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Exposure assessment of 3-monochloropropane-1, 2-diol esters from edible oils and fats in China

Chang Li, Shao-Ping Nie, Yong-qiang Zhou, Ming-Yong Xie *

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, Jiangxi 330047, China

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ABSTRACT

3-monochoropropane-1, 2-diol (3-MCPD) esters from edible oils are considered to be a possible risk factor for adverse effects in human. In the present study, the exposure assessment of 3-MCPD esters to Chinese population was performed. A total of 143 edible oil and fat samples collected from Chinese markets were determined for the concentrations of 3-MCPD esters. The concentration data together with the data of fats consumed were analyzed by the point evaluation and probabilistic assessment for the exposure assessment. The point evaluation showed that the mean daily intake (DI) of 3-MCPD esters were lower than the value of provisional maximum tolerable daily intake (PMTDI) of 3-MCPD (2 μg/kg BW/d). The mean DI values in different age groups obtained from probabilistic assessment were similar to the results of the point evaluation. However, in high percentiles (95th, 97.5th, 99th), the DI values in all age groups were undesirably higher than the value of PMTDI. Overall, the children and adolescents exposed more to 3-MCPD esters than the adults. Uncertainty was also analyzed for the exposure assessment. Decreasing the level of 3-MCPD esters in edible oils and consuming less oil were top priority to minimize the risk of 3-MCPD esters.

1. Introduction

In 1978, 3-monochoropropane-1, 2-diol (3-MCPD) was identified as a contaminant in acid-hydrolyzed vegetable proteins (HVP) and soy sauce (Velíšek et al., 1978). In the following years, free 3-MCPD was detected in various types of processed food. 3-MCPD was categorized as a "possible human carcinogen" – category 2B by International Agency for Research on Cancer (IARC) (IARC, 2012). Tolerable Daily Intake (TDI) of 2 μg/kg body weight (BW) for 3-MCPD was derived by the European Commission's Scientific Committee for Food (SCF of EC, 2001). The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) established the provisional maximum TDI (PMTDI) of 2 μg/kg BW (JECFA, 2002).

Even early in 1980, it was observed that certain amounts of mono- and di-esters of 3-MCPD were formed during the acid-hydrolysis of HVP (Davidek et al., 1980). Nevertheless, 3-MCPD esters were not recognized as food contaminants in the following 20 years. Surveys have shown that 3-MCPD esters occurred in many heat processed foodstuffs (Doležal et al., 2005; Hamlet and Sadd, 2004; Svejkovska et al., 2004; Zelinková et al., 2006, 2009a, 2009b), even in human breast milk (Zelinkova et al., 2008), with greater amounts than their free forms, especially in refined edible oils and fats. It has been found that formation of fatty acid esters of 3-MCPD can occur during deodorization of edible oils (Franke et al., 2009; Zelinková et al., 2006). They can release free 3-MCPD with the catalysis of lipase. It has been reported that 3-MCPD esters were hydrolyzed by lipases from *Aspergillus oryzea* (Hamlet and Sadd, 2004). Another study showed that various 3-MCPD fatty acid esters were hydrolyzed within minutes by using an *in vitro* system based on the activity of pancreatic lipase in the presence of porcine bile extract (Seefelder et al., 2008). Toxicology research had revealed their toxic effects (Bakhiya et al., 2011; Barocelli et al., 2011; Liu et al., 2012; Tee et al., 2011). Therefore, 3-MCPD esters were regarded as contaminant in foods in recent years.

Recently, dietary surveys of esters of 3-MCPD in food have been conducted for the estimation of exposure (BfR, 2007; Chung et al., 2013). In previous surveys, exposure estimates were made based on the assumption that 3-MCPD esters are hydrolyzed completely

Abbreviations: BfR, Das Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment); BW, body weight; CCCF, Codex Committee on Contaminants in Foods; DGF, Deutsche Gesellschaft für Fettwissenschaft e.V (German Society for Fat Research); DI, daily intake; EFSA, European Food Safety Authority; EPA, U.S. Environmental Protection Agency; GC–MS, gas chromatograph–mass spectrometry; HVP, hydrolyzed vegetable protein; IARC, International Agency for Research on Cancer; ISO, International Standard Organization; JECFA, The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives; MCPD, monochloropropanediol; PBA, phenyboronic acid; PMTDI, Provisional Maximum Tolerable Daily Intake; SCF, European Commission's Scientific Committee on Food; *T-BME*, tert-butyl methyl ether; TDI, Tolerable Daily Intake.

