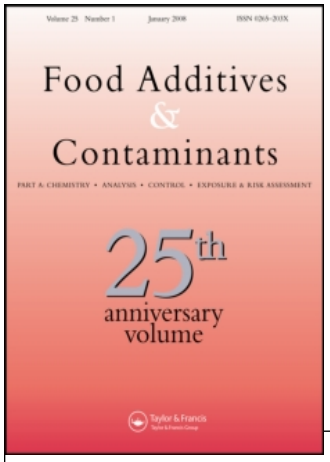

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Occurrence of 3-MCPD fatty acid esters in human breast milk

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A series of twelve breast milk samples were analysed by gas chromatography-mass spectrometry (GC/MS) operated in selected ion monitoring mode for 3-chloropropane-1,2-diol (3-MCPD). Whilst none of the samples contained 3-MCPD above the limit of detection of $3\,\mu g\,kg^{-1}$ milk, all contained high amounts of 3-MCPD esterified with higher fatty acids. The levels of 3-MCPD released by hydrolysis of these esters (bound 3-MCPD) ranged from the limit of detection ($300\,\mu g\,kg^{-1}$, expressed on a fat basis) to $2195\,\mu g\,kg^{-1}$; with a mean level of bound 3-MCPD of $1014\,\mu g\,kg^{-1}$, which corresponded to $35.5\,\mu g\,kg^{-1}$ milk. The presence of bound 3-MCPD was confirmed using orthogonal gas chromatography coupled with high-speed time-of-flight mass spectrometry analysis for four randomly selected breast milk samples. Six breast milks collected from one of the nursing mothers 14–76 days after childbirth contained bound 3-MCPD within the range of 328–$2078\,\mu g\,kg^{-1}$ fat (mean $930\,\mu g\,kg^{-1}$ fat). The calculated bound 3-MCPD content of these samples was within the range of 6 and $19\,\mu g\,kg^{-1}$ milk (mean of $12\,\mu g\,kg^{-1}$ milk). The major types of 3-MCPD esters were the symmetric diesters with lauric, palmitic, and oleic acids, and asymmetric diesters with palmitic acid/oleic acid among which 3-chloro-1,2-propanediol 1,2-dioleate prevailed.

Keywords: breast milk; 3-chloropropane-1,2-diol (3-MCPD); bound 3-MCPD; 3-MCPD fatty acid esters; organochlorine contaminants; organohalogens

Introduction

3-Chloropropane-1,2-diol (3-MCPD) is representative of the so-called food-borne or food-processing contaminants. It was identified in acid-hydrolysed vegetable protein (acid-HVP) in 1981 (Davídek et al. 1982) where it originates as a reaction product of phospholipids, acylglycerols, and glycerol with hydrochloric acid. More recently, it has been shown that 3-MCPD occurs as a racemic mixture of its enantiomers, (*R*)- and (*S*)-3-MCPD (Velíšek et al. 2002). In view of its toxicity, the European Commission's Scientific Committee on Food (SCF) has proposed a provisional total daily intake (TDI) level of $2\,\mu g\,kg^{-1}$ body weight day^{-1} for the amount of 3-MCPD that can be consumed daily over a lifetime without appreciable harm to a consumer's health (SCF 2001). The TDI was adopted on 8 March 2001 and applies from 5 April 2002. Similarly, the Joint FAO/WHO Expert Group on Food Additives (JECFA) set a provisional maximum tolerable daily intake (PMTDI) of $2\,\mu g\,kg^{-1}$ body weight day^{-1} in 2001 (JECFA 2001). A regulatory limit of $20\,\mu g\,kg^{-1}$, based on a 40% dry matter content, has been adopted for 3-MCPD in liquid acid-HVP and soy sauce and came into force in the European Union in 2002 (European Commission 2001).

Several recent studies have demonstrated that 3-MCPD is not only the contaminant typical for acid-HVP, soy sauces and related products, but also it occurs in a wide range of retail outlet and home-made foods as well as in various food ingredients formulated without the addition of acid-HVP (Crews et al. 2001, 2002; Hamlet et al. 2002, 2004a, b; Breitling-Utzmann et al. 2003; Divinová et al. 2004; Doležal et al. 2005).

In raw acid-HVP, 3-MCPD occurs mainly as a free compound and, to a minor extent, esterified with higher fatty acids (Velíšek et al. 1980). Rather surprising was a finding of fatty acid esters of 3-MCPD in the neutral fraction of goat's milk lipids (Cerbulis et al. 1984); at that time, their occurrence was attributed to the use of chlorine-based sanitizers.

