different U.S infant formula varieties but found no such trend. In terms of manufacturer differences for specialized formula, the lowest concentrations of 2-MCPDEs (8.7 µg/kg), 3-MCPDEs (18.3 µg/kg), and GEs (1.0 µg/kg) were found in products from manufacturer A. On the other hand, one product from manufacturer F showed the highest concentrations of both MCPDEs and GEs at 32.9 µg/kg for 2-MCPDEs, 102.8 µg/kg for 3-MCPDEs, and 30.7 µg/kg for GEs.

Overall, the occurrence data for MCPDEs and GEs showed that products from manufacturer A tend to contain less amount of these processing contaminants, and specialized powder infant formula is significantly higher in MCPDEs than standard formula products. Despite the elevated concentrations of MCPDEs in specialized products, the levels of both MCPDEs and GEs in infant formula in this study are still among the lowest reported worldwide (Arisseto et al., 2017; Becalski et al., 2015; Beekman et al., 2020; Wöhrlin et al., 2015). For instance, a

recent study in Brazil showed up to 600 µg/kg of 3-MCPDEs and 750 µg/kg of GEs in infant formula available in this country (Arisseto et al., 2017).

3.2. Comparison between different powder formula varieties

Due to the lack of specialized liquid formula samples, in this section, only a comparison between standard and specialized powder formula was made. For standard powder formula, there were three varieties namely normal, premium, and organic. Within a brand, normal products are the most basic ones, premium products are branded as such and often packed with more ingredients for various purposes (e.g. digestive improvement, cognitive development enhancement, etc.), and organic products are produced with organic ingredients. Specialized formulas were also divided into three varieties according to their medical purposes: premature for premature-newborn, sensitive for babies with sensitivity toward lactose, and hypoallergenic for infants with allergies toward cow milk.

Previously, Leigh & MacMahon, 2017 studied different infant formula varieties in the United States and found hypoallergenic ones contained much lower levels of 3-MCPDE and GEs than other varieties. This was, however, not the case in this study. In fact, hypoallergic infant formula showed a similar amount of GEs and higher MCPDEs concentrations than other varieties (Table 2). In specific, the average concentrations of 2- and 3-MCPDEs in the hypoallergenic formula were 18.6 and 51.9 µg/kg, respectively. These values were slightly higher than those found in other specialized products intended for premature babies and infants with sensitive stomach, and significantly higher than all varieties in standard infant formula (t-test at 95% confidence level, $p < 0.05$). Interestingly, organic products showed the lowest levels of 2- and 3-MCPDEs at 7.5 and 22.6 µg/kg, respectively. From the perspective of GEs contamination, premature formula showed the lowest level at 6.4 µg/kg which were quite similar to premium and organic products, while normal and sensitive products contained the highest GEs concentration.

Overall, the MCPDEs contamination patterns among different varieties were observed as follows: hypoallergenic $\approx$ sensitive $>$ premature $>$ normal $\approx$ premium $>$ organic. For GE, the trend was: normal $\approx$ sensitive $>$ hypoallergenic $>$ premium $\approx$ organic $>$ premature.

3.3. Exposure estimation and risk assessment

The exposure of exclusively formula-fed infants to different types of formula was estimated according to our previous publication (Nguyen & Fromberg, 2020). Briefly, the exposure estimation was carried out for two groups of infants according to their age. The first group is newborn children up to 1 month old with 4.2 kg average body weight, and the second group is infants from 2 to 5 months old with an average body weight of 7.3 kg (Danish Health Authority, 2019). The suggested infant formula consumption per day is 170 mL/kg body weight (bw) for the first group, and 110 mL/kg bw for the second one (Danish Health Authority, 2019). In order to convert powder formula to ready-to-consume liquid formula, a factor of 7.7 was used (EFSA CONTAM Panel, 2016). Unless stated otherwise, the exposure estimations were carried out using the average concentrations of the chemicals in different type-variety combinations. The estimations of MCPDEs and GE exposure of Danish infants receiving formula only are described in Table 3.

