Assessment of Synthetic Musk Fragrances in Seven Wastewater Treatment Plants of Beijing, China

Zhengjun Hu · Yali Shi · Shengxiao Zhang · Hongyun Niu · Yaqi Cai

Abstract Seven typical synthetic musks were measured in influents, effluents and sewage sludge from seven wastewater treatment plants of Beijing in three seasons. Galaxolide and tonalide were the dominant musks, and the levels were medium compared with those from other regions. Musk concentrations and galaxolide/tonalide ratios were low in warm seasons relative to cold seasons in both water and sludge samples. The removal efficiencies of galaxolide and tonalide ranged in <14.3%–98.0% and <18.5%–98.7%, respectively. The discharge of galaxolide and tonalide through effluents into environment were 1.8–685.6 g/d (mean: 70.1 g/d) and 1.6–195.3 g/d (mean: 22.9 g/d), respectively.

Keywords Synthetic musk fragrances · Wastewater treatment plants (WWTPs) · Beijing

Synthetic musk fragrances (SMs) have been widely used as fragrances in a variety of personal care and household products (Carballa 2004). Polycyclic musks gradually become the main fragrances in the world (Peck and Hornbuckle 2006), because many nitro musks have been identified toxic. In recent years, toxic effects of polycyclic musks are also of great concern. The potential estrogenic and anti-estrogenic effects of the polycyclic musks have been proved by various in vitro *or vivo* experiments (Toivanen et al. 2008). The continuous growth in human population has created a corresponding increase in the demand for the personal care products including synthetic musks. It is reported that 77% of the musks would drain into the sewer system after used, and then reach wastewater treatment plants (WWTPs) (Reiner et al. 2007). The conventional sewage treatment plants can not be considered as effective barriers to SMs due to their low concentrations and specific metabolic properties of these compounds. During the treatment process, the SMs would be transformed or degraded, but some of them might still remain in aqueous phase or into sludge. They would enter into ecosystem again with the reuse of effluents and sludge. For example, disposal of sludge in landfills and/or application as biosolids to agriculture can contribute to contamination of the terrestrial environment. Effluents were often discharged into river, used to irrigate crops, or used in fish culture. Therefore, there is considerable significance to investigate the levels of musk concentrations in WWTPs which may be beneficial to assess the possible risk of SMs to the environment.

In China, there are only several studies about the musks samples which were from the WWTPs in Guangdong province and Shanghai (Zeng et al. 2005; Zeng et al. 2007; Zhang et al. 2008), located in the southern and southeastern of China. There is little information about temporal trends of SMs in WWTPs in the northern cities of China, such as Beijing where people might have the different living style from those in Guangdong and Shanghai. Beijing, located in northern China, is the second largest city and capital of China, densely populated with up to 20 million inhabitants (including native and nonnative). In the present study, samples from seven main WWTPs of Beijing were collected in three seasons and seven typical SMs were

Z. Hu · Y. Shi · H. Niu · Y. Cai (✉)
State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China
e-mail: caiyaqi@rcees.ac.cn

S. Zhang
School of Chemistry and Materials Science, Ludong University, Yantai 264025, China

measured. The object of the present study is to provide the fundamental data on the levels of SMs, understand the removal efficiencies of SMs in WWTPs of Beijing and investigate the mass flows of musks discharged into environment through effluents. Moreover, seasonal variations and relationships between musk concentrations and some factors, such as the size of the treatment plant and the size of population, were also examined.

Materials and Methods

The standard solutions containing Tonalide (AHTN; 7-acetyl-1,1,3,4,4,6- hexamethyl-1,2,3,4-tetrahydronaphthalene), Galaxolide (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6, 7, 8, 8-hexamethylcyclopenta (g) -2-benzopyran), Celestolide (ADBI; 4-acetyl-1,1-dimethyl-6-tert- butylindan), Phantolide (AHMI; 6-acetyl-1,1,2,3,3,5-hexamethylindan), Traseolide (ATII; 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindan), musk ketone (MK) and musk xylene (MX) were obtained from Dr. Ehrestorfer (Augsburg, Germany). Surrogate standard d_3-AHTN and internal standard (HCB) $^{13}C_6$ were purchased from Dr. Ehrestorfer (Augsburg, Germany). Dichloromethane (DCM), n-hexane, acetone were of high-performance liquid chromatography (HPLC) grade and purchased from J. T. Barker (Phillipsburg, NJ, USA). C-18 discs were from Supeclo (Ø 47 mm, Bellefonte, USA). Anhydrous sodium sulfate, neutral Al_2O_3 and silica gel (100–200 mesh) were baked at 450°C for 4 h prior to use.

