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

Jan Velisek, Zuzana Zelinkova, Ondrej Novotny, Jakub Schurek, Jana Hajslova, Marek Dolezal

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

Journal:	*Food Additives and Contaminants*
Manuscript ID:	TFAC-2007-140.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	05-Nov-2007
Complete List of Authors:	Velisek, Jan; Institut of Chemical Technology, Food Chemistry and Analysis Zelinkova, Zuzana; ICT Prague, Food Chemistry and Analysis Novotny, Ondrej; ICT Prague, Food Chemistry and Analysis Schurek, Jakub; ICT Prague, Food Chemistry and Analysis Hajslova, Jana; ICT Prague, Food Chemistry and Analysis Dolezal, Marek; ICT Prague, Food Chemistry and Analysis
Methods/Techniques:	Chromatography - GC/MS, GC/MS, Risk assessment, Toxicology
Additives/Contaminants:	3-MCPD, Nutrition, Process contaminants - 3-MCPD
Food Types:	Baby food, Milk

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

Z. Zelinková, O. Novotný, J. Schůrek, J. Velíšek*, J. Hajšlová & M. Doležal
Department of Food Chemistry and Analysis, Institute of Chemical Technology Prague, Technická 1905, 166 28 Prague, Czech Republic

Abstract

A series of twelve breast milk samples were analysed by GC/MS operated in SIM mode for 3-chloropropane-1,2-diol (3-MCPD). Whilst none of the samples contained 3-MCPD above the limit of detection of 3 μg/kg milk, all of them 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 μg/kg, expressed on fat basis) to 2195 μg/kg; with a mean level of bound 3-MCPD of 1014 μg/kg, which corresponded to 35.5 μg/kg milk. The presence of bound 3-MCPD was confirmed using GC×GC-TOF-MS 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 μg/kg fat (mean 930 μg/kg fat). The calculated bound 3-MCPD content of these samples was within the range of 6 μg/kg milk and 19 μg/kg milk (mean 12 μg/kg 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.

Key words: *Brest 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 (known as 3-MCPD) is a representative of the so-called food borne or food processing contaminants. 3-MCPD 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*)-3-MCPD 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 μg/kg body weight/day for the amount of 3-MCPD that can be consumed daily over a lifetime

without appreciable harm to consumers 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 μg/kg body weight/day in 2001 (JECFA 2001). A regulatory limit of 20 μg/kg, 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 (EC 2001).

Several recent studies have demonstrated that 3-MCPD is not only the contaminant typical for acid-HVP, soy sauces and related products but it also occurs in a wide range of retail outlet and home-made foods as well as in various food ingredients formulated without addition of acid-HVP (Crews *et al*. 2001, 2002, Breitling-Utzmann et al. 2003, Hamlet et al. 2002, 2004a, 2004b, 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 (Svejkovská et al. 2004, Hamlet & Sadd 2004, Doležal et al. 2005, Svejkovská et al. 2006, 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).

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. The study of Svejkovská et al. (2004) reports on the presence of bound 3-MCPD in 20 selected retail food products. High levels (in the range of 280-2420 μg/kg) of bound 3-MCPD were found in salty crackers, pickled herrings, doughnuts, crisp bread, dark malt and French fries. Compared to the free form, they were 5 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 μg/kg) and toast (160 μg/kg) while in the crumb they were much lower amount (26 μg/kg) and

