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# Levels of synthetic musk compounds in municipal wastewater for potential estimation of biota exposure in receiving waters

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We analyzed water samples from the confluence of three municipal sewage treatment effluent streams, surface water, and whole carp (*Cyprinus carpio*) for synthetic musks for a period of 7–12 months. The lipid content of each fish was determined and compared with the concentration of musks in the whole fish tissue. Enhanced methods were used for water sampling and musk extraction. The data presented here provide insight as to the relationship between concentrations of synthetic musks in the municipal effluent and associated biota. This study confirmed the presence of polycyclic and nitro musk compounds in sewage effluent, Lake Mead water, and carp. The concentrations were found to be considerably lower than previous studies conducted in other countries. This study also provides data for polycyclic and nitro musk compounds, as well as some of the nitro musk metabolites in sewage treatment plant effluent, lake water, and carp.

## Introduction

Synthetic musk compounds are consumer chemicals manufactured as fragrance materials and consumed in very large quantities worldwide. Due to their high use and release, they have become ubiquitous in the environment.<sup>1,2</sup> These compounds are produced and sold by the fragrance industry as fixatives for perfumes and as aroma enhancing additives to bath soaps, household cleaners, laundry detergents, fabric softeners, shampoos, after-shave lotions, fishing bait, and even in herbicides in Japan.<sup>3,4</sup> Despite the high production levels, the levels of synthetic musk compounds in the environment and their impact on ecosystems have only recently been addressed by the scientific community.<sup>2</sup>

The nitro musks in Table 1 include compounds with two nitro groups [e.g., musk ambrette (MA), musk ketone (MK), and musk tibetene (MT)] or three nitro groups [e.g., musk xylene (MX)]. These compounds were discovered and developed by Baur in 1888.<sup>5</sup> Nitro musk metabolites used in this study are shown in Table 2. Polycyclic musks (lacking nitro groups) were introduced in 1945. This group includes the compounds in Table 3, Galaxolide<sup>®</sup> (HHCB), Tonalide<sup>®</sup> (AHTN), Traseolide<sup>®</sup> (ATII), Celestolide<sup>®</sup> (ADBI), Cashmeran<sup>®</sup> (DPMI), Phantolide<sup>®</sup> (AHMI), and Versalide<sup>®</sup> (AETT). Their success in the fragrance industry may be attributed to their outstanding stability towards alkali and light, their moderate cost (they are produced from inexpensive raw materials), and finally, their excellent odor and fixative properties, which nearly duplicate the odor of the naturally occurring macrocyclic musks.<sup>5</sup> Most of these colorless solids are readily soluble in most common solvents, and have excellent persistence<sup>5</sup> and high Henry's law constants.<sup>6</sup>

The logarithm of the *n*-octanol–water partition coefficient (log  $K_{ow}$ ) of synthetic musk compounds, and some of their metabolites, ranges from 3.8 to 6.3, while the molecular weights range from 206 to 297 g mol<sup>-1</sup>. By virtue of their chemical structures and physicochemical properties, synthetic musk compounds have the potential to bioconcentrate and bioaccumulate in the adipose tissues of aquatic and terrestrial organisms.<sup>2</sup> The primary release of synthetic musk compounds to surface waters is from

municipal sewage systems.<sup>7</sup> The fraction of these compounds removed by sewage treatment plants depends on the nature and level of treatments used by the sewage treatment plant managers.<sup>8</sup> In the United States and Europe, the percent removal of fragrance materials by primary wastewater treatment ranged from 14.6% to 50.6%, while percent removals increased to 87.8 to 99.9% with secondary treatment.<sup>8</sup> Even so, synthetic musk compounds at concentrations between 0.3 and 410 ng L<sup>-1</sup> were detected in sewage treatment plant effluent, downstream effluent, and receiving waters.<sup>1,9–13</sup>

**Table 1** Trade, CAS names, and structures for five nitro musks

Trade and CAS name (acronym)	Chemical structure
Musk ketone, 1- <i>tert</i> -butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene (MK)	
Musk moskene, 4,6-dinitro-1,1,3,3,5-pentamethylindane (MM)	
Musk ambrette, 2,6-dinitro-3-methoxy-4- <i>tert</i> -butyltoluene (MA)	
Musk xylene, 1- <i>tert</i> -butyl-3,5-dimethyl-2,4,6-trinitrobenzene (MX)	
Musk tibetene, 1- <i>tert</i> -butyl-2,6-dinitro-3,4,5-trimethylbenzene (MT)	

**Table 2** Trade, CAS names, and structures for three nitro musk metabolites

Trade and CAS name (acronym)	Chemical structure
2-Amino musk ketone, 2-amino-1- <i>tert</i> -butyl-3,5-dimethyl-6-nitro-4-acetylbenzene (2-AMK)	
4-Amino musk xylene, 4-amino-1- <i>tert</i> -butyl-3,5-dimethyl-2,6-dinitrobenzene (4-AMX)	
2-Amino musk xylene, 2-amino-1- <i>tert</i> -butyl-3,5-dimethyl-4,6-dinitrobenzene (2-AMX)	

