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Chemosphere 57 (2004) 987–996

CHEMOSPHERE

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Considerations about the enantioselective transformation of polycyclic musks in wastewater, treated wastewater and sewage sludge and analysis of their fate in a sequencing batch reactor plant

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Received 23 September 2003; received in revised form 7 July 2004; accepted 14 July 2004

Abstract

The present work consists of two distinct parts: in the first part enantioselective GC was used to separate the different enantiomeric/diastereomeric polycyclic musks, PCMs (HHCB, AHTN, AHDI, ATII and DPMI) including the main transformation product of HHCB, HHCB-lactone, in wastewater and sewage sludge. After optimization all PCMs were resolved on a cyclodextrin containing Rt-BDEXcst capillary GC column. Enantiomeric ratios of PCMs in a technical mixture were determined and compared to those obtained from enantioselective separation of wastewater and sewage sludge samples. In general, enantiomeric ratios were similar for most materials in influent, effluent and stabilized sewage sludge. However, the ratios for HHCB, AHDI and particularly ATII suggest some stereospecific removal of these compounds. In the second part, a field study was conducted on a wastewater treatment plant comprising a sequencing batch reactor. Concentrations of HHCB, AHTN, ADBI, AHDI, ATII, DPMI and HHCB-lactone were determined by non-enantioselective GC in daily samples of influent, effluent and activated sludge during one week. Mean concentrations in influent were 6900 and 1520 ng/l for HHCB and AHTN, respectively. The other PCMs exhibited contents $\leqslant$200 ng/l. Mean percent removal was between 61% (AHDI) and 87% (HHCB) resulting in mean effluent concentrations below 860 ng/l. HHCB-lactone concentration increased during wastewater treatment with a mean in the influent of 430 ng/l and in the effluent of 900 ng/l, respectively, indicating a degradation of HHCB.

Keywords: Artificial fragrances; Synthetic musks; HHCB-lactone; Enantioselective GC; Enantioselective biodegradation; Removal

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doi:10.1016/j.chemosphere.2004.07.020

1. Introduction

Polycyclic musks (PCMs) are widely used as fragrance ingredients in washing and cleaning agents, products for personal care and in other consumer products. HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran) and AHTN (1-[5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl]-ethanone) represent the most frequently used compounds. After use of fragrance containing products PCMs are mainly released into wastewater. Due to their lipophilic property with $\log K_{ow}$ values of 5.4–6.3 (Osemwengie and Steinberg, 2001), they are predominantly adsorbed onto suspended matter during wastewater treatment. The ease of analysis, the high consumption volumes and low degradability of PCMs are responsible that these compounds have been detected in treatment plant influent and effluent, in surface and sea water as well as in fish (Bester et al., 1998; Gatermann et al., 1999; Heberer et al., 1999; Rimkus, 1999; Fromme et al., 2001a,b; Dsikowitzky et al., 2002; Heberer, 2002; Gatermann et al., 2002a) and in sediment (Winkler et al., 1998; Dsikowitzky et al., 2002). Additionally, transformation products of HHCB and AHTN have been described in biota samples (Franke et al., 1999; Gatermann et al., 2002b) and HHCB-lactone has recently been quantified in sewage sludge (Kupper et al., 2004). This gives evidence for degradation processes in spite of the low biodegradability of PCMs. Finally, there is still an increasing concern about potential hazards associated with their ubiquitous distribution in the environment.

PCMs contain one (AHTN; AHDI: 1-[2,3-dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl]-ethanone; DPMI: 1,2,3,5,6,7-hexahydro-1,1,2,3,3- pentamethyl- 4H-inden-4-one) or two (HHCB; HHCB-lactone: 1,3,4,6,7,8-hexahydro- 4,6,6,7,8,8- hexamethylcyclopenta[g]-2- benzopyran-1-one; ATII: 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methyl-ethyl)-1H-inden-5-yl]-ethanone) chiral C atoms. ADBI (1-[6-(1,1-dimethylethyl)-2,3-dihydro-1,1-methyl-1H-inden-4-yl]-ethanone) is not chiral. They are used as racemic mixtures in commercial products. Franke et al. (1999) have described an enantioselective and species-dependent transformation of HHCB and AHTN in the aquatic environment. It can therefore be concluded that enantioselective transformation might also occur during wastewater treatment.

The aim of the present study was to develop an enantioselective GC allowing the separation of all PCMs including the main transformation product of HHCB, HHCB-lactone, in order to study the enantiomeric ratios (ERs) in selected wastewater and sewage sludge samples and to compare them to ER in a technical mixture (part 1). Furthermore, a field study on a wastewater treatment plant (WWTP) was conducted to determine the fate of PCMs (part 2).