exceeded the free 3-MCPD levels 6 to 52 times. The level of bound 3-MCPD in roasted coffee was relatively low and varied between 6 μg/kg (soluble coffee) and 390 μg/kg (decaffeinated coffee) and exceeded the free 3-MCPD level 8 to 33 times (Doležal et al. 2005). Coffee surrogates contained bound 3-MCPD in the range of 145-1184 μg/kg, the highest level was found in roasted barley (Divinová et al. 2007). In this case the bound 3-MCPD levels were higher 32 to 81 times. In malts (Divinová et al. 2007), the bound 3-MCPD levels ranged from 4 to 650 μg/kg and the highest amount was found in roasted malts (463-650 μg/kg). The bound 3-MCPD levels exceeded the free 3-MCPD levels 0.4 to 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 μg/kg to 2462 μg/kg 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 μg/kg 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 e.g. 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 top of which are humans, and create a lasting toxic body burden. During lactation, transfer of POPs into human brest 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 minimise 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 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 (> 98%, 3-MCPD) was purchased from E. Merck (Darmstadt, Germany), 3-MCPD-d_5 (99.4%) from Dr. Ehrenstorfer (Augsburg, Germany), phenylboronic acid (PBA, ≥ 97%) 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 synthesised 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-*d5* 1,2-dipalmitate, i.e. was synthesised employing 3-MCPD-d_5 and purified using the same procedures. The GC purity of symmetric 3-MCPD diesters was in the range of 94.4% and 99.4%. The asymmetric 3-MCPD diesters were mixtures with the corresponding symmetric 3-MCPD diesters and their content was in the range of 44.4% and 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, 7 samples within 1-2 months, 1 sample after 4 months and 1 sample after 11 months after childbirth. Ages of the mothers were within the range of 18-36 years (mean 28.1, median 28.5). One of the mothers (29 years old) provided us with a series of 6 samples collected 14, 49, 70, 71, 74 and 76 days after childbirth. The breast milk samples were collected in cleaned glass containers and stored at -20^{o}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 minutes and the aqueous layer was 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 shaked for additional 5 minutes. 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, 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-*d5* 1,2-dipalmitate (7.26 µg) was placed onto the top of a silica gel column 330 x 20 mm (silica gel 60, 70-230 mesh, Merck, Darmstadt, Germany). The flask was washed out 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 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 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 derivatised 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), neutralised using saturated $NaHCO_3$ solution, spiked with 3-MCPD-d_5 and derivatised with phenylboronic acid. Three parallel examinations of each milk sample were made.

Orthogonal gas chromatography (GC x GC) 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 x 0.25 mm x 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. Helium at a flow rate of 0.8 ml/min 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* 91and 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 milk fat, RSD (3 injections, 0.3282 μg 3-MCPD/ml) = 5.9%, linearity (0.005-8.575 μg 3-MCPD/ml), $r^2 = 0.9998$.

The GC×GC/TOF-MS system, used for examination of 4 selected samples, consisted of a HP 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with split-splittles injector. The detector was Pegasus III, high speed time-of-flight mass spectrometer (LECO, St. Joseph, MI, USA) with 10 ml/min pumping capacity and operated in electron ionisation mode (EI). 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, secondary 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, Palo Alto, CA, USA) and a secondary column BPX50 (2.2 m × 0.1 mm × 0.1 μm; SGE Ringwood, Australia). The oven temperature program was as follows: 80°C for 1 min, 10°C/min to 250°C, 5 min at 250°C, secondary oven was held 5°C above the main oven; helium flow rate was 1.0 ml/min; injection mode: splittles for 1.0 min; injection temperature: 270°C; modulation time: 5 s (hot pulse 1.5 s); modulation temperature offset: 15°C. The instrumental of the mass spectrometer was as follows: solvent delay 350 s; acquisition rate 125 Hz; mass range 45-500 amu; ion source temperature 220°C; transfer line temperature 270°C; detector voltage -1700V. The performance characteristics were as follows: LOD = 3

µg 3-MCPD/kg milk fat, RSD (5 injections, 0.0515 µg 3-MCPD/ml) = 6 .5%, linearity (0.01-0.515 µg 3-MCPD/ml), $r^2 = 0.9942$.

GC/MS analysis of 3-MCPD diesters was performed on a capillary column DB-1HT (15 m x 0.25 mm i.d., film thickness 0.1 µm, Agilent Technologies, Palo Alto, CA, USA). 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 and then to 400°C (10 min) at a rate of 40°C/min. Helium at a flow rate of 0.7 ml/min was used as the carrier gas, 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 $[M\text{-}RCOOH]^+$ 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-*d5* 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 $[M\text{-}RCOOH+2]^+$ and $[RCO]^+$ were monitored for confirmation.

Statistical methods

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

Results and discussion

The advantages of breast-feeding 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 efficiently excrete lipids and correspondingly also POPs are 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 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 conventional GC/MS procedure (unit resolution, single quadrupole mass analyser operated in a selected ion monitoring mode) for analysis of derivatised 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 target analyte presence 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 analysis of randomly selected 4 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. In Figure 1, there is shown an example of the chromatographic record obtained by GCxGC-TOF-MS analysis of sample no. 1; the presence of bound 3-MCPD in breast milk fat (analyte released from the respective esters by hydrolysis) was unambiguously demonstrated. The content of bound 3-MCPD determined by this technique in samples no. 1, 2, 6 and 11 was 1147, 771, 618, and 705 µg/kg fat, respectively. These results were in good agreement with data obtained by routine GC/MS method that was used for analysis of the whole set of samples (Table I). As shown in Table I, free 3-MCPD was not detected in any of milk samples, while the bound 3-MCPD was present in all of them. Its levels ranged from the LOD (100 µg/kg fat) up to 2195 µg/kg fat, the mean value was 1014 µg/kg fat. Considering a large variability of fat content in mother's milk (1.7% to 7.2%, mean 3.8%), then the calculated bound 3-MCPD content of the breast milks lays within the range of <11 µg/kg and 76 µg/kg (mean 35.5 µg/kg milk).

