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Occurrence of Pharmaceuticals and Personal Care Products in German Fish Tissue: A National Study

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Supporting Information

ABSTRACT: German Environment Specimen Bank (GESB) fish tissue samples, collected from 14 different GESB locations, were analyzed for 15 pharmaceuticals, 2 pharmaceutical metabolites, and 12 personal care products. Only 2 pharmaceuticals, diphenhydramine and desmethylsertraline, were measured above MDL. Diphenhydramine (0.04−0.07 ng g^{-1} ww) and desmethylsertraline (1.65−3.28 ng g^{-1} ww) were measured at 4 and 2 locations, respectively. The maximum concentrations of galaxolide (HHCB) (447 ng g^{-1} ww) and tonalide (AHTN) (15 ng g^{-1} ww) were measured at the Rehlingen sampling site in the Saar River. A significant decrease in HHCB and AHTN fish tissue concentrations was observed from 1995 to 2008 at select GESB sampling sites (r^2 = 0.69−0.89 for galaxolide and 0.89−0.97 for tonalide with $p < 0.003$). Galaxolide and tonalide fish tissue concentrations in Germany were ∼19× and ∼28× lower, respectively, as compared to fish tissue concentrations measured in a United States nationwide PPCP study conducted in 2006. Proximity of the sampling locations to the upstream wastewater treatment plant discharging point and mean annual flow at the sampling location were found to significantly predict galaxolide and tonalide fish tissue concentrations (HHCB: r^2 = 0.79, p = 0.021 and AHTN: r^2 = 0.81, p = 0.037) in Germany.

INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) are increasingly used for human and animal applications. PPCPs and their metabolites are typically introduced to the environment through wastewater treatment plant (WWTP) effluent.[1−4] The continuous loading of PPCPs to aquatic systems by effluent discharges causes PPCPs to behave as pseudopersistent despite their short environmental half-life.[5−7] As a result, PPCPs and their metabolites have been measured in surface waters, sediments, and groundwater at ng L^{-1} to μg L^{-1} levels.[8]

Select PPCPs have shown to accumulate in fish tissue collected from surface waters receiving effluent discharges.[9,10] PPCPs have been measured in different fish tissues such as fillet, liver, blood plasma, and brain.[10−17] For example, the pharmaceuticals fluoxetine, sertraline (STL), carbamazepine (CBZ), diltiazem, diphenhydramine (DPH), and gemfibrozil, and PCPs galaxolide (HHCB), tonalide (AHTN), and triclosan, were recently reported in fish from five U.S. rivers in the U.S. Environmental Protection Agency's national pilot study of PPCPs in fish tissue.[10] Previous PCPs studies have measured synthetic musk,[18] alkylphenols and their monoethoxylates,[19] and triclosan and one of its metabolites,[20] in the German Environment Specimen Bank (GESB) fish tissue samples collected from 1994 to 2003. However, a more comprehensive screening study including pharmaceuticals in GESB fish tissue has not been reported.

The primary objective of this research was to determine PPCPs fish tissue concentrations from 13 GESB river sampling sites and one GESB lake site. Spatial distribution of select PPCPs in fish was examined. PPCPs fish tissue concentrations from this study were compared to those of previous studies in Germany and the United States. Finally, predictors of PPCPs in GESB fish tissue concentrations were explored.

EXPERIMENTAL SECTION

Sampling Locations. Representative bream (*Abramis brama*) fish samples were collected in 2007−2008 at 14 different GESB sampling locations (Supporting Information (SI), Figure S1).[19,20] Sampling locations include 13 river sites

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Figure 1. Distribution of HHCB and AHTN concentrations in fish tissue (n = 2) at GESB sampling locations in 2008. HHCB = galaxolide; AHTN = tonalide; MDL = method detection limit; lw = lipid weight.

