The occurrence of synthetic musks in human breast milk in Sichuan, China

Jie Yin, Hao Wang, Jing Zhang, Naiyuan Zhou, Fudie Gao, Yongning Wu, Jie Xiang, Bing Shao

Abstract

Human breast milk samples collected from mothers (n = 110) who lived in Chengdu, Sichuan Province, southwestern China in 2009 were analyzed to determine the concentrations of 13 musk compounds. Possible relationships between musk concentrations and some personal characteristics were also studied. Only five target analytes were detected in the milk samples analyzed, with median concentration values of 16.5, 11.5, 7.85, <1.5 and <1.4 ng g⁻¹ lipid weight for AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene), HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopentla[γ]-2-benzopyran), HHCB-lactone (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran-1-one), OTNE ([1,2,3,4,5,6,7,8-octahydro-2,3,8-tetramethyl-naphthalene-2-yl]ethan-1-one) and musk ketone (4-tert-butyl-2,6-dimethyl-3,5-dinitroacetophenone, MK), respectively. Mothers who reported high use of hand-cleaning agents, body-cleaning agents, shampoo and hair conditioners, hair dyes and hair gels had significantly elevated milk concentrations of HHCB whereas elevated milk concentrations of AHTN were observed among mothers reporting high use of body-cleaning agents, body lotions, shampoos, hair dyes and hair gels. Younger age showed a significantly positive effect on milk concentrations of both HHCB and AHTN whereas BMI after delivery, the number of children nursed and place of residence (urban or rural) had no significant effect. The estimated median daily intakes of synthetic musks for breast-fed infants were considerably lower than the current provisional tolerable daily intake amounts suggested for adults.

1. Introduction

Synthetic musks, including nitro musks, polycyclic musks and macrocyclic musks, are extensively used in a variety of personal care products and cleaning products as fragrance ingredients. These compounds have been reported to be ubiquitously present in the environment (Rimkus, 1999, 2004; Heberer, 2002; Fromme et al., 2004; Osemwengie and Gerstenberger, 2004; Bester et al., 2008; Guo et al., 2010) and can accumulate in animal and human tissues because of their lipophilic nature (Rimkus and Wolf, 1995; Muller et al., 1996; Hutter et al., 2005; Nakata, 2005). Because of the potential toxicity of synthetic musks observed in both in vivo and in vitro studies (IARC, 1996; Api and Ford, 1999; Schrauwers et al., 2002; Salvito, 2005), human exposure to these compounds has been a great concern in recent years.

Human milk is widely used for identifying and monitoring the body burden of lipophilic and persistent environmental pollutants because relatively large volumes of samples can be collected non-invasively compared with blood and adipose tissue. Furthermore, the concentrations of organic pollutants in human milk can reflect the exposure levels of breastfed infants (WHO, 2009), as well as the contamination status of the local environment. Over the last few decades, the levels of synthetic musks in human milk have been analyzed to evaluate the infant exposure in many countries. Musk xylene (1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene, MX), musk ketone (4-tert-butyl-2,6-dimethyl-3,5-dinitroacetophenone, MK), ADBI (4-acetyl-1,1-dimethyl-6-tert-butylindan), HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran) and AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene) were found in human milk samples from Europe, the United States and Japan (Rimkus et al., 1994; Rimkus and Wolf, 1996; Liebl et al., 2000; Duedahl-Olesen et al., 2005; Reiner et al., 2007; Lignell et al., 2008; Ueno et al., 2009). Some European countries, such as Sweden and Germany, have been routinely monitoring the concentrations of synthetic musks in breast milk for many years (Solomon and Weiss, 2002). However, only one study on the synthetic musk compounds in human milk from China has been published. Zhang et al. (2011) have detected four musk compounds in human milk samples collected from the Yangtze River Delta area in eastern China. Because of China’s huge
territory and regional differences of environmental contaminants in human tissues, women from different geographic locations should be investigated in further studies to elucidate the background exposure to synthetic musks across the country.