2. Sample characteristics, materials, methods and field study

2.1. Characterization of WWTPs

Samples of WWTP influent, effluent and sewage sludge for part 1 were obtained from four WWTPs of a monitoring network (Kupper et al., 2004). Information about treatment technology of the WWTPs and treatment performance is given in Table 1.

The field study regarding the fate of PCMs during wastewater treatment (part 2) was conducted on a small rural WWTP called Chevilly. The wastewater treatment comprises a screen, a degritter and a sequencing batch reactor (SBR) (for further description: see Kupper et al., 2004). This plant was chosen due to the high percentage of domestic sewage in influent considered as the

Table 1
Main characteristics of monitoring sites and wastewater treatment plants for 2001

Name	Connected inhabitants	Daily influent (m^3)	Sewage[a] (%)			Sewer system	Wastewater treatment[b]	Sludge processing[c]	Solids retention time (days)	Removal (%)[d]	
			dom	ind	sto					BOD	Ptot
Prahins[e]	214	36	88	0	12	Separate	EA	AES	20	99	95
Echallens[e]	5700	2460	35	5	60	Unitary	AS	ANS	4	98	92
Wohlen[e]	8460	5050	27	0	73	Unitary	AS	ANS	3	90	80
Konolfingen[e]	7860	6320	19	12	69	Unitary	AS	ANS, H	7	90	80
Chevilly[f]	210	60	60	0	40	Separate	SBR	AES	20	99	97

[a] dom: domestic; ind: industrial; sto: stormwater and infiltration water.
[b] EA, extended aeration; AS, activated sludge system and SBR, sequencing batch reactor.
[c] AES, aerobic stabilization; ANS, anaerobic stabilization and H, hygienization.
[d] Ptot, total phosphorus.
[e] Included in part 1 of this study.
[f] Included in part 2 of this study.

main source of PCMs and due to lack of data for removal of PCMs in a SBR.

2.2. Sampling

Samples of WWTP influent, effluent and sewage sludge for part 1 were collected between March and July 2000. The influent sample from WWTP Prahins consisted of one grab sample, the effluent was sampled over 24 h with a peristaltic pump operated time proportional. Sampling of stabilized sewage sludge on the WWTPs was conducted as described in Kupper et al. (2004). All samples were analyzed the day after sampling.

Within the field study on the WWTP Chevilly (part 2), sampling was conducted during one week in April 2001. For sampling the inflow, a flow related sampler (type "ISCO 3710") was used. The sampling pipe was installed at the influent channel in front of the screen. The sampling of the outflow was conducted with a peristaltic pump operated time proportional. For the analysis of the activated sludge 10 grab samples of 3 l each were taken directly from the aeration tank during an aeration period once a day.

Samples were protected from sunlight and cooled immediately after sampling. In the laboratory, the composite water and activated sludge samples were mixed. Aliquots were taken out and stored in the freezer at −20 °C. Samples of stabilized sewage sludge were stored at 4 °C and analyzed within 48 h. No preservatives or stabilizers were added.

2.3. Laboratory contamination with polycyclic musks

PCMs might be present in the laboratory (soaps, hand cream, towels, etc.). Therefore, it is important to remove all possible sources which might contain such compounds in order to avoid any contamination of the environmental samples. To monitor PCM contamination tap water from the laboratory was extracted and analyzed together with each series of wastewater and sludge samples ($n = 6$) using the same glassware, solvents and filters as for the environmental samples. The concentrations of the PCMs detected were always below LOQs (see Section 2.9).

2.4. Extraction of wastewater, treated wastewater and activated sludge

2.4.1. Non-filtered influent and effluent

To 600–700 ml of sample, 20 g of NaCl and 100 ml of *n*-hexane were added. The mixture was vigorously stirred for 2 h at room temperature (RT). The organic phase was decanted, dried over Na_2SO_4 and a few drops of dodecane (keeper) were added. An aliquot of 50–80 ml of the *n*-hexane solution was used for final analysis. This aliquot was concentrated to 5–10 ml by rotary evaporation and further reduced in volume in a gentle stream of N_2. The extract was redissolved in 1 ml isooctane and submitted to GC–MS analysis.

2.4.2. Activated sludge

To 500 ml of homogenized sample (dry weight 1–1.5%), 20 g of NaCl and 200 ml of *n*-hexane were added and the mixture stirred for 2 h at RT. The *n*-hexane phase was decanted, dried over Na_2SO_4 and 50–100 ml taken for the final analysis. This aliquot of solution was prepared for GC–MS analysis as described for wastewater.