located downstream from WWTP discharges along the Rhine, Danube, and Elbe rivers and their tributaries in Germany. Sampling location names include the actual location name followed by the river name, for example, Weil/Rhine describes the Weil site along the Rhine River. The river sites include Weil/Rhine, Iffezheim/Rhine, Bimmen/Rhine, Ulm/Danube, Kelheim/Danube, Jochenstein/Danube, Prossen/Elbe, Barby/Elbe, Blankenese/Elbe, Güdingen/Saar, Rehlingen/Saar, Wettin/Saale, and Dessau/Mulde. Fish were also collected from Lake Belau, a reference lake site, which does not receive WWTP effluent. Bream were sampled according to the standard procedures of the GESB.[21] About 20 healthy individuals of mixed sex and of a defined age class (8−12 years) are collected annually at well-characterized sites after the spawning period.[22,23] Subsamples of pooled fillet samples prepared by cryo-milling are provided for analysis. Bream are secondary consumers in the limnic food web[22,24] and differences in lipid content reflect different nutrient availability (e.g., high in large rivers like the Rhine, low at Lake Belau).[25,26] Generally, unless different distribution information is available, data normalization to total lipid content is preferred.[27] Thus it is assumed that for lipophilic compounds the lipid-normalized concentration is a good measure for comparisons between sites. Percentage lipid contents, ranging from 1.79 to 7.51% (Table S8), were obtained from the GESB database.[23] Previous studies revealed the potency of GESB bream samples for retrospective investigation of temporal and spatial trends of lipophilic compounds.[19,20] For this study, duplicate subsamples were retrieved from the archive and shipped on dry ice to Baylor University where they were stored at −80 °C prior to PPCPs analysis. The cold chain was maintained throughout all steps.

Chemical Analysis. Target analytes, surrogates, internal standards, and derivatizing agent are listed in the SI. Analytical methodologies used for the analysis of PCPs[28] and pharmaceuticals[29] in fish tissue have been previously described. Briefly, PCPs were analyzed using ∼2.5 g wet weight (ww) fish fillet composite homogenized with ∼65 g of anhydrous Na_2SO_4. Fish tissue homogenates were fortified with isotopically labeled PCPs standards and were extracted using a pressurized liquid extraction combined with silica gel cleanup technique. PCPs target analytes were separated from high molecular weight interferences using gel permeation chromatography. Eluates were concentrated, derivatized, fortified with internal standard, and analyzed for 12 PCPs using a gas chromatography system coupled with ion trap tandem mass spectrometry. Quality assurance and quality control data are provided in Tables S1−S6.

Pharmaceuticals were analyzed using ∼1 g ww of fish fillet composite fortified with isotopically labeled internal standards prior to extraction.[29] Extracts were analyzed for 17 pharmaceuticals using isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC system was coupled to a triple-quadrupole mass analyzer via an electrospray ionization interface. Labeled and unlabeled structures, precursor and product ions, and optimal collision energies of monitored pharmaceuticals are reported elsewhere.[29]

Statistical Analysis. Influences of WWTP effluent discharges on sample locations, and hence PCPs fish tissue concentration, were investigated in terms of proximity of the sampling locations (PSL, km) to the upstream WWTP effluent discharge point, mean annual flow (MAF, $m^3\ s^{-1}$) of the river at the sampling locations, and total WWTP capacity inhabitants equivalent (CIE) (Table S7). PSL has been previously identified as a predictor of water and fish tissue concentrations for alkylphenols[30] as well as fluoroquinolone water concentrations.[31] PPCP water concentrations can be proportional to the discharge capacity of WWTP effluents[32] and instream flows of the receiving waterbodies.[32,33] In this study, MAF at the

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sampling locations was selected as a surrogate for the mean flow of the receiving system at WWTP discharge point. Though MAF was the most robust parameter available for these locations, this analysis may not include additional dilution associated with minor tributaries located between the WWTP and the sampling site. CIE was selected as a potential surrogate for WWTP discharge capacity and was examined at 20, 30, and 50 km upstream. Minimum CIE of respective classes were considered for WWTPs where absolute CIE could not be acquired. The predictive ability of PSL, MAF, and CIE to estimate fish tissue concentrations was examined with linear regression (LR) analysis and multiple linear regression (MLR) analysis utilizing SigmaPlot (Version 11, Systat Software, Inc., San Jose, CA, USA).

RESULTS AND DISSCUSSION

Personal Care Products. PCPs were measured in fish tissue collected from 13 GESB sampling sites that received WWTP effluent. PCPs were not detected in fish tissue collected from Lake Belau. HHCB and AHTN were the only PCPs measured in fish fillet composites. Polycyclic musk fragrances, HHCB and AHTN, were detected at a frequency of 100% and 69%, respectively, in sites receiving WWTP discharges (Figure 1). PCPs concentrations were lipid normalized (Figure S2), and mean HHCB and AHTN concentrations for duplicate samples are reported herein unless stated otherwise. HHCB fish tissue concentrations ranged from 268 to 11 100 ng g^{-1} lipid weight (lw), while AHTN concentrations ranged from 98 to 382 ng g^{-1} lw (Figure 1 and Table S8). HHCB was <MDL (1.6 ng g^{-1} ww) in one of the duplicate fish tissue samples at Dessau/Mulde, and AHTN was <MDL (3.0 ng g^{-1} ww) in one of the duplicate fish tissue samples at Kelheim/Danube, Dessau/Mulde, Prossen/Elbe, and Barby/Elbe. AHTN was not detected in fish tissue collected from Blankenese/Elbe (Table S8).