In the first group of newborn and up to 1-month-old babies, the daily exposure to 2-MCPDEs ranged from 0.2 to 0.41 $\mu\text{g/kg bw/day}$. These ranges for 3-MCPDEs and GE were 0.52 – 1.14 $\mu\text{g/kg bw/day}$ and 0.09 – 0.17 $\mu\text{g/kg bw/day}$, respectively. In the second group of 2 – 5 months old babies, the respective daily exposure to 2-MCPDEs, 3-MCPDEs, and GE were: 0.11 – 0.24, 0.3 – 0.66, and 0.09 – 0.17 $\mu\text{g/kg bw/day}$. These estimated exposures were in good agreement with what we reported in our preliminary study on infant formula (Nguyen & Fromberg, 2020). Among all different types of formula, the highest estimated exposure to MCPDEs was via consumption of hypoallergenic products, which were approximately two times higher than the lowest exposure in organic products. For instance, newborn to 1-month

231 babies were estimated to expose to 3-MCPDEs at 1.14 $\mu\text{g/kg bw/day}$ if they consumed solely
232 hypoallergenic formula. This value dropped to 0.52 $\mu\text{g/kg bw/day}$ if the organic formula was
233 chosen. Regardless of the variety formula of choice, their estimated daily exposures were still
234 below the Tolerable Daily Intake for 3-MCPDEs (TDI) at 2 $\mu\text{g/kg bw/day}$ set by EFSA (EFSA
235 CONTAM Panel, 2018). However, it is important to note that on an individual product level,
236 one hypoallergenic powder formula (item number 35, Table 1) showed an 3-MCPDEs exposure
237 value of 2.27 $\mu\text{g/kg bw/day}$ for newborn to 1-month infants. This was higher than the TDI
238 established by EFSA and further monitoring is needed to warrant a safe 3-MCPDEs intake
239 level from this product.

240 In terms of GE exposure, the lowest estimated exposures were found in premature products at
241 0.14 $\mu\text{g/kg bw/day}$ for 0 – 1 month group, and 0.08 $\mu\text{g/kg bw/day}$ for 2 – 5 months group. The
242 highest exposures to GEs were observed in normal products, specifically at 0.31 $\mu\text{g/kg bw/day}$
243 for 0 – 1 month group, and 0.18 $\mu\text{g/kg bw/day}$ for 2 – 5 months group. Since there is no TDI
244 available for GEs, a margin of exposure (MoE) calculation approach was employed to assess
245 the exposure of infants to GEs. The T25 value of 10.2 mg/kg bw per day for peritoneal
246 mesothelioma in male rats was used as the no-observed-adverse-effect level for MoE
247 calculation according to EFSA (EFSA CONTAM Panel, 2016). A MoE of 25,000 or higher is
248 considered to of low health concern for GEs exposure. In this study, the estimated MoEs to
249 GEs for 0 – 1 month babies ranged between 14000 to 460000 for different individual infant
250 formula products. Specifically, one standard liquid formula product (item number 10, Table 1),
251 two standard powder formula (items number 12 and 19, Table 1), and one specialized powder
252 formula (item number 35, Table 1) showed MoEs smaller than 25000 which indicated a health
253 concern. For the 2 – 5 months group, the MoEs were between 22000 to 710000 and two powder
254 formula products (items number 19 and 35, Table 1) showed MoEs smaller than 25000 in this
255 age group. With the assumption that parents are highly loyal to a certain infant formula brand

or product, the GEs health concern in relation to items number 10, 12, 19, and 35 might require further investigations. This is even more true for item number 35 since this item also possesses a higher than TDI for estimated 3-MCPDEs exposure in 0 – 1 month group. Overall, most standard or specialized infant formula showed no particular health concern for both GEs and 3-MCPDEs exposure with a few exceptions as discussed above.