**Table 3** Trade, CAS names, and structures for seven polycyclic musks

Trade and CAS name (acronym)	Chemical structure
Galaxolide, 1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta[ <i>g</i> ]-2-benzopyran (HHCB)	
Tonalide, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphthalen-2-yl)ethanone (AHTN)	
Traseolide, 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1 <i>H</i> -inden-5-yl]ethanone (ATII)	
Celestolide, 1-[6-(1,1-dimethylethyl)-2,3-dihydro-1,1-dimethyl-1 <i>H</i> -inden-4-yl]ethanone (ADBI)	
Cashmeran, 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4 <i>H</i> -inden-4-one (DPMI)	
Phantolide, 1-(2,3-dihydro-1,1,2,3,3,6-hexamethyl-1 <i>H</i> -inden-5-yl)ethanone (AHMI)	
Versalide, 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin (AETT)	

Nitro musks were found in higher concentration in sewage treatment plant (STP) influents compared with effluents.<sup>14</sup> Synthetic musks and their metabolites were removed from STPs mostly in sewage sludge. This sludge was often used as agricultural fertilizer. In Switzerland,  $1.07 \times 10^8$  kg of sludge, which represented 51% of sludge produced, was applied to agricultural fields.<sup>14</sup> In the fields, rainwater could leach synthetic musks and their metabolites into surface waters. Amino metabolites of the nitro musks, amino musk xylene (AMX), amino musk ketone (AMK), and amino musk moskene (AMM), were detected in domestic and industrial sewage sludge in higher concentrations compared with their

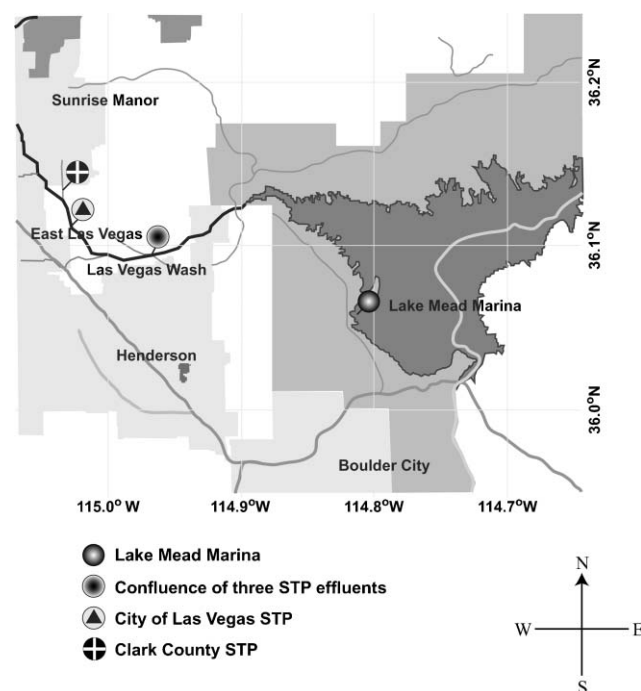
parent compounds.<sup>15</sup> These amino metabolites were released into effluents from sewage treatment facilities, along with their unreduced parent compounds. With this in mind, the nitro musk metabolites in Table 2 (2-amino musk xylene, 4-amino musk xylene, and amino musk ketone) were examined in this study. HHCB, and AHTN were the major polycyclic musks found in sewage sludge<sup>8</sup> and were also examined.

Studies in the Netherlands<sup>16,17</sup> have determined nitro- and polycyclic musk fragrances in a few sewage sludge samples, but no efforts have been made to undertake a long-term study of the fate and transport of these compounds in the United States. Levels of these compounds may be different in the municipal sewage effluent in the United States, due to increasing use of tertiary sewage treatment systems. This contrasts with certain countries of Europe where tertiary treatment is not typically used. Therefore, we conducted a long-term study examining the levels of synthetic musks in municipal sewage effluent, and evaluated these data for suitability in estimating biota exposure in the receiving waters.

## Experimental

### Chemicals, water sampling procedures, and analytical methods

All chemicals, water sampling procedures, and analytical methods have been previously described.<sup>11</sup> In brief, on a monthly basis (November 2000 through December 2001), sampling equipment was assembled at the following sampling sites: 1) the confluence of effluents from three Nevada sewage treatment plants (STPs) (City of Las Vegas, Clark County, and City of Henderson) at N 36° 05' 22" and W 114° 56' 30"; and 2) Lake Mead, Nevada at N 36° 03' 05" and W 114° 48' 35" (Fig. 1). For a duration of 6 to 8 h, large volumes of water from municipal sewage treatment effluent and lake water were extracted on-site with a train of extraction devices connected in series.<sup>11</sup> The sewage effluents were sampled from a dedicated effluent receiving stream (one that receives only sewage effluent and rarely runoff). The volume extracted varied from 45 L when the water was turbid to 85 L when the water was clear. A coarse filter composed of glass wool, deactivated with 5% dimethyldichlorosilane (DMDCS), removed large particles and algae, and a 400 cm<sup>2</sup>, 5.0 μm pore-size disposable filter removed



