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1 | **Monochloropropanediol and glycidyl esters in infant formulae and baby food products**
2 **on the Danish market: Occurrence and preliminary risk assessment**

3

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Abstract

A GC/MS-MS method for determination of monochloropropanediol (MCPDEs) and glycidol (GEs) was developed, validated, and applied to analyze ~~6054~~ infant formula and baby food products commercially available on the Danish market. The contaminants were quantifiable in almost all sample categories with the exception of fruit or vegetable based purees. The highest concentrations of 3-MCPDEs and 2-MCPDEs were found in a baby biscuit product at 102.6 µg/kg and 52.8 µg/kg, respectively. Meanwhile, a baby puff item contained GEs as high as 149 µg/kg. MCPDEs and GEs concentrations are well correlated with each others in all sample types except puffs and biscuits. Occurrence data suggested that GEs formation might have taken place during production of baby puffs by unknown mechanisms. Preliminary exposure and risk assessment showed no apparent risk of Danish infants to these chemicals in formulae and baby foods. However, special attention should be paid when feeding babies with puffs or similar items due to their relatively high level of GEs.

Keywords

MCPD esters, glycidyl esters, infant formulae, baby food, processing contaminants, risk assessment

1. Introduction

Process contaminants are chemicals that are generated when food constituents undergo chemical changes during processing. Some of their prime examples are the well-studied acrylamide, polyaromatic hydrocarbons, or furan and furan derivatives¹⁻³. Fatty acid esters of monochloropropanediol (MCPDEs) and glycidol (GEs) are emerging process contaminants that are often found in oil-containing products⁴. According to the position of chlorine, MCPDEs are divided into two types: 3-monochloro-1,2-propanediol esters (3-MCPDEs) and 2-monochloro-1,3-propanediol esters (2-MCPDEs). The presence of 3-MCPDEs in some foodstuffs was firstly reported in 2004⁵. This isomer of MCPDEs then came into attention in 2006 after it was detected at up to 2462 µg/kg in refined seed and olive oils⁶. Both 2- and 3-MCPDEs are formed by reactions of lipids and chlorines at high temperature, most notably during oil deodorization process (Cao et al., 2019). Under the same condition, lipids can also transform into GEs by different mechanisms than MCPDEs (Cheng et al., 2016). In many countries, MCPDEs and GEs have been detected in refined vegetable oils and products contains refined vegetable oils such as shortenings, infant formulae, bakeries, confectionaries, etc. ~~in many countries~~⁹⁻¹². Recent studies found very high concentrations of these contaminants in refined vegetable oils, up to 3770 µg/kg for 3-MCPDEs, 1910 µg/kg for 2-MCPDEs, and 1880 µg/kg for GEs¹³. Similarly, up to 510 and 720 µg/kg for 3-MCPDEs and GEs have been detected in Brazilian infant ~~formulae~~formula, respectively¹¹. Significant levels of GEs can also be generated during heat treatment of meat patties, especially at high temperature using on open fire charcoal grill¹⁴.

Upon consumption, 2-MCPDEs, 3-MCPDEs, and GEs are readily hydrolyzed by the gastrointestinal tract into their corresponding free forms (2-MCPD, 3-MCPD, and glycidol, respectively)^{15,16}. This is of high concern due to their potential toxicological effects on humans. Glycidol is classified as a genotoxic carcinogen by both EFSA¹⁷ and the

International Agency for Research on Cancer – IARC ¹⁸ -while 3-MCPD is listed as a non-genotoxic carcinogen by IARC ¹⁹. Currently very limited toxicological data of free 2-MCPD or 2-MCPDEs ~~is~~^{are} available. A recent *in vivo* research suggested that 2-MCPD exerts mild toxicity on rat kidney via different molecular mechanisms ~~s~~ than 3-MCPD ²⁰.

Ubiquitous in food products with high toxicity, 3-MCPDEs and GE~~s~~ have drawn a global concerns~~s~~. In a scientific report in 2016 by EFSA, infant~~s~~ receiving only ~~formulae~~^{formula} were estimated~~d~~ to be at high risks of exposure to 3-MCPD (total of free and bound forms) and glycidol (from GE~~s~~) ¹⁷. Specifically, estimated daily exposure of such infants based on mean consumption and mean occurrence were 2.4 µg/kg b~~.~~w for 3-MCPD and 1.9 µg/kg b~~.~~w for glycidol. In this report, EFSA has also established a Tolerable Daily Intake (TDI) of 0.8 µg/kg b~~.~~w for 3-MCPD but later increased ~~it~~ to 2 µg/kg b~~.~~w in 2017 after some revisions ²¹. Even higher risks ~~s~~ ~~have~~^{have} been recently reported for infants in U.S.A and Brazil ^{11,22}.

Against this background, we aim to investigate the occurrence of MCPDEs and GE~~s~~ in infant formulae~~e~~ and baby foods available on the Danish market. From th~~is~~^{ese} occurrence data, dietary exposure and potential risks from consumption of infant formulae~~e~~ and baby foods will be estimated.