Humans can be exposed to synthetic musk through percutaneous absorption, ingestion of contaminated food and inhalation (Müller et al., 1996). For adult females, dermal absorption from perfumed products, including personal care products and cleaning products, has been suggested to be a major route of exposure to musk compounds (Müller et al., 1996; Liebl et al., 2000). Thus, the musk levels in maternal milk should positively correlate with the individual usage rates of perfumed products before and during the lactation period. Surprisingly, only limited studies have investigated this possible relationship between these two factors. In 2008, Lignell et al. (2008) reported relationships between musk concentrations and lifestyle factors of mothers in Sweden. A significant positive association was found between the use of perfumed products and mothers’ milk concentrations of HHCB (perfumes) and AHTN (perfumed laundry detergents) whereas no significant associations were found for MX. However, the number of milk samples in Swedish study (n = 44) was small, which makes the results of the study relatively uncertain. Further investigations are required to verify the findings of this research.

In the present study, the occurrence of twelve synthetic musks and one musk metabolite in breast milk samples collected from southwestern China was determined to elucidate the current contamination status of human milk in China. In addition, we investigated possible relationships between musk concentrations of maternal milk and the usage rates of perfumed products. To assess the health risk of these contaminants on breast-feeding infants in China, the daily intake amounts of synthetic musk compounds through breast milk were calculated and compared with proposed tolerable intake amounts.

2. Materials and methods

2.1. Donor recruitment and sample collection

Convenience sampling was employed to recruit donors at several general hospitals in Chengdu, Sichuan Province, located in southwestern China. Mothers were asked to participate in our study when they took their children to the hospital for vaccine injections. Finally, 110 out of 135 mothers agreed to donate breast milk for chemical analysis, with a response rate of 81.5%. All mothers donating milk were informed of the nature and purpose of the study and signed consent forms. The study had been approved by the Institutional Review Board of the Beijing Center for Disease Control and Prevention before recruitment.

The milk sampling was conducted from May to June in 2009. To avoid contamination, the mothers were asked to wash their breasts with fresh water (not using soaps and other perfumed detergents) before sampling. Approximately 50 mL of milk was collected from each mother using a manual suction pump and transferred to a pre-washed polyethylene bottle. The milk samples were frozen immediately and stored at −80 °C until analysis. A self-reported questionnaire was answered by each mother to provide information on her residence record, age, height, body weight, date of delivery, number of children nursed and habits of perfumed-products use. The habits of perfumed-products use were scaled as frequency of use per day, per week or per month. The perfumed products investigated in our study included facial cleaners, facial creams, hand-cleaning agents, hand creams, body-cleaning agents, body lotions, shampoos, hair dyes and hair gels. Some personal characteristics of the mothers, such as residence, age, body mass index (BMI) and number of children nursed, are shown in Table 1.

2.2. Chemicals

MX, MK, musk moskene (1,3,5-pentamethyl-4,6-dinitroindane, DM), musk ambrette (1-(1,1-dimethyl)-2-methoxy-4-methyl-3,5-dinobenzene, MA), musk tibetene (1-tert-butyl-3,4,5-trimethyl-2,6-dinitrobenzene, MT), HHCB, AHTN, ADHI (6-acetyl-1,2,3,5-hexahexylindan), ATII (5-acetyl-1,1,2,6-tetramethyl-3-isopropylindan), OTNE (1,2,3,4,5,6,7,8-octahydro-2,3,8-tetramethylphenanthralen-2-yl)[1]tetranth-1-one) and HHCB-lactone (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[1]1-2-benzopyran-1-one) were provided by Prof. Jean-Daniel Ber- set. Musk T (1,4-dioxyacycloheptadecane-5,17-dione), deuterated musk xylene (MX-d15), deuterated AHTN (AHTN-d3) and deuterated phenanthrene (phenanthrene-d10) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All solvents used in the extraction and analysis procedure were HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.3. Sample analysis