4. Conclusion

This study investigated a more in-depth occurrence of MCPDEs and GEs in different infant formula types and varieties available in Denmark, including both standard formula and specialized formula for medical purposes. Our finding indicated overall higher levels of MCPDEs but similar levels of GEs in specialized infant formula products in comparison with the standard ones. Among all different varieties, the concentrations of MCPDEs were the highest in the hypoallergenic formula and lowest in the organic formula. Meanwhile, the highest levels of GEs were observed in the normal formula and lowest in the premature formula. Despite the higher levels of MCPDEs in specialized products, on average our estimation of MCPDEs and GEs exposure via formula consumption for Danish infants showed no particular risks. However when individual products were assessed, a hypoallergenic powder formula showed health concerns for both 3-MCPDEs and GEs exposure, while three other standard formulas showed GEs exposure risk. Further investigations in the future might be needed to warrant safety for infants exclusively fed with these products.

Declarations of interest: none

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References

- Arisseto, A. P., Silva, W. C., Scaranelo, G. R., & Vicente, E. (2017). 3-MCPD and glycidyl esters in infant formulas from the Brazilian market: Occurrence and risk assessment. *Food Control*, 77, 76–81. <https://doi.org/10.1016/j.foodcont.2017.01.028>
- Becalski, A., Zhao, T., Feng, S., & Lau, B. P. Y. (2015). A pilot survey of 2- and 3-monochloropropanediol and glycidol fatty acid esters in baby formula on the Canadian market 2012-2013. *Journal of Food Composition and Analysis*, 44, 111–114. <https://doi.org/10.1016/j.jfca.2015.08.004>
- Beekman, J. K., Grassi, K., & MacMahon, S. (2020). Updated occurrence of 3-monochloropropane-1,2-diol esters (3-MCPD) and glycidyl esters in infant formulas purchased in the United States between 2017 and 2019. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 37(3), 374–390. <https://doi.org/10.1080/19440049.2019.1706002>
- BLE (The German Federal Office for Agriculture and Food for the Federal Ministry of Food and Agriculture). (2016). *Investigation into the presence of 3-MCPD esters and related compounds in foods*. Retrieved from https://service.ble.de/ptdb/index2.php?detail_id=56944&site_key=141&stichw=2815HS002
- Cao, R., Wang, S., Li, C., Liu, W., Zhou, H., & Yao, Y. (2019). Molecular Reaction Mechanism for the Formation of 3-chloropropanediol Esters in Oils and Fats. *Journal of Agricultural and Food Chemistry*. <https://doi.org/10.1021/acs.jafc.8b06632>
- Cheng, W., Liu, G., & Liu, X. (2016). Formation of Glycidyl Fatty Acid Esters Both in Real Edible Oils during Laboratory-Scale Refining and in Chemical Model during High Temperature Exposure. *Journal of Agricultural and Food Chemistry*, 64(29), 5919–

303 5927. <https://doi.org/10.1021/acs.jafc.6b01520>

304 Danish Health Authority. (2019). Ernæring til spædbørn og småbørn. Retrieved August 8,
 305 2019, from [https://www.sst.dk/da/sundhed-og-](https://www.sst.dk/da/sundhed-og-livsstil/ernaering/~/_media/2986643F11A44FA18595511799032F85.ashx)
 306 [livsstil/ernaering/~/_media/2986643F11A44FA18595511799032F85.ashx](https://www.sst.dk/da/sundhed-og-livsstil/ernaering/~/_media/2986643F11A44FA18595511799032F85.ashx)

307 Dipasquale, V., Serra, G., Corsello, G., & Romano, C. (2020). Standard and Specialized
 308 Infant Formulas in Europe: Making, Marketing, and Health Outcomes. *Nutrition in*
 309 *Clinical Practice*, 35(2), 273–281. <https://doi.org/10.1002/ncp.10261>

310 EFSA CONTAM Panel. (2016). Risks for human health related to the presence of 3- and 2-
 311 monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid
 312 esters in food. *EFSA Journal*, 14(5). <https://doi.org/10.2903/j.efsa.2016.4426>

313 EFSA CONTAM Panel. (2018). Update of the risk assessment on 3-monochloropropane diol
 314 and its fatty acid esters. *EFSA Journal*, 16(1), 1–48.
 315 <https://doi.org/10.2903/j.efsa.2018.5083>