2. Materials and methods

2.1. Chemicals and standards

Ammonium sulfate (99.5%), diethyl ether (97%), isooctane (99.8%), n-hexane (99.3%), n-heptane (99.3%), sodium sulfate anhydrous (99%), and sulfuric acid (95-97%) were purchased from Merck (Darmstadt, Germany). Sodium hydrogen carbonate (99.7%), sodium bromide (99.5%), phenylboronic acid (PBA, 97%), and tetrahydrofuran (THF, 99.9%) were obtained from Sigma-Aldrich Chemical (Missouri, U.S.A). Ethyl acetate

(99.9%), methanol (MeOH, 99.9%) and toluene (99.8%) were provided by Rathburn Chemicals Ltd. (Walkerburn, United Kingdom). Water was obtained from a Millipore Milli-Q system (Merch, Darmstadt, Germany).

Rac 1,2-bis-palmitoyl-3-chloropropanediol (PP-3-MCPD, 98%), Rac 1,2-bis-palmitoyl-3-chloropropanediol-d5 (PP-3-MCPD-d5, 98%), 1,3-bis-palmitoyl-2-chloropropanediol (PP-2-MCPD, 98%), 1,3-bis-palmitoyl-2-chloropropanediol-d5 (PP-2-MCPD-d5, 98%), Glycidyl palmitate (Gly-P, 98%), Glycidyl palmitate d5 (Gly-P-d5, 97%) were purchased from Toronto Research Chemicals (Ontario, Canada). All stock solutions and working solutions were prepared in toluene.

2.2. Sample collection

A total of ~~6054~~ samples from different manufacturers were purchased at various supermarkets in Zealand, Denmark during the spring of 2019. The samples comprised of different commercial food products intended for infants and babies: powder infant formulae, liquid infant formulae, cereal based and vegetable based baby foods, smoothies, puffs, and biscuits. The sample details can be seen in Table 1. Samples were homogenized and stored at -18 °C until analysis.

2.3. Analytical method

The analytical method was developed and validated based on the European Commission's Joint Research Center method report EUR 27288 ²³, and AOCS Official First Action Method 2018.03 ²⁴ with some modifications.

2.3.1. Sample preparation

Since MCPDEs and GEs are highly lipophilic, a fat extraction step was necessary to extract these contaminants out of food matrices. Based on the fat content information on the food packages, an amount of sample containing approximately 100-110 mg of fat was

weighed into a 50 ml centrifuge tube (maximum 5 g of sample). The sample was spiked with 50 μ L of an internal standard mixture (5 ng/ μ L) containing PP-3-MCPD-d5, PP-2-MCPD-d5, and Gly-P-d5. Then, 12 mL of Milli-Q water and 12 mL of ethyl acetate were added to the tube and the mixture was vortexed for 10 s before sonication for 15 min at 50 °C in an ultrasonicator (Branson 5510, Branson Ultrasonics, U.S.A). Afterwards, the samples were placed on a ~~high-high~~ speed vertical shaker (Geno Grinder 2100, SPEX SamplePrep, U.S.A) and extracted at 1500 rpm for 5 min. For better phase separation and salting out effect, 10 g of sodium sulfate anhydrous was added before the sample was shaken again at 1500 rpm for 3 min follow~~ed~~ing by centrifugation at 4800 rpm²⁵. The upper layer was collected and an ~~additional ether~~ 12 mL of ethyl acetate was added to the tube for a second extraction (including ultrasonication and shaking). Extracted fat from the sample was obtained by evaporation of the combined upper layers to dryness under a nitrogen stream at 40 °C.

It has been reported that food items contain a significant amount of partialglycerides, especially monoacylglycerides (MAGs), can cause ~~artefact-artifact~~ formation of GEs during bromination of GEs²⁶. Therefore, a cleanup step by aminopropyl SPE cartridge was employed to remove MAGs²⁶. Extracted fat was re-dissolved in 1 mL of mixture A containing n-hexane and ethylacetate at a ratio of 85:15 (v/v) then loaded on a SPE cartridge previously conditioned with 2 mL of mixture A. The cartridge was eluted with 10 mL of mixture A into a collection test tube then the sample was evaporated to dryness under a nitrogen stream at 40 °C.

The bromination of GEs to 3-monobromopropanediol monoesters (3-MBPDEs), transesterification, and derivatizations were performed according to Dubois et al., 2018. Briefly, the procedure included bromination of GEs by acidic sodium bromide solution; acid methanolysis; salting out of fatty acid methyl esters by ammonium sulfate and n-heptane; extraction of free forms of 3-MCPDEs, 2-MCPDEs, and 3-MBPDEs by ethyl acetate; and

derivatization by PBA. After the derivatization process, the sample was carefully evaporated to dryness under a gentle nitrogen stream at 40 °C then re-dissolved in 300 µL of isooctane. While PBA is a good derivatization reagent for diols, the chemical is quite harsh and sticky on GC columns, leading to rapid column degradation. Therefore, the isooctane containing derivatized analytes ~~w~~as~~s~~ transferred into a Mini Uni-Prep PTFE housing (Whatmann, U.S.A) and stored at -18 °C for 1-2 hours to precipitate excess PBA. Afterwards, the Mini Uni-Prep 0.45 µm filter (PTFE media) was pressed into the housing to filter out precipitated PBA. The filtered supernatant was ~~trans~~ferred to a GC glass vial for analysis by GC-MS/MS.