Synthetic musks in milk samples were analyzed according to the method described in our previous paper (Wang et al., 2011). Briefly, 15 g of breast milk was freeze-dried and thoroughly mixed with Celite. After 100 mL of internal standard solution (1 mg L−1) was added to the mixtures, the mixtures were packed into 22 mL-stainless steel accelerated solvent extraction (ASE) cells and extracted with approximately 30 mL of hexane/methylene chloride (DCM) (1:1, v/v). Two cycles of static extraction were performed at a constant pressure of 1500 psi and a temperature of 120 °C for 8 min after the extraction temperature was reached. The extracts were concentrated to near dryness with a rotary evaporator in a temperature controlled bath (38 °C). The residual was weighed to determine lipid amounts and redissolved with 5 mL of ethyl acetate (EA)/cyclohexane (1:1, v/v). This organic solution was then subjected to a gel permission chromatography (GPC) column (225 × 2.0 mm i.d., J2 Scientific) packed with BioBeads SX3 and eluted with EA/cyclohexane (1:1, v/v) at a flow rate of 5 mL min−1. After the first 42.5 mL of the eluate was discarded, the subsequent 50 mL fraction was collected and concentrated to near dryness. For further clean-up, the residual was reconstituted with 5 mL of cyclohexane, loaded onto a Sep-Pak Florisil cartridge (500 mg, 6 mL) that was preconditioned with 6 mL of 10% EA in pentane and eluted with 15 mL of 10% EA in pentane. The eluate was dried and dissolved in a 0.5 mL solution of isooctane that contained 20 µg L−1 of phenanthrene-d10 for gas chromatography/tandem mass spectrometry (GC–MS/MS) analysis.

2.4. Quality control

Performance of the entire analytical procedure was assessed using blank cow milk samples spiked at three different levels. Recoveries of the analytes based on the isotopic internal standard correction ranged from 82.4% to 112.0%, with relative standard
deviations less than 20.0%. The calibration standards were prepared ranging from 1 to 1000 µg L\(^{-1}\) with 200 µg L\(^{-1}\) of internal standard, resulting in acceptable lineairities for all compounds (correlation coefficients of \(r^2 > 0.99\)). The method quantification limits (MQLs) were 0.6 to 5.4 ng g\(^{-1}\) lipid.

Special precautions were taken to prevent intralaboratory contamination that could occur from perfumes, deodorants, and so forth. The chemists involved in this analysis were asked to not use any cream during the extraction and clean-up procedure. Background contamination was evaluated during sample preparation using procedural blanks that were treated along with every set of processed milk (ten samples). All glassware was rinsed in a washing machine and baked at 400 °C for 4 h after being dried at 100 °C.

2.5. Statistics analysis

According to the literature, the concentrations of environmental pollutants are distributed log normally in biological systems (WHO, 1995). However, the musk concentrations in milk samples analyzed in this study were not normally distributed after being log-transformed. Thus, all statistical analyses were performed using nonparametric tests. Concentration values below the MQL were assigned a value of half the MQL. Spearman’s rank correlation was employed to investigate possible associations between the musk concentrations and the age of the mother, the body mass index after delivery, the number of children nursed and the use of different perfumed products. The Mann–Whitney \(U\) test was used to compare differences in musk concentrations between mothers from rural and urban areas. To reduce uncertainty of the results, only musk compounds with more than 50% of the concentration values above the MQL were included in the statistical analysis. A \(p\)-value less than 0.05 was chosen to indicate statistical significance. All analyses were performed with the statistical software SPSS 11.0 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Contamination status