316 Franke, K., Strijowski, U., Fleck, G., & Pudel, F. (2009). Influence of chemical refining
 317 process and oil type on bound 3-chloro-1,2-propanediol contents in palm oil and
 318 rapeseed oil. *LWT - Food Science and Technology*, 42(10), 1751–1754.
 319 <https://doi.org/10.1016/j.lwt.2009.05.021>

320 IARC. (2000). Glycidol. *IARC Monographs*, 1–18. Retrieved from
 321 <https://monographs.iarc.fr/ENG/Monographs/vol77/mono77-19.pdf>

322 IARC. (2013). 3-Monochloro-1,2-propanediol. *IARC Monographs*, 101.

323 Kuhlmann, J. (2011). Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound
 324 monochloropropanediol (MCPD) in refined oils. *European Journal of Lipid Science and*
 325 *Technology*, 113(3), 335–344. <https://doi.org/10.1002/ejlt.201000313>

- Leigh, J., & MacMahon, S. (2017). Occurrence of 3-monochloropropanediol esters and glycidyl esters in commercial infant formulas in the United States. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 34(3), 356–370. <https://doi.org/10.1080/19440049.2016.1276304>
- Nguyen, K. H., & Fromberg, A. (2020). Monochloropropanediol and glycidyl esters in infant formula and baby food products on the Danish market: Occurrence and preliminary risk assessment. *Food Control*, 110(November 2019), 106980. <https://doi.org/10.1016/j.foodcont.2019.106980>
- Oey, S. B., van der Fels-Klerx, H. J., Fogliano, V., & van Leeuwen, S. P. J. (2019). Mitigation Strategies for the Reduction of 2- and 3-MCPD Esters and Glycidyl Esters in the Vegetable Oil Processing Industry. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 349–361. <https://doi.org/10.1111/1541-4337.12415>
- Wöhrlin, F., Fry, H., Lahrssen-Wiederholt, M., & Preiß-Weigert, A. (2015). Occurrence of fatty acid esters of 3-MCPD, 2-MCPD and glycidol in infant formula. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 32(11), 1810–1822. <https://doi.org/10.1080/19440049.2015.1071497>
- Zhang, Z., Gao, B., Zhang, X., Jiang, Y., Xu, X., & Yu, L. (2015). Formation of 3-monochloro-1,2-propanediol (3-MCPD) Di- and monoesters from tristearoylglycerol (TSG) and the potential catalytic effect of Fe²⁺ and Fe³⁺. *Journal of Agricultural and Food Chemistry*, 63(6), 1839–1848. <https://doi.org/10.1021/jf5061216>

Table 1. Concentrations of 2-MCPDEs, 3-MCPDEs, and GEs in standard and specialized infant formula available on the Danish market

Item	Brand	Specific variety	Fat content (%) [*]	2-MCPDEs (µg/kg) ^{**}	3-MCPDEs (µg/kg) ^{**}	GEs (µg/kg) ^{**}
Liquid formula, standard						
1	A	Normal	3.6	1.9	6.2	1.9
2	A	Organic	3.6	1.6	3.1	1.0
3	A	Normal	3.2	1.7	5.3	1.6
4	A	Organic	3.2	1.2	3.3	0.7
5	A	Normal	3	1.2	3.9	0.5
6	B	Normal	3.5	1.9	5.1	1.0
7	B	Normal	2.9	2.1	5.9	1.0
8	C	Normal	3.6	3.2	7.7	2.2
9	C	Normal	3.1	3.3	7.9	2.3
10	D	Normal	3.6	2.5	5.9	4.3
<i>Mean</i>				<i>2.1</i>	<i>5.4</i>	<i>1.7</i>
Powder formula, standard						
11	A	Premium	26	14.5	36.7	9.4
12	A	Normal	28	15.9	43.7	21.5
13	A	Organic	28	1.9	5.3	8.1
14	A	Organic	24	1.1	8.1	3.0
15	A	Premium	23	11.1	30.7	7.3
16	A	Normal	28	7.6	22.4	3.8
17	A	Normal	22	20.1	42.3	8.5
18	B	Normal	27	13.3	32.5	15.4
19	B	Normal	27	22.3	50.1	21.8
20	B	Normal	19	13.2	35.6	14.5
21	B	Normal	19	8.0	26.0	13.7
22	B	Normal	20	9.7	23.6	13.6
23	C	Organic	28	22.7	59.6	13.4
24	C	Organic	22.5	4.3	17.2	7.6
<i>Mean</i>				<i>11.8</i>	<i>31</i>	<i>11.5</i>
Powder formula, specialized						
25	A	Premature	26.4	22.1	63.7	11.7
26	A	Premature	18	8.9	18.3	1.0
27	A	Sensitive	28	26.5	69.2	13.5
28	A	Sensitive	25	18.5	47.5	15.8
29	A	Hypoallergenic	26	19.6	45.3	13.6
30	A	Hypoallergenic	23	8.7	32.2	8.2
31	A	Hypoallergenic	26	20.6	65.9	14.8
32	A	Hypoallergenic	24.6	17.2	47.9	8.8
33	B	Sensitive	27	13.5	36.0	11.1
34	B	Sensitive	19	11.4	39.1	13.0
35	F	Hypoallergenic	26	32.9	102.8	30.7
36	F	Hypoallergenic	25	20.3	55.6	9.9