To visualise the relationships between the bound 3-MCPD in individual breast milk samples and the other individual parameters (variables), partial correlation analysis was done using the data presented in Table I. 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 (Pearson correlation was significant at the 0.01 level, 2 tailed). The fat content or mother's age as 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 samples no. 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 no. 1 were symmetric diesters with lauric, palmitic, and oleic acids and asymmetric diesters with palmitic acid/oleic acid (Table II). The asymmetric diesters bearing 2 different acyl groups (i.e. oleoyl/palmitoyl) can be seen in 2 GC/MS records corresponding to *m/z* 331 and *m/z* 357, which are formed by the fission of $[M\text{-}RCOOH]^+$ involving either palmitic or oleic acid. As it can be seen, the major 3-MCPD diester of this breast milk sample was 1,2-dioleoyl-3-chloro-1,2-propanediol (3117 µg/kg fat), which corresponds to the average fatty acid composition of human milk lipids (western diet) where the weight % and range of oleic acid is 31.0% and 22.6-38.7%, respectively (Donangelo & 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 µg/kg fat, which is in a good agreement (134%) with the value given in Table I (1263 µg/kg fat). Analogous results were obtained analyzing breast milk samples no. 2 (505 µg/kg fat, 69%), no. 6 (603 µg/kg fat, 90%), and no. 11 (851 µg/kg fat, 113%), respectively. Figure 2 is an example of chromatograms obtained analysing the individual diesters in the breast milk sample no. 1.

Table III summarises 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 no. 13-18). As shown here, 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 of 328 µg/kg fat and 2078 µg/kg fat (mean 930 µg/kg fat). The calculated bound 3-MCPD content of these samples was within the range of 6 µg/kg milk and 19 µg/kg milk (mean 12 µg/kg milk) but no meaningful correlation between any variables was found.

Question arises about the origin of 3-MCPD esters in human breast milk. It has been already 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 (Svejkovská et al. 2004, Hamlet and Sadd 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 metabolised, to which extent are they hydrolysed or biosynthesised 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 the average content of bound 3-MCPD of 35.5 μg/kg (Table I) 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) with a range of 570-900 ml per day (Figure 3). Under these conditions the amount of 3-MCPD taken by the baby per day is 26.625 μg. Considering tolerable daily intake (TDI) of 2 μg/kg body weight/day (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 is exceeded after the consumption of 183 ml of such breast milk. The situation significantly improves with the age of the baby (the 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.

Acknowledgement

This work was substantially part of the Research Project MSM6046137305 supported by the Ministry of Education and Youth of the Czech Republic. Development of analytical methods aimed at confirmation of bound 3-MCPD was supported by the project no. 2B06168 –National program of research.

References

Bauchner E. 2003. Environmental contaminants and human milk. LEAVEN 39:123-125.

Breitling-Utzmann CM, Köbler H, Herbolzheimer D, Maier A. 2003. 3-MCPD-occurrence in bread crust and various food groups as well as formation in toast. Deutsche Lebensmittel-Rundschau 99:280-285.

Cerbulis J, Parks OW, Liu RH, Piotrowski EG, Farrell HM, Jr. 1984. Occurrence of diesters of 3-chloro-1,2-propanediol in the neutral lipid fraction of goat´s milk. Journal of Agricultural and Food Chemistry 32:474-476.

Crews C, Brereton P, Davies A. 2001. Effect of domestic cooking on the levels of 3-monochloropropane-1,2-diol in food. Food Additives & Contaminants 18:271-280.

Crews C, Hough P, Brereton P, Harvey D, Macarthur R, Matthews W. 2002. Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999-2000. Food Additives & Contaminants 19:22-27.

Davídek J, Velíšek J, Kubelka V, Janíček G. (1982). New chlorine-containing compounds in protein hydrolysates. In: Baltes W., Czedik-Eysenberg P.B., Pfannhauser W., editors. Recent Developments in Food Analysis. Proc. Euro Food Chem I. Vienna, Austria, 17-20 Feb, 1981, Weinheim:Deerfield Beach, Florida, USA, pp 322-325.