**Fig. 1** Map of Las Vegas (Nevada) Basin showing Lake Mead and Las Vegas Wash sampling sites.

most remaining particulate matter. The twice-filtered water was drawn by and passed through a peristaltic or diaphragm pump and finally through a cartridge containing the sorbent, 6 g of a 1 : 1 (polymethyl methacrylate) : (polystyrene cross-linked with 50% divinylbenzene) sorbent (NEXUS, Varian Inc., Harbor City, CA, USA). The sorbent retained most organic compounds. Previous laboratory experiments determined that breakthrough did not occur for any target analyte after 100 L of spiked deionized water passed through the cartridge.

The cartridge was wrapped in aluminium foil, transported to the laboratory on ice and the analytes were immediately desorbed from the solid-phase of the cartridge with successive portions of 25 mL of ethyl acetate and 25 mL of *n*-hexane. The enriched extracts were concentrated; solvent exchanged to toluene, and analyzed using gas chromatography-mass spectrometry (GC/MS) in the full scan mode. The low concentrations of the analytes in the Lake Mead extracts diminished the analytes' signals. For this reason, Lake Mead extracts were analyzed using GC/MS in the selected-ion monitoring mode.

### Fish sample collection and preparation

Seven to eight carp (*Cyprinus carpio*), averaging 2 kg each, were collected by net from Lake Mead on a monthly basis for 12 months.<sup>18</sup> The fish were collected from the same site as the lake water samples, approximately 200 m from the drinking water intake area of the lake (Fig. 1). A detailed description of the fish sample collection, preparation, and clean-up procedures has been previously described.<sup>18</sup> In brief, an average of 2.3 g of fish tissue was weighed and transferred into a smooth-surface mortar that had been silanized with 5% DMDCS in toluene. Twenty-five  $\mu\text{L}$  of the solution of surrogate standards (200  $\mu\text{g mL}^{-1}$  each of 2,6-dinitrotoluene, pentachloronitrobenzene, and 2,2'-dinitrobiphenyl) was added to the fish sample in the mortar. Eight samples from the same homogenized fish tissue were also spiked with the surrogate standards mixture and 100  $\mu\text{L}$  of a mixture of musk standards and amino metabolites. This solution contained 15 musk or musk related compounds, each at a concentration of 20  $\mu\text{g mL}^{-1}$ . To each fish sample in the mortar, 5 g of diatomaceous earth hydromatrix was added, and the mixture was homogenized using a silanized pestle until a free flowing powder was achieved. The fish samples were selectively extracted with deactivated basic alumina, using pressurized liquid extraction (PLE). The selective extraction of fish samples by PLE has been described elsewhere.<sup>19</sup> Each PLE extract was concentrated to 300  $\mu\text{L}$  using a Turbo Vap II solvent evaporator at 35 °C under a gentle stream of nitrogen. To each 300  $\mu\text{L}$  extract, 3 mL of methylene chloride was added, and the volume was then concentrated to 1 mL for gel permeation chromatography, using a Turbo-Vap II solvent evaporator.

### Extract clean-up

Bulk lipids from all fish samples were removed by gel permeation chromatography (GPC).<sup>18</sup> Subsequent cleanup was carried out by eluting the extract from 1 g of 3-aminopropyl derivatized silica in a 6 mL polypropylene cartridge (Strata  $\text{NH}_2$ , Phenomenon, Torrance, CA, USA) using 10 mL of methylene chloride in a Supelco 12-position Visiprep-DL solid-phase extraction vacuum manifold.

### Total lipid extraction and determination

Hydrophobic compounds have the tendency to partition into biological tissues in proportion with the lipid contents.<sup>20</sup> In order to compare synthetic musk concentrations in individual carp tissue with the lipid content, bulk lipid (glycerol esters of fatty acids) as confirmed by the presence of the carbonyl group in the IR spectrum ( $1750\text{ cm}^{-1}$ ),<sup>18</sup> was extracted from all fish samples. The same homogenized fish used for PLE were also used for the total lipid determination. The following procedure was used to extract lipids from carp tissue. Fish samples ranging from 4 to 9 g were extracted by the PLE system, using 100% chloroform as the extraction solvent. Each fish sample was weighed, mixed with approximately 10 g of diatomaceous earth hydromatrix in a mortar and pestle, and then loaded into a 33 mL stainless steel PLE extraction cell containing a cellulose filter.<sup>18</sup> Each extract was concentrated to 2 mL using a Turbo-Vap II, under a gentle stream of nitrogen gas, and quantitatively transferred to a pre-weighed small porcelain bowl and allowed to air dry for 48 hours or until all subsequent weighing remained constant. The percent lipid for each extract was calculated as a ratio of the final lipid weight to the initial fish sample weight, multiplied by 100. The data used for lipid content determination are available upon request.