High frequency of detection and relatively high concentrations of HHCB and AHTN in fish tissues have been previously reported in Germany.[9,18] Frequent measurements of HHCB and AHTN in fish tissue may be due to their relatively high production/consumption volume combined with their unique physical/chemical properties such as high bioconcentration factor (BCF), high solid-water distribution coefficient, and low photo- or biodegradation constants. In 2000, 1427 t of HHCB and 358 t of AHTN were consumed in Europe, which highlights the high production and consumption volume of these compounds.[34] In 1995, the average use per capita of HHCB and AHTN in Europe were 11.1 and 4.40 mg d^{-1}, respectively.[35] Additionally, higher lipophilicity of HHCB and AHTN (Figure S2) comparable to that of some persistent organic pesticides and PCBs (log K_{ow}: HHCB = 5.90, AHTN = 5.70, *p,p′*-DDT = 5.80−6.90, PCB 101 = 6.65) results in relatively higher bioconcentration and bioaccumulation.[36] For example, the BCF values for HHCB and AHTN are 1584 and 597 L kg^{-1}, respectively.[34] In the present study, log K_{ow} values of HHCB and AHTN are the highest among all of the target analytes, except octocrylene,[10] which likely resulted in the highest tissue concentrations of HHCB and AHTN.

The highest HHCB and AHTN fish tissue concentrations were measured in the Saar River at the Güdingen (French−German border) and Rehlingen sites (Figure 1). At Güdingen/Saar and Rehlingen/Saar, HHCB was measured at 9680 ± 601 and 11 100 ± 1270 ng g^{-1} lw, while AHTN was measured at 288 ± 59 and 382 ± 93 ng g^{-1} lw, respectively. The average HHCB tissue concentration for these two sites (10 400 ng g^{-1} lw) was approximately an order of magnitude higher than the average tissue concentrations of the remaining 11 sites (1010 ng g^{-1} lw). Similarly, the average AHTN tissue concentration measured for these two sites (335 ng g^{-1} lw) was 2.8× the average tissue concentration of the remaining seven sites (119 ng g^{-1} lw). Güdingen/Saar and Rehlingen/Saar may be influenced by two WWTPs <7 km and <15 km upstream, respectively (Table S7). The annual flows at Güdingen/Saar and Rehlingen/Saar are ∼60 and ∼80 m^3 s^{-1}, respectively, which are two of the lowest MAFs in this study besides Dessau/Mulde (∼64 m^3 s^{-1}). Higher HHCB and AHTN fish tissue concentrations in the Saar River may be a result of the close proximity of sampling locations to the WWTP discharging points (<7 km) and low MAF (<80 m^3 s^{-1}). HHCB and AHTN fish tissue concentrations in the Saar River (10 400 ng g^{-1} lw) were an order of magnitude lower than fish tissue concentrations reported in a WWTP effluent-impacted pond study in Germany (100 000 ng g^{-1} lw).[9]

HHCB and AHTN fish tissue concentrations in the Rhine River were highest at Iffezheim/Rhine (1740 ± 184 and 156 ± 37 ng g^{-1} lw, respectively). HHCB at Weil/Rhine (Swiss−German border) was lower (1035 ± 106 ng g^{-1} lw) than at Bimmen/Rhine (German−Dutch border) (1370 ± 57 ng g^{-1} lw). However, fish tissue levels of AHTN was higher at Weil/Rhine (124 ± 5.0 ng g^{-1} lw) than at Bimmen/Rhine (80 ± 16 ng g^{-1} lw). Synthetic musk concentrations were measured in fish tissue at Iffezheim/Rhine > Bimmen/Rhine > Weil/Rhine, which suggests no downstream concentration trends. The different PCPs fish tissue concentration may be attributed to variations in local effluent discharge volumes combined with pseudopersistence resulting from continuous discharges to these aquatic systems.

The measured HHCB fish tissue concentrations in the Danube River were 1240 ± 42, 847 ± 260, and 805 ± 202 ng g^{-1} lw at Ulm/Danube, Kelheim/Danube, and Jochenstein/Danube, respectively. AHTN tissue concentrations measured at Ulm/Danube and Jochenstein/Danube were 160 ± 55 and 98 ± 42 ng g^{-1} lw, respectively. At the Kelheim/Danube site, AHTN was measured at 98 ng g^{-1} lw in one of the duplicate samples and <MDL in the other. The distribution of HHCB and AHTN fish tissue concentrations at these three Danube River sites was similar from Ulm/Danube to downstream Jochenstein/Danube (German−Austrian border).

