Spatial and seasonal distribution of synthetic musks in sewage treatment plants of Shanghai, China

Wenjing Sang, Yalei Zhang, Xuefei Zhou and T. C. Zhang

ABSTRACT

As one kind of emerging contaminant, synthetic musks (SMs) are commonly used in varying amounts in many personal care products and have been detected in different environmental systems. Occurrence and fate of four common SMs [galaxolide (HHCB), tonalide (AHTN), musk xylene (MX) and musk ketone (MK)] were studied in different sewage treatment plants (STPs) of Shanghai, China among different seasons. Results showed that total dissolved concentrations of the four SMs were 536–3,173 ng/L in influent, 351–2,595 ng/L in effluent and 147–6,839 μg/kg dry weights in sludge. The SM concentrations varied with input sources, STP treatment processes, usage patterns, and different seasons or surveyed years of consumption. There was no significant removal of SMs in most of the sewage samples of the four STPs. Significant positive correlations were observed between concentrations of HHCB and AHTN ($R^2 = 0.9062$, $n = 12$, $p < 0.05$), HHCB and MK ($R^2 = 0.7471$, $n = 8$, $p < 0.05$), as well as AHTN and MK ($R^2 = 0.9321$, $n = 8$, $p < 0.05$) in sludge samples.

Key words | effluent, influent, sewage treatment plant, sludge, synthetic musks

INTRODUCTION

Synthetic musks (SMs) are a group of anthropogenic chemicals, including nitro musks, macrocyclic musks and polycyclic musks. SMs are widely used in many personal care products (Heberer 2003; Reiner et al. 2007a). Concerns over the toxicity of nitro musks led to restrictions on their use since the late 1980s (Yamagishi et al. 1981, 1983; Yurawecz & Puma 1985). Recently, nitro musks have been replaced successively by polycyclic musks in some countries (e.g., Germany). However, a small amount of musk xylene (MX) and musk ketone (MK) are used continuously (Heberer 2003). Conversely, the production and use of polycyclic musks have rapidly increased in the last decade (Sommer 2004). In 1996, more than 6,000 t of polycyclic musk were been produced worldwide, 90–95% of which were galaxolide (HHCB) and tonalide (AHTN) (Geyer et al. 2000; Sommer 2004).

Recently, SMs have raised considerable attention because of their persistence and bioaccumulation potential. Physicochemical properties of SMs are similar to non-polar and highly lipophilic compounds (Eschke et al. 1994), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Therefore, SMs can be easily accumulated in sediments, sludge and suspended solid matrix (Heberer 2003). SMs can be used as anthropogenic markers to assess the impacts of domestic wastewater in surface water (Zhang et al. 2008). Moreover, studies have shown that both HHCB and AHTN can bind to estrogen receptors in the cell and are capable of interacting with hormone systems (Bitsch et al. 2002), which characterizes them as one kind of emerging contaminant.

After use, SMs enter the environments through sewage systems and sewage treatment plants (STPs). SMs have been regularly detected/quantified in untreated/treated domestic wastewaters, sewage sludge (Lee et al. 2005), rivers, lakes, estuaries (Heberer et al. 1999), fish/mussels (Kannan et al. 2005), the atmosphere, as well as human milk, blood, fat, and adipose tissue (Kannan et al. 2005; Reiner et al. 2007b). Significant differences in concentrations and occurrence of SMs are observed between different geographical areas. However, most of the SM studies have been carried out in the USA and EU; data from developing countries are scarce (Zeng et al. 2007, 2008; Shek et al. 2008; Zhang et al. 2008; Hu et al. 2011), which need further studies in different geographical areas.

Shanghai, with a population of 19 million, discharges a large amount of SMs (1.26 t HHCB and 0.38 t AHTN in…
2007) into the aquatic environment every year (Zhang et al. 2008). Therefore, it would be interesting to evaluate spatial and seasonal distribution of four main SMs (HHCB, AHTN, MK and MX) in STPs in Shanghai, China to fill the current knowledge gap.

**EXPERIMENTAL**

**Test substances**

HHCB, AHTN, MX, MK and phenathrene-d10 (Phe-D10) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and hexamethyl benzene (HMB) from Aldrich (Milwaukee, WI, USA). Standard solutions of the test substances with concentrations of 10–200 and 200–1,000 μg/L were prepared in n-hexane, stored at 4°C, and renewed weekly.