### GC/MS-SIM analysis

Fish tissue extracts from PLE were reconstituted in toluene and concentrated to 90  $\mu\text{L}$ , after which 10  $\mu\text{L}$  of the internal standard, naphthalene- $\text{d}_8$  (100  $\mu\text{g mL}^{-1}$  concentration) was added. Due to low analyte signals, fish extracts were analyzed using GC/MS, in the selected-ion monitoring mode. A HP Chemstation using Microsoft Windows<sup>7</sup> (Microsoft Corp., Bellevue, WA) based environmental analysis software (Agilent Technologies) was used to control the instrument and to acquire and analyze the data.

## Results

### Synthetic musk compounds detected in STP effluent

The average monthly concentrations of various musk compounds detected at the confluence of three STP effluents from Las Vegas Wash are presented in Table 4. High monthly

**Table 4** Monthly mean concentrations of synthetic musks in STP effluent from the Las Vegas Wash ( $\text{ng L}^{-1}$ ) ( $n = 2$  per month)

Month	HHCB	AHTN	AHMI	ATII	ADBI	MX	MK	4-AMX	2-AMX	AMK
Jan	— <sup>a</sup>	—	—	—	—	—	—	—	—	—
Feb	36.6	27.8	2.6	6.9	0.6	0.6	22.3	33.0	nd <sup>b</sup>	nd
Mar	54.0	30.1	3.2	27.8	1.0	0.4	21.5	5.5	nd	nd
Apr	—	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—	—
Jun	32.8	21.7	2.7	nd	0.9	0.1	20.2	nd	nd	nd
Jul	44.2	39.4	2.6	20.6	1.0	nd	18.0	17.9	1.5	nd
Aug	32.6	22.2	1.0	15.2	0.4	nd	19.1	9.6	nd	nd
Sept	42.7	26.9	2.7	17.8	3.8	0.5	28.0	4.2	nd	1.8
Oct	74.4	40.2	3.3	28.5	4.7	0.9	38.6	9.6	1.7	nd
Nov	97.3	49.7	3.8	44.9	6.0	1.6	45.7	21.3	nd	4.7
Dec	97.9	47.6	3.2	51.0	5.9	1.3	33.2	7.0	nd	nd
Annual mean <sup>c</sup>	56.9 (26.4)	34.0 (10.6)	2.80 (.78)	26.6 (15.0)	2.7 (2.4)	1.1 (0.5)	27.4 (9.8)	13.5 (9.9)	1.6 (0.1)	3.2 (2.1)

<sup>a</sup> — = Not sampled. <sup>b</sup> nd = Not detected (see ref. 15 for MDL). <sup>c</sup> Mean ( $\pm 1$  standard deviation).

**Table 5** Monthly mean concentrations of synthetic musks in Lake Mead Water ( $\text{ng L}^{-1}$ ) ( $n = 2$  per month)

Month	HHCB	AHTN	AHMI	ATII	ADBI	MX	MK	4-AMX	2-AMX	AMK
Jan	— <sup>a</sup>	—	—	—	—	—	—	—	—	—
Feb	—	—	—	—	—	—	—	—	—	—
Mar	—	—	—	—	—	—	—	—	—	—
Apr	—	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—	—
Jun	0.12	0.08	nd <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd
Jul	0.22	0.08	nd	nd	nd	nd	nd	0.51	nd	0.30
Aug	0.06	0.08	nd	nd	nd	nd	nd	0.45	0.69	0.31
Sept	0.21	nd	nd	nd	nd	nd	nd	0.21	nd	nd
Oct	0.35	0.12	nd	nd	nd	nd	nd	0.47	nd	nd
Nov	0.52	nd	nd	nd	nd	nd	nd	0.68	nd	nd
Dec	1.02	0.57	nd	0.57	nd	nd	nd	0.28	nd	nd
Annual mean <sup>c</sup>	0.36 (0.3)	0.19 (0.2)	nd	0.57	nd	nd	nd	0.43 (0.2)	0.69	0.31 (0.0)

<sup>a</sup> — = Not sampled. <sup>b</sup> nd = Not detected (see ref. 15 for MDL). <sup>c</sup> Mean ( $\pm 1$  standard deviation).

concentrations of HHCB (32.6 to 97.9  $\text{ng L}^{-1}$ ), AHTN (21.7 to 49.7  $\text{ng L}^{-1}$ ), ATII (6.9 to 51.0  $\text{ng L}^{-1}$ ), and lower concentrations of AHMI (1.0 to 3.8  $\text{ng L}^{-1}$ ) and ADBI (0.4 to 6.0  $\text{ng L}^{-1}$ ) were detected (see Table 4). HHCB and AHTN were consistently detected in higher concentration relative to the other polycyclic musks, and seemed to increase in concentration in about the same proportion from February through the month of December, except for August and September when slight declines were observed.

