

Synthetic Musk Fragrances in Human Breast Milk and Adipose Tissue from Japan

D. UENO¹, M. MORIBE¹, K. INOUE¹, T. SOMEYA¹, N. RYUDA¹, M. ICHIBA²,
T. MIYAJIMA³, T. KUNISUE⁴, H. IN⁵, K. MARUO⁵ and H. NAKATA⁶

¹*Faculty of Agriculture, Saga University, Honjo-chou, Saga 840-0528, Japan*

²*Faculty of Medicine, Saga University, Japan*

³*Faculty of Science and Engineering, Saga University, Japan*

⁴*Wadsworth Center, New York State Department of Health, U.S.A.*

⁵*Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan*

⁶*Graduate School of Science and Technology, Kumamoto University, Japan*

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Abstract—Concentrations of synthetic polycyclic musks (SPMs: HHCB and AHTN) were determined in the samples of human breast milk and adipose tissues collected from Japan. HHCB and AHTN were detected in half of the human breast milk ($n = 20$) and adipose tissue ($n = 3$) samples analyzed. Concentrations of HHCB and AHTN in milk samples ranged from <50 to 440 ng g^{-1} and from <50 to 190 ng g^{-1} on a lipid weight basis, respectively. Concentrations of HHCB and AHTN in adipose tissues ranged from <10 to 33 ng g^{-1} and from <10 to 13 ng g^{-1} on a lipid weight basis, respectively. This is the first report to determine SPMs in human tissue samples from Japan. Concentrations of HHCB and AHTN in human milk samples in this study were comparable to those from Europe and USA. The daily intakes of HHCB and AHTN by infants through breast-feeding were estimated based on the concentrations of SPMs in it. Daily intake of HHCB ranged from 240 to $930 \text{ ng kg}^{-1} \text{ day}^{-1}$ and those were more than 3 orders of magnitude lower than adverse effect levels obtained by animal studies.

Keywords: musk, HHCB, AHTN, human milk, adipose tissue

INTRODUCTION

Large quantities of synthetic musk fragrances have been manufactured and used in a variety of personal care products, such as perfumes, skin cream, deodorants, soaps, and detergents. Among the various classes of synthetic fragrances, synthetic polycyclic musks (SPMs) are dominant in terms of production volume which comprised 60% of the total amount of synthetic musk compounds produced in the world (7000 t) in 1987 (Sommer, 2004). In particular, high production and usage of HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl cyclopenta[g]2-benzopyrane) and AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl tetrahydro naphthalene) were reported in Europe and the United States in the 1990s among

SPMs. It was reported that industrial usage of those SPMs (HHCB and AHTN) were 3300 t in 2000 (HERA, 2004).

Since potential toxicity of SPMs have been reported in *in vivo* and *in vitro* study (Api and Ford, 1999; Christian *et al.*, 1999; Schreurs *et al.*, 2002; Api *et al.*, 2004), human exposure to those compounds has been of great concern. Humans may be exposed to SPMs through the use of personal care products and cleaning products. For adults, dermal sorption from personal care products can be a major route of exposure to synthetic musks (Kannan *et al.*, 2005). SPMs are expected to be accumulated in lipid-rich tissues of human because those compounds have lipophilic nature (Kannan *et al.*, 2005). Hence, human breast milk and human adipose tissue would be the valuable samples to elucidate the exposure levels of SPMs in human which contain relatively higher lipid among collectable tissues. In particular, contaminations of breast milk by toxic chemicals are of concern because of its implications for childhood exposures.

Although HHCB and AHTN have been found in human adipose tissue and breast milk samples from European countries and the United States (Rimkus and Wolf, 1996; Muller *et al.*, 1996; Duedahl-Olesen *et al.*, 2005; Reiner and Kannan, 2006; Reiner *et al.*, 2007), no information has been available for human exposure of SPMs in Japan. This study attempts to elucidate current contamination status of human in Japan, and to evaluate daily intake of these chemicals by infant through breast milk by analyzing those compounds in human breast milk and adipose tissue samples collected from Japan.

MATERIALS AND METHODS

Sample collection

Human breast milk samples were collected from primiparae ($n = 4$) and multiparae ($n = 1$) in Saga prefecture, Japan during 2006–2008. All the samples were collected during a month after childbirth, and five samples were collected monthly from each donor. Ages of primiparae and multiparae were 27–38 and 28, respectively. Samples were collected using polyethylene bottles and stored at -20°C until chemical analysis. Adipose tissue (subcutaneous fat) samples were collected from 3 donors (male; $n = 2$, female; $n = 1$) at Kumamoto University hospital during surgical operation in 2007. Approvals for the analysis of human tissues were obtained from the institutional review boards of ethics committee in Kumamoto University and Saga University, and informed consent were taken from all the donors.

Chemical analysis

Synthetic polycyclic musks (SPMs: HHCB and AHTN) and PCBs were analyzed following method described previously (Nakata *et al.*, 2007) with slight modifications. Briefly, sample (10 g of milk; 1 g of adipose tissue) was grounded with sodium sulfate and extracted with mixed solvents of dichloromethane and hexane (8:1) for 5 h using a Soxhlet apparatus. An aliquot of the extract was used

for determination of crude fat by measuring total non-volatile extract. A internal standards, d_{10} -phenanthrene and $^{13}\text{C}_{12}$ -PCBs mix (from mono- to deca-CB), were spiked into the extract, and it was subjected to gel permeation chromatography (GPC) for lipid removal. The GPC fraction containing target compounds was concentrated and passed through an activated silica gel packed glass column for further cleanup and separation. The first fraction eluted with hexane was collected for PCBs analysis, and the second fraction eluted with 90% dichloromethane in hexane was collected for SPMs analysis. These fractions were micro-concentrated and injected into a gas chromatograph coupled with a mass spectrometer (GC-MS) using selected ion monitoring (SIM) mode. The oven temperature was programmed from 80 to 160°C at a rate of 10°C/min and held for 10 min, and then the temperature was increased to 300°C at a rate of 3°C/min, with a final hold time of 20 min. The temperatures of injector and detector were set at 270 and 300°C, respectively. BPX-5 (SGE Co. Ltd. Australia: 30 m 0.25 mm i.d., 0.25 μm film thickness) were used for analysis.