37	F	Hypoallergenic	20	16.9	47.1	5.4
38	F	Hypoallergenic	28	18.6	67.5	6.2
39	G	Hypoallergenic	25.7	24.3	56.8	12.0
40	G	Hypoallergenic	21.8	16.7	40.8	9.5
41	H	Hypoallergenic	22	16.6	33.9	7.9
42	H	Hypoallergenic	24.5	10.2	26.4	9.2
<i>Mean</i>				<i>18.0</i>	<i>49.8</i>	<i>11.2</i>

*: information specified on the labels

**: µg/kg product expressed as free form equivalent

Table 2. Mean concentrations of 2-MCPDEs, 3-MCPDEs, and GEs in Danish powder infant formula categorized by variety

Type	Variety	2-MCPDEs (µg/kg)*	3-MCPDEs (µg/kg)*	GEs (µg/kg)*
Standard	Normal (n=8)	13.8	34.5	14.1
	Premium (n=2)	12.8	33.7	8.3
	Organic (n=4)	7.5	22.6	8.0
Specialized	Premature (n=2)	15.5	41.0	6.4
	Sensitive (n=4)	17.5	47.9	13.3
	Hypoallergenic (n=12)	18.6	51.9	11.3

*: µg/kg product expressed as free form equivalent

Table 3. Estimated exposure of Danish infants to 2-MCPDEs, 3-MCPDEs, and GEs via formula consumption according to different formula varieties (µg/kg bw/day)

Compound	2-MCPDEs		3-MCPDEs		GEs	
Age group (months)	0-1	2-6	0-1	2-6	0-1	2-6
Standard, Normal	0.34	0.20	0.89	0.51	0.31	0.18
Standard, Premium	0.28	0.16	0.74	0.43	0.18	0.11
Standard, Organic	0.20	0.11	0.52	0.30	0.16	0.09
Specialized, Premature	0.34	0.20	0.91	0.52	0.14	0.08
Specialized, Sensitive	0.39	0.22	1.06	0.61	0.29	0.17
Specialized, Hypoallergenic	0.41	0.24	1.14	0.66	0.25	0.14

Table S.1. Concentrations of 2-MCPDEs, 3-MCPDEs, and GEs in FAPAS® 2656 infant formula samples analyzed along with real samples in different batches (µg/kg, free form equivalent)

	FAPAS® assigned value	In-house assigned value*	Range for $ z ^{**} \leq 2$	Batch 1	Batch 2	Batch 3
2-MCPDEs	16.4	16.6	9.16 – 23.6	15.8	17.4	15.0
3-MCPDEs	44	40	24.6 – 63.4	43.6	40.3	40.6
GEs		20.7		18.4	22.7	16.7

*: Determined from in-house analysis of seven sub-samples of FAPAS® 2656 material

**: z-score derived from a proficiency test using FAPAS® 2656 reference material