Recent findings document that 3-MCPD esters occur in a wide variety of both unprocessed and processed foods and in various food ingredients (Hamlet and Sadd 2004; Svejkovská et al. 2004, 2006; Doležal et al. 2005; Zelinková et al. 2006; Divinová et al. 2007). Fatty acid esters of 3-MCPD thus represent a new class of food contaminants as 3-MCPD can be easily released from these compounds by a lipase-catalysed hydrolysis reaction (Hamlet and Sadd 2004).

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Generally, the amount of 3-MCPD in any food or food ingredient released from 3-MCPD esters by hydrolysis (bound 3-MCPD) largely exceeds that of free 3-MCPD. Svejkovská et al. (2004) reported on the presence of bound 3-MCPD in 20 selected retail food products. High levels (in the range 280–2420 $\mu g\,kg^{-1}$) of bound 3-MCPD were found in salty crackers, pickled herrings, doughnuts, crisp bread, dark malt and French fries. Compared with the free form, they were five to 157 times higher. Analysis of white bread (Hamlet and Sadd 2004) showed that the highest levels of bound 3-MCPD were found in the crust (547 $\mu g\,kg^{-1}$) and toast (160 $\mu g\,kg^{-1}$), while in the crumb they were much lower amount (26 $\mu g\,kg^{-1}$) and exceeded the free 3-MCPD levels by six to 52 times. The level of bound 3-MCPD in roasted coffee was relatively low and varied between 6 $\mu g\,kg^{-1}$ (soluble coffee) and 390 $\mu g\,kg^{-1}$ (decaffeinated coffee) and exceeded the free 3-MCPD level by eight to 33 times (Doležal et al. 2005). Coffee surrogates contained bound 3-MCPD in the range of 145–1184 $\mu g\,kg^{-1}$; the highest level was found in roasted barley (Divinová et al. 2007). In this case, the bound 3-MCPD levels were higher by 32–81 times. In malts (Divinová et al. 2007), the bound 3-MCPD levels ranged from 4 to 650 $\mu g\,kg^{-1}$, and the highest amount was found in roasted malts (463–650 $\mu g\,kg^{-1}$). The bound 3-MCPD levels exceeded the free 3-MCPD levels by 0.4–36 times. Recently, it has been shown (Zelinková et al. 2006) that edible oils, notably the refined edible oils including refined olive oils, contain relatively high levels of bound 3-MCPD. Amounts ranging from <300 to 2462 $\mu g\,kg^{-1}$ have been found. Analysis of coffee creamers, cream aerosols, and bouillon cubes produced using refined vegetable oils revealed that these products did not contain free 3-MCPD, but their bound 3-MCPD levels ranged from 110 to 730 $\mu g\,kg^{-1}$ product (Karšulínová et al. 2007).

Considering the above facts, the esterified 3-MCPD extends the list of toxic chlorinated chemicals to which humans can be exposed through diet. The bound 3-MCPD content in food lipids is comparable or even higher than levels of chlorinated persistent organic pollutants (POPs) found, for instance, in some fish, which is in most cases their major dietary source. It should be noted that POPs represented, for example, by dioxins, organochlorine pesticides and/or polychlorinated biphenyls (PCBs) are of great concern since they bioaccumulate in adipose tissues of biota throughout the food chain, at the top of which are humans, and create a lasting toxic body burden. During lactation, transfer of POPs into human breast milk occurs, thus breastfeeding provides a significant source of exposure to POPs early in infant life, the effects of which are unknown, and is the subject of a growing body of research. However, despite the possibility of harm from environmental contaminants in breast milk, experimental evidence suggests that, barring certain health issues, human breast milk is the best source of nourishment for human infants. In any case, measures aimed at reduction of toxic chemicals, regardless of how they are formed during processing or are due to environmental pollution, have to be adopted to minimize the risk of toxic effects.

While many data illustrating bioaccumulation of various halogenated environmental pollutants in human milk are available (e.g. Heifetz et al. 1989; Golding 1997; Hooper and Jianwen 2002; Bauchner 2003; Kalantzi et al. 2004), the preliminary information on the occurrence of persistent chlorine-containing processing contaminants, such as 3-MCPD esters, has been mentioned only recently (Velíšek 2006). Based on the assumption of similarity in the bioaccumulation potential in human fat tissue and possible transfer of lipophilic 3-MCPD esters into human milk, we attempted to analyse them in the isolated breast milk fat.