Divinová V, Svejkovská B, Novotný O, Velíšek J. (2004). Survey of 3-chloropropane-1,2-diol and its precursors in foods in the Czech Republic. Czech Journal of Food Sciences, Special Issue 22:230-234.

Divinová V, Doležal M, Velíšek J. (2007). Free and bound 3-chloropropane-1,2-diol in coffee surrogates and malts. Czech Journal of Food Sciences 25:39-47.

Doležal M, Chaloupská M, Divinová V, Svejkovská B, Velíšek J. 2005. Occurrence of 3-chloropropane-1,2-diol and its esters in coffee. European Food Research & Technology 221:221-225.

Donangelo CM, Trugo NMF (2003): Human milk: composition and nutritional value. In: Encyclopedia of food sciences and nutrition, 2nd ed. (B. Caballero, LC Trugo, PM Finglas, eds.), Academic Press, p. 3453.

EC 2001: European Commission Regulation No. 466/2001. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities L77/1, 16 March, Luxembourg: Office for Official Publications of the European Communities.

Golding J. 1997. Unnatural constituents of breast milk – medication, lifestyle, pollutants, viruses. Early Human Development 49 Suppl:S29-S43.

Hamlet CG, Sadd PA. (2004). Chloropropanols and their esters in cereal products. Czech Journal of Food Sciences, Special Issue, 22:229-262.

Hamlet CG, Sadd PA, Crews C, Velíšek J, Baxter DE. (2002): Occurrence of 3-chloro-propane-1,2-diol (3-MCPD) and related compounds in foods: a review. Food Additives & Contaminants 19:619-631.

Hamlet CG; Sadd PA; Gray DA. 2004a. Generation of monochloropropanediols (MCPDs) in model dough systems. 1. Leavened doughs. Journal of Agricultural and Food Chemistry. 52:2059-2066.

Hamlet CG; Sadd PA; Gray DA. 2004b. Generation of monochloropropanediols (MCPDs) in model dough systems. 2. Unleavened doughs. Journal of Agricultural and Food Chemistry. 52:2067-2072.

Heifetz M, Taylor R, Sharon S. 1989. Mother's milk or mother's poison? Pesticides in breast milk. Journal of Pesticide Reform. 9:15-17.

Hooper K, Jianwen S. 2002. Lessons from the polybrominated diphenyl ethers (PBDEs): Precautionary principle, primary prevention, and the value of community-based body-burden monitoring using breast milk. Environmental Health Perspectives 111:109-114.

JECFA 2001: Joint FAO/WHO Expert Committee on Food Additives, Fifty-seventh meeting Rome, 5-14 June 2001.

Kalantzi OL, Martin FL, Thomas GO, Alcock RE, Tang HR, Drury SC, Carmichael PL, Nicholson JK, Jones KC. 2004. Different levels of polybrominated diphenyl ethers (PBDEs) and chlorinated compounds in breast milk from two UK regions. Environmental health Perspectives 112:1085-1091.

Karšulínová L, Folprechtová B, Doležal M, Dostálová J, Velíšek J. (2007). Analysis of the lipid fraction of coffee creamers, cream aerosols and bouillon cubes. Czech Journal of Food Sciences 25:in press.

Kraft R., Brachwitz H., Etzold G., Langen P., Zöpel H.J. 1979. Massenspektrometrische Strukturuntersuchung stellungsisomerer Fettsäureester der Halogenpropandiole (Desoxy-glyceride). Journal für praktische Chemie 321:756-768.

SCF 2001: Opinion of the Scientific Committee on food on 3-monochloro-propane-1,2-diol (3-MCPD) updating the SCF opinion of 1994. Adopted on 30 May 2001.

Schenzler C., Their H.-P. 2001. European standardization of methods for pesticide residue analysis in foods – current status. Food Additives & Contaminants 18:875-879.

Svejkovská B, Novotný O, Divinová V, Réblová Z, Doležal M, Velíšek J. 2004. Esters of 3-chloropropane-1,2-diol in foodstuffs. Czech Journal of Food Sciences 22:190-196.

Svejkovská B., Doležal M., Velíšek J. 2006. Formation and decomposition of 3-chloropropane-1,2-diol esters in models simulating processed foods. Czech Journal of Food Sciences 24:172-179.