2.5. Synthesis of HHCB-lactone

2.5.1. Solvents, chemicals and instruments

Dichloromethane (DCM), *n*-hexane, isopropylether were residue grade obtained from Merck, Dietikon, Switzerland, d_3-chloroform ($CDCl_3$) was spectroscopy grade and was from Fluka. $CuSO_4 \times 5H_2O$, $KMnO_4$ and celite were from Fluka. Silicagel Si60 was from Merck. Instruments: ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer (Bruker Biospin, Rheinstetten, Germany) at 298 K, operating at 500.13 MHz for ^1H. The compound (20–30 mg) was dissolved in $CDCl_3$ and a few drops of $CDCl_3$ doped with tetramethylsilan (TMS) was added to refer ^1H and ^{13}C chemical shifts to this internal standard.

2.5.2. Synthesis and spectroscopic characterization

The HHCB-lactone was synthesized according to Franke et al. (1999) with slight modifications. 2 g (3.87 mMol HHCB) of a 1:1 mixture of racemic HHCB and benzylbenzoate was dissolved in 20 ml of DCM. A mixture of 5 g of $KMnO_4$ and $CuSO_4 \times 5H_2O$ was slowly added to the starting material. The reaction mixture was refluxed for 17 h and then filtered over celite. The residue was washed with 60 ml of DCM and 20 ml of isopropylether and the combined organic phases evaporated to dryness. The pure racemic HHCB-lactone was obtained after purification using silicagel chromatography (column dimensions: 330 × 22 mm. ID, 50 g, used without any deactivation) and DCM 100% as elution solvent. Thin layer chromatography: Si60, 100% DCM, $R_f = 0.33$ for HHCB-lactone. Crystallization from *n*-hexane gave 0.85 g (3.12 mM, 80%) of colorless crystals of racemic HHCB-lactone. Low resolution mass spectrometry (70 eV): *m/z* 272 (15, M^+), 257 (100, $M^+ - 15$), 239 (12), 197 (13), ^1H NMR: see Franke et al. (1999). ^{13}C NMR ($CDCl_3$): 8.38 (C17), 16.9, 16.95 (d, C(18)), 25.62, 25.65, 28.68, 28.74, 28.95, 29.05 (Me–C(10,11,12,13)), 32.01, 32.03 (d, C(16)), 44.57 (C(7)), 45.19 (C(9)), 54.11, 54.15 (d, C(8)), 72.43 (C(15)), 119.76 (C(4)), 122.95, 122.98 (d, C(3)), 124.98, 125.01 (d, C(1)), 143.38, 143.43 (d, C(2)), 151.12 (C(5)), 158.3 (C(6)), 165.74 (C(14)).

2.6. Enantioselective GC

Enantioselective GC was performed on a 30 m × 0.25 mm ID, 0.25 μm film OV 1701 capillary column (14% cyanopropylphenyl/86% dimethyl polysiloxane) doped with proprietary amounts of cyclodextrin material (Rt-BDEXcst fused silica from Restek company, obtained from BGB Analytik, Anwil, Switzerland) using the same GC–MS equipment as described in Section 2.8 and the same samples without a further clean-up. Separation of the enantiomers/diastereomers was achieved using the following temperature program: 85 °C, 2 min hold, 30 °C/min to 120 °C, 0.2 °C/min to 130 °C, 220 min hold, 30 °C/min to 150 °C, 50 min hold, 10 °C/min to 220 °C, 40 min hold. Helium was used as a carrier gas at a linear velocity of 35 cm/s at 85 °C in the constant flow mode (0.9 ml/min). Standards and extracts were injected (1.5 μl) on-column in the oven tracking mode. Oven tracking keeps the inlet temperature 3 °C higher than the oven temperature and follows the same oven temperature program as the analytical column. This improves retention time stability. Data were acquired in the EI-SIM mode using the same ions as described in Section 2.8. MS temperature parameters: transfer line: 230 °C, quadrupole, ion source: see Section 2.8.

2.7. Standards, chemicals and solvents

PCMs (HHCB, AHTN, ADBI, AHDI, ATII and DPMI) were obtained from Promochem, Wesel, Germany. Purities of the compounds were as follows: HHCB 75% (GC), AHTN (98%), ADBI (98%), AHDI (94.5%), ATII (90%) and DPMI (90%). Concentrations of standard solutions were corrected for purity. *n*-Hexane and isooctane, residue grade, were purchased from Merck, Dietikon, Switzerland. Anhydrous sodium sulfate (Na_2SO_4) was from Merck as well. Filters (Nylon 66 membranes, 0.2 μm, diameter 47 mm) were from Supelco, Buchs, Switzerland, NaCl was from Fluka, Buchs, Switzerland.