STP effluent contained MX and MK the predominant nitro musks in the highest concentrations. MK was detected in higher monthly concentrations (18.0 to 45.7  $\text{ng L}^{-1}$ ) relative to MX (0.1 to 1.6  $\text{ng L}^{-1}$ ) (Table 4). MX shows a sharp increase in concentration from the months of September to December.

The nitro musk metabolites detected in the same STP effluent are 4-AMX, 2-AMX and AMK (Table 4). The annual mean concentration of 4-AMX (13.5  $\text{ng L}^{-1}$ ) was higher than the mean concentrations of AMK (3.2  $\text{ng L}^{-1}$ ) and 2-AMX (1.6  $\text{ng L}^{-1}$ ) by a factor of 4.2 to 8.4 (Table 4). The mean value for 2-AMK (3.2  $\text{ng L}^{-1}$ ) obtained in this study is about one half the 2-AMK value (7  $\text{ng L}^{-1}$ ) obtained for similar studies in the Elbe River in Hamburg, Germany.<sup>21</sup> The concentrations of polycyclic synthetic musk compounds in STP effluent are about one to two orders of magnitude greater than those found in Lake Mead water (Table 5).

#### Synthetic musk compounds in lake mead water

In Lake Mead water, HHCB, AHTN, and ATII were detected in low concentrations with (mean) values of  $0.36 \pm 0.3$ ,  $0.19 \pm 0.2$ , and  $0.57 \text{ ng L}^{-1}$  respectively (Table 5) compared with their concentrations,  $56.9 \pm 26.4$ ,  $34.0 \pm 10.6$ , and  $26.6 \pm 15.0 \text{ ng L}^{-1}$  in STP effluent (Table 4). While no nitro musks were detected in Lake Mead water, some of their metabolites, 4-AMX,

2-AMX, and AMK, were detected in low (mean) concentrations,  $0.43 \pm 0.2$ ,  $0.69$ , and  $0.31 \pm 0.0 \text{ ng L}^{-1}$  respectively (Table 5).

#### Synthetic musk compounds in fish tissue

In the whole fish tissue, five polycyclic musks, HHCB, AHTN, AHMI, ATII, and ADBI were detected in varying concentrations (Table 6). HHCB was detected (mean concentration of  $3.0 \pm 1.0 \text{ ng g}^{-1}$ ) in 98% of the fish samples, while AHTN was detected (mean concentration of  $2.4 \pm 0.8 \text{ ng g}^{-1}$ ) in 99% of the fish samples analyzed. HHCB and AHTN are the two most important products, produced in highest quantities by the fragrance industry.<sup>15</sup> MX, MK, 4-AMX, 2-AMX, and AMK were the nitro musks and nitro musk metabolites detected in the whole fish tissue (Table 6). While MX and MK were detected at annual mean concentrations of  $0.6 \pm 0.2 \text{ ng g}^{-1}$  and  $2.7 \pm 2.2 \text{ ng g}^{-1}$  in the fish tissue respectively, MX and MK metabolites were detected in much higher concentrations (4-AMX, 2-AMX, and AMK mean concentration of  $21.1 \pm 9.2$ ,  $4.4 \pm 2.4$ , and  $10.4 \pm 12.5 \text{ ng g}^{-1}$  respectively) (Table 6).

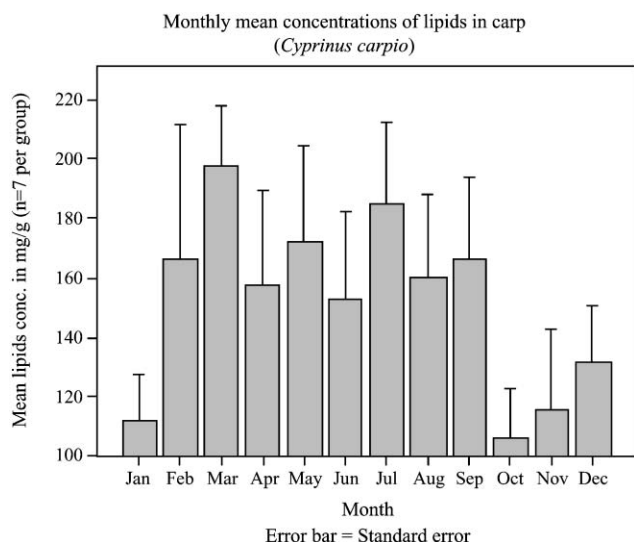
#### Correlation analysis of fish lipid content and synthetic musks concentrations

Correlation analyses of fish lipid content and synthetic musk compound concentrations were done with SAS (Version 8, Cary, North Carolina). The concentrations of synthetic musk compounds detected in fish tissue (wet weight) and lipid content were summarized using the collected raw data. Statistical examination of the raw data showed that the lipid content in carp correlated well with only the stable and highly used polycyclic musk compounds. A significant correlation ( $r = 0.79$ ;  $p = 0.002$ ) between the lipid content and the concentration of HHCB in Lake Mead carp was observed. AHTN concentration also correlated with percent lipid ( $r = 0.58$ ;  $p < 0.05$ ). The concentrations of all other musk compounds are not