**Sample collection and preparation**

Four STPs (referred to as STPs A, B, C and D) located in Shanghai (Table 1) were sampled from December 2008 to June 2009 (to reflect seasonal variations in STPs), including influents (after bar screen), effluents (after advanced treatment) and waste sludge (from the sludge dewatering unit). Aqueous grab samples were collected in amber glass bottles, immediately adjusted to pH 3 using 1% orthophosphoric acid solution to reduce biological activity, and then stored in the dark at +4°C until analysis. Sludge samples were collected and brought back to the laboratory, where they were freeze-dried, ground, homogenized by sieving through a stainless-steel (40-mesh), mixed thoroughly and then stored in a sealed plastic bottle at 4°C until extraction.

**Sample extraction**

**Sewage**

Prior to extraction, aqueous samples were filtered using a water membrane (0.45 μm), and adjusted to pH 7–7.5 by NaOH (1 M) (Ternes et al. 2005). SMs (HHCB, AHTN, MX and MK) were extracted from the aqueous samples (volume = 300 mL) by solid-phase extraction (SPE) with an ENVIC18 cartridge. The optimum SPE method for the extraction of SMs from the aqueous samples has been reported previously (Sang et al. 2009). The eluent was evaporated under a gentle flow of high purity nitrogen and was then dissolved into 1 mL HEX and stored at 4°C until analysis.

**Sludge**

For extraction, an automated Soxtec Avanti 2050 from FOSS Analytical AB (Foss Tecator, Sweden) was used. Sludge samples were extracted with the same methods used for river sediments by Sang et al. (2012).

**Analytical methods**

SMs were determined by gas chromatography-mass spectrometry (GC-MS), using a Finnigan Voyager quadrupole MS under electron impact ionization (EI, 70 eV, 250°C) and full scan (m/z 35–400 amu, 0.08 s/scan, nominal mass resolution) or selected-ion monitoring (SIM) conditions. Analytes were separated with a 50 m HP-5MS column (0.25 mm i.d. and 0.25 μm film thickness) with helium as the carrier gas (1.0 mL/min). The oven temperature program was 60°C for 5 min, 10°C/min to 250°C, 20°C/min to 280°C, and then 280°C for 5 min. The sample (1 μL) was injected into GC-MS by the splitless mode for 0.75 min and an inlet temperature of 280°C. Quantification ions were used as follows: m/z = 243 for HHCB; 243 for AHTN; 282 for MX; and 279 for MK. All samples were analyzed in triplicates. Limit of quantification (LOQs) and relative standard deviation (RSD) are shown in Table 2.

**RESULTS AND DISCUSSION**

**Concentrations and distribution patterns of SMs in sewage**

The total dissolved SM concentrations in the sewage samples varied widely, ranging from 536 to 3,173 ng/L in...
the influents and from 351 to 2,595 ng/L in the effluents (Table 3). The mean concentrations of HHCB in influent of STP A were higher than that of the other STPs. No significant difference in the concentrations of AHTN and MK were found among the four STPs. Among the four SMs, HHCB was dominant and was detected in all aqueous samples, while AHTN was detected in 10, MK in nine, and MX in five out of 12 STP samples. These results coincide well with the consumption pattern of SMs.

Most of the previous research was about HHCB and AHTN due to their greater production and high usage (Balk & Ford 1999; Heberer 2003; Horii et al. 2007). The detected concentrations among the four STPs in this study were 356–2,185 ng/L HHCB and 42–811 ng/L AHTN in influents, as well as 351–1,707 ng/L HHCB and 85–704 ng/L AHTN, which are comparatively lower than those in most of the STPs in USA and EU countries (Simonich et al. 2002; Artola-Garicano et al. 2003a; Bester 2004; Lishman et al. 2006). Artola-Garicano et al. (2003a, b) measured 0.54–1.76 μg/L AHTN and 1.18–4.50 μg/L HHCB in influent from STPs in the Netherlands. Similarly, 6.9 μg/L HHCB and 1.52 μg/L AHTN were detected in STPs in Switzerland (Berset et al. 2004) and 1.9 μg/L HHCB and 0.58 μg/L AHTN were found in a typical STP receiving 50% domestic sewage and 50% industrial wastewater in Germany (Bester 2004). Lower concentrations have also been measured in raw sewage from a STP located in Canada, in the range between 4.8 and 390.2 ng/L for six polycyclic musks, with HHCB (390.2 ng/L) and AHTN (85.9 ng/L) being the main components; the results might be attributed to the fact that less people were inhabitants of this area (Yang & Metcalfe 2006). As mentioned above, the occurrence of HHCB and AHTN does not show a specific geographical distribution because it mainly depends on SMs’ production and usage volume in each country.