2.3.2. Instrument analysis

The analysis of 3-MCPDEs, 2-MCPDEs, and GEs (in the form of derivatized 3-MCPD, 2-MCPD, and 3-MBPD, respectively) was performed on a GC-MS/MS system (Thermo Fisher Scientific, U.S.A) comprised of a Trace 1300 gas ~~chromatograph~~chromatography, a TSQ 8000 EVO triple quadrupole mass spectrometer, and a Triplus RSH autosampler. Chromatographic separation of the target analytes was achieved on 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). A 1 µL aliquot of sample was injected in PTV splitless mode using helium as carrier gas at a constant flow rate of 1.5 mL/min. The PTV program was: 70 °C for 0.02 min; increased to 280 °C at a rate of 8.5 °C/s for 8 min; then increased ~~d~~ to 330 °C at a rate of 10 °C/s for 14.5 min. The splitless time was 0.5 min, after that a split flow of 30 mL/min was applied. ~~The b~~Backflush feature was utilized to remove residues of excess PBA and reduce ~~maintain~~-maintenance frequency. The following oven program was used: initial temperature of 60 °C for 1 min; increased 30 °C/min to 120 °C; ramped to 250 °C at 8 °C/min; then increased 30 °C/min to 260 °C, held for 3 min, for a total run time of 22.6 min. The transfer line and ion source were held constant at 300 °C and 280 °C, respectively.

Detection was performed in positive electron impact ionization (70 eV) with selected reaction monitoring (SRM) mode. Detailed SRM scans are shown in Table S1.

2.4. Method validation

The method was validated in-house by two persons in terms of linearity, precision (intra-lab reproducibility and inter-day repeatability), accuracy (recovery), limit of detection (LOD), and limit of quantification (LOQ) according to the guidelines by ISO 17025. Linearity was assessed using an eight point procedural calibration curve over the range of 0 - 1000 ng (bound forms). Calibrants were prepared by spiking native standards (at the levels of 2, 6, 20, 50, 100, 300, 500, and 1000 ng) and labeled standards (250 ng) into 5 μ L of extra virgin olive oil and proceeded to bromination, transesterification, and derivatization as normal samples. The linear procedural calibration curve was plotted between the peak area ratio of an analyte with its corresponding internal standard on the Y-axis versus the amount of that analyte on the X-axis using 1/X weighting to ensure fitting across the whole calibration range.

Precision and accuracy were evaluated by spiking pseudo-blank vegetable oil, infant ~~formulae~~formula and baby food samples (containing low levels of MCPDEs and GEs) with native standards at two concentration levels (Table 2). Each level of concentration was analyzed in eight replicates (four replicates on the first day by the first ~~technician~~personnel and another four on the second day with a second ~~personnel~~technician). Additionally, a reference material of vegetable oil (FAPAS T2646QC) was used to further assess the trueness of analysis results for 3-MCPDEs and GEs. LODs and LOQs were calculated based on the standard deviations of MCPDEs and GEs in 10 pseudo-blank replicates (Std_{blk}) as following: $\text{LOD} = 3.3 \times \text{Std}_{\text{blk}}$; $\text{LOQ} = 3.3 \times \text{LOD}$. The pseudo-blank for each assessed matrix in Table 2 was a low-contaminated sample of the corresponding matrix. Since the method utilized an indirect analysis approach, concentrations of MCPDEs₇ and GEs were calculated and

expressed as µg/kg of ~~free form equivalent~~bound MCPDs and bound glycidol for all samples in this study.

3. Results and discussion

3.1. Method validation

Procedural calibration curves showed excellent linearity throughout the whole calibration range ($R^2 > 0.9997$). The calibration residual plots of MCPDEs and GE~~s~~ showed a random pattern and the average standardized residual of less than 8% was observed for all calibration levels (data not shown). Since there are no available performance criteria set by the European Union (EU) for methods of analysis for MCPDEs and GE~~s~~, we adapted the performance criteria for analysis of free 3-MCPD set by EU regulation with some modifications²⁷. Specifically, the precision has to be smaller than 15% and the recovery must fall between 75 to 110% at all levels of concentration.

The precision and accuracy in this study expressed by the coefficient of variation under intra-lab reproducibility (CV_{LR} , %), coefficient of variation under intra-day repeatability (CV_{DR} , %), and recovery (R, %) showed very good results and met all the set criteria which~~as~~ can be seen in Table 2. Briefly, the CV_{LR} for 2-MCPDEs, 3-MCPDEs, and GE~~s~~ ranged from 1.1 to 7.8% while the CV_{DR} ranged from 1.1 to 9.5%. The method also provided good recovery for interested analytes which ranged from 84 to 106%. Analysis of 3-MCPDEs and GE~~s~~ in the reference vegetable oil material FAPAS T2646QC further confirmed trueness of the method (Table S.2). The LOQs for 2-MCPDEs, 3-MCPDEs, and GE~~s~~ in baby food were 1, 2, and 1.2 µg/kg, respectively, while those for infant ~~formulae~~formula were 1.2, 1, and 0.5 µg/kg, respectively (Table 2).

In consideration of the achieved linearity, precision, accuracy, LODs, and LOQs, the validated method is suitable for monitoring ~~of~~ 2-MCPDEs, 3-MCPDEs, and GE~~s~~ in infant

formulae and baby foods. The method was accredited and then applied to analyze 6054 infant formulae and baby food samples commercially available on the Danish market.