Of the 13 target compounds, only AHTN, HHCB, HHCB-lactone, OTNE and MK were detected in the milk samples (Table 2). AHTN and HHCB were the predominant compounds detected and were observed in 83% and 88%, respectively, of the milk samples analyzed. HHCB-lactone was found in a relatively lower proportion of the samples (60%) compared with HHCB. OTNE was identified in 34% of the human milk samples, and MK was identified in 42% of the samples. Concentration levels of AHTN, HHCB and HHCB-lactone ranged from <0.6 to 794.2, from <1.1 to 456.7 and from <0.7 to 258.3 ng g\(^{-1}\) lipid weight, respectively. The concentrations of OTNE were below the MQL in all detected milk samples whereas MK was quantifiable in only one sample at a concentration of 11.0 ng g\(^{-1}\) lipid weight. Among the five detected musk compounds, AHTN showed the highest median concentration (16.5 ng g\(^{-1}\)), followed by HHCB (11.5 ng g\(^{-1}\)) and HHCB-lactone (7.85 ng g\(^{-1}\)).

3.2. Level comparisons

A comparison with the previous literature (Table 3) indicates that the concentration ranges of AHTN and HHCB in milk samples from southwestern China in this study were of the same order of magnitude as those from Germany, Sweden, Denmark, Japan, the USA and eastern China (Rimkus and Wolf, 1996; Liebl et al., 2000; Duedahl-Olesen et al., 2005; Reiner et al., 2007; Lignell et al., 2008; UENO et al., 2009; Zhang et al., 2011). The higher levels and detection ratios of AHTN and HHCB compared with the other musks correspond to their higher production and usage rates in recent decades; these results are similar to those reported in previous studies (Liebl et al., 2000; Reiner et al., 2007; Lignell et al., 2008; Zhang et al., 2011). However, the median concentration of HHCB measured in the present study was three- to tenfold lower than the concentrations detected in milk samples from other countries and from eastern China. Interestingly, to the best of our knowledge, median concentrations of HHCB in human milk were always higher than those of AHTN in previous reports, but the reverse was observed in the breast milk analyzed in the present study. In addition to HHCB and AHTN, the other three polycyclic musks, ADBI, AHDI and ATII, were not detected in any of the milk samples in the present study whereas these compounds were found in human milk from Denmark, Sweden and Germany at low concentration levels (Liebl et al., 2000; Duedahl-Olesen et al., 2005; Lignell et al., 2008). HHCB-lactone, the oxidation product of HHCB, was detected in 59% of our milk samples. This detection ratio was much higher than that reported in the USA (Reiner et al., 2007), where HHCB-lactone was found in only two of 39 milk samples. Because of its potential toxicological impact on human health, the German Cosmetic, Toiletry, Perfumery and Detergent Association recommended in 1993 that MX not be used in household products; MX was later regulated in Europe (Liebl et al., 2000). Hence, its concentrations in human milk from European countries has decreased significantly over the past decade (Ott et al., 1999; Liebl et al., 2000; Duedahl-Olesen et al., 2005; Lignell et al., 2008; Raab et al., 2008). In milk samples collected from the USA and eastern China in recent years, this compound was detected with median concentrations of 30 and 17 ng g\(^{-1}\) lipid, respectively (Reiner et al., 2007; Zhang et al., 2011). However, in the present study, MX was found in none of the 110 analyzed samples of breast milk at a MQL of 3.5 ng g\(^{-1}\) lipid weight. Similarly, the concentrations of MK measured in this study were also much lower than those in milk samples from Denmark, the USA and eastern China (Duedahl-Olesen et al., 2005; Reiner et al., 2007; Zhang et al., 2011). These differences of musk concentrations in human milk may be caused by the different usage patterns of these compounds in perfumed products, which results from differences in both location and time. The lower musk levels observed in the present study suggest lower usage of perfumed products in south-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum concentration (ng g(^{-1}) lipid)</th>
<th>Maximum concentration (ng g(^{-1}) lipid)</th>
<th>Number of samples &lt; MDLa</th>
<th>Number of samples &gt; MDL but &lt; MQLb</th>
<th>Number of samples &gt; MQL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHTN</td>
<td>16.5</td>
<td>794.2</td>
<td>19</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>HHCB</td>
<td>11.5</td>
<td>456.7</td>
<td>13</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>HHCB-</td>
<td>7.85</td>
<td>258.3</td>
<td>44</td>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>lactone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTNE</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>73</td>
<td>37</td>
<td>–</td>
</tr>
<tr>
<td>MK</td>
<td>&lt;1.4</td>
<td>11.0</td>
<td>64</td>
<td>45</td>
<td>1</td>
</tr>
</tbody>
</table>