Along the Elbe River, the highest HHCB tissue concentrations were measured near the Czech−German border at Prossen/Elbe (1375 ± 177 ng g^{-1} lw) followed by Blankenese/Elbe (near the mouth of the river) (403 ± 50 ng g^{-1} lw). HHCB fish tissue concentration at Barby/Elbe (268 ± 24 ng g^{-1} lw) was the lowest of the three Elbe River sites. A WWTP in close proximity of Blankenese/Elbe within 4 km (capacity: >1 641 600 m^3 d^{-1} and 2 960 000 IE) may have contributed to the higher tissue concentration of HHCB and AHTN at Blankenese/Elbe than at Barby/Elbe (PSL = 15.6 km, capacity: ∼8100 m^3 d^{-1} and 27 000 IE). AHTN was <MDL in one of the duplicate fish tissue samples at Prossen/Elbe and Barby/Elbe, and was not detected in fish tissue collected from the Blankenese/Elbe site (Figure 1). In the Saale River, a tributary of Elbe, the HHCB and AHTN fish tissue concentrations were 1360 ± 99 and 120 ± 11 ng g^{-1} lw, respectively, at Wettin/Saale. Similarly, HHCB fish tissue concentration in the Mulde River, another tributary of Elbe, was 636 ng g^{-1} lw and <MDL at Dessau/Mulde. HHCB tissue concentrations at Wettin/Saale

and Dessau/Mulde were approximately 5× and 2.4× higher than that at Barby/Elbe, respectively. Based on HHCB fish tissue concentrations and the overall HHCB/AHTN ratio of 15:1 (for this study) the predicted AHTN fish tissue concentrations were $<^1/_2$ MDL at Prossen/Elbe, Barby/Elbe, Blankenese/Elbe, and Dessau/Mulde.

Pharmaceuticals. Desmethylsertraline (DMS) and DPH were the only pharmaceutical analytes measured in fish fillet composites with concentrations ranging approximately from 0.01 to 3.0 ng g^{-1} ww. In this study, pharmaceutical fish tissue concentrations were 1−6 orders of magnitude lower than PCPs concentrations. DMS, an active metabolite of STL, was measured at Ulm/Danube and Güdingen/Saar (Figure 2).

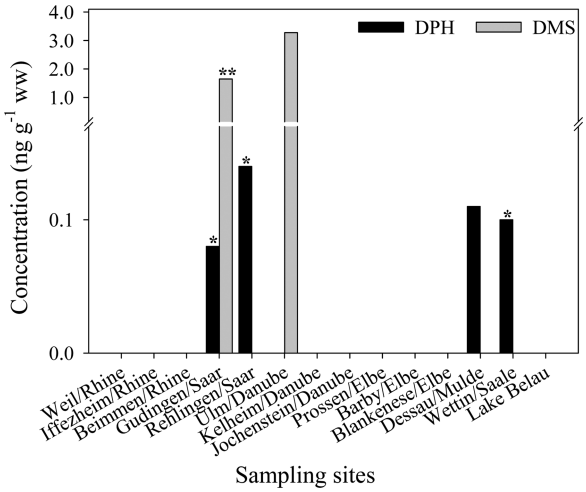

Figure 2. Pharmaceuticals concentrations in fish tissues (n = 2) from various GESB sampling locations. DPH = diphenhydramine; DMS = desmethylsertraline; *DPH measured < MDL in one of the duplicate samples; ** DMS was not detected in one of the duplicate samples.

The mean tissue concentration of DMS at Ulm/Danube was 3.28 ng g^{-1} ww. In Güdingen/Saar, DMS was observed at 1.65 ng g^{-1} ww in one of the duplicate fish tissue samples. DPH was measured at Dessau/Mulde, Rehlingen/Saar, Güdingen/Saar, and Wettin/Saale. The mean tissue concentration of DPH at Dessau/Mulde was 0.055 ng g^{-1} ww. DPH was measured in one of the duplicate samples from Rehlingen/Saar (0.07 ng g^{-1} ww), Güdingen/Saar (0.04 ng g^{-1} ww), and Wettin/Saale (0.05 ng g^{-1} ww) (Figure 2). All other pharmaceutical tissue concentrations were <MDL (Table S8).