A total of 45 samples, including 17 influent samples, 16 effluent samples and 12 sludge samples were collected from seven municipal wastewater treatment plants in Beijing. Sampling was performed once in each WWTP on January 15, May 28 and September 24 in 2008, respectively. In this study, the concentrations presented are for 24 h composite samples at every sampling time. The water samples were filtered through nylon film (0.45 μm). The sewage sludge was freeze-dried, ground, and homogenized by sieving through a stainless-steel, 75-mesh (pore size, 0.5 mm) sieve. The filtered sewage and freeze-dried sludge were stored at −20°C until analysis.

The extraction method of water sample is as follows. Briefly, after the addition of 10 ng d_3-AHTN as surrogate standard, water samples (500 ml) were extracted and concentrated by solid phase extraction using a C-18 disc. The disc was conditioned with 25 ml of methanol and 20 ml of ultrapure water subsequently before use, followed by loading steps, and dried under gentle N_2 flow. The enriched compounds were eluted with 25 ml of n-hexane and 15 ml of n-hexane/DCM (1:1, V/V) consecutively. The two fractions were combined and concentrated to 1 ml. (HCB) $^{13}C_6$ was added as internal standard prior to GC–MS analysis. The sewage sludge was extracted using

an accelerated solvent extraction system (ASE) (Dionex 350, Dionex Corporation). 34 ml of ASE extraction cell was first filled with approximately 4 g of activated silica (100–200 mesh) followed by approximately 28 g of anhydrous Na_2SO_4/0.1 g of sludge mixture. The surrogate standard (20 μl of a 100 μg/L solution of deuterated d_3-AHTN in acetone) was added at the top of extraction cell. The ASE cell was placed on the ASE unit and extracted with C_6H_{14}:CH_2Cl_2 (1:1 V/V) at 60°C and 1500 psi in static mode for 15 min. The process was repeated twice. The extract was cleaned up with activated copper (activated with 10% hydrochloric acid, analytical grade), then passed through a glass chromatography column (1 cm i.d. × 15 cm) which was equiped with Teflon stopcock and packed with glass wool and 6 g anhydrous sodium sulfate to removal copper granules and water. The final extract was concentrated to 0.5 ml and solvent was exchanged into n-hexane. The n-hexane phase was passed through a glass chromatography column (1 cm i.d. × 15 cm) which was fitted with Teflon stopcock and packed consecutively with glass wool, 2 g activated neutral Al_2O_3, 2 g activated silica gel and 0.5 g anhydrous sodium sulfate for cleaning up. Prior to use, the column was conditioned with 5 ml of n-hexane. Elution was performed with n-hexane (5 ml), hexane/dichloromethane (2:1) (20 ml), hexane/dichloromethane (1:2) (30 ml) and hexane/dichloromethane (1:3) (20 ml) subsequently. The combined eluate was collected and concentrated to 1 ml. Prior to GC–MS analysis, the internal standard HCB $^{13}C_6$ was added. The GC–MS analysis was carried out by an Agilent 7890GC/5975MSD system with electron ionization and a quadrupole mass detector using selected ion monitoring (SIM) mode (Agilent, USA). The ions monitored for ADBI were m/z 229, 224 and 173; for AHMI they were m/z 229, 224, and 187; for HHCB they were m/z 243, 258 and 213; for AHTN they were m/z 258, 243 and 148; for ATII they were m/z 215, 258, and 173; for MX they were m/z 282, 297, and 207; for MK they were 294 and 279 and for d_3-AHTN they were m/z 246 and 261. The first ion in each group was used for quantification. Besides, the ion for HCB $^{13}C_6$ was 290. A 30 m 5% phenyl methyl siloxane capillary column (HP-5MS; 0.25 mm ID, 0.25 μm film thickness) was used for separation of target analyte. The column temperature was programmed as follows: run at 90°C, held for 2 min, with a rate of 10°C/min to 170°C, 1°C/min to 180°C, 30°C/min to 270°C and held for 7 min. One microlitre was injected splitless at 250°C. The transfer line temperature was 280°C, and source temperature was 230°C.