3.2. Occurrence

Table 1 shows the levels of the processing contaminants found in infant formulae and baby food in Denmark together with details about each product including sample type, fat content, and intended usage age. In all fruit/vegetable based purees, 2-MCPDEs, 3-MCPDEs, and GEs presented at less than LOQ level with the only exception of GEs in one sample at 1.7 µg/kg. This is reasonable as these contaminants arise from exposure of fat to chloride and/or high temperature^{7,28} while the fat contents in fruit/vegetable based puree are minimal (less than 2%). For cereal based purees, the three chemicals showed quantification frequencies (the percentage of samples above LOQs) at 50% for 2-MCPDEs, 73% for 3-MCPDEs, and 41% for GEs. In these products, 2-MCPDEs and GEs presented at similar levels (<LOQ – 3.8 µg/kg) and both of their concentrations are significantly lower (P-value < 0.001) than 3-MCPDEs in the same samples (<LOQ – 9.9 µg/kg). A similar contaminants profile was also observed in puree powder made from cereal (2-MCPDEs ≈ GEs < 3-MCPDEs), however with slightly higher concentrations. The higher concentrations in puree powder is likely because of their higher fat content (Table 1), most of which comprised of vegetable oils such as rapeseed oil or sunflower oil. Among eight analyzed puree powder samples, the contaminants were not quantifiable in only one product which contained mostly dried wholegrain rye with naturally occurring fat (2%) and no added vegetable oils.

MCPDEs and GEs have been detected in infant formulae products in many countries (Table 3). In this study, we found these chemicals in all test samples of infant formulae, both in liquid and powder form. In liquid formulae, the mean concentrations of 2-MCPDEs, 3-MCPDEs, and GEs were 2.3, 5.6, and 1.1 µg/kg,

respectively, while those for powder ~~formulae~~formula were 15.5, 38, and 12.7 µg/kg, respectively. The lowest levels of MCPDEs and GEs were observed in an infant formula from manufacturer C, which were at least 3 times lower than other products. On the other hand, the highest concentrations of all contaminants were detected in a product from manufacturer B intended for newborn babies. While 3-MCPDEs and GEs have been extensively assessed in vegetable oils, the occurrence data of MCPDEs and GEs in infant ~~formulae~~formula is still somewhat limited. In comparison with previous studies, ~~it~~ is clear from Table 3 that the levels of these contaminants are lowest in infant ~~formulae~~formula products from this study ~~in comparison with previous studies~~. This might be due to the fact that the oil and food industry have been constantly working on mitigation of processing contaminants including MCPDEs and GEs ⁴.

On 26 February 2018, the European Union ~~has~~ published its Regulation No 2018/290 (amending its Regulation No 1881/2006) to set the maximum levels of GEs in vegetable oils and fats, powder/liquid infant ~~formulae~~formula and baby foods ²⁹. Specifically, the concentrations of GEs in infant ~~formulae~~formula and baby foods have to be lower than 50 µg/kg in powder form and 6.0 µg/kg in liquid form. All of the analyzed infant ~~formulae~~formula and purees in this study contained complied~~safe~~ levels of GEs in accordance ~~to~~ with EU regulations. Currently, ~~regulations~~ for maximum levels of 2- or 3-MCPDEs are not available.

Along with infant ~~formulae~~formula and puree products, in this study, we also assessed the levels of MCPDEs and GEs in baby finger foods (biscuits or puffs) which are currently not under EU regulations. These products were intended for babies over 6 months old (puffs) or over 10-12 months old (biscuits). The processing contaminants were detected in eight ~~among of~~ nine assessed samples. Similar to puree powder, the only puff item does not contain MCPDEs and GEs comprises of only green peas (80%) and corn (20%) without any added

vegetable oils. These observations further confirm ~~the fact~~ that the presence of MCPDEs and GEs is highly associated with usage of refined vegetable oils in food processing ^{4,28}. Baby biscuits contained the highest levels of MCPDEs among all studied infant ~~formulae~~formula and baby food products. Specifically, 2-MCPDEs presented at the concentration range between 28.6 to 52.8 µg/kg while the ranges for 3-MCPDEs and GEs were 59.6 – 102.6 µg/kg and 16.2 – 35 µg/kg, respectively. The two biscuit products with the highest concentrations of 2- and 3-MCPDEs (Table 1) contained only palm oil while the other two used a mix of palm and sunflower oil. This might be the reason for their differences in MCPDEs levels, as palm oil is well known for its potential to form MCPDEs and GEs during refining due to its high diacylglycerides content ⁷. Indeed, the highest levels of 3-MCPDEs were found in palm oil among all analyzed vegetable oils (including sunflower oil) in a recent study conducted by EFSA (EFSA CONTAM Panel, 2016). Currently, there ~~are~~are limited ~~studies~~very limited study about the occurrence of MCPDEs and GEs in baby foods rather than infant ~~formulae~~formula. Sadowska-Rociek et al. (2018) ~~has~~have found the presence of 3-MCPDEs in baby biscuits purchased in Poland during 2016-2017. In comparison with this study, 3-MCPDEs in Polish baby biscuits showed higher concentrations, ranging between 88 – 443 µg/kg. Meanwhile, GEs ~~was~~were not detected in their samples.

Baby puffs showed similar concentrations of 2-MCPDEs and 3-MCPDEs to cereal based puree powder, ranging between <LOQ – 8.7 µg/kg and <LOQ – 26.5 µg/kg, respectively. Surprisingly, GEs were found at much higher levels than MCPDEs in baby puffs with the average concentration of 90.7 µg/kg (27.5 – 149 µg/kg). In fact, GEs concentrations were the highest in these items among all assessed products in this study. Apart from the green peas based puff, all other puffs comprised of mostly cornstarch (71-75%) and sunflower oil (13-15%). It is unclear whether original GEs content in oil is the sole contributor to the spiked level of GEs in puffs, or if puff production processes also is a

contributing factor. Puffs are usually made by food extrusion machines where raw ingredients are continuously mixed and fed into a heated extruder, then discharged through a confined volume opening forming puffy products. In order to properly cook the ingredients, ~~high~~ temperatures ~~s~~ as ~~highmuch~~ as 200 °C must be employed at the extruder ³⁰. At this temperature, GEs can form rapidly from partial acylglycerides presented in vegetable oils ⁸.