\(a\) MDL: method detection limit.
\(b\) MQL: method quantification limit.
and laundry detergent (Zhang et al., 2008). Higher levels of HHCB and AHTN were found in toothpaste, perfume, fabric softener, body wash, shampoo, facial/body cream and hand creams. This result may be due to the absence of HHCB and AHTN in these products. Nevertheless, this explanation could not be confirmed in the present study because information on the musk content of these types of products used by the milk donors was not available. A survey of synthetic musk compounds in household commodities performed in Shanghai, China makes it impossible to verify this in our work.

3.3. Relationships of musk concentrations and perfumed products use

The use of body-cleaning agents, shampoos, hair dyes and hair gels showed a significant positive effect on milk concentrations of both HHCB and AHTN (Table 4). In addition, a positive correlation was found between HHCB concentration and the use of body lotion and AHTN milk concentration was observed. However, milk concentrations of HHCB and AHTN did not correlate with any of the data on the use of facial cleaners, facial creams and hand creams. This result may be due to the absence of HHCB and AHTN in these products. Nevertheless, this explanation could not be confirmed in the present study because information on the musk content of these types of products used by the milk donors was not available. A survey of synthetic musk compounds in household commodities performed in Shanghai, China provided average concentrations of HHCB and AHTN in toothpaste, perfume, fabric softener, body wash, shampoo, facial/body cream and laundry detergent (Zhang et al., 2008). Higher levels of HHCB and AHTN were measured in shampoo and body wash compared with other personal care products, which supports our finding of a significant positive correlation between concentrations of HHCB and AHTN in human milk and the use of body-cleaning agents and shampoos. In addition, the survey also indicated that the mean concentrations of AHTN in most household commodities were considerably lower than those of HHCB. This finding is contrary to the result observed in our study that the median concentration of AHTN in milk samples was higher than the median concentration of HHCB. There are several possible interpretations of these inconsistent results. First, the musk composition and concentrations in perfumed products of the same type may differ substantially among different regions. Second, some additional sources of synthetic musk exposure may exist other than dermal absorption of HHCB and AHTN. Third, HHCB may exhibit lower absorptivity through human skin than AHTN. The existence of a positive association found here between the use of certain perfumed products and milk concentrations of AHTN and HHCB has also been reported by Lignell et al. (2008). In their study, milk concentrations of HHCB and AHTN were shown to be

Table 3
The recently reported concentrations of synthetic musk compounds (ng g⁻¹ lipid) in breast milk from some countries and regions.