Influence of WWTPs on PCPs Fish Tissue Concentrations. Relationships among PSL, MAF, and CIE to estimate HHCB and AHTN fish tissue concentrations in GESB samples were examined using LR and MLR. However, PSL, MAF, and CIE data could not be acquired for the Iffezheim/Rhine, Barby/Elbe, and Prossen/Elbe locations. LR analysis showed no significant relationships between the independent predictor variables (PSL, MAF, and CIE). In addition, no significant relationships were observed between HHCB and AHTN fish tissue concentration with PSL ($r^2 \leq 0.20$ and $p \geq 0.210$), MAF ($r^2 \leq 0.31$ and $p \geq 0.246$), or CIE ($r^2 \leq 0.10$ and $p \geq 0.470$). MLR analysis of HHCB and AHTN fish tissue concentrations with PSL, MAF, and CIE (<50 km) provided r^2 of 0.39 ($p \leq 0.372$) and 0.56 ($p \leq 0.308$), respectively. The same analysis performed using CIE (<30 km) instead of CIE (<50 km) provided r^2 for HHCB of 0.48 ($p \leq 0.325$) and AHTN of 0.56 ($p \geq 0.308$). Replacing CIE (<30 km) with CIE (<20 km) in the same analysis provided similar results (data not shown). Excluding CIE, the least significant predictor variable, from the MLR analysis of HHCB and AHTN fish tissue concentrations with PSL and MAF provided r^2 of 0.44 (p = 0.177) and 0.56 (p = 0.131) (SI). Potential correlations of pharmaceuticals fish tissue concentration to the PSL, MAF, and CIE could not be calculated due to the limited data points.

The MLR was further examined by omitting statistical outliers identified using Cook's distance measurement through SigmaPlot 11. The largest MAF (2000 m^3 s^{-1}) reported at Bimmen/Rhine was a statistical outlier (Cook's distance >3) and was omitted in further MLR analysis. MLR analysis of HHCB and AHTN fish tissue concentrations with PSL, MAF (omitting Bimmen/Rhine), and CIE (<50 km) provided r^2 of 0.59 ($p \leq 0.182$) and 0.59 ($p \leq 0.069$), respectively. The overall regression was improved again by replacing CIE (<50 km) with CIE (<30 km), which provided r^2 for HHCB of 0.79 (p = 0.075) and AHTN of 0.81 (p = 0.129) (SI). MLR analysis of HHCB and AHTN fish tissue concentrations with PSL and MAF (omitting Bimmen/Rhine), while excluding CIE, provided the highest r^2 values with p values <0.05 (eqs 1 and 2, respectively). Despite the fact that r^2 values were similar when CIE was included as a predictor variable along with PSL and MAF, the corresponding p-values increased from <0.05 to >0.05.

$$\text{HHCB} = 9854 - (629*\text{PSL}) - (9.47*\text{MAF}) \quad (r^2 = 0.79,\ p = 0.021) \tag{1}$$

$$\text{AHTN} = 334.0 - (14.9*\text{PSL}) - (0.26*\text{MAF}) \quad (r^2 = 0.81,\ p = 0.037) \tag{2}$$

PSL and MAF were found to be significant predictors of HHCB and AHTN fish tissue concentrations in GESB fish tissues examined in this study. Approximately 79% of the variability in spatial distribution of HHCB fish tissue concentrations (p = 0.021) and 81% of that of AHTN (p = 0.037) in German rivers was predicted by PSL and MAF (eqs 1 and 2). The negative coefficients of PSL and MAF in MLR analyses indicate that the predicted HHCB and AHTN fish tissue concentrations are higher at the sampling locations that are closer to the WWTP effluent discharges and in rivers with low MAF. PSL has also been shown to be a inversely related to alkylphenols fish tissue concentrations.[30] Based on known BCF and BSAFs, higher PCP fish tissue concentrations at sampling sites near WWTP effluent discharge points with low MAF suggest that these sites may also have increased PCP water and/or sediment concentrations as compared to other sampling sites.

Comparison with the Historical GESB PCP Measurements. Temporal relationships of HHCB and AHTN fish tissue concentrations at 14 GESB sampling locations were examined by comparing tissue concentrations from the current study (samples collected in 2008) with historical measurements (1995−2003).[18] A significant decrease in HHCB and AHTN fish tissue concentrations was observed from 1995 to 2008 at Barby/Elbe, Wettin/Saale, Rehlingen/Saar, and Weil/Rhine sampling sites (r^2 = 0.69−0.89 for HHCB and 0.89−0.97 for AHTN with p < 0.003) (Figure 3). Environmental halving times for HHCB levels in fish at Barby/Elbe, Wettin/Saale, Rehlingen/Saar, and Weil/Rhine were subsequently calculated to be 3.0, 4.1, 5.9, and 7.0 yr, respectively. Similarly,