**Table 6** Monthly mean concentrations of synthetic musks in Lake Mead carp tissue ( $\text{ng g}^{-1}$ ) ( $n = 7$  per month)

Month	HHCB	AHTN	AHMI	ATII	ADBI	MX	MK	4-AMX	2-AMX	AMK
Jan	3.3 (0.8)	3.1 (0.8)	1.3 (0.0)	2.2 (0.6)	1.1 (1.0)	0.5 (0.2)	3.4 (2.0)	24.8 (8.1)	5.8 (3.0)	nd
Feb	4.1 (1.4)	3.6 (1.1)	1.4 (0.0)	3.0 (1.1)	nd <sup>a</sup>	0.6 (0.2)	nd	23.3 (10.9)	4.0 (0.7)	4.4 (4.0)
Mar	4.5 (2.4)	3.1 (1.4)	0.7 (0.0)	2.6 (0.9)	0.8 (0.1)	0.6 (0.1)	3.3 (1.4)	19.6 (11.5)	3.7 (2.4)	3.4 (0.0)
Apr	3.1 (2.3)	2.4 (0.7)	0.5 (0.0)	1.9 (0.6)	0.4 (0.0)	0.7 (0.2)	0.8 (0.3)	15.0 (4.7)	6.1 (1.1)	3.1 (1.8)
May	4.1 (1.7)	3.3 (1.3)	1.8 (0.0)	3.2 (4.1)	1.0 (0.0)	0.9 (0.5)	7.4 (5.8)	20.3 (14.2)	5.2 (1.2)	35.0 (0.0)
Jun	2.7 (1.7)	1.8 (1.1)	1.0 (0.8)	1.8 (1.2)	0.9 (0.0)	0.6 (0.2)	2.3 (0.5)	20.8 (11.0)	2.6 (1.0)	4.7 (0.0)
Jul	3.4 (1.1)	2.6 (0.5)	nd	2.4 (1.4)	1.4 (1.2)	0.7 (0.2)	2.8 (1.9)	20.4 (10.5)	4.5 (1.0)	nd
Aug	2.7 (1.4)	2.0 (0.7)	nd	nd	nd	0.5 (0.2)	0.5 (0.0)	35.7 (0.0)	nd	12.0 (0.0)
Sept	3.4 (1.7)	2.7 (1.7)	1.5 (1.4)	4.0 (3.1)	2.7 (0.0)	0.9 (0.1)	5.6 (2.0)	39.6 (4.5)	10.0 (1.7)	nd
Oct	1.5 (0.5)	1.4 (0.4)	1.0 (0.3)	2.0 (1.8)	nd	0.7 (0.2)	2.1 (0.7)	16.9 (18.7)	2.9 (1.2)	nd
Nov	1.4 (0.6)	1.4 (0.3)	0.7 (0.0)	2.8 (1.3)	0.5 (0.0)	0.5 (0.1)	0.4 (0.0)	9.3 (6.7)	1.7 (0.6)	nd
Dec	1.9 (0.6)	1.7 (1.1)	nd	1.2 (0.5)	0.6 (0.3)	0.4 (0.0)	1.3 (1.4)	8.0 (6.3)	2.0 (1.1)	nd
Annual mean <sup>b</sup>	3.0 (1.0)	2.4 (0.8)	1.1 (0.4)	2.5 (1.1)	1.0 (0.7)	0.6 (0.2)	2.7 (2.2)	21.1 (9.2)	4.4 (2.4)	10.4 (12.5)

<sup>a</sup> nd = Not detected (see ref. 18 for MDL). <sup>b</sup> Mean ( $\pm 1$  standard deviation).



**Fig. 2** Monthly mean concentrations of lipids in Lake Mead carp and standard error.

correlated with percent lipid to the  $p < \text{or} = 0.05$  level. Fig. 2 shows the monthly variation in the lipid content ( $\text{mg lipid g}^{-1}$  fish tissue) in carp (*Cyprinus carpio*).

#### Cross-correlation of synthetic musks in fish tissue

A cross-correlation analysis of synthetic musk concentrations in fish tissue was performed using SAS (Table 7). The concentration of 4-AMX tends to increase linearly in the tissue of fish as 2-AMX in fish increases. The linear positive correlation coefficient ( $r$ ) was found to be 0.83 and  $p < 0.01$  (Table 7). This is not surprising because the parent compound, MX, is probably being metabolically reduced by the nitroreductases in the fish liver to produce 4-AMX and 2-AMX metabolites.<sup>22</sup> A strong correlation was found between MK and MX ( $r = 0.81$ ;  $p < 0.01$ ). This may be due to the fact that both MX and MK are present in similar products, such as detergents and cosmetics.<sup>23</sup> Cosmetics, bath soap, and detergents are discharged daily into the domestic sewage system. Their high use and discharge, and their high  $n$ -octanol-water partition coefficients suggest that these two compounds may partition similarly into the lipid contents of biological tissue. The correlation of AHTN and MK ( $r = 0.78$ ;  $p < 0.01$ ), and 2-AMX and ADBI ( $r = 0.90$ ;  $p < 0.01$ ) are not surprising for similar reasons. For 2-AMX and ADBI, their correlation may be attributed to the biotransformation of MX in the fish tissue, and the direct partitioning of ADBI from Lake Mead water into fish tissue. There seemed to be a very strong linear correlation between AHMI and MK ( $r = 0.96$ ;  $p < 0.001$ ), and HHCB and AHTN ( $r = 0.96$ ;  $p < 0.001$ ). Similarly, a correlation is observed with HHCB and MK ( $r = 0.75$ ;  $p < 0.05$ ), ATII and AHMI ( $r = 0.77$ ;  $p < 0.05$ ), AHTN and AHMI ( $r = 0.67$ ;  $p < 0.05$ ),