3.3. Correlations among MCPDEs, GEs, and fat content

To examine the relationship between an individual contaminant with another or with fat content of the product, Spearman rank correlation was employed. Despite various type, composition, and content of vegetable oils in infant ~~formulaeformula~~ and baby foods from different manufacturers, Spearman rank correlation test revealed that all three contaminants are greatly correlated to fat content ($p < 0.001$ in all cases) (Table S.3). The test also showed high positive correlation coefficients between fat content and levels of MCPDEs and GEs, meaning the more fat in the products ~~is~~, the higher concentrations of these contaminants. This is in agreement with Zelinková et al. (2009) where the concentrations of 3-MCPDEs were proportioned with fat content of infant ~~formulaeformula~~ in ~~the~~ Czech Republic. However, a recent study in infant ~~formulaeformula~~ in Brazil found no such correlations ¹¹.

Previous studies have proposed that, at high temperature, MCPDEs precursors such as tri-, di-, and mono-acylglycerides can undergo multiple mechanisms to form both 2-MCPDEs and 3-MCPDEs at the same time, albeit at different ratio ^{32,33}. It is therefore hypothesized that the two isomers co-present in contaminated samples. As expected, significant correlation between 2-MCPDEs and 3-MCPDEs was found at very high degree ($p < 0.001$, Spearman rank's correlation coefficient = 0.972). Regression analysis also detected ~~a~~ strong linear relationship between the concentrations of 2- and 3-MCPDEs in this study (adjusted $R^2 = 0.99$) (Figure 1). ~~Similar-A similar~~ finding was reported previously in infant ~~formulaeformula~~ ³⁴. The concentration ratios between 2- and 3-MPCDEs in quantifiable samples from our

study mostly ranged from 0.31 to 0.51 with a median of 0.39 (Table 1). Others found comparable ratio 2- to 3-MCPDEs in infant formulae from different countries: 0.45 in Germany³⁴ and 0.38 in Canada³⁵. These ratios are also in good agreement with values found in different vegetable oils such as palm, coconut, corn, or peanut oil^{36,37}. Assessment of MCPDEs isomerization model on crude palm oil confirmed the direct relation of 2- to 3-MCPDEs ratio with deodorization temperature³⁸. At 180 °C, the 2- to 3-MCPDEs ratio in refined palm oil was about 0.13, increased to 0.29 at 220 °C and reach equilibrium of 0.5 at 240-260 °C deodorization temperature³⁸. Low deodorization temperature is also an industrial approach to reduce the final levels of MCPDEs and GEs in refined vegetable oils⁴. In this survey, the only product showed 2- to 3-MCPDEs ratio out of the 0.31-0.51 range was a powder formulae produced by manufacturer C (item number 46, Table 1) at the ratio of 0.15. This product contained sunflower and rapeseed oil and showed the lowest concentrations of all three contaminants among the powder formulae group, even at comparable levels with liquid formulae samples despite having much higher fat content (Table 1). It also has the lowest concentrations of MCPDEs and GEs on fat basis among all quantifiable samples in this study (data not shown). This is an indication that low temperature deodorization together with other possible mitigation strategies (i.e. by using different types of edible oils than palm oil) might have been applied to significantly reduce the level of MCPDEs and GEs in item number 46.

Despite the significant correlation between GEs and the two MCPDEs isomers in the samples, no linear relationships could be observed by regression analysis (Figures 2A and 3A). Visual inspection of the scatter plots suggested biscuit and puff products might be the reason for this non-linearity behavior. By removing biscuit and puff samples from regression analysis models, decent linearity could be detected with $R^2 \approx 0.85$ between the content of GEs and either 2-MCPDEs or 3-MCPDEs (Figures 2B and 3B). Interestingly enough, the

relationship between MCPDEs and GEs in biscuits and puffs products are opposite to each other. In biscuits, the higher MCPDEs concentration is, the lower GEs level that product contains. On the contrary, in puff items, the amount of MCPDEs and GEs seems to be proportional to each other. This implies the production of biscuits or puffs lead to changes in concentration profiles of MCPDEs and GEs in different manners, and also different from those in infant ~~formulae~~formula and baby puree (both ready to consume and powder forms). More studies are therefore needed to investigate the formation of MCPDEs and GEs in snacks such as biscuits or puffs, especially those intended for babies.

3.4. Preliminary exposure estimation and risk assessment

The Danish Health Authority recommended that an infant should be over 4 months old before starting on solid foods, however, it is best to wait until the child is 6 months of age ³⁹. Since there is no available data on baby food consumption after 6 months, we estimated the exposure and risk for 0-5 months babies who receive ~~formulae~~formula only. In consideration of neither TDI nor MOE threshold for 2-MCPDEs was established, only the risk assessment of 3-MCPDEs and GEs were discussed.