<table>
<thead>
<tr>
<th>Location</th>
<th>Survey time</th>
<th>n</th>
<th>Statistic</th>
<th>HHCB</th>
<th>AHTN</th>
<th>ADBI</th>
<th>ATII</th>
<th>AHDI</th>
<th>MX</th>
<th>MK</th>
<th>HHCB-lactone</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>China (three eastern cities)</td>
<td>2006–2007</td>
<td>110</td>
<td>Median</td>
<td>63</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
<td>4</td>
<td>–</td>
<td>Zhang et al. (2011)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Detection</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Japan (Saga prefecture)</td>
<td>2006–2008</td>
<td>20</td>
<td>Median</td>
<td>50</td>
<td>&lt;5–40</td>
<td>&lt;5–190</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Ueno et al. (2009)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>&lt;50–440</td>
<td>&lt;50–90</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Detection</td>
<td>–</td>
<td>60%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Germany (Middle Hesse)</td>
<td>1995</td>
<td>55</td>
<td>Mean</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>41</td>
<td>10</td>
<td>–</td>
<td>Ott et al. (1999)</td>
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<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100%</td>
<td>87%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Germany (Schleswig-holstein)</td>
<td>1996</td>
<td>5</td>
<td>Mean</td>
<td>22</td>
<td>6</td>
<td>n.d.</td>
<td>n.d.</td>
<td>–</td>
<td>10</td>
<td>5</td>
<td>–</td>
<td>Rimkus and Wolf (1996)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Detection</td>
<td>–</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Germany (Munich)</td>
<td>1997–1998</td>
<td>40</td>
<td>Median</td>
<td>64</td>
<td>22</td>
<td>1.6</td>
<td>1.5</td>
<td>3.2</td>
<td>6.1</td>
<td>4.6</td>
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<td>Liebl et al. (2000)</td>
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<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>21–1316</td>
<td>16–148</td>
<td>1.0–14.1</td>
<td>1.1–51.3</td>
<td>1.0–19.8</td>
<td>1.3–47.9</td>
<td>2.1–82.9</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Detection</td>
<td>88%</td>
<td>33%</td>
<td>38%</td>
<td>25%</td>
<td>25%</td>
<td>95%</td>
<td>45%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Germany (Bavaria)</td>
<td>2005</td>
<td>39</td>
<td>Median</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8</td>
<td>n.d.</td>
<td>–</td>
<td>Raab et al. (2008)</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;LOQ-240</td>
<td>&lt;LOQ-6</td>
<td>–</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Detection</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>21%</td>
<td>8%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Sweden (Uppsala County)</td>
<td>2000–2003</td>
<td>101</td>
<td>Median</td>
<td>63.9</td>
<td>10.4</td>
<td>&lt;2.0</td>
<td>&lt;3.0</td>
<td>&lt;3.0</td>
<td>9.5</td>
<td>5.0</td>
<td>–</td>
<td>Lignell et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>2.9–268.0</td>
<td>&lt;3.0–53.0</td>
<td>&lt;2.0–11.0</td>
<td>&lt;3.0–12.6</td>
<td>&lt;3.0–6.5</td>
<td>&lt;6.0–8.39</td>
<td>&lt;5.0–24.4</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N &lt; LOQ</td>
<td>0</td>
<td>26</td>
<td>75</td>
<td>77</td>
<td>70</td>
<td>31</td>
<td>83</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Denmark (Hvidovre)</td>
<td>1999</td>
<td>10</td>
<td>Median</td>
<td>147</td>
<td>17.5</td>
<td>5.98</td>
<td>&lt;0.22</td>
<td>&lt;1.0</td>
<td>3.1</td>
<td>&lt;5.0</td>
<td>–</td>
<td>Duedahl-Olesen et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>38.0–422</td>
<td>5.58–37.9</td>
<td>&lt;0.39–11.2</td>
<td>&lt;0.22–2.58</td>
<td>&lt;1.0–9.94</td>
<td>&lt;3.1–46.4</td>
<td>&lt;5.0–26.9</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>USA (Massachusetts)</td>
<td>2004</td>
<td>38</td>
<td>Mean</td>
<td>220</td>
<td>46.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>30.0</td>
<td>74.5</td>
<td>58.3</td>
<td>Reiner et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>&lt;5–917</td>
<td>&lt;5–144</td>
<td>&lt;2–150</td>
<td>&lt;2–238</td>
<td>&lt;10–88</td>
<td>85%</td>
<td>5%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Detection</td>
<td>97%</td>
<td>56%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>36%</td>
<td>85%</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* n.d.: not detected.