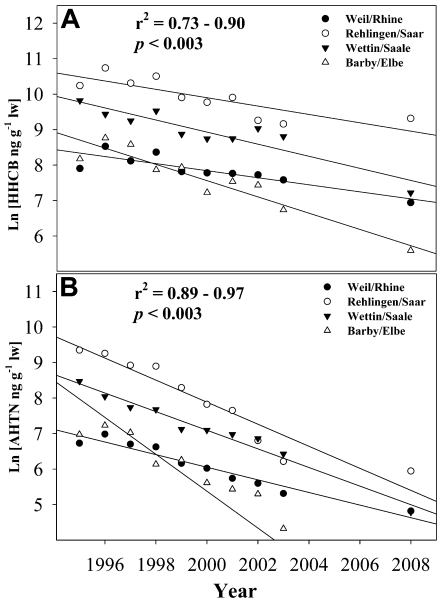

Figure 3. Fish tissue concentrations from this study (2008) and those reported by Rüdel et al. (1995–2003) at Weil/Rhine, Rehlingen/Saar, Wettin/Saale, and Barby/Elbe: (A) HHCB and (B) AHTN.

environmental halving times for AHTN were estimated to be 1.3, 2.6, 2.2, and 3.9 yr at Barby/Elbe, Wettin/Saale, Rehlingen/Saar, and Weil/Rhine, respectively. At these four sites, the rate of dissipation of AHTN in fish tissue is ~2× higher than HHCB. Temporal trends and changes in HHCB/AHTN ratio from 1995–2008 in select GESB sampling locations suggest a change in HHCB and AHTN production and/or consumption pattern. Similar trends in HHCB and AHTN consumption patterns and changes in the HHCB/AHTN ratio, increasing from 2.7 to 4.0 over the period of 1992 to 2000, were observed in Europe.[34]

For samples examined in this study (from 2008), a HHCB/AHTN tissue concentration ratio for the Saar River was ~31 which is ~3× the ratio that was measured at any other GESB sampling location. The HHCB/AHTN ratio measured in fish tissue at Güdingen/Saar from 1995 to 2001 provided a linear relationship with a slope of 0.75 (r^2 = 0.79); however, from 2001 to 2008 there was a noticeable increase in the slope of this relationship to 3.7 (r^2 = 0.98; Figure S3A). A similar change in slope from 1995 to 2001 (0.98; r^2 = 0.85) and 2001 to 2008 (2.8; r^2 = 0.94) was observed at Rehlingen/Saar (Figure S3B). From 2001 to 2003, both Saar sites experienced a 2-fold increase in HHCB/AHTN ratio. Typically, changes in HHCB/AHTN ratios are associated with differences in HHCB and AHTN sorption to sediment and differences in degradation rates such as biodegradation and photodegradation.[34,37,38] In March 2001, a new WWTP began operations at Brebach at the Güdingen/Saar site with a PSL of 37 km downstream to the Rehlingen/Saar.[39] The operation of this new plant improved the overall water quality within the Saarland watershed.[40] In 2001, the HHCB/AHTN ratio in fish tissue measured at Weil/Rhine, Iffezheim/Rhine, Bimmen/Rhine, and Wettin/Saale was 8.9.[18] However, after the new Brebach WWTP began operations in 2001, the HHCB/AHTN ratio in fish tissue from the Saar River increased from 8.4 in 2001 to ~31 in 2008. Excluding the Saar sampling locations, the mean HHCB/AHTN ratio was ~10 in 2008. This observation suggests that the new Brebach WWTP potentially contributed to the noticeable change in HHCB/AHTN ratio measured in fish tissue at both Güdingen/Saar and Rehlingen/Saar.

Observations of the present study are similar to findings of retrospective monitoring of synthetic musks by Rüdel et al.[18] For example, the HHCB tissue concentrations were measured in the order of $HHCB_{Saar}$ > $HHCB_{Saale}$ > $HHCB_{Rhine}$ > $HHCB_{Elbe}$ in both studies. In case of AHTN, tissue concentrations were measured in the order of $AHTN_{Saar}$ > $AHTN_{Saale}$ > $AHTN_{Rhine}$ in both studies. However, AHTN was not measured in the Elbe River in this study. In the Rhine River, the highest concentration of HHCB and AHTN were found at Iffezheim/Rhine in both studies. Similarly, higher level of HHCB was measured at Blankenese/Elbe than at Barby/Elbe in the Elbe River.