d_3-AHTN and HCB $^{13}C_6$ were used as surrogate standard and internal standard, respectively. Recoveries of the d_3-AHTN in sewage and sludge were in the range of 89.7%–104.9%. HCB $^{13}C_6$ was used to quantify musk concentrations in sample extracts. The reported concentrations

of samples had been corrected using recovery of d_3-AHTN, which would be spiked into samples before pretreatment. For calibration purposes, six different concentrations of synthetic musk compounds were analyzed by GC–MS. A standard calibration was performed. Correlation coefficient R^2 are >0.99. Matrix spike recovery studies were carried out. In sludge, recoveries of seven spiked synthetic musk fragrances (2 ng each of standard) ranged from 83.6% to 105.1%, and RSD ranged from 3.2% to 9.8%. The limits of detection (LODs) were based on a signal-to-noise ratio of 5. LODs were 3.3 ng/g (sludge samples) and 1.2 ng/l (sewage samples), respectively. To avoid the possible contamination, all glassware was soaked in $K_2Cr_2O_7-H_2SO_4$ lotion for at least 12 h, and all equipment was rinsed with acetone in advance. A procedural blank was processed with each batch of 6 samples. Furthermore, a field blank was included during sampling in each treatment plant every time. The concentrations of all synthetic musk fragrance were below detection limits in all of the blank samples.

Differences in musk concentrations in sewage and sludge samples between different groups (such as sampling time and plants) were calculated using the nonparametric tests (U Kruskal–Wallis test). A p-value below 0.05 was considered significant. All the statistical analyses were performed by the software of SPSS 13.0.

Results and Discussion

Two polycyclic musks, HHCB and AHTN, were observed in all the water samples. The concentrations of HHCB and AHTN in influents were in the ranges of 30.9–3,038.97 ng/l, and 28.61–1,486.1 ng/l, respectively. This result was similar with those reported in Guangdong province of China (Zeng et al. 2007)(HHCB: 1,010–3,080 ng/l and AHTN: 120–160 ng/l) and in Austria (HHCB: 830–4,443 ng/l; AHTN 210–1,106 ng/l) (Clara et al. 2005) and slightly higher than those in Netherlands (Artola-Garicano et al. 2003)(HHCB 790–1,630 ng/l; 210–480 ng/l AHTN). Besides, Carballa et al. (2004) have reported similar values (HHCB: 2,100–3,400 ng/l; AHTN: 900–1,700 ng/l) in raw influents from Spanish WWTPs which included particle and filtered sewage. As a result, the HHCB and AHTN concentrations of raw influents in Beijing would be higher than those in Spain. Effluent dissolved concentrations (HHCB; 30.4–685.62 ng/l and AHTN 14.26–195.3 ng/l) in the present study also exhibited comparable values with the raw concentrations in Spain (Carballa et al. 2004) and dissolved concentrations in Austria and Netherlands (Clara et al. 2005; Artola-Garicano et al. 2003). Therefore, our total concentrations would be higher than those from Spain. Nevertheless, the results in the present study were slightly low relative to those in WWTPs in Guangdong (HHCB;