Velíšek J. 2006. Historical perspective on 3-MCPD in foods and recent research. 3-MPCD Stakeholders' Meeting on Heat Generated Formation of 3-MCPD in Foods. Food Standards Agency, London, GB, September 22.

Velíšek J, Davídek J, Kubelka V, Janíček G, Svobodová Z, Šimicová Z. 1980. New chlorine-containing organic compounds in protein hydrolysates. Journal of Agricultural and Food Chemistry 28:1142-1144.

Velíšek J, Doležal M, Crews C, Dvořák T. 2002. Optical isomers of chloropropanediols: mechanisms of their formation and decomposition in protein hydrolysates. Czech Journal of Food Sciences 20:161-170.

Zelinková Z, Svejkovská B, Velíšek J, Doležal M. 2006. Fatty acids esters of 3-chloropropane-1,2-diol in edible oils. Food Additives & Contaminants 23:1290-1298.

Zelinková Z, Velíšek J, Doležal M. 2007. Unpublished results.

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

Sample no.	Age [years]	Collected* [months]	Fat content [%]	Bound 3-MCPD [µg/kg fat]	RSD [%]	Bound 3-MCPD [µg/kg 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

* After childbirth.

Table II. Levels of individual 3-MCPD diesters in breast milk sample no. 1.

Fatty acids in 3-MCPD diesters	Diesters content [µg/kg fat]	Bound 3-MCPD [µg/kg fat]
Lauric/Lauric	662	154
Myristic/Myristic	nd	nd
Palmitic/Palmitic	2816	530
Palmitic/Oleic	2580	465
Oleic/Oleic	3117	539
Stearic/Stearic	nd	nd
Total	9175	1688

nd=not detected.

Table III. The dynamics of bound 3-MCPD during breast feeding (39 years old mother).

Sample no.	Collected* [days]	Fat content [%]	Bound 3-MCPD [µg/kg fat]	RSD [%]	Bound 3-MCPD [µg/kg 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

* After childbirth.

Figure 1. Detection and identification of 3-MCPD released from breast milk fat (sample no. 1) by CGxGC-TOF-MS. A=contour plot of particular part of chromatogram with target analyte, B=mass spectrum of 3-MCPD after derivatization using 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane, C=3D chromatogram showing target analyte (zoom).

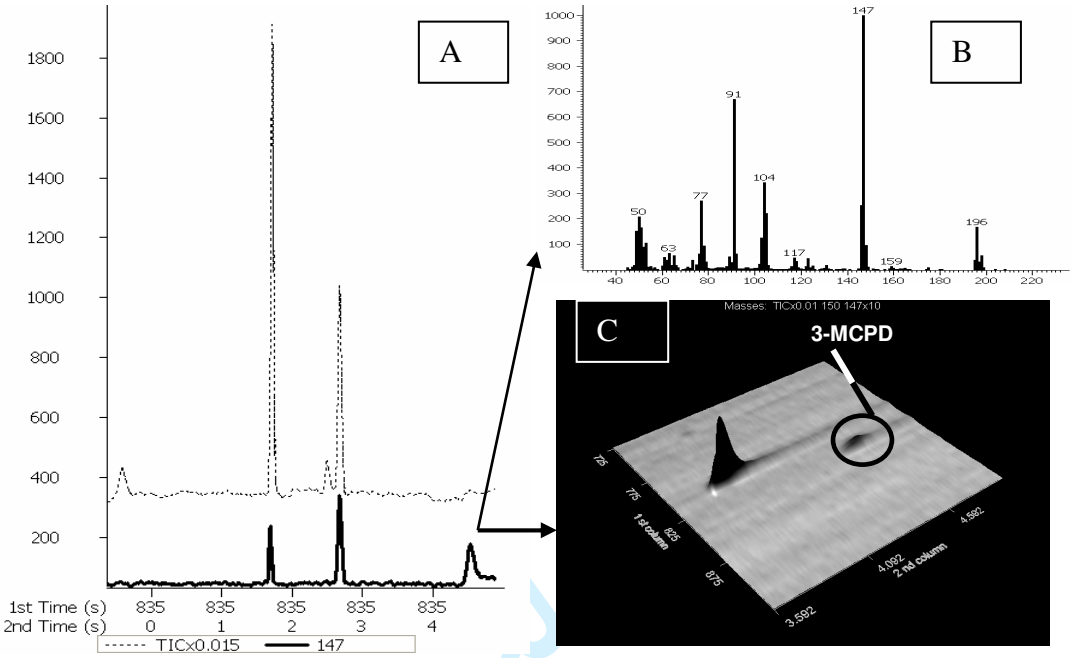

Figure 2. GC/MS analysis of 3-MCPD fatty acid esters in breast milk sample no. 1.