Quality control

Recovery tests of HHCB and AHTN for whole analytical procedure were conducted using vegetable oil mixed with standard mixture of SPMs. The average recoveries of HHCB and AHTN were 99% and 109%, respectively. The recovery percentages of d_{10} -phenanthrene in samples ranged from 82 and 121%. A procedural blank was analyzed with every set of five samples. Method detection limits (DLs) were defined as three times of the standard deviation for procedural blank values ($n = 5$). DLs of HHCB and AHTN were 1 ng g⁻¹ and 1 ng g⁻¹ for milk sample and 0.1 ng g⁻¹ and 0.1 ng g⁻¹ for adipose tissue sample (wet weight basis). Values lower than the DL were treated as zero to calculate mean values.

RESULTS AND DISCUSSIONS

Contamination status

Synthetic polycyclic musks (SPMs) were detected in breast milk and adipose tissue samples collected from Japan (Fig. 1). HHCB and AHTN were detected from 60% and 30% of milk samples ($n = 20$) and 60% and 30% of adipose tissue samples ($n = 3$) analyzed in this study, respectively. Concentration ranges of HHCB and AHTN in milk samples were from <50 to 440 ng g⁻¹ and from <50 to 190 ng g⁻¹ (lipid weight basis), respectively. Concentration ranges of HHCB and AHTN in adipose tissue samples were from <11 to 33 ng g⁻¹ and from <9.0 to 13 ng g⁻¹ (lipid weight basis), respectively. Concentration levels of HHCB detected in breast milk were comparable to those of PCBs in same samples (Fig. 1). PCBs were detected from 100% of breast milk in Japan, and those predominant compounds among the group of persistent organic compounds (POPs) reported (Kunisue *et al.*, 2006). This result suggests that HHCB concentrations seem to be one of the major synthetic chemicals in breast milk in Japan.

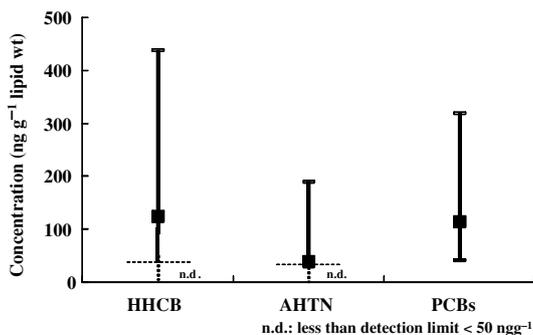


Fig. 1. Concentrations of SPMs and PCBs in breast milk collected from Japan.

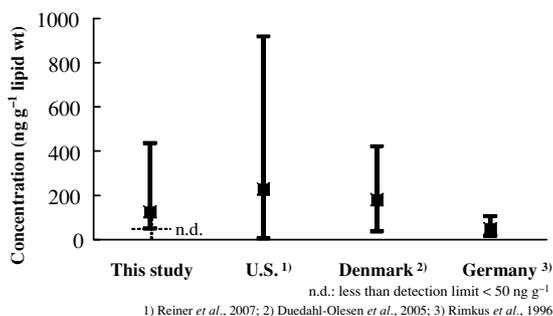


Fig. 2. International comparison of HHCB concentrations in breast milk.

International comparison

In order to evaluate the contamination levels of SPMs in breast milks from Japan, concentrations of HHCB in this study were compared with those levels previously reported (Fig. 2). Concentration ranges of HHCBs in milk samples from United States (Reiner *et al.*, 2007), Denmark (Duedahl-Olesen *et al.*, 2005) and Germany (Rimkus and Wolf, 1996) were comparable to those from Japan in this study. HHCB concentrations in breast milk in Japan seem to be similar levels to other countries.

Daily intake by infant

The detection of SPMs in breast milk suggests that these compounds can be transferred to breast-fed infants. Daily intake of SPMs by infant through breast milk were estimated using intake rates of breast milk by an infant (700 g milk day⁻¹ by 5 kg body weight infant) (Van Oostdam *et al.*, 1999). It was estimated that an infant would ingest 360 (from 240 to 930) ng HHCB and 280 (from 150

to 400) ng AHTN day⁻¹. Using estimated intake of SPMs, toxic effects for infant were evaluated. Estimated intake of HHCb by infant through breast milk were more than 3 order magnitude lower than adverse effect levels by *in vivo* and *in vitro* studies reported (Api and Ford, 1999; Christian *et al.*, 1999; Schreurs *et al.*, 2002; Api *et al.*, 2004). Since toxicological information has been limited, additional toxicological study, especially for fetus and infant, is needed for risk assessment for them. Also, in order to reduce the intake of these chemicals by fetus and infant, further investigations to elucidate intake routes are required.

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D. Ueno (e-mail: uenod@cc.saga-u.ac.jp), M. Moribe, K. Inoue, T. Someya, N. Ryuda, M. Ichiba, T. Miyajima, T. Kunisue, H. In, K. Maruo and H. Nakata