In STP A, HHCB and AHTN concentrations in influent were higher in cold season (December 2008 and March 2009, 110–2,185 ng/L) as compared with the warm season (June 2009, 295–932 ng/L). This was probably caused by rain water dilution. The rainy season of Shanghai is in June and July, so the warm season has more rain water than cold season. STP A was connected to some open sewage system which would be diluted by rain water in rainy season, and eventually SM concentrations in influent would be decreased. Similar results were reported in previous research (Yang & Metcalfe 2006). In STPs B, C and D, the concentrations of HHCB and AHTN in influent didn’t have obvious differences between the cold and warm seasons (Figure 1).

The reported overall removal rates of SMs in activated sludge of STPs ranged from 63 to 88% for HHCB, and 64 to 89% for AHTN (Simonich et al. 2002; Berset et al. 2004; Bester 2004; Kupper et al. 2004, 2006; Horii et al. 2007; Zhang et al. 2008). High removal rates (98–99%) were reported in Canada when soluble concentrations alone in wastewater were analyzed (Lishman et al. 2006). Table 3 shows that the highest removal efficiencies of HHCB, AHTN and MK are found to be 60, 44 and 61%, respectively; in some samples, the effluent concentrations were even higher than influent ones. The results of the current study agree well with those reported (Artola-Garicano et al. 2003a, b), who concluded that the stable dissolved concentrations in the aqueous phase were dependent on particles loading and the partition coefficient of the analytes between particle and the aqueous phase, and the difference between the free and the total concentration of SMs can be substantial even in the effluent. The removal efficiency in this study might be underestimated as only the dissolved concentrations in influent and effluent were examined.

Three different biological treatment processes (CAS, Unitank and A^2O) were investigated in this study. Decrease in effluent concentrations of polycyclic musks as compared with the influent ones indicates that SMs are removed during treatment processes. Decrease in dissolved concentrations of polycyclic musks suggests biodegradation during the treatment process (Artola-Garicano et al. 2003a,

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**_table2** Limit of quantification (LOQ) and recovery of SMs with different matrices

<table>
<thead>
<tr>
<th>Substance</th>
<th>LOQ (ng/L)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Sludge (μg/kg dw)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Sludge (μg/kg dw)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHCB</td>
<td>30</td>
<td>86.99 ± 5.31</td>
<td>3.61</td>
<td>83.98 ± 6.31</td>
<td>4.30</td>
<td>81.15 ± 6.05</td>
<td>7.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHTN</td>
<td>30</td>
<td>99.62 ± 5.70</td>
<td>3.60</td>
<td>92.50 ± 6.97</td>
<td>7.55</td>
<td>70.75 ± 5.98</td>
<td>8.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MX</td>
<td>42</td>
<td>96.89 ± 10.75</td>
<td>11.10</td>
<td>87.88 ± 9.25</td>
<td>10.52</td>
<td>66.64 ± 8.62</td>
<td>12.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK</td>
<td>30</td>
<td>117.81 ± 4.68</td>
<td>3.97</td>
<td>85.54 ± 6.00</td>
<td>7.02</td>
<td>71.33 ± 11.46</td>
<td>16.06</td>
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</table>