950–2,250 ng/l and AHTN 100–140 ng/l) (Zeng et al. 2007). Unlike HHCB and AHTN, other SMs were not frequently detected. Especially, AHMI, ADBI and ATII were not detected in all sewage specimens in the study. MK was found in five of influent and five of effluent samples, and MX could be detected in six influents. Surprisingly, the existence of MK was not regular. MK could be detected in certain effluent samples, while it was absent in the corresponding influent samples. MX concentrations in influents were in the range of < LOQ-22.95 ng/l. MK ranged in 52.25–165.8 and 22.77–91.6 ng/l in influents and effluents, respectively. Furthermore, we investigated the difference of musk influent concentrations between plants, and no apparent statistical significance was discerned ($p > 0.05$). In the present study, five of the seven SMs (except AHMI and ADBI) have been detected in sludge samples. HHCB, AHTN and MK were found in all the samples. The level of the three SMs followed the order of HHCB > AHTN > MK, and the corresponding concentrations were in the ranges of 0.26–12.59 mg/kg (dw), 0.01–2.56 mg/kg (dw) and 0.13–0.53 mg/kg (dw), respectively. The concentrations of HHCB and ATHN in this study were similar with those in Guangdong of China (HHCB; 5.4–21 mg/kg and AHTN; 0.72–6.2 mg/kg) (Zeng et al. 2005) and slightly higher than those in Nertherlands (HHCB: 0.03–0.234; AHTN: 0.012–0.234) (Artola-Garicano et al. 2003). The feature agreed well with those observed in influents and effluents. Furthermore, the results in the present study were similar with those in Kentucky and Georgia (HHCB; 0.02–36 mg/kg and AHTN; 0.02–7.2 mg/kg) (Horii et al. 2007) and lower than those in Switzerland (HHCB; 7.4–36 and AHTN; 2.5–11 mg/kg) (Kupper et al. 2004) and Korean (HHCB; 0.52–82.0 mg/kg and AHTN; 0.12–28.8 mg/kg) (Guo et al. 2010). MX and ATII were detected at low concentrations and frequencies. ATII could be detected in ten of twelve samples, ranging from 0.015 to 0.3 mg/kg. MX could be found only in five of twelve samples and all of them were under LOQ. On Sep 24, the highest of HHCB and AHTN sludge concentrations was found in samples of BJ_6 followed by BJ_2, while the lowest was from BJ_1. On Jan 15, the highest concentrations were from BJ_3 and BJ_7, while the lowest were from BJ_6. Furthermore, AHMI and ADBI were not nearly detected in the sludge samples. The character was similar with those found in Guangdong province of China.

To understand the relationships between HHCB and AHTN, correlation tests were conducted. The results indicated that significant positive correlation coefficients existed not only in influent water samples but also in sewage sludge samples in all the sampling seasons ($p < 0.05$), suggesting the co-exposure of HHCB and AHTN in Beijing. There was no significant difference between the ratios of HHCB to AHTN concentrations in

different plants ($p > 0.05$). The ratios of HHCB to AHTN concentrations were 5.34 ± 1.39, 2.02 ± 0.85 and 3.09 ± 0.52 in influents on Sep 24, May 28 and Jan 15, respectively. They were 4.61 ± 2.83 and 2.22 ± 0.51 in sewage sludge on Sep 24 and May 28, respectively. It should be noted that ratios of HHCB to AHTN concentrations were similar between in influents and sewage sludge in the same season. This feature is similar with those observed by Yang and Metcalfe (2006) and different from those reported by Horii et al. (2007) who presented that the ratios in sewage sludge were larger than those in sewage water. Moreover, we could found that the ratio of HHCB to AHTN concentrations in June were smaller than that from other sampling seasons. The phenomena were in good agreement with those found by Ricking et al. (2003) that HHCB is easier biodegradability than AHTN. The similar conclusion has been also obtained in the air samples in the North Sea and Arctic (Xie et al. 2007). Compared with other results, our values (2–6) of HHCB/AHTN concentrations were similar with those found in Canadian samples (2.7–6.1) (Yang and Metcalfe 2006), which were collected in all the seasons. Interestingly, we found that the most ratios obtained in environmental matrixes, such as influents, effluents, sewage sludge, sediments, rivers, streams, air and so on were nearly not more than 6 (Xie et al. 2007; Lee et al. 2010; Yang and Metcalfe 2006; Zeng et al. 2007). In effluent samples, there was no significant relationship between concentrations of HHCB and AHTN ($p > 0.05$). The phenomena indicated that the removal efficiency of HHCB and AHTN was different during the treatment process.