Materials and methods

Chemicals

3-Chloropropane-1,2-diol (3-MCPD; >98%) was purchased from E. Merck (Darmstadt, Germany); 3-MCPD-d_5 (99.4%) was from Dr. Ehrenstorfer (Augsburg, Germany); and phenylboronic acid (PBA; ≥97%) was from Fluka Chemie (Buchs, Switzerland). 1,2-Diacyl-3-chloro-1,2-propanediols, symmetric and asymmetric (mixed) diesters of 3-MCPD with lauric, myristic, palmitic, stearic, and oleic acids, respectively, were synthesized according to Kraft et al. (1979) and purified on a silica gel column using light petroleum ether/diethyl ether mixtures (see below). Isotopically labelled 3-MCPD-d_5 1,2-dipalmitate was synthesized employing 3-MCPD-d_5 and purified using the same procedures. The GC purity of symmetric 3-MCPD diesters was in the range 94.4–99.4%. The asymmetric 3-MCPD diesters were mixtures with the corresponding symmetric 3-MCPD diesters and their content was in the range 44.4–49.3%. All other reagents and solvents were of analytical purity.

Samples

Human breast milk samples were collected from healthy native Czech mothers living in the Prague and East Bohemia regions. Three samples were collected within 1–2 weeks, seven samples within 1–2 months, one sample after 4 months, and one sample after 11 months after childbirth. The ages of the mothers were within the range 18–36 years (mean = 28.1, median = 28.5). One 29-year-old mother provided a series of six samples collected at 14, 49, 70, 71, 74 and 76 days after childbirth. The breast milk samples were collected in

cleaned glass containers and stored at –20°C until analysis, then thawed and used for isolation of milk fat.

Isolation of milk fat

Milk (about 20 ml) was transferred to a 100-ml separatory funnel by a volumetric cylinder, weighed and 2 ml of saturated solution of potassium oxalate, 20 ml of ethanol (96%, v/v) and 40 ml of a mixture of hexane–diethyl ether (1:1, v/v) were added. The mixture was shaken for 15 min and the aqueous layer transferred to a second separatory funnel. To this funnel, 10 ml of ethanol and 20 ml of hexane–diethyl ether mixture were added and the content was shaken for an additional 5 min. The aqueous layer was discarded and the upper layer combined with the upper organic layer from the first separatory funnel. The extract was then twice partitioned with 5 ml of water, the aqueous layer was again discarded, and the upper layer was dried over anhydrous sodium sulphate and evaporated to dryness at 40°C using a rotary vacuum evaporator (Schenzler and Their 2001). The residue was redissolved in hexane (2 ml) and a 0.5-ml aliquot was dried at 80°C to determine the lipid content gravimetrically.

Analysis of 3-MCPD diesters

The isolated milk fat (about 200 mg) dissolved in hexane (1 ml) containing 3-MCPD-d_5 1,2-dipalmitate (7.26 μg) was placed onto the top of a silica gel column 330 × 20 mm (silica gel 60, 70-230 mesh; Merck, Darmstadt, Germany). The flask was washed with 2 ml of a mixture of light petroleum ether (b.p.; 40–65°C) with diethyl ether (95:5, v/v) and the column was eluted at a flow rate of 4 ml min^{-1} using 500 ml of the solvent. The eluent containing 3-MCPD diesters was evaporated using a rotary evaporator and the residue was dissolved in tetrahydrofurane (200 μl). An aliquot of this solution (1 μl) was analysed by gas chromatography-mass spectrometry (GC/MS).

Determination of free and bound 3-MCPD

Free and bound 3-MCPD were analysed following the method described earlier (Zelinková et al. 2006). Briefly, the milk fat (5 g) with added 3-MCPD-d_5 (internal standard) was extracted with a hexane–acetone (1:1, v/v) mixture and obtained extract was derivatized with phenylboronic acid and used for the determination of free 3-MCPD by GC/MS. For the determination of bound 3-MCPD by GC/MS, the milk fat (100 mg) was dissolved in tetrahydrofurane, treated with 1.8 ml sulphuric acid solution (98%, 1.8 ml in 100 ml methanol), neutralized using saturated $NaHCO_3$ solution, spiked with 3-MCPD-d_5 and derivatized with phenylboronic acid. Three parallel examinations of each milk sample were made.

Orthogonal gas chromatography (GCxGC) coupled with high-speed time-of-flight mass spectrometry (TOF-MS) was used for confirmation of results obtained by the above method. Four randomly selected samples were used for this purpose.