Besides HHCB and AHTN, fish tissue concentration of celestolide, musk ketone, and musk xylene have also been measured previously in GESB samples from the Rhine, Saar, Elbe, Saale, and Mulde rivers (1993–1999).[18] In this study, temporal relationships for celestolide, musk ketone, and musk xylene were examined at Prossen/Elbe, Barby/Elbe, Rehlingen/Saar, and Weil/Rhine. At these sites, celestolide tissue concentrations were observed decreasing from 1993 to 1999 with r^2 values ranging from 0.78 to 0.99 (p = 0.005 – 0.119). The calculated environmental halving times for celestolide fish tissue concentrations at Prossen/Elbe, Barby/Elbe, Rehlingen/Saar, and Weil/Rhine were 2.8, 3.2, 3.8, and 8.5 yr, respectively. Celestolide regressions were then extrapolated to 2008 for the four sites. Predictive tissue concentrations ranged from 0.57 to 4.0 ng g^{-1} lw, which are <MDL in this study. Musk ketone and musk xylene tissue concentrations were also observed decreasing from 1993 to 1999 at Prossen/Elbe, Barby/Elbe, Rehlingen/Saar, and Weil/Rhine (r^2 = 0.57–0.95, p = 0.026–0.266). These regressions were also extrapolated to 2008, and musk ketone and musk xylene tissue concentrations were predicted to be <MDL (Table S2).

Though alkylphenols ethoxylates were not observed in the present study, alkylphenols, including *p*-octylphenol, *p*-nonylphenol, and their ethoxylates, were previously quantified in GESB fish samples (1993–2001).[19] There was a significant decrease in *p*-nonylphenol tissue concentrations at Barby/Elbe (r^2 = 0.86 and p = 0.002) and Weil/Rhine (r^2 = 0.67, p = 0.046) from 1993 to 2001, with environmental halving times of *p*-nonylphenol fish tissue concentrations of 2.9 and 5.3 yr, respectively. Again, regressions were extrapolated to 2008 and predicted the *p*-nonylphenol tissue concentrations of 1.0 and 0.9 ng g^{-1} ww at Barby/Elbe and Weil/Rhine, respectively, which are similar to our MDL of 1.2 ng g^{-1}. There was a significant decrease in *p*-octylphenol tissue concentrations at Barby/Elbe from 1993 to 2001 (r^2 = 0.70, p = 0.02) with environmental halving times of *p*-octylphenol fish tissue concentrations of 3.5 yr. Regression was extrapolated to 2008 and predicted a *p*-octylphenol tissue concentration of 2.7 ng g^{-1} ww, which is similar to our MDL of 3.1 ng g^{-1}.

Comparison with the USEPA National Pilot Study. PPCP fish tissue levels from the present study (collected in 2008) in Germany were also compared with a similar PPCP national study in the United States (2006) (Figure 4).[10] Ramirez et al. reported PPCP fish tissue concentrations in five major WWTP effluent-dominated U.S. streams,[10] which represent worst case scenarios for studying PPCP accumulation in fish.[41] Mean HHCB fish tissue levels in the United States (48 700 ng g^{-1} lw) were ~19 times than that observed in Germany (excluding nondetects at Lake Belau). Similarly, mean

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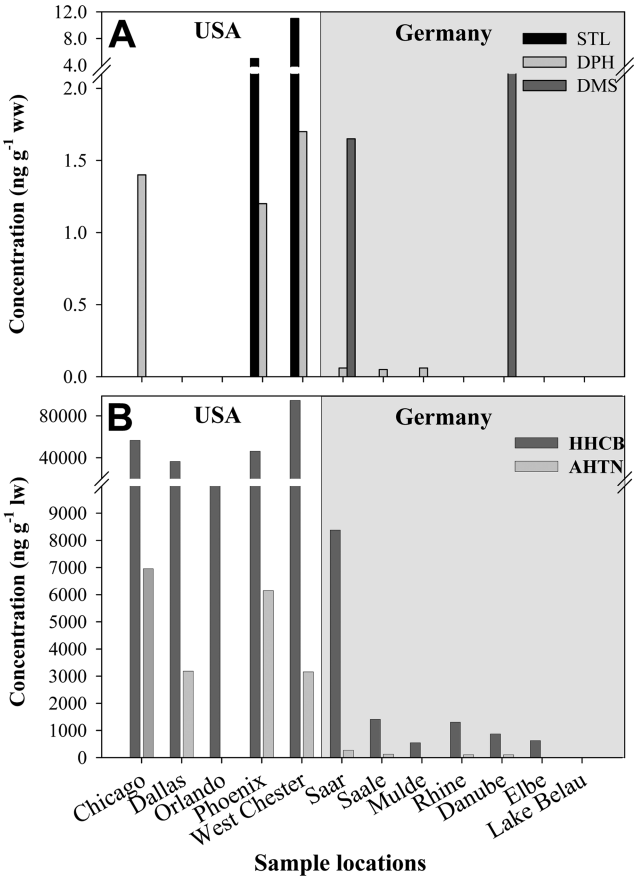