Table 4
Correlation coefficients between musk concentrations and the use of perfumed products, mother’s age, BMI after delivery and number of children nursed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HHCB</th>
<th>AHTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of perfumed products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facial cleaner</td>
<td>0.128</td>
<td>0.167</td>
</tr>
<tr>
<td>Facial cream</td>
<td>−0.107</td>
<td>−0.102</td>
</tr>
<tr>
<td>Hand-cleaning agent</td>
<td>0.234</td>
<td>0.166</td>
</tr>
<tr>
<td>Hand cream</td>
<td>−0.034</td>
<td>0.059</td>
</tr>
<tr>
<td>Body-cleaning agent</td>
<td>0.478**</td>
<td>0.417**</td>
</tr>
</tbody>
</table>
| Body lotion                        | 0.157| 0.218*
| Shampoo                            | 0.336**| 0.199**|
| Hair dyes                          | 0.778**| 0.606**|
| Hair gel                           | 0.610**| 0.648**|
| Age                                | −0.292**| −0.279**|
| BMI after delivery                 | 0.176| 0.101|
| Number of children nursed          | 0.031| −0.171|

*p < 0.05. ** p < 0.01.

western China compared with eastern China, the USA and European countries. However, a lack of information on the habits of perfumed-products use among different countries and regions makes it impossible to verify this in our work.
positively correlated with the use of perfumes and perfumed laundry detergents, respectively, among mothers in Sweden. In addition to human breast milk, similar relationships were also observed for other human tissues such as blood. For instance, Hutter et al. (2005) reported statistically significantly associations between HHCB levels in plasma samples and the use of perfume and body lotion among young adults in Austria. Furthermore, Hutter and his colleagues investigated the influence of the use of cosmetics on synthetic musk concentrations in the blood of healthy young adults and found that the use of lotion and perfume had a significant positive effect on the levels of polycyclic musks in blood (Hutter et al., 2009). Together, these studies, including the present study, suggest that the dermal application of perfumed products may be the most relevant source of synthetic musk exposure for adults. Nevertheless, the number of investigated persons in each of these studies was small, not more than two hundred, and the musk content and usage amount of perfumed products were not quantified by the authors. Moreover, some important confounding factors, e.g., diet and air exposure to musks, were not carefully considered in these studies. All these limitations make our assumption relatively uncertain.

HHCB concentrations in milk samples analyzed in our study were significantly correlated with AHTN concentrations ($r = 0.46$, $p < 0.001$) (Fig. 1). This result was consistent with those previously reported by Zhang et al. (2011) and Lignell et al. (2008). A similar correlation between HHCB and AHTN was also found in human adipose tissues by Kannan et al. (2005). These results suggest that HHCB and AHTN had identical sources of exposure in the investigated subjects. However, the same correlation between HHCB and AHTN was not observed in human breast milk from American women (Reiner et al., 2007), which implies that the exposure routes of these two synthetic musks may be multiple for some populations. In addition, HHCB-lactone showed a strong association with HHCB concentrations in human milk in the present study ($r = 0.61$, $p < 0.001$) (Fig. 1). This association suggests that HHCB was mainly transformed into HHCB-lactone in the human body.

3.4 Relationships between musk concentrations and other personal characteristics

The influence of the mother’s age on the concentrations of synthetic musks was considered in this paper. Older mothers were found to have significantly lower milk concentrations of both HHCB and AHTN (Table 4). This result was similar to a previous finding reported by Hutter et al., in which a significantly inverse relationship was found between age and polycyclic concentrations in human plasma from Australia (Hutter et al., 2009). This age-related decrease of synthetic musks in blood has been explained by speculation that the use of cosmetics may be reduced in older mothers and that the bioaccumulation of musks in human tissues may vary with age. In contrast to our result, age showed no significant correlation with HHCB and AHTN concentrations in milk samples from Sweden, the USA and eastern China (Reiner et al., 2007; Lignell et al., 2008; Zhang et al., 2011). This inconsistency may be caused by the different habits of cosmetics use among women from different regions. Other factors, including the mother’s BMI after delivery and the number of children nursed, were also examined. No correlation was found between these two factors and the concentrations of HHCB or AHTN in breast milk (Table 4).