2.8. Separation and quantification of PCMs of the field study using non-enantioselective HRGC-EI-SIM mass spectrometry

HRGC was performed on a HP 6890 GC (Agilent Technologies, Waldbronn, Germany) equipped with an HP 7683 Series automatic injector (enhanced parameters) and a HP 5973 mass selective detector (MSD) in the electron impact (EI) mode (ionization energy 70 eV). Separation was achieved on a 50 m HT-8 column (SGE Scientific, distributed by BGB Analytik AG, Anwil, Switzerland) × 0.22 mm ID, 0.25 μm film thickness. The analytical column was protected with a 5 m × 0.53 mm ID deactivated retention gap using the following temperature program: 80 °C, 1 min hold, 10 °C/min to 175 °C, 1 min hold, 5 °C/min to 240 °C and 10 °C/min to 290 °C, 10 min hold. Helium was used as a carrier gas at a linear velocity of 28 cm/s at 80 °C in the constant flow mode (1 ml/min). Calibration standards and extracts were injected (1 μl) on-column in the oven track mode. After 10–15 injections, the precolumn was cut about 30 cm to restore chromatographic resolution.

For quantification of the compounds data were acquired in the single ion monitoring (SIM) mode using the following characteristic ions (*m*/*z*): HHCB: 243, 258, AHTN: 243, 258, ADBI: 229, 244, AHDI: 229, 244, ATII: 215, 258, DPMI: 191, 206 and HHCB-lactone: 257, 272. Quantification was performed using standard solutions containing the target compounds in isooctane. MS temperature parameters: transfer line: 290 °C, quadrupole: 150 °C and ion source: 230 °C.

Final concentrations were calculated as described in Kupper et al. (2004).

2.9. Recovery data and LOD/LOQ

Recovery experiments were performed by spiking tap water at two concentrations (10, 100 ng/l, $n = 3$) and submitting the samples to the whole analytical procedure. Average recovery rates were as follows: HHCB: 78% (±8% RSD), AHTN: 83% (±7% RSD), ADBI: 92% (±9% RSD), AHDI: 82% (±8% RSD), ATII: 87% (±11% RSD), DPMI: 79% (±7% RSD), HHCB-lactone: 94% (±11% RSD). Data acquisition in the EI-SIM-MS allowed detection of 2–10 pg on column (LOD, signal/noise (S/N) ≈ 3) of the different compounds. LOQ (S/N ≈ 10) were as follows: HHCB/AHTN/ADBI: 20, AHDI, DPMI: 10, ATII: 15 ng/l.

Recovery experiments with activated sewage sludge were performed as described in Kupper et al. (2004).

3. Results and discussion

3.1. Development of an enantioselective separation scheme for PCMs and determination of enantiomeric ratios in influent, effluent and sewage sludge (part 1)

3.1.1. Optimization of enantioselective GC

Enantioselective gas chromatography has been described as a potent method to separate enantiomeric or diastereomeric compounds (Buser and Müller, 1997; Finizio et al., 1998; Wiberg et al., 1998; Hühnerfuss, 2000). In order to determine enantiomeric ratios (ERs) in wastewater and sewage sludge samples an enantioselective GC was developed allowing the separation of the different enantiomeric/diastereomeric PCMs with sufficient resolution, including the main transformation product of HHCB, HHCB-lactone, in the same chromatographic run. As a stationary phase a commercially

available Rt-BDEXcst capillary GC column was chosen which incorporates unknown amounts of modified cyclodextrins into 14% cyanopropylphenyl/86% dimethylpolysiloxanes. This stationary phase was developed specifically for the fragrance industry which is frequently using chiral compounds as starting material for their products. Recently, the 4 pure diastereomers of HHCB were obtained via preparation of organo-chromium complexes (Frater et al., 1999). The olfactory evaluation showed that mainly isomer *cis-4S,7R*-HHCB exhibited the most intense odour of Galaxolide. The elution order of the HHCB stereoisomers has already been described (Biselli et al., 1999) and could be confirmed in our study by injecting pure reference compounds on the Rt-BDEXcst column: G1: *trans-4S7S*, G2: *cis-4S7R*, G3: *cis-4R7S* and G4: *trans-4R7R*-HHCB (Fig. 1).

Optimization of the enantioselective separation was performed considering the following parameters: linear velocity of the helium carrier gas, temperature ramp rates, initial GC temperature and sample capacity. It is well known that resolution between enantiomeric compounds might be improved by increasing the linear velocity of the mobile phase. Several GC runs were made using optimized temperature ramp rates and increasing the linear velocity from 35 to 70 cm/s. However, no significant improvement in peak resolution was observed. The most profound effect on resolution could be attributed to the temperature ramp rate applied during the GC run. Typically, slow rates of 1–2 °C/min are used. In the present study, optimum resolution of the PCMs was however reached using mainly isothermic temperature programming. Only DPMI elutes as a well separated doublet signal during the first temperature ramp at the