The Danish Health Authority suggested 170 ml/kg bodyweight (b.w) of ~~formulae~~formula per day during an infant's first month of life, then slowly decreased to 110 ml/kg b.w for 1-5 months infants ⁴⁰. Therefore, we calculated the MCPDEs and GEs intake for two groups of infants: 0-1 months and 2-5 months using mean concentrations of MCPDEs and GEs in ~~formulae~~formula products intended for this age group. A factor of 7.7 was used to convert powder ~~formulae~~formula into ready to use liquid ~~formulae~~formula as previously done by EFSA ¹⁷. The average infant body weight was 4.2 kg for 0-1 months and 7.3 kg for 2-5 months ⁴⁰. In this study, we assumed 100% conversion of the esters into their free forms upon consumption ¹⁷. The preliminary exposure assessment to 3-MCPDEs and GEs of Danish infants receiving infant ~~formulae~~formula only (expressed as their corresponding free form

equivalent) is shown in Table 4. The 2-MCPDEs daily intake was 0.3 µg/kg b.w/day for 0-1 months and 0.19 µg/kg b.w/day for 2-5 months babies. Meanwhile, 0-1 months and 2-5 months infants were exposed to 0.75 and 0.49 µg/kg b.w/day of 3-MCPDEs, respectively. In both age groups, the estimated 3-MCPDEs exposure values were considerably lower than the TDI of 2 µg/kg b.w/day published by EFSA ²¹. The estimated 3-MCPDEs exposure for children from 0 to 5 months in this study was also the lowest in comparison with previous studies in Europe, U.S.A, ~~and~~ Brazil (Table 4).

Similarly, the GEs exposure for 0-1 months and 2-5 months children were calculated at 0.23 and 0.15 µg/kg b.w/day, respectively. These values were 5 to 10 times lower ~~that~~ than those reported in other countries ^{11,17,22}. Since no TDI is currently available for GEs exposure, the margin of exposure to GEs (MoE) was calculated according to a previous study by EFSA where the T25 value of 10.2 mg/kg b.w per day for peritoneal mesothelioma in male rats were used ¹⁷. A MoE smaller than 25,000 is of health concern for GEs exposure. In both assessed age groups in this study, the MoEs for GEs exposure were larger than 25,000 (data not shown). In general, our estimation indicated that under average consumption scenario using recommended daily intakes, the exposure of Danish infants to 3-MCPDEs and GEs in infant ~~formulae~~ formula is considered of low health concern.

4. Conclusion

This study provided the first investigation on the occurrence of 2- and 3-MCPD esters and glycidyl esters in infant ~~formulae~~ formula and baby foods in Denmark. Overall, low levels of MCPDEs and GEs were observed in most vegetable oil containing products while samples with no added oil showed <LOQs levels. The degree of MCPDEs and GEs contamination was in the following order: cereal based puree ≈ liquid ~~formulae~~ formula < cereal based puree powder ≈ baby puffs < powder ~~formulae~~ formula < baby biscuits, with the exception for GEs where baby puffs showed the highest concentrations. Industrial mitigation strategies for

MCPDEs and GEs have demonstrated the effectiveness in reducing these chemicals' levels in baby puree and infant ~~formulae~~formula. However, more attention should be paid on the formation, and possibly also mitigation measures, of MCPDEs and GEs in baby biscuits, puffs, or any other similar products where dry heating is applied. Preliminary exposure estimation and risk assessment revealed that there ~~are~~is no apparent risk of exposure to 3-MCPDEs and GEs from infant ~~formulae~~formula and baby foods for Danish infants.

Declarations of interest: none

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Highlights for the manuscript: “Monochloropropanediol and glycidyl esters in infant formula and baby food products on the Danish market: Occurrence and preliminary risk assessment”

- 2- and 3-MCPDEs and GEs were analyzed in Danish infant formula and baby foods
- Most products contained low levels of MCPDEs and GEs
- Highest concentrations of MCPDEs were in baby biscuit and of GEs were in baby puff
- GEs formation might take place during puff production
- Consumption of infant formula does not cause an apparent risk for infants

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1. Concentrations of 2-MCPDEs, 3-MCPDEs and GEs in infant formula and baby food products purchased in Denmark

Item	Sample type	Oil composition*	Brand	Intended age of use (months)	Fat content (%)*	2-MCPDEs (µg/kg)	3-MCPDEs (µg/kg)	GEs (µg/kg)	Ratio 2-/3- MCPDEs
Biscuits									
1		palm, sunflower	E	12	12.7	28.6	59.6	35.0	0.48
2		palm	E	10	11.2	44.7	96.3	16.2	0.46
3		palm	E	10	12.9	52.8	102.6	23.9	0.51
4		palm, sunflower	F	10	13.5	34.8	71.7	24.6	0.49
Cereal based puree									
5		rapeseed	D	6	2.7	1.2	3.2	2.2	0.37
6		rapeseed	D	8	2.5	1.3	3.7	1.7	0.35
7		rapeseed	D	6	3	2.7	6.9	<LOQ	0.39
8		rapeseed	D	8	2.1	1.7	4.4	1.8	0.38
9		canola	D	8	3	1.3	3.7	1.5	0.35
10		rapeseed	A	6	2.5	1.4	3.6	<LOQ	0.39
11		rapeseed	A	8	2.8	1.2	3.0	<LOQ	0.42
12		no added oil	E	7	1.4	<LOQ	<LOQ	<LOQ	NA
13		no added oil	E	7	1	<LOQ	<LOQ	<LOQ	NA
14		rapeseed	A	6	3.2	1.8	6.0	<LOQ	0.31
15		rapeseed	A	6	3	1.3	3.7	1.5	0.35
16		rapeseed	A	6	2.5	<LOQ	4.2	<LOQ	NA
17		rapeseed	A	6	3	1.5	5.0	1.3	0.31
18		rapeseed	A	6	3	<LOQ	3.1	1.9	NA
19		rapeseed	D	8	2.8	3.8	9.9	2.4	0.39
20		rapeseed	D	6	0.6	<LOQ	<LOQ	<LOQ	NA
21		rapeseed	D	6	0.6	<LOQ	2.2	1.5	NA
22		canola	F	6	0.5	<LOQ	<LOQ	<LOQ	NA

