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3-MCPD and glycidyl esters in infant formulas from the Brazilian market: Occurrence and risk assessment

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ABSTRACT

High concentrations of esters of 3-monochloropropane-1,2-diol (3-MCPDE) and glycidol (GE) have been reported in infant formulas due to the use of refined vegetable oils that may contain high levels of these contaminants. Commercial infant formulas available on the Brazilian market (n = 40) were analyzed for the first time for 3-MCPDE and GE using a gas chromatography-mass spectrometry method. For 3-MCPDE, the limits of detection (LOD) and quantitation (LOQ) were 0.08 mg/kg and 0.16 mg/kg, respectively. For GE, the LOD and LOQ were 0.10 mg/kg and 0.20 mg/kg, respectively. Mean recoveries varied from 93 to 108% for 3-MCPDE and from 82 to 97% for GE. Levels of 3-MCPDE in the products ranged from not detected to 0.60 mg/kg whereas concentrations of GE ranged from not detected to 0.75 mg/kg. A theoretical preliminary exposure assessment showed that 3-MCPDE and GE intakes were up to 5.81 and 10.46 μg/kg body weight/day, respectively, in a worst case scenario (95th percentile). According to the results obtained in this study, the levels of 3-MCPDE and GE in infant formulas marketed in Brazil may pose a potential risk to the health of the consumers of these products and need to be constantly monitored.

1. Introduction

Infant formulas are products for special dietary use only, as a complete or partial substitute for human milk. These products are recommended for children whose mothers cannot breastfeed or do not produce enough milk (FDA, 2015). Infant formulas are developed with ingredients aiming to mimic the composition of human milk and ensure the nutritional needs of children (Carroll, 2004). A mixture of vegetable oils is added to the product to obtain a fatty acid composition similar to the human milk. However, most of the vegetable oils present in these formulas are refined and may represent an important food safety concern due to the presence of high levels of chemical contaminants such as esters of 3-monochloropropane-1,2-diol (3-MCPDE) and glycidol (GE) (Franke, Strijowski, Fleck, & Pudel, 2009; Hrncirik & van Duijn, 2011; Pudel et al., 2011; Zelinková, Svejkovská, Velísek, & Dolezal, 2006). In palm oil and palm olein, which are usually employed in this kind of product, the levels of these compounds can reach up to 10 mg/kg (Arisseto, Marcolino, & Vicente, 2014; Karšulínová, Folprechtová, Doležal, Dostálová, & Velišek, 2007; Kuhlmann, 2011; MacMahon, Begley, & Diachenko, 2013; Weiβhaar, 2011).

3-MCPDE comprise a group of chemical contaminants derived from glycerol (1,2,3-propanetriol) which have been identified in various foods and food ingredients since 2004 (EFSA, 2016; Svejkovská et al., 2004; Zelinková et al., 2006). These compounds are formed from lipids and chlorides during the oil refining process, especially under the high temperatures employed in the deodorization step. GE is also formed under similar conditions in the refining process of vegetable oils, but through different precursors and mechanisms (Pudel et al., 2011).

The presence of 3-MCPDE and GE in the diet is a potential concern since these esters are effectively hydrolyzed by enzymes in the gastrointestinal tract, releasing their free forms, 3-MCPD and glycidol, which are potentially toxic (Abraham et al., 2013). 3-MCPD has already shown testicular and renal toxicity as well as potential to induce cancer in experimental animals while glycidol is considered a genotoxic carcinogen. According to the International

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Agency for Research on Cancer (IARC), 3-MCPD is classified as a possible human carcinogen (group 2B) and glycidol as a probable human carcinogen (group 2A) (IARC, 2000; IARC, 2012).

In the last years, significant levels of 3-MCPDE and GE have been found in infant formula available on the market (Becalski, Zhao, Feng, & Lau, 2015; EFSA, 2016; Jędrkiewicz, Głowacz-Różyńska, Gromadzka, Kloskowski, & Namieśnik, 2016; Zelinková, Doležal, & Velíšek, 2009). According to data reported by EFSA (2016), the dietary exposure estimate to 3-MCPD of children fed exclusively with infant formula corresponds to 2.4 μg/kg body weight (bw) per day (mean occurrence) and 3.2 μg/kg bw per day (P95 occurrence). Considering the Tolerable Daily Intake (TDI) of 0.8 μg/kg bw established by EFSA (2016), potential risks to health could not be excluded. For glycidol, the dietary exposure values varied from 1.8 to 2.1 μg/kg bw per day (mean occurrence) and 4.9 μg/kg bw per day (P95 occurrence), which also suggest a concern.

Taking into account the potential health risks of 3-MCPDE and GE as well as the lack of occurrence and intake data in Brazil from this type of food, the objective of this study was to perform an in-house validation of a gas chromatography – mass spectrometry (GC-MS) method to analyze simultaneously 3-MCPDE and GE in infant formulas, determine the levels of these contaminants in samples available on the Brazilian market, perform a preliminary exposure assessment, and evaluate the potential risks associated with the consumption of infant formulas containing these compounds.