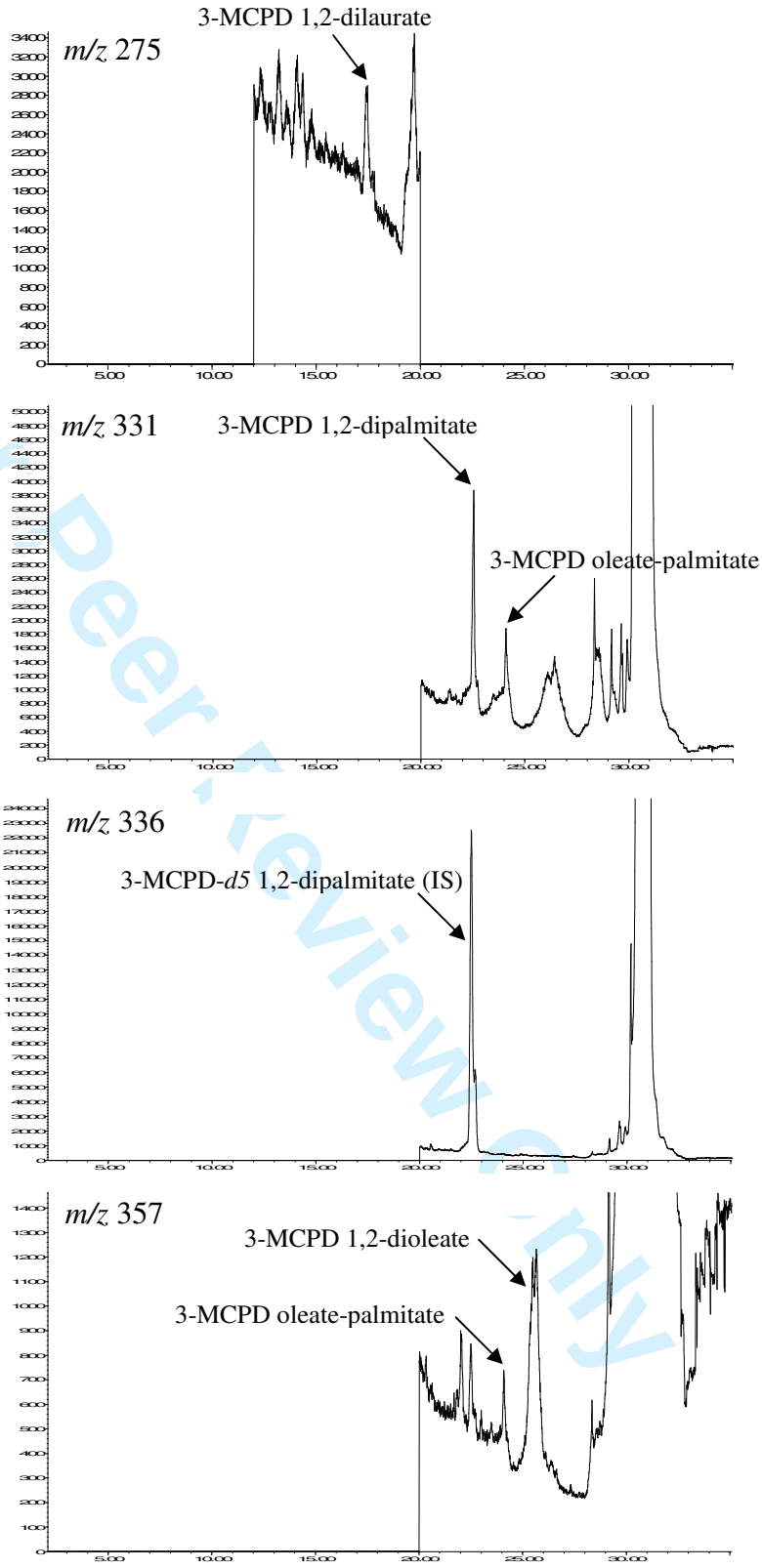

Figure 3. Ideal weight score, TDI and 3-MCPD/TDI during the first four months after childbirth. ♦ = ideal weight score in kg (calculated from: http://www.medindia.net/patients/calculators/ ideal_weight_result.asp), ■ = TDI in µg/day, ● = 3-MCPD in µg/day/TDI in µg/day