23	Cereal based puree powder	canola	F	6	1.8	<LOQ	<LOQ	<LOQ	NA
24		rapeseed	A	6	1.9	<LOQ	2.2	<LOQ	NA
25		rapeseed	A	6	2	<LOQ	2.2	<LOQ	NA
26		rapeseed, sunflower	A	6	20	5.0	15.7	7.6	0.31
27		rapeseed, sunflower	A	8	20	7.9	20.9	5.5	0.38
28		rapeseed, sunflower	A	6	20	9.2	23.3	5.2	0.40
29		rapeseed, sunflower	A	6	20	6.0	16.2	3.8	0.37
30		no added oil	A	6	2	<LOQ	<LOQ	<LOQ	NA
31		rapeseed, sunflower	A	4	18	7.5	21.1	8.9	0.36
32		canola, sunflower	A	6	20	6.1	17.4	10.0	0.35
33	Liquid formula	palm, sunflower, canola	D	6	14.3	3.3	10.0	3.7	0.33
34		palm, rapeseed, coconut, sunflower	A	0	3.5	1.5	3.7	0.6	0.41
35		palm, sunflower, rapeseed, coconut, fish	A	6	2.9	2.3	5.3	0.9	0.44
36		palm, sunflower, rapeseed, coconut, fish	C	0	3.6	2.2	5.9	1.5	0.37
37		palm, sunflower, rapeseed, coconut, fish	C	6	3.6	1.2	3.4	0.7	0.35
38		palm, rapeseed, soybean, coconut	B	0	3.6	3.0	6.9	1.6	0.44
39		palm, rapeseed, soybean, coconut	B	6	3.1	3.7	8.5	1.2	0.43
40	Powder formula	sunflower, rapeseed, coconut, fish	C	0	28	18.6	50.2	8.7	0.37

41		palm, palm kernel, sunflower, rapeseed, fish	A	0	19	20.1	46.5	17.3	0.43
42		palm, palm kernel, sunflower, rapeseed, fish	A	6	19	11.1	26.9	9.4	0.41
43		palm, sunflower, rapeseed, coconut, fish	A	12	20	12.4	28.8	8.8	0.43
44		palm, canola, coconut, soybean	B	0	23	29.0	65.0	31.2	0.45
45		palm, canola, coconut, soybean	B	6	22.5	16.1	40.2	10.3	0.40
46		sunflower, rapeseed, fish	C	6	24	1.3	8.6	3.1	0.15
	Puffs								
47		no added oil	F	6	3.4	<LOQ	<LOQ	<LOQ	NA
48		sunflower	F	6	14.4	3.4	7.4	27.5	0.46
49		sunflower	E	6	13.8	7.6	20.9	128	0.36
50		sunflower	E	6	15.8	8.7	21.8	149	0.40
51		sunflower	E	6	14	8.4	26.5	58.0	0.32
	Fruit/vegetable based puree								
52		no added oil	E	4	0.1	<LOQ	<LOQ	<LOQ	NA
53		no added oil	E	4	0.1	<LOQ	<LOQ	<LOQ	NA
54		no added oil	F	4	0.1	<LOQ	<LOQ	<LOQ	NA
55		no added oil	F	6	0.2	<LOQ	<LOQ	<LOQ	NA
56		no added oil	B	6	0.3	<LOQ	<LOQ	<LOQ	NA
57		no added oil	B	6	0.5	<LOQ	<LOQ	<LOQ	NA
58		no added oil	C	6	0.7	<LOQ	<LOQ	<LOQ	NA
59		no added oil	C	6	0.8	<LOQ	<LOQ	<LOQ	NA
60		no added oil	A	6	2	<LOQ	<LOQ	1.7	NA

*: information specified on the labels

Table 2. Validation results, LODs, and LOQs for 2-MCPDEs, 3-MCPDEs and GEs in oil, infant formula, and baby food

Matrices	Parameters	2-MCPDEs		3-MCPDEs		GEs	
Oil	Spike level ($\mu\text{g/kg}$)*	98.8	988	98.8	988	117	1174
	R (%)	93	101	94	94	91	84
	CV _{LR} (%)	2.0	1.3	2.8	1.8	3.4	2.9
	CV _{DR} (%)	2.0	1.2	2.8	1.8	3.4	2.9
	LOD ($\mu\text{g/kg}$)*	2.3		3.2		1.9	
	LOQ ($\mu\text{g/kg}$)*	7.6		10.7		6.2	
Infant formula	Spike level ($\mu\text{g/kg}$)*	3.3	33	3.3	33	3.9	39.1
	R (%)	99	99	95	95	94	95
	CV _{LR} (%)	1.2	1.3	1.1	1.4	1.1	2.2
	CV _{DR} (%)	1.2	1.3	1.1	1.4	2.1	2.4
	LOD ($\mu\text{g/kg}$)*	0.4		0.3		0.1	
	LOQ ($\mu\text{g/kg}$)*	1.2		1.0		0.5	
Baby food	Spike level ($\mu\text{g/kg}$)*	3.3	33	3.3	33	3.9	39.1
	R (%)	106	100	93	91	98	99
	CV _{LR} (%)	7.8	3.3	3.0	1.9	5.9	2.4
	CV _{DR} (%)	2.7	2.3	3.0	1.8	5.9	1.3
	LOD ($\mu\text{g/kg}$)*	0.3		0.6		0.4	
	LOQ ($\mu\text{g/kg}$)*	1		2		1.2	