RSD: relative standard deviation.
<table>
<thead>
<tr>
<th>STP</th>
<th>HHCB</th>
<th>AHTN</th>
<th>MX</th>
<th>MK</th>
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<tr>
<td></td>
<td>Influent</td>
<td>Effluent</td>
<td>Sludge</td>
<td>Influent</td>
</tr>
<tr>
<td>A</td>
<td>Dec-08</td>
<td>2,184.6</td>
<td>1,475.6</td>
<td>470.0</td>
</tr>
<tr>
<td></td>
<td>Mar-09</td>
<td>919.2</td>
<td>1,706.6</td>
<td>1,434.0</td>
</tr>
<tr>
<td></td>
<td>Jun-09</td>
<td>931.4</td>
<td>1,086.4</td>
<td>369.4</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1,345.1</td>
<td>1,422.9</td>
<td>757.8</td>
</tr>
<tr>
<td>B</td>
<td>Dec-08</td>
<td>1,100.4</td>
<td>596.4</td>
<td>211.7</td>
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<tr>
<td></td>
<td>Mar-09</td>
<td>1,479.2</td>
<td>658.4</td>
<td>3,492.5</td>
</tr>
<tr>
<td></td>
<td>Jun-09</td>
<td>800.6</td>
<td>562.4</td>
<td>817.1</td>
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<tr>
<td></td>
<td>Mean</td>
<td>1,126.7</td>
<td>605.7</td>
<td>1,507.1</td>
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<tr>
<td>C</td>
<td>Dec-08</td>
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<td>350.8</td>
<td>268.2</td>
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<td>Mar-09</td>
<td>1,106.6</td>
<td>445.2</td>
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<td>Jun-09</td>
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<td>292.5</td>
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<tr>
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<td>Mean</td>
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<td>703.6</td>
<td>610.0</td>
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<tr>
<td>D</td>
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<td>477.4</td>
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<td>2,216.9</td>
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<td></td>
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<td>914.6</td>
<td>194.4</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>965.5</td>
<td>965.2</td>
<td>840.9</td>
</tr>
</tbody>
</table>

ND: no detection; LOQ: limit of quantification.
b). It was reported that the major removal pathway of polycyclic musks in STPs is the transfer of SMs to sludge due to sorption onto particles in wastewater, while biodegradation might play a considerable role in elimination of HHCB (Bester 2004). The removal efficiency may be enhanced with adding coagulation reagents, such as FeCl₃, Al₂(SO₄)₃ and aluminum polychloride (PAX) during primary treatment (Carballa et al. 2005), and ozonation may be another efficient technique for removing polycyclic musks (Ternes et al. 2006).

SMs in sludge

SM concentrations in sludge collected from the four STPs ranged from 147 to 6,839 µg/kg dw (Table 3). More SMs were detected at the level above LOQ in sludge samples. Meanwhile, the polycyclic musks were also more frequently detected in sludge samples than nitro musks due to their high usage. HHCB and AHTN were detected in all sludge samples. MK was found in eight, while MX was found in four out of 12 samples.

The composition profiles of SMs in sludge from different STPs and different sampling periods were compared and shown in Figure 2. Hierarchical cluster analysis was performed with SMs concentrations in sludge samples to investigate their distribution patterns. Generally, HHCB was dominant in all sludge samples; it accounted for 40.1–84.4% of the four SMs, followed by AHTN (15.6–43.1%), MK (0–10.1%) and MX (0–7.9%). These results coincide with the reported values of SMs in the USA and EU countries (Herren & Berset 2004; Horii et al. 2007), but pollution levels in Shanghai are slightly below the EU, which may be due to the living habits in different regions and different countries. In EU countries, the mean concentrations of HHCB and AHTN in sludge were measured at 27 and 4.70 mg/kg in England (Stevens et al. 2005), 8.26 and 3.56 mg/kg in Germany (Heberer 2004), and 20.3 and 7.3 mg/kg in Switzerland (Kupper et al. 2004). The content of SMs in cosmetic products in Europe is generally higher than in China, with per capita use of SMs being larger. Compared with Shanghai, Hong Kong owns a high degree of internationalization, small region, and the high population density, and therefore, the concentrations of SMs in domestic sludge of Hong Kong were relatively high, measured at 7 and 4.70 mg/kg (Shek et al. 2008).

The sludge with the largest proportion of HHCB (84.4%) was observed in samples from STP C, in December 2008, while the highest percentage of AHTN (43.1%) was found in sludge from STP D, in March 2009. Both MX (4.9–7.9%) and MK (2.3–10.1%) contributed small proportions to the four SMs in sludge samples. The sludge with the largest proportion of MX and MK was observed in March 2009. Although the dominant analyst in sludge was still HHCB and AHTN, the SMs profiles in sludge

![Figure 1](https://iwaponline.com/wst/article-pdf/66/1/201/442812/201.pdf)
were obviously different from sewage. The greatest concentration of the four SMs (6.839 µg/kg dw) was detected in the sludge samples from STP B in March 2009 (Figure 2). SMs have high log $K_{ow}$ values and are lack of ready biodegradability, and therefore, may transport easily from daily chemical products via the sewage system and STP to sludge (Heberer 2002). The ratios of AHTN and MX increased more in March 2009.