Instrumentation and operating conditions

GC/MS analyses were generally carried out on an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Series 5975 quadrupole mass selective detector Agilent 5973 MSD (70 eV) and data-processing system (MSD Productivity ChemStation, Revision D.02.00 SP1).

4-Chloromethyl-2-phenyl-1,3,2-dioxaborolane, the product obtained by derivatization of 3-MCPD with phenylboronic acid, was analysed using a GC capillary column Equity-1 (30 m × 0.25 m × 1 μm; Supelco, Bellefonte, PA, USA). The injector was held at 250°C (splittles); the column temperature was programmed from 80°C (1 min) to 300°C (37 min) at a rate of 10°C min^{-1}. Helium at a flow rate of 0.8 ml min^{-1} was used as the carrier gas; 1 μl sample was injected. For quantification purposes, single-ion monitoring was used to monitor ions at m/z 147 (3-MCPD) and at m/z 150 (3-MCPD-d_5). Ions at m/z 91 and 196 (3-MCPD) and at m/z 93 and 201 (3-MCPD-d_5) were used as qualifiers. The performance characteristics were as follows: the limit of detection (LOD) was 100 μg kg^{-1} milk fat; the relative standard deviation (RSD) (three injections, 0.3282 μg 3-MCPD ml^{-1}) was 5.9%; and linearity (0.005–8.575 μg 3-MCPD ml^{-1}), $r^2 = 0.9998$.

The GCxGC TOF-MS system used for the examination of four selected samples consisted of an HP 6890 (Agilent Technologies) gas chromatograph with a split–splittles injector. The detector was Pegasus III, high-speed TOF mass spectrometer (LECO, St Joseph, MI, USA) with 10 ml min^{-1} pumping capacity and operated in electron ionization (EI) mode. Inside the GC oven, a dual-stage jet modulator and the secondary oven were mounted. Resistively heated air was used as a medium for hot jets, while cold jets were supplied by gaseous nitrogen, secondarily cooled by liquid nitrogen. The instrumental set-up of GC consisted of a primary column DB5ms (30 m × 0.25 mm × 0.25 μm; Agilent Technologies) and a secondary column BPX50 (2.2 m × 0.1 mm × 0.1 μm; SGE, Ringwood, Australia). The oven temperature programme was as follows: 80°C for 1 min, 10°C min^{-1} to 250°C, 5 min at 250°C, the secondary oven was held at 5°C above the main oven; the helium flow rate was 1.0 ml min^{-1}; injection mode: splittles for 1.0 min;

injection temperature: 270°C; modulation time: 5 s (hot pulse of 1.5 s); and a modulation temperature offset of 15°C. The instrumental of the mass spectrometer was as follows: solvent delay of 350 s; acquisition rate of 125 Hz; mass range of 45–500 amu; ion source temperature of 220°C; transfer line temperature of 270°C; and detector voltage of –1700V. The performance characteristics were as follows: LOD = 3 μg 3-MCPD kg^{-1} milk fat, RSD (five injections, 0.0515 μg 3-MCPD ml^{-1}) = 6.5%, and linearity (0.01–0.515 μg 3-MCPD ml^{-1}), $r^2 = 0.9942$.

GC/MS analysis of 3-MCPD diesters was performed on a capillary column DB-1HT (15 m × 0.25 mm i.d., film thickness 0.1 μm; Agilent Technologies). The injector was held at 300°C (pulsed splittles), the column temperature was programmed from 170°C (1 min) to 305°C at a rate of 5°C min^{-1} and then to 400°C (10 min) at a rate of 40°C min^{-1}. Helium at a flow rate of 0.7 ml min^{-1} was used as the carrier gas; a 1-μl sample was injected. Ionization was performed by electron impact at 70 eV and temperature of 250°C. For quantification purposes, single-ion monitoring was used to monitor the ions $[M - RCOOH]^+$ at *m/z* 275 (3-MCPD 1,2-dilaurate), *m/z* 303 (3-MCPD 1,2-dimyristate), *m/z* 331 (3-MCPD 1,2-dipalmitate), *m/z* 336 (3-MCPD-d_5 1,2-dipalmitate), *m/z* 331 (mixed palmitate and oleate of 3-MCPD), *m/z* 357 (3-MCPD 1,2-dioleate), and *m/z* 359 (3-MCPD 1,2-distearate). The ions $[M - RCOOH + 2]^+$ and $[RCO]^+$ were monitored for confirmation.

Statistical methods

Statistical evaluation of the obtained results was performed employing the computer program SPSS for Windows, Release 11.0.0, Standard Version (SPSS, Inc., Chicago, IL, USA).