2. Materials and methods

2.1. Standards

3-MCPD-1,2-dipalmitoyl ester (PP-3-MCPD), 3-MCPD-1,2-dipalmitoyl-d5 ester (PP-3-MCPD-d5), glycidyl palmitate (P-Gly) and glycidyl palmitate-d5 (P-Gly-d5) with a purity >98% were purchased from Toronto Research Chemicals (North York, ON, Canada). Stock solutions of PP-3-MCPD and PP-3MCPD-d5 were prepared individually at 0.5 mg/mL in tetrahydrofuran (THF). Stock solutions of P-Gly and P-Gly-d5 were prepared at 1 and 0.2 mg/mL, respectively, in toluene. Stock solutions of PP-3-MCPD and P-Gly were combined and diluted to prepare calibration solutions at 55 μg/mL of PP-3-MCPD and 100 μg/mL of P-Gly, and at 5.5 μg/mL of PP-3-MCPD and 10 μg/mL of P-Gly, both in THF. Similarly, a combined solution of internal standards was prepared in THF at 40 μg/mL of PP-3-MCPD-d5 and 50 μg/mL of P-Gly-d5.

2.2. Solvents and reagents

Methanol (HPLC grade) was purchased from Tedia Company Inc. (Fairfield, OH, USA). Hexane, acetone, sulfuric acid, sodium hydrogencarbonate, and ammonium sulfate (analytical grade) were obtained from Labsynth (Diadema, SP, Brazil). Tetrahydrofuran (THF), toluene, methyl *tert*-butyl ether (MTBE) and sodium bromide (analytical grade), and phenylboronic acid (PBA) 97% purity, were supplied by Sigma-Aldrich (Sigma-Aldrich Corp., Steinheim, Germany). Ultrapure water was obtained from a Milli-Q Plus system (Millipore, Bedford, MA, USA).

2.3. Samples

Forty infant formula products from four different manufacturers were purchased from retail outlets in Campinas, São Paulo, Brazil, in 2015. Sampling was made considering the availability of the products in the market and included first infant and follow-on formula containing cow and soy milk as well as different ingredients such as prebiotics, nucleotides and essential fatty acids, among others. The analyses were carried out in the powdered product, non-reconstituted in water.

2.4. Determination of 3-MCPDE and GE

Sample preparation for the extraction of the compounds was performed according to Ermacora and Hrnčiřík (2014) with some modifications. For that, 350 mg of the sample were weighed into 50 mL centrifuge tubes and 50 μL of the combined solution of internal standards were added. Then, 3 mL of hexane:MTBE (1:2) solution and 2 mL of water were added into the tube followed by vigorous mixing (vortex, 20–30 s). The mixture was incubated at 60 °C for 10 min, homogenized (vortex, 10 s), sonicated during 10 min and centrifuged for 5 min (3000 rpm) at room temperature. The supernatant (organic layer) was transferred to a test tube and then evaporated to dryness under a nitrogen stream (at approximately 85–90 °C).

The conversion of GE to 3-monobromopropanediol monoesters (3-MBPDE) was performed according to the American Oil Chemists Society Official Method Cd 29a-13 (AOCS, 2013). After the addition of 2 mL of anhydrous THF to the oil residue (approximately 100 mg) and vigorous mixing (vortex, 15 s), 30 μL of an acid aqueous solution of sodium bromide was added to the sample, which was incubated in water bath at 50 °C for 15 min. The reaction was stopped by adding 3 mL of a solution of sodium hydrogencarbonate 0.6%. Then, 2 mL of hexane were added to separate phases. The upper phase was transferred to another test tube and evaporated to dryness under a nitrogen stream for 15 min at 40 °C. The residue was diluted with 1 mL of anhydrous THF.

Transesterification and derivatization steps were performed according to Arisseto et al. (2014). Briefly, the procedure included acid transesterification (H_2SO_4 in methanol), neutralization with sodium hydrogencarbonate, salting-out of fatty acid methyl esters (FAMEs) using ammonium sulfate solution and hexane, and derivatization with PBA. Since an indirect analytical approach was used, individual esters could not be identified and quantified and, therefore, the total concentration of 3-MCPDE and GE was expressed as free 3-MCPD and 3-MBPD equivalents, respectively.

2.5. GC-MS analysis

GC-MS analyses were carried out on a HP 7890A gas chromatograph coupled to a MSD 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). An aliquot of 1 μL of the extract was injected at 180 °C in splitless mode. The separation was carried out on a capillary column VF-1ms 30 m × 0.25 mm (0.25 μm) (Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The following temperature program was used in the oven: 60 °C (held for 1 min), 6 °C/min to 190 °C, 20 °C/min to 280 °C (held for 30 min). Detection was performed by selected ion monitoring (SIM) after positive electron impact ionization (70 eV). The following ions were monitored: *m/z* 147, 196 and 198 for 3-MCPD derivative, *m/z* 150, 201 and 203 for the internal standard 3-MCPD-d5 derivative, *m/z* 147 and 240 for 3-MBPD derivative, and *m/z* 150 and 245 for the internal standard 3-MBPD-d5 derivative.

2.6. Method validation

The method was in-house validated in terms of linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy (recovery), and precision (repeatability and within-laboratory reproducibility) according to the guidelines established by the Brazilian Institute of Metrology, Quality and Technology (INMETRO, 2011). Linearity was evaluated in the range 0–2.60 mg/kg for 3-MCPDE and 0–5.97 mg/kg for GE (nine calibration points for both solvent and matrix