The concentrations of SMs in sludge are related to influent wastewater sources, sewage treatment processes and capacity, among other factors (Herren & Berset 2000; Shek et al. 2008). If the flow rate of domestic sewage into a STP is large, SM concentrations in sludge will be relatively high. This deduction is supported by the results of this study, that is, STP C with the lowest ratio of residential sewage (50% RS) has lower concentrations of the SMs in sludge as compared with other STPs, especially in March 2009 (Figure 2 and Table 3). This result is consistent with other studies (Heberer 2002; Stevens et al. 2003; Kupper et al. 2004). Shek et al. (2008) and Carballa et al. (2005) reported that SMs concentrations in sludge have a certain relationship with STP processes, and sludge tends to absorb more SMs (Carballa et al. 2005; Shek et al. 2008).

Concentrations of the two most prevalent polycyclic musks (HHCB and AHTN) measured in the sludge samples of the current study were generally greater than that reported in the other areas (Herren & Berset 2000; Heberer 2003; Stevens et al. 2003; Kupper et al. 2004; Shek et al. 2008). The environmental effect of HHCB and AHTN on earthworms and springtails has been studied; they reported that the no-effect concentration (PNEC) was 320 µg/kg soil for both substances (Balk & Ford 1999). Meanwhile, AHTN and MK can stay in the soil for one year (Difrancesco et al. 2004). In this study, the concentration range of HHCB and AHTN were 111–3,493 and 55–2,402 µg/kg, respectively. All of the four selected STPs use landfill for sludge disposal. Thus, if the SM content in sludge was higher than the PNEC, land application of sludge may influence the soil eco-system and result in longer-term adverse effects.

**Behavior of SMs in STPs**

Ratios of each SM to the other SMs in water samples have normally been used to identify their fate in the water system (Lee et al. 2010). In this study, we investigated the $R^2$ value among the three SMs (i.e. HHCB, AHTN and MK) to examine their inter-relations; MX was excluded because it was only detected in five influent samples. Linear regression analysis was performed using each concentration level of the SM concentrations in all sewage and sludge samples (Figure 3).

For sewage samples, there was no significant relationship between HHCB and AHTN, HHCB and MK, or AHTN and MK (Figure 3(a)). These results were different from previous reports (Artola-Garicano et al. 2003a; Horii et al. 2007; Lee et al. 2010). Lee et al. (2010) investigated SMs (HHCB, AHTN, MX, MK) in 10 STPs and 14 surface water sites and a close relationship was observed between AHTN and HHCB; the concentration of either one in a water system can be predicted from that of the other. Horii et al. (2007) also found positive correlations between HHCB and AHTN. All of these studies used liquid–liquid extraction with no filtration before extraction. Thus, they could measure the total SMs concentrations, but only dissolved SMs were detected in the current study, which might be the reason why no significant pairwise relationship was found.

For sludge samples, significant positive correlations were observed between concentrations of HHCB and AHTN ($R^2 = 0.9062$, $n = 12$, $p < 0.05$), HHCB and MK ($R^2 = 0.7471$, $n = 8$, $p < 0.05$), as well as AHTN and MK ($R^2 = 0.9321$, $n = 8$, $p < 0.05$) (Figure 3(b)). Positive
correlations between HHCB and AHTN concentrations in sludge samples were also reported in previous studies (Reiner et al. 2007a; Shek et al. 2008), indicating that the polycyclic musks in sludge may come from the same source. The polycyclic musk concentrations in sludge may be attributed to their concentrations in the original products, the extensive uses, and discharges of those products into the environment. Thus, the HHCB to AHTN ratio can be used as a tracer for source discrimination and for the degree of degradation in the environment (Zeng et al. 2008).

CONCLUSIONS

Concentrations of four SMs were investigated in influent, effluent and waste sludge of four STPs in Shanghai in different seasons. HHCB and AHTN were found to be the main pollutants. For sewage samples, there was no significant difference in concentrations of HHCB, AHTN and MK in influent samples between warm and cold seasons except STP A. For sludge samples, the result was totally different with the SMs concentrations of four STPs in March 2009 being much higher than that of other seasons. The ratios of AHTN and MX increased more in March 2009. Based on the dissolved concentrations of SMs in influent and effluent, there was no significant removal of SMs in the four STPs. Significant positive correlations were observed between concentrations of HHCB and AHTN, HHCB and MK, as well as AHTN and MK in sludge, but not in aqueous samples.

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