Results and discussion

The advantages of breastfeeding both for infant and mother have been widely acknowledged: it offers superior nutrition, protection against infection, enhancement of the immune system, a contraceptive effect while lactating, economic benefits, and emotional support. At the same time, mother's milk is a good pollution indicator. The pharmacokinetics of POPs transfer from mother to infant via breastfeeding is a complex process that is strongly influenced by a particular chemical characteristic of all POPs: their distinct affinity for fat. When a woman begins lactating, her fat stores are mobilized to excrete lipids efficiently and correspondingly POPs are also excreted to breast milk. She effectively transfers her own body burden of pollutants to her newborn. The lipid content in breast milk may have concentrations of POPs ten times higher than lipids of ordinary food (Heifetz et al. 1989; Bauchner 2003).

Considering high amounts of lipophilic 3-MCPD esters in various foodstuffs, we hypothesized on the potential transfer of these chemicals into breast milk in a similar way as known for POPs. It should be noted that, until now, no information on the excretion of chlorinated food contaminants such as 3-MCPD esters into human milk has been available. The preliminary experiments employing a conventional GC/MS procedure (a unit resolution, single quadrupole mass analyser operated in a selected ion-monitoring mode) for the analysis of derivatized extract obtained from milk fat hydrolysate indicated the presence of 3-MCPD. Nevertheless, due to a high chemical noise in such a complex matrix and relatively low selectivity of selected ions, unbiased proof of the presence of the target analyte in human milk fat was needed. For this purpose, a novel approach represented by comprehensive-orthogonal gas chromatography (GCxGC) coupled with high-speed time of flight mass spectrometry (TOF-MS) was employed for the analysis of four randomly selected breast milk samples. This challenging technique offers both high-resolution power on the GC side and full mass spectral information, even at low analyte level, enabling identification. Figure 1 shows an example of the chromatographic record obtained by GCxGC TOF-MS analysis of sample number 1; the presence of bound 3-MCPD in breast milk fat (the analyte released from the respective esters by hydrolysis) was demonstrated unambiguously. The content of bound 3-MCPD determined by this technique in sample numbers 1, 2, 6, and 11 was 1147, 771, 618, and 705 μg kg^{-1} fat, respectively. These results were in good agreement with data obtained by the routine GC/MS method used for the analysis of the whole set of samples (Table 1). As shown in Table 1, free 3-MCPD was not detected in any milk samples, while the bound 3-MCPD was present in all of them. Its levels ranged from the LOD (100 μg kg^{-1} fat) up to 2195 μg kg^{-1} fat; the mean was 1014 μg kg^{-1} fat. Considering a large variability of fat content in mother's milk (1.7–7.2%, mean = 3.8%), the calculated bound 3-MCPD content of the breast milks lies within the range < 11–76 μg kg^{-1} (mean = 35.5 μg kg^{-1} milk).

To visualize the relationships between the bound 3-MCPD in individual breast milk samples and the other individual parameters (variables), partial correlation analysis was performed using the data presented in Table 1. The only meaningful positive correlation ($r = 0.735$) was obtained between the level of bound 3-MCPD content in breast milk and its fat content (the Pearson correlation was significant at the 0.01 level, two-tailed). The fat content or mother's age as