Figure 4. Comparison of PPCPs fish tissue concentrations measured in this study and the USEPA pilot study: (A) pharmaceuticals and (B) personal care products. PPCPs fish tissue concentrations from the USEPA study are presented as site averages from North Shore Channel (Chicago), the Trinity River (Dallas), Little Econlockhatchee (Orlando), the Salt River (Phoenix), and Taylor Run (West Chester).[10] PPCPs fish tissue concentrations from this study are presented as river average concentrations. STL = sertraline; DPH = diphenhydramine; DMS = desmethylsertraline; HHCB = galaxolide; AHTN = tonalide. HHCB and AHTN plotted data are lipid normalized. No bar is shown where the tissue concentrations were < MDL and not detected.

AHTN tissue concentration (4860 ng g^{-1} lw) in the United States (excluding nondetects at Little Econlockhatchee, Orlando) was ~28 times greater than that observed in Germany (excluding nondetects at Elbe, Mulde, and Lake Belau). In fact, HHCB and AHTN fish tissue concentrations from the United States[10] were similar to fish tissue levels measured in the Saar River, Germany a decade earlier.[18] However, HHCB and AHTN fish tissue concentration have been decreasing over the past decade in the Saar River (Figure 4B). Though HHCB and AHTN production/consumption volumes in Europe from 1995 to 2000 decreased by 4 and 39%, respectively,[34] production/consumption volume of polycyclic musks increased by ~25% from 1996 to 2000 in the United States.[42] HHCB and AHTN represent ~90% of the United States polycyclic musk market.[43] Thus, the different HHCB and AHTN fish tissue concentrations in Germany and the United States may be influenced by these production/consumption patterns; however, a more comprehensive comparison of Germany and the United States production/consumption patterns could not be obtained due to limited polycyclic musk market data.

DPH was the only pharmaceutical measured in the U.S. and German pilot studies (Figure 4A). Similar to observations for musks, levels of DPH in fish tissue (1.2−1.7 ng g^{-1} ww) from the U.S.[10] were 17 times greater than those observations of the present study from Germany (0.04−0.07 ng g^{-1} ww). DMS, a relatively stable metabolite of STL, was not included in the U.S. study, but was measured in Germany with a mean tissue concentration of 3.28 ng g^{-1} ww at Ulm/Danube and 1.65 ng g^{-1} ww in one of the duplicate samples at Güdingen/Saar. These concentrations are comparable to levels of DMS previously reported by Brooks et al.,[11] which subsequently stimulated a number of recent studies of the consequences of fish bioaccumulation of SSRIs, their active metabolites, and other PPCPs.[16]

Because thresholds of pharmaceutical exposure resulting in adverse effects on fish and other wildlife are poorly understood, particularly when chronic responses resulting from therapeutic modes/mechanisms of action are examined,[44] several groups have explored use of human therapeutic concentrations (e.g., C_{max} values) to potentially identify adverse thresholds of exposure for fish models.[13,16,45−47] For example, Fick et al. observed that when fish were exposed to effluent discharges, a number of pharmaceuticals accumulated in fish plasma, and levongestrel levels exceeded human therapeutic dose levels.[45] Further, Valenti et al. recently coupled physiological based pharmacokinetic modeling and adverse outcome pathway approaches to predict bioaccumulation and adverse effects of sertraline in fish.[47,48] When sertraline accumulation in fish plasma reached human therapeutic plasma concentrations, the pharmacological target of SSRIs and ecologically relevant behaviors were significantly altered.[47]

Though human therapeutic doses vary over 8 orders of magnitude with 10% of pharmaceuticals possessing C_{max} values below 0.00347 μg mL^{-1},[16] more potent pharmaceuticals are expected to correspondingly exhibit higher potency in fish.[49] A recent prediction suggested that 10% of pharmaceuticals may be expected to accumulate to human therapeutic levels in fish at the environmentally relevant surface water concentration of 29 ng L^{-1}.[50] Thus, it appears clear that additional studies relating bioaccumulation to toxicological thresholds are necessary to support future environmental risk assessments of PPCPs in aquatic systems. In fact, a recent international horizon scanning exercise identified relationships between bioaccumulation and ecological effects as major areas of research need for understanding the risks of PPCPs in the environment.[51]

■ ASSOCIATED CONTENT

Supporting Information

Text, tables, and figures describing GESB sample locations, chemicals and reagents, quality assurance and quality control data, and lipid determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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