* Corresponding author. State Key Laboratory of Food Science and Technology, Nanchang University, 235 Nanjing East Road, Nanchang 330047, China. Tel.: +0086 791 83969009; fax:+0086 791 83969009.
E-mail address: myxie@ncu.edu.cn (M.-Y. Xie).

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in the human gut. According to European Food Safety Authority (EFSA), there is no any evidence to dispute the assumption that 100% of 3-MCPD is released from its esters in the human body (EFSA, 2008). In 2010, the Codex Committee on Contaminants in Foods (CCCF) regarded 3-MCPD fatty acid esters as one of the priorities for evaluation by JECFA for toxicological assessment and exposure assessment. However, the data of concentration and exposure level of 3-MCPD esters are insufficient around the world so far.

Currently, edible oils and fats are contaminated more seriously than other oil-containing foods, to some extent, the 3-MCPD esters in other processed foodstuffs are from edible oils (Weißhaar, 2011). In order to narrow the information gaps on both levels and intake exposure of 3-MCPD esters in China, in the present study, 3-MCPD esters content in the edible oils and fats from China were determined, by combining the data of China Health and Nutrition Survey (CHNS), the dietary exposure of 3-MCPD esters for Chinese population was estimated. The output of exposure was compared with the value of PMTDI of 3-MCPD.

2. Materials and methods

2.1. Reagents and chemicals

D_5-3-MCPD-1,2-bis-palmitoyl ester and 3-MCPD-1,2-bis-palmitoyl ester were bought from Toronto Research Chemicals, Canada.

Toluene, tert-butyl methyl ether, methanol, isohexane, ethyl acetate, diethyl ether isooctane, sulfuric acid were purchased from Sinopharm Chemical Reagent (Shanghai) Co., Ltd.

Sodium methoxide, Sodium sulfate anhydrous, sodium bromide, phenylboronic acid were obtained from Aladdin Reagents (Shanghai) Co., LTD.

2.2. Samples collection

Edible oils and fats were selected as the samples in the present study, since edible oils and fats are major sources of dietary exposure to 3-MCPD esters. These samples included 102 refined oils and 41 crude oils (unrefined oils). A total of 102 refined oil and fat samples from 11 varieties were collected from the markets in China. Considering the fact that crude oils are consumed in some rural areas in China, they were also collected for the study. Each sample was selected from different brand. For the commercial interests of the producers, the information on the sample packages and brand was not mentioned in this article. Sampling method in this study was based on ISO 5555 (ISO, 2001).

2.3. Determination of fatty acid esters of 3-MCPD in edible oils and fats

The concentration of 3-MCPD esters in edible oils and fats was determined by gas chromatograph–mass spectrometry (GC–MS) using a standardized, widely used DGF method C-VI 18 (10) (DGF, 2011). DGF method C-VI 18 (10) was documented as a working draft (WD) by ISO (ISO, 2012) and the document is at the status of *"under development"* to Draft International Standard(DIS)(ISO/DIS18363-1,2014). For the determination of 3-MCPD esters, edible oil or fat was dissolved in tert-butyl methyl ether (*t*-BME) and spiked with a surrogate standard d_5-3-MCPD-1, 2-bis-palmitoyl ester; free 3-MCPD and free glycidol were released by addition of sodium methoxide (in methanol). The reaction was stopped by addition of excess amount of an acidic NaBr solution. After removing undesired compounds and double extracting of the aqueous phase with isohexane, the analyte together with the surrogate standard was transferred into organic phase by multiple extraction of aqueous phase with the mixture of diethyl ether and ethyl acetate. Phenyboronic acid (PBA) was then added into the organic phase to react with free 3-MCPD. The derivative was dried by nitrogen blowing, and then resolved in isooctane. The solution was measured by GC–MS after being filtered by microfiltration membrane. GC–MS was carried out on a 7890 A Agilent gas chromatograph equipped with a 5975C mass detector and a split/splitless injector including an auto sampler. Each derivatized calibration solution was injected in splitless injection mode and detected in selected ion monitoring mode. A HP 5 MS capillary column (Agilent, Waldbronn, Germany; 5% phenyl, 95% dimethylpolysiloxane; 30m × 0.25 mm, 0.25 μm film thickness) was used for separation. The injector temperature was kept at 280 °C, and ultrapure grade helium was used as carrier gas with a flow of 1.0 mL/min. GC oven temperature was programmed from an initial temperature of 85 °C (isothermal 0.5 min), with 6 °C/min to 150 °C, with 12 °C/min to 180 °C, with 25 °C to 280 °C, isothermal 7.16 min. Quantitative analysis was carried out by monitoring characteristic ions (quantifier) at m/z147 (3-MCPD) and m/z150 (3-MCPD-d5). Ions at m/z196 (3-MCPD), and m/z201 (3-MCPD-d5) were used as qualifiers. Quantification of the 3-MCPD esters was carried out by multiplying the ratio of signal areas of the analyte and the isotopic labeled surrogate standard based on corresponding ion traces with the spiking level of the isotopic labeled surrogate standard in each assay. Tests were performed in triplicate. The means ± SD of levels were expressed as microgram weights of 3-MCPD to kilogram of oil or fat (μg/kg).