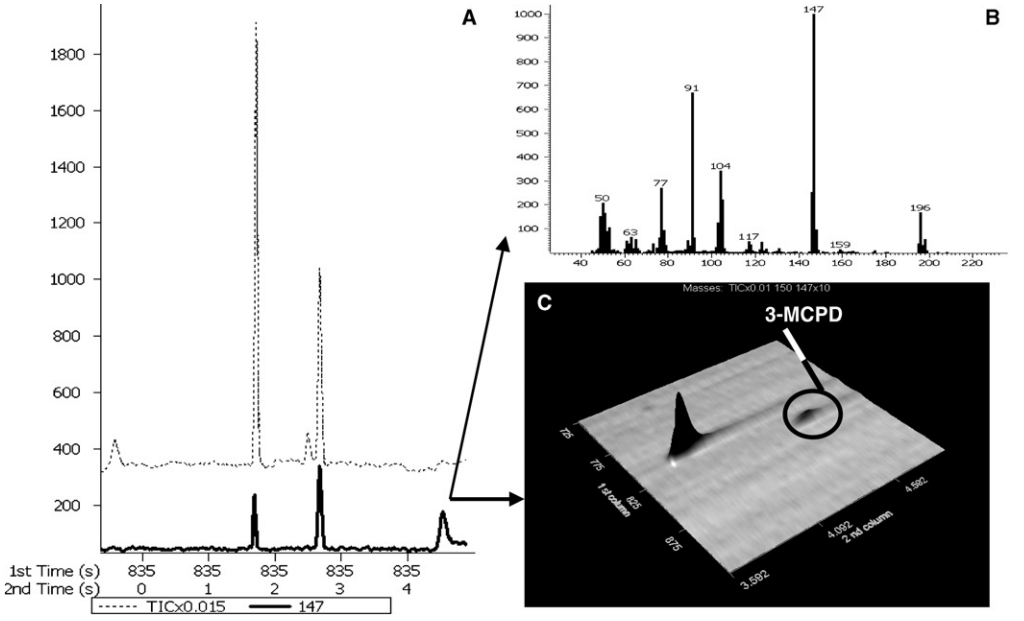

Figure 1. Detection and identification of 3-MCPD released from breast milk fat (sample number 1) by CGxGC TOF-MS. A, contour plot of a particular part of the chromatogram with the target analyte; B, mass spectrum of 3-MCPD after derivatization using 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane; C, three-dimensional chromatogram showing the target analyte (zoom).

Table 1. Characteristics of breast milk samples, their content of fat and bound 3-MCPD.

Sample number	Age (years)	Collected* (months)	Fat content (%)	Bound 3-MCPD ($\mu g\,kg^{-1}$ fat)	RSD (%)	Bound 3-MCPD ($\mu g\,kg^{-1}$ breast milk)
1	18	1	5.00	1263	8.8	63
2	24	4	3.40	729	3.9	25
3	26	1	2.40	471	4.1	11
4	27	0.25	4.27	1789	1.0	76
5	28	11	1.70	916	3.5	16
6	28	1	3.90	671	6.0	26
7	29	0.25	2.02	2195	7.2	44
8	29	0.5	2.93	1952	3.5	57
9	29	1	3.75	<300	–	<11
10	30	2	6.60	<300	–	<20
11	33	2	7.20	753	5.3	54
12	36	2	2.80	833	13.5	23

Note: *After childbirth.

well as the date of milk sample collection after childbirth did not correlate with any other variable.

In the final phase of this study, the individual naturally occurring 3-MCPD esters were investigated in sample numbers 1, 2, 6, and 11 employing the GC/MS method for examination of the diester fraction isolated from human milk fat. According to our previous results, the 3-MCPD diesters prevail over the 3-MCPD monoesters (Zelinková et al. 2007). The major types of 3-MCPD diesters in breast milk number 1 were symmetric diesters with lauric, palmitic, and oleic acids and asymmetric diesters with palmitic acid/oleic acid (Table 2). The asymmetric diesters bearing two different acyl groups (i.e. oleoyl/palmitoyl) can be seen in two GC/MS records corresponding to m/z 331 and m/z 357, which are formed by the fission of $[M - RCOOH]^+$ involving either palmitic or oleic acid. As can be seen, the major 3-MCPD diester of this breast milk sample was 1,2-dioleoyl-3-chloro-1,2-propanediol ($3117\,\mu g\,kg^{-1}$ fat), which corresponds to the average fatty acid composition of human milk lipids (Western diet) where the weight percentage and range of oleic acid is 31.0% and 22.6–38.7%, respectively (Donangelo and Trugo 2003). It is highly probable that several other minor 3-MCPD diesters and monoesters occur in the analysed sample as well. The bound 3-MCPD content calculated using the determined levels of the individual 3-MCPD diesters was $1668\,\mu g\,kg^{-1}$ fat, which is in a good agreement (134%) with the value given in

Table 1 (1263 μg kg^{-1} fat). Analogous results were obtained analysing breast milk sample numbers 2 (505 μg kg^{-1} fat, 69%), 6 (603 μg kg^{-1} fat, 90%), and 11 (851 μg kg^{-1} fat, 113%), respectively. Figure 2 is an example of chromatograms obtained analysing the individual diesters in the breast milk sample number 1.

Table 3 summarizes the results obtained by analysing the second series of samples collected from one of the nursing mothers 14, 49, 70, 71, 74 and 76 days after childbirth (sample numbers 13–18). As shown herein, their fat content was relatively low and ranged from 0.92 to 1.93% (mean = 1.48%). All these samples contained bound 3-MCPD within the range 328–2078 μg kg^{-1} fat (mean = 930 μg kg^{-1} fat). The calculated bound 3-MCPD content of these samples was within the range 6–19 μg kg^{-1} milk (mean - 12 μg kg^{-1} milk), but no meaningful correlation between any variables was found.