In urban areas, the higher usage of cosmetics and perfumes can lead to higher levels of exposure to musk compounds and, consequently, to higher musk concentrations in human milk. This assumption was verified by comparing musk concentrations in milk samples between women who lived in urban and rural areas. However, no statistical differences were found in the residual levels of AHTN, HHCB or HHCB-lactone in breast milk between rural and urban areas in our study ($p > 0.05$). This result implies that the difference in the usage of perfumed products between rural and urban areas in the investigated city has been greatly narrowed with the rapid development of urbanization in rural areas of China. To confirm this explanation, we further compared differences in the frequencies of perfumed products use between rural and urban areas, which were recorded in the mothers’ self-reported questionnaires. As we expected, no significant difference was observed in the usage of each perfumed product between rural and urban areas in the present study ($p > 0.05$).

3.5 Health risk assessment for infants

The presence of synthetic musks in human breast milk has been of great concern because these compounds are transferred to infants by breast feeding. To assess the exposure level of infants to synthetic musks, the daily intake amounts of musk compounds via mother’s milk were assessed based on an assumption that the daily milk consumption of an infant is 700 mL (lipid content of 3.7%). The total daily intake values (median (range)) were 295 (14–11829) ng for HHCB, 427 (8–20570) ng for AHTN and 203 (9–6690) ng for HHCB-lactone. For an infant with a body weight of 5 kg, the maximum intake values were 2.37, 4.11 and 1.34 µg kg$^{-1}$ body weight for HHCB, AHTN and HHCB-lactone, respectively. The estimated maximum daily intake amounts of...
HHCB and AHTN through lactation in the present study are much higher than those reported for the USA, Sweden and Japan (Reiner et al., 2007; Lignell et al., 2008; Ueno et al., 2009). To date, the toxicological effects of synthetic musks on human health have not been completely elucidated. No tolerable daily intake values of musk compounds have been officially proposed by any governmental or scientific organization. Slanina reviewed the toxicological literature on musk compounds and suggested provisional tolerable daily intake amounts (PTDIs) of 500 μg kg⁻¹ body weight for HHCB and 50 μg kg⁻¹ body weight for AHTN (Slanina, 2004). These PTDIs were one to two orders of magnitude higher than our calculated maximum daily intake amounts, and hence, the health risks to infants from musk exposure are low. However, the PTDIs proposed by Slanina were based on toxicological studies for adults, which makes the comparison between infant intake amounts and current PTDIs questionable because infants are usually more susceptible to chemical contaminants. Additional toxicological studies regarding infant exposure to synthetic musks are needed to establish special limits for this population. Furthermore, there are still routes of musk exposure for infants other than breast milk, such as the application of a perfumed napkin, soaps and lotions. The contribution of these exposure routes should also be considered in future investigations to enable a reliable risk assessment of the synthetic musk compounds.

4. Conclusions

In the present study, we investigated the concentrations of 13 musk compounds in human milk collected from Chengdu in Sichuan Province in southwestern China, analyzed the relationships between milk musk concentrations and the use of perfumed products as well as personal characteristics and evaluated possible health risks for infants. Five musks—AHTN, HHCB, HHCB-lactone, OTNE and MK—were detected in analyzed samples, with AHTN and HHCB being the predominant compounds. The median concentrations of all target analytes except AHTN and OTNE were lower than those reported in previous studies. The personal habits of using specific perfumed products showed a significant positive association with AHTN and HHCB concentrations whereas the mother’s age had an inverse relationship to concentrations of these two compounds. No significant correlation was observed between milk concentrations and the variables of BMI after delivery, the number of children nursed and place of residence (urban or rural) in our study. The musk exposure of infants by human breast milk feeding was low according to current toxicological data. However, further studies should be conducted to evaluate the long-term toxicity of currently used synthetic musks on infants. Continuous surveillance on synthetic musks in human milk from general populations is also needed to correctly assess the health risks of these compounds in the future.

Acknowledgments

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References