2.4. Consumption data

Data of edible oils and fats consumption were obtained from CHNS. CHNS was jointly carried out by Carolina Population Center in University of North Carolina (USA) and Institute of Nutrition and Food Safety of CDC (China) (Cui and Michael, 2012; Zhai et al., 2009). The surveys were conducted by an international team of researchers whose expertise includes nutrition, public health, economics, sociology, Chinese studies and demography. The CHNS collected health data from 228 communities in nine provinces including Guangxi, Guizhou, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Liaoning and Shandong from 1989 to 2009. Using multistage, random cluster sampling, counties in the nine provinces were stratified by income and weighted sampling was used to randomly select four counties in each province. Villages and small towns within counties and urban and suburban neighborhoods within cities were selected randomly into primary sampling units that were politically and geographically classified by State Statistical Office. The surveyed provinces represent 56% of the Chinese population. Survey procedures were described elsewhere (Popkin et al., 2010). All data are freely available online. The latest data available were from the survey in 2009. The latest data were extracted from the CHNS data sets and analyzed by SAS V.9.0 for the exposure assessment in this study.

2.5. Exposure assessment

Based on the levels of 3-MCPD esters determined in this study and the newest survey data on consumption of edible oils and fats of Chinese residents mentioned above, the dietary exposure to 3-MCPD esters was assessed. All the exposure assessments were based on the assumption that the ester forms would be hydrolyzed completely *in vivo*. For the exposure assessment, the respondents were grouped in five age categories: children (7–10 years), adolescents (11–13 years and 14–17 years), adults (18–49 years), middle and old adults (>50 years). In each category, they were analyzed separately by gender. Non-detected data were considered to be 0.5 LOD for data analysis. The concentration of 3-MCPD esters in sample was presented as μg/kg. The exposure of Chinese population to 3-MCPD esters was expressed as μg/kg BW/d. Daily intake (DI, μg/kg BW/d) was calculated according to the following equation:

$$\mathrm{DI} = \frac{x \cdot c}{BW \cdot 1000}$$

where x is the fats consumption (g/d); c is the concentration of 3-MCPD esters in different oils and fats (μg/kg); BW is the bodyweight (kg); 1000 is the conversion factor.

The point evaluation and the Monte Carlo assessment model were combined to assess risk of 3-MCPD esters in the present study.

2.5.1. Point exposure assessment

The point evaluations of intake of 3-MCPD esters were calculated by multiplying the mean concentration by the mean and different percentiles of the consumption data. The results were compared with the value of PMTDI of 3-MCPD.

2.5.2. Probabilistic exposure assessment

Probabilistic analysis combined distributions of food consumption data with distributions of 3-MCPD esters in tested edible oils and fats, which involves a scenario in which both the levels of 3-MCPD esters and the intake of edible oils and fats were modeled as distributions. Best fitted distributions were selected for the assumption defining both the concentration and the consumption amounts. Monte Carlo simulation technique was used for the probabilistic analysis. The Oracle Crystal Ball Fusion Edition v11.1.2.1.00 (Oracle, USA), an add-in for Microsoft Office Excel, in combination with Microsoft Office Excel 2007, was applied to run the simulation. First order Monte Carlo simulations were run for 50,000 iterations. Then, the DI values under different percentile intakes, i.e., 50th, 75th, 90th, 95th, 97.5th and 99th, were consecutively obtained, and compared to the value of PMTDI of 3-MCPD.

3. Results and discussion

3.1. Concentration of 3-MCPD esters in edible oils and fats

A total of 143 edible oils and fats were collected for the analysis of occurrence of 3-MCPD esters. Concentrations of 3-MCPD esters