A question arises about the origin of 3-MCPD esters in human breast milk. It has already been shown that various foodstuffs such as crackers, donuts (doughnuts), cookies, French fries (chips), baked goods, snack foods, fried foods, many other processed foods, and, especially, the refined vegetable oils may contain elevated levels of 3-MCPD esters (Hamlet and Sadd 2004; Svejkovská et al. 2004; Doležal et al. 2005; Zelinková et al. 2006; Divinová et al. 2007). It is therefore highly probable that these and some other dietary items become the major sources of 3-MCPD esters occurring in breast milk. Currently, there is no information available on how are these esters metabolized, to which extent are they hydrolysed or biosynthesized in the body, to which extent they deposit in tissues, and how do they influence the properties and functions of tissues (if they really do it) is not known.

Regarding dietary intake, the following scenario could be considered. The baby is breastfed for up to 4 months with only his/her mother's milk with an average content of bound 3-MCPD of 35.5 μg kg^{-1} (Table 1) and 3-MCPD esters of the milk are totally hydrolysed in his/her body by lipases so that 3-MCPD is released in its free form. An average daily intake of mother's milk by the baby is about 750 ml (approximate density of 1 g ml^{-1}) with a range of 570–900 ml day^{-1} (Figure 3). Under these conditions the amount of 3-MCPD taken by the baby is 26.625 μg day^{-1}. Considering a tolerable daily intake (TDI) of 2 μg kg^{-1} body weight day^{-1} (JECFA 2001) and the ideal weight score of a newborn baby 3.25 kg (on average boys are 0.3 kg heavier and girls 0.3 kg lighter than the ideal weight score), then his/her daily intake of 3-MCPD is 4.1 times higher than that corresponding to TDI. The TDI level of 6.5 μg day^{-1} is exceeded after the consumption of 183 ml of such breast milk. The situation significantly improves

Table 2. Levels of individual 3-MCPD diesters in breast milk sample number 1.

Fatty acids in 3-MCPD diesters	Diesters content (μg kg^{-1} fat)	Bound 3-MCPD (μg kg^{-1} fat)
Lauric/lauric	662	154
Myristic/myristic	n.d.	n.d.
Palmitic/palmitic	2816	530
Palmitic/oleic	2580	465
Oleic/oleic	3117	539
Stearic/stearic	n.d.	n.d.
Total	9175	1688

Note: n.d., Not detected.