Concentrations of synthetic musks in the WWTPs would vary over three sampling time, Jan 15, May 28 and Sep 24. Different WWTPs exhibit different characters. According to the Kruskal–Wallis test, there was significant difference in HHCB and AHTN concentrations in influent samples of five plants (except BJ_1 and BJ_5) between different sampling time ($p < 0.05$) (see Fig. 1). The five plants exhibit low HHCB and AHTN concentrations in influent samples on May 28 relative to other sampling time. In Beijing, the average temperature when we sampled were -4.3, 22 and $16°C$ on Jan 15, May 28 and Sep 24, respectively. Therefore, we could conclude that the levels of SMs were low in warm seasons compared with cold seasons. This feature was similar with those reported by Yang and Metcalfe (2006), and similar phenomena were also found in streams (Kristin and Wilhelm 2008) and lakes (Peck and Hornbuckle 2006). Peck and Hornbuckle (2006) attributed lower concentrations in warm seasons to higher temperature and higher intensity of solar radiation. This explanation may be identified by the fact that HHCB and AHTN concentrations were also lower in all of effluent samples in warmer seasons than those in other seasons ($p < 0.05$) (see Fig. 1). Analysis of air samples in WWTPs may provide further information in this regard. However, HHCB and AHTN concentrations in influent samples of Plant BJ_1 and BJ_5 were higher in warm season than those in cold seasons. These phenomena had also been found in Plant A in Kentucky and Georgia reported By Horii et al. (2007). The character may be explained by the possible presumption that musks were so much frequently used in warmer seasons that influence of musk loss was neglected in the two Plants. Besides, although the concentrations of HHCB and AHTN in influents of Plant BJ_1 and BJ_5 were slightly higher than those of other plants, there was no significant difference ($p > 0.05$). In the case of sludge samples, HHCB and AHTN concentrations on Sep 24 were also significantly higher than those on May 28 ($p < 0.05$). This pattern is similar with those in influent samples and observed by Müller et al. (2006) who contribute the low concentrations in warm seasons to lower degradation and volatilization in cold seasons.

The removal efficiencies of the musks in the different plants may be different because of the different treatment

Fig. 1 Comparisons of Musk concentrations collected in different seasons
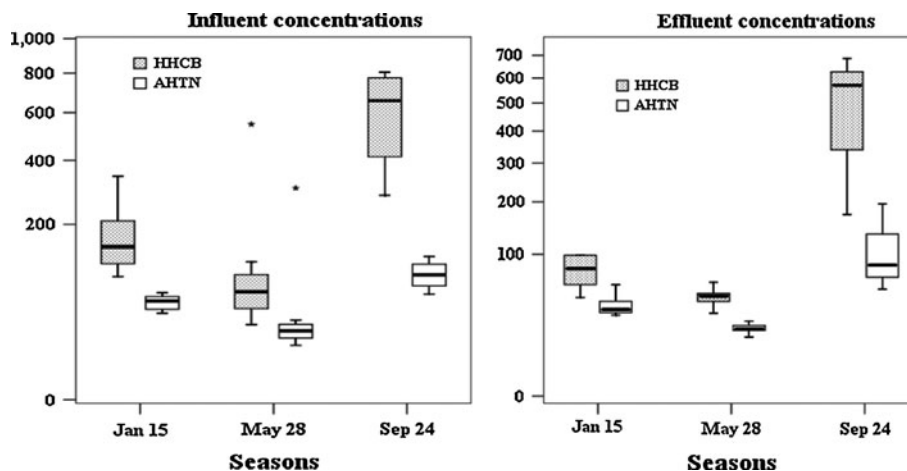
processes and the hydraulic retention time (HRT). In this study, the removal efficiencies of synthetic musk fragrances by conventional treatment processes were calculated using the following equation: $R = 100 \times (C_{influent} - C_{effluent})/C_{influent}$, where R is the removal %, $C_{influent}$ is the concentration of influent water and $C_{effluent}$ is the concentration of treated water. Due to low detection frequencies of other musks except HHCB and AHTN, only the removal efficiencies of HHCB and AHTN were discussed in the study. The removal efficiencies of the two compounds were in the ranges of $< 14.3\% - 98.0\%$ (HHCB) and $< 18.5\% - 98.7\%$ (AHTN), respectively. It should be mentioned that the concentrations of HHCB and AHTN in effluents were comparable or even higher than those in influents in some WWTPs. For example, the effluent concentrations of HHCB in BJ_7 and BJ_3 were similar with those in influents on May 28, and AHTN influent concentrations were lower than those in effluents in BJ_2 of Sep 24 and BJ_7 of Jan 15. The phenomena might indicate that strongly effective removal methods for SMs might be needed in the treatment plants.