4-AMX and MX ( $r = 0.68$ ;  $p < 0.05$ ), 2-AMX and AHMI ( $r = 0.67$ ;  $p < 0.05$ ) and AMK and MX ( $r = 0.89$ ;  $p < 0.05$ ) (Table 7). The correlation of MX and 4-AMX is expected because 4-AMX is a major metabolite of MX in biological tissues.<sup>22</sup> For MK, the acetyl group is assumed to make the metabolism and elimination of MK in fish tissue easier than for other nitro musk compounds.<sup>1</sup> The same ease of metabolism and elimination may apply to most polycyclic musk compounds, since most possess the acetyl functional group. The metabolism rate of MK and AHMI may be similar, and hence the concentrations correlate. HHCB and AHTN are both used together in a certain proportion in fine-fragrances.<sup>24</sup> Carp tissue concentrations of HHCB correlated strongly with those of AHTN, with HHCB values being slightly higher than AHTN ( $r = 0.96$ ;  $p < 0.001$ ) (Table 7). The presence of 9 out of 15 (60%) of the musk compounds in carp tissue, and their similar rate of accumulation, irrespective of their different classes, may also be attributed to their concentrations in the original products and the high use and discharge of those products into the environment.

#### Regression analysis results

Regression analysis was performed, using AXUM (Version 7, Cambridge, Massachusetts). The regression analysis of the concentrations of synthetic musk compounds from the confluence of three STP effluents versus musk compounds in Lake Mead water showed a significant linear relationship for HHCB with coefficient of determination ( $r^2 = 0.78$ ;  $p = 0.01$ ). There seems to be a positive relationship between Tonalide<sup>®</sup> concentration in STP effluent and Lake Mead water ( $r^2 = 0.73$ ;  $p = 0.06$ ). A strong positive relationship was also observed between the concentrations of 4-AMX in Lake Mead water and in STP effluent ( $r^2 = 0.85$ ;  $p = 0.01$ ). However, there was no significant relationship between synthetic musk concentrations in Lake Mead water and in carp tissue. There is evidence of a relationship between HHCB in STP effluent and in carp tissue ( $r^2 = 0.65$ ;  $p = 0.03$ ). We expected a positive correlation from the regression analysis of municipal effluent vs. whole fish, but experimentally we observed a negative correlation. One possible explanation for this could be that the concentrations in biota and in the aqueous phase are not in equilibrium all of the time. A change in the concentration in the aqueous phase (the source or effluent) may not reflect instant corresponding change in the concentrations in biota in the effluent receiving waters, if the data for effluent and biota samples from the same month are selected for analysis. This may be due to the slow elimination process of hydrophobic compounds in carp. Alternatively, the limited number of experimental data points may not be sufficient to elucidate this relationship. Similarly, there seems to be a stronger relationship between MX in STP effluent and in carp tissue ( $r^2 = 0.83$ ;  $p = 0.03$ ). There was no statistically significant relationship between all other synthetic musk compounds in STP effluent and in carp tissue.

**Table 7** Cross-correlation of synthetic musks in fish tissue, using data from 12 months values shown are the correlation coefficients ( $r$ )

	MX	MK	HHCB	AHMI	ADBI	ATII	AHTN	4-AMX	2-AMX	AMK
MX	—	—	—	—	—	—	—	—	—	—
MK	0.81 <sup>b</sup>	—	—	—	—	—	—	—	—	—
HHCB	0.46	0.75 <sup>a</sup>	—	—	—	—	—	—	—	—
AHMI	0.51	0.96 <sup>c</sup>	0.67	—	—	—	—	—	—	—
ADBI	0.57	0.61	0.41	0.60	—	—	—	—	—	—
ATII	-0.10	-0.29	-0.06	0.77 <sup>a</sup>	0.43	—	—	—	—	—
AHTN	0.35	0.78 <sup>b</sup>	0.96 <sup>c</sup>	0.67 <sup>a</sup>	0.37	-0.14	—	—	—	—
4-AMX	0.68 <sup>a</sup>	0.64	0.53	0.38	0.75	0.49	0.49	—	—	—
2-AMX	0.44	0.43	0.48	0.67 <sup>a</sup>	0.90 <sup>b</sup>	0.50	0.39	0.83 <sup>b</sup>	—	—
AMK	0.89 <sup>a</sup>	0.91	0.48	0.69	0.66	0.08	0.32	0.47	0.18	—

<sup>a</sup>  $p < 0.05$ . <sup>b</sup>  $p < 0.01$ . <sup>c</sup>  $p < 0.001$ .