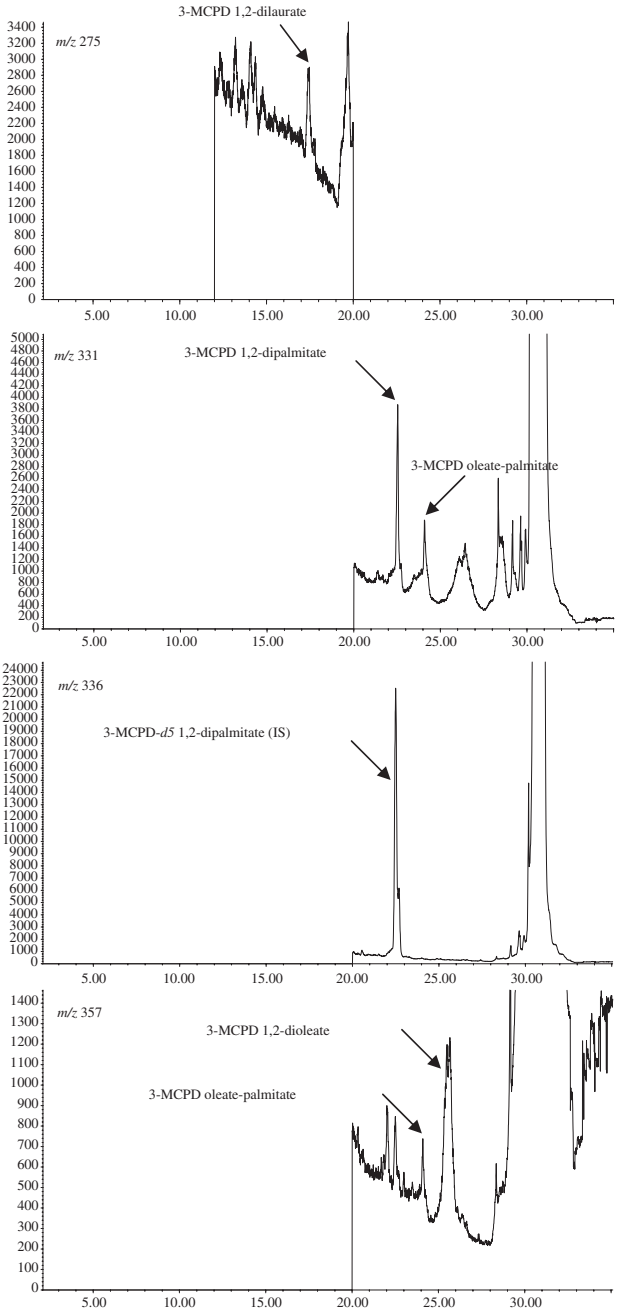

Figure 2. GC/MS analysis of 3-MCPD fatty acid esters in breast milk sample number 1.

Table 3. Dynamics of bound 3-MCPD during breast feeding (in a 39-year-old mother).

Sample number	Collected* (days)	Fat content (%)	Bound 3-MCPD ($\mu g\,kg^{-1}$ fat)	RSD (%)	Bound 3-MCPD ($\mu g\,kg^{-1}$ breast milk)
13	14	1.67	612	4.7	10
14	49	1.28	1450	2.1	19
15	70	1.76	328	3.0	6
16	71	0.92	2078	21.4	19
17	74	1.31	617	9.4	8
18	76	1.93	493	5.2	10

Note: *After childbirth.

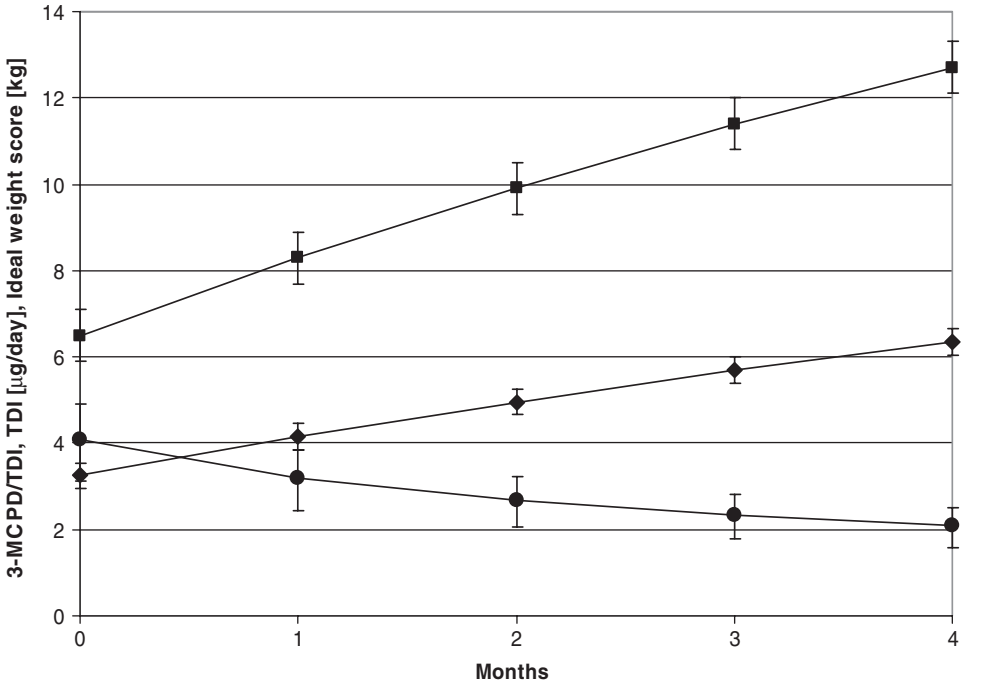

Figure 3. Ideal weight score, TDI and 3-MCPD/TDI during the first 4 months after childbirth. ♦, Ideal weight score (kg) (calculated from: http://www.medindia.net/patients/calculators/ideal_weight_result.asp), ■, TDI in $\mu g\ day^{-1}$; ●, 3-MCPD in $\mu g\,day^{-1}$/TDI in $\mu g\,day^{-1}$.

with the age of the baby (whose body weight increases) as at the age of 4 months he/she takes daily only 2.1 times higher amount of 3-MCPD than the amount corresponding to TDI (Figure 3).

MCPD esters are principally processing contaminants. It has been shown, for example, that appropriate manufacturing controls the levels of MCPD esters in edible oils, but strategies to reduce these compounds in other food products have not yet been fully explored. Identifying primary routes of 3-MCPD esters exposure, their mitigation, metabolism and/or biosynthetic pathways and biological effects are subjects for further research.

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