It was reported that high temperatures would reinforce evaporation of the compounds from water phase, and increasing intensities of solar radiation could enhance the photo-degradation of synthetic musks (Horii et al. 2007). Therefore, strong evaporation and photo-degradation might result in high removal efficiencies of musks in warm seasons. However, there was no significant difference in the removal efficiencies between seasons ($p > 0.05$). Besides, musk concentrations were not significantly relative with the population observed ($p > 0.05$). Moreover, mass loadings were calculated, based on the daily flow rates and concentrations of HHCB and AHTN in effluents. The results show that the outflow for HHCB and AHTN through effluents discharge into environment was 1.8–685.6 g/d (mean: 70.1 g/d) and 1.6–195.3 g/d (mean: 22.9 g/d) in treatment plants in Beijing. The mean values were higher than those obtained by Horii et al. (2007) (HHCB: 0.67–2.9 g/d; AHTN: 2.0–7.4 g/d) and slightly lower than those observed by Reiner et al. (2007) (HHCB: 258–319 g/d; AHTN:53.0–67.9 g/d).

References

Artola-Garicano E, Borkent I, Hermens JLM, Vaes WHJ (2003) Removal of two polycyclic musks in sewage treatment plants: freely dissolved and total concentrations. Environ Sci Technol 37:3111–3116

Carballa M, Omil F, Lema JM, Llompart M, Garcí-Jares C, Rodríuez I, Gómez M, Ternes T (2004) Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res 38:2918–2926

Clara M, Strenn B, Gans O, Martinez E, Kreuzinger N, Kroiss H (2005) Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Res 39:4797–4807

Guo R, Lee IS, Kim UJ, Oh JE (2010) Occurrence of synthetic musks in Korean sewage sludges. Sci Total Environ 408:1634–1639

Horii Y, Reiner JL, Loganathan BG, Senthil Kumar K, Sajwan K, Kannan K (2007) Occurrence and fate of polycyclic musks in wastewater treatment plants in Kentucky and Georgia, USA. Chemosphere 68:2011–2020

Kristin Q, Wilhelm P (2008) Organophosphates and synthetic musk fragrances in freshwater Streams in Hessen/Germany. Clean-Soil Air Water 36:70–77

Kupper T, Berset JD, Etter-Holzer R, Furrer R, Tarradellas J (2004) Concentrations and specific loads of polycyclic musks in sewage sludge originating from a monitoring network in Switzerland. Chemosphere 54:1111–1120

Lee I-S, Lee S-H, Oh JE (2010) Occurrence and fate of synthetic musk compounds in water environment. Water Res 44:214–222

Müller J, Böhmer W, Th.litz N (2006) Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants. Part 1: Behaviour of polycyclic musks in sewage sludge of different treatment plants in summer and winter. J Soils Sediments 6:231–235

Peck AM, Hornbuckle KC (2006) Synthetic musk fragrances in urban and rural air of Iowa and the great lakes. Atmos Environ 40:6101–6111

Reiner J, Berset J, Kannan K (2007) Mass flow of polycyclic musks in two wastewater treatment plants. Arch Environ Contam Toxicol 52:451–457

Ricking M, Schwarzbauer J, Hellou J, Svenson A, Zitko V (2003) Polycyclic aromatic musk compounds in sewage treatment plant effluents of Canada and Sweden-first results. Mar Pollut Bull 46:410–417

Toivanen N, Toimela T, Tähti H (2008) Effect of polycyclic musks on the aromatase activity in JEG-3 chorion carcinoma cells. Toxicol Lett 180:S118–S118

Xie Z, Ebinghaus R, Temme C, Heemken O, Ruck W (2007) Air-Sea Exchange Fluxes of synthetic polycyclic musks in the North Sea and the Arctic. Environ Sci Technol 41:5654–5659

Yang JJ, Metcalfe CD (2006) Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. Sci Total Environ 363:149–165

Zeng X, Sheng G, Xiong Y, Fu J (2005) Determination of polycyclic musks in sewage sludge from Guangdong, China using GC-EI-MS. Chemosphere 60:817–823

Zeng X, Sheng G, Gui H, Chen D, Shao W, Fu J (2007) Preliminary study on the occurrence and distribution of polycyclic musks in a wastewater treatment plant in Guandong, China. Chemosphere 69:1305–1311

Zhang XY, Yao Y, Zeng X, Qian G, Guo Y, Wu M, Sheng GY, Fu JM (2008) Synthetic musks in the aquatic environment and personal care products in Shanghai, China. Chemosphere 72:1553–1558