The lack of a statistically significant relationship of MK, 4-AMX, and AHTN in STP effluent and in carp collected from Lake Mead may be due to microbial degradation in the effluent stream, and biotransformation in carp tissue.

The estimations of the levels of musk compounds in biota (carp, *Cyprinus carpio*) using levels determined in real world municipal sewage treated effluent for both Galaxolide<sup>®</sup> and musk xylene were found to be statistically significant ( $r^2 = 0.65$ ;  $p = 0.03$ ) and ( $r^2 = 0.83$ ;  $p = 0.03$ ) respectively, both with negative slopes. Attempts to correlate the levels of all other musk compounds in sewage treatment effluent with those in carp proved futile.

## Discussion

Galaxolide (a polycyclic musk) and musk xylene (a nitro musk) are both produced in high volumes by the fragrance industry.<sup>15</sup> Since they represent both classes of musk compounds and are ubiquitous in the environment, they may serve as the best candidates for environmental monitoring. The low concentrations of musk compounds in the lake water, compared with their concentrations in carp, suggests they potentially sorb to either the lake sediments or to the suspended particulate matter, including algae. Since carp are bottom dwellers, the high concentrations of musk compounds found in the carp tissue relative to those found in the lake water suggests bioconcentration. The presence of HHCB, AHTN, and MX in all monthly fish samples, suggests their relative resistance to abiotic (*i.e.*, photolysis and hydrolysis) and biotic (biotransformation) degradation.

The low concentrations of musk compounds found in Lake Mead, compared with the levels found in the municipal sewage effluent may be due in part to the dilution of the discharge of the combined STPs effluent, along with their synthetic musk loadings, into Lake Mead. This lake has the largest surface area of any reservoir in the northern hemisphere.<sup>25</sup> The effluent volume so discharged, constitutes only 1.37% of the total lake volume.<sup>25</sup> It may also be attributed to the following physical or chemical processes: 1) volatilization, or sorption onto suspended particulate matter that settles to form sediments; 2) abiotic reduction by reducing agents present in the effluent; and 3) potential biological incorporation of musks into algae cells or other aquatic fauna and flora. These complexities may not be accounted for in a laboratory setting. Therefore, to predict the concentrations of these compounds in biota (carp), using values obtained from the source (sewage effluent), a real-world determination of musk compound concentrations in the source, receiving water, and fish tissue was necessary.

### Synthetic musk concentrations (ng L<sup>-1</sup>) in lake mead: a predictive equation

Based on our experimental data, we developed correlation equations [eqn. (1) and (2)] that may have predictive values, under the assumption that the concentration-altering processes of HHCB and 4-AMX in STP effluent, Lake Mead water, and sediment, would remain fairly constant over the next several years.

$$\frac{\text{HHCB in lake (ng L}^{-1}\text{)}}{\text{HHCB in STP effluent}} \times 0.01 - 0.24; r^2 = 0.78 \quad (1)$$

$$\frac{\text{4-AMX in lake (ng L}^{-1}\text{)}}{\text{4-AMX in STP effluent}} \times 0.02 + 0.16; r^2 = 0.85 \quad (2)$$

In making predictions based on eqn. (1) and (2), the following restrictions must be taken into consideration: 1) the above equation should only be used to make predictions about the receiving water in the geographical location from which the samples were originally collected; 2) the equations

should be used only over the sample concentration domain intervals shown in Tables 5 and 6. Any input variable outside the values shown in Tables 5 and 6 may yield questionable results; and 3) any change in the methods and procedures used for wastewater treatment at any of the three sewage treatment plants renders this predictive equation questionable.

### Synthetic musk concentrations in fish: a predictive equation

Because of the low  $Y$ -intercepts ( $b_0$ ), and negative slopes that were derived from our regression analyses of the collected data (concentration of musks in carp vs. concentration in effluent), we could not provide convincing predictive equations that may be used to predict future concentrations of musk compounds in carp relative to the source.

## Conclusion

Based on the tables, graphs, and text of this paper and the collected data, the following conclusions may be drawn:

This study confirmed the presence of polycyclic and nitro musks in STP effluent, Lake Mead water, and carp. The concentrations of the polycyclic and nitro musks found in Lake Mead carp were considerably lower than previous studies in Germany, other European countries, and Japan. The carp samples were found to have mostly the mono-amino-metabolites of the nitro musks and intact polycyclic musks, principally Galaxolide<sup>®</sup> and Tonalide<sup>®</sup>. Finally, the determination of sufficiently high levels of Galaxolide<sup>®</sup> and 4-amino musk xylene concentrations in STP may be used to infer the presence of trace levels of other classes of musk compounds in the lake water.

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