R: recovery; CV_{LR}: coefficient of variation under intra-lab reproducibility; CV_{DR}: coefficient of variation under intra-day repeatability

*: free form equivalents

Table 3. Concentrations of MCPDEs and GEs (µg/kg) in infant formula from this study and comparison with other studies

Region	Sampling year	Sample type	2-MCPDEs		3-MCPDEs		GEs		Reference
			Range	Mean	Range	Mean	Range	Mean	
Czech	2006	Powder			62-588	312 (median)			Zelinková et al., 2009
Germany*	2013	Powder	24-85	48.5	67-177	109	40-213	93	Wöhrlin et al., 2015
Europe	2012-2013	Powder		44		108		87	EFSA CONTAM Panel, 2016
U.S.A	2013-2016	Normalized to liquid form**		54	3-119	13	1-50		Spungen et al., 2018
Brazil	2015	Powder		150	ND-600	220	ND-750		Arisseto et al., 2017
China	NA, before 2015	Powder	ND-52	41	ND-316	185			Wang et al., 2016
Denmark	2019	Powder	1.3-29	15	8.6-65	36	3.1-31	13.4	This study
Denmark	2019	Liquid	1.5-3.7	2.7	3.7-8.5	6.4	0.6-1.6	1.2	This study

*Data was calculated based on fat content declared on packaging

**Normalized by conversion of concentrations in fat basis into product basis assuming 3.5% fat in liquid form

Table 4. Estimated intake of MCPDEs and GEs in infant formula from this study and comparison with other studies

Region	Sampling year	Age group (months)	2-MCPDEs intake* (µg/kg b.w/day)	3-MCPDEs intake* (µg/kg b.w/day)	GEs intake* (µg/kg b.w/day)	Reference
Europe	2012-2013	1-4	1	2.4	1.9	EFSA CONTAM Panel, 2016
U.S.A	2013-2016	0-1		10	2	Spungen et al., 2018
		2-3		8	2	
		5-6		7	2	
Brazil	2015	0-5		2.49	3.65	Arisseto et al., 2017
Denmark	2019	0-1	0.3	0.75	0.49	This study
		2-5	0.19	0.23	0.15	

*Data was calculated using mean concentrations of the contaminants

Figure

Figure 1. Scatter plot and linear regression analysis between the concentrations of 2-MCPDEs and 3-MCPDEs in infant formula and baby foods

Figure 2. Scatter plot and linear regression analysis between the concentrations of 2-MCPDEs and GEs in infant formula and baby foods with (A) or without (B) biscuit and puff product

Figure 3. Scatter plot and linear regression analysis between the concentrations of 3-MCPDEs and GEs in infant formula and baby foods with (A) or without (B) biscuit and puff product

Table S.1. Selected reaction monitoring parameters for the analysis of 2-MCPDEs, 3-MCPDEs and GEs by GC-MS/MS in positive EI mode.

Compound	Analysis form	Transition	Collision Energy	Dwell time (sec)
3-MCPDEs	3-MCPD-PBA	196 ⇒ 147	8	0.2
		198 ⇒ 147	8	0.2
PP-3-MCPD-d5	3-MCPD-d5-PBA	201 ⇒ 150	8	0.2
		150 ⇒ 93	20	0.2
2-MCPDEs	2-MCPD-PBA	196 ⇒ 104	16	0.2
		198 ⇒ 104	16	0.2
PP-2-MCPD-d5	2-MCPD-d5-PBA	201 ⇒ 104	20	0.2
		201 ⇒ 107	12	0.2
GEs	3-MBPD-PBA	240 ⇒ 147	8	0.2
		242 ⇒ 147	8	0.2
Gly-P-d5	3-MBPD-d5-PBA	247 ⇒ 150	8	0.2
		150 ⇒ 93	20	0.2

Transitions in **bold** are quantifying transitions

Table S.2. Concentrations of 3-MCPDEs and GEs in FAPAS T2646QC vegetable oil sample by duplicated analysis on various days

	Assigned value [*]	Range for z ^{**} ≤ 2	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3	Day 4	Day 4	Day 5	Day 5
3- MCPDEs [*]	586	325- 821	533	530	524	539	539	600	502	556	495	490
GEs [*]	255	153- 357	219	213	198	215	218	229	225	249	210	215

^{*}: µg/kg (free form equivalent)

^{**}: z-score derived from a proficiency test using T2646QC reference material

Table S.3. Spearman's rank correlation coefficients between fat content and the levels of MCPDEs and GEs in infant formula and baby food products ($p < 0.001$ in all cases)

	2-MCPDEs	3-MCPDEs	GEs
Fat content	0.863	0.887	0.815
2-MCPDEs		0.972	0.866
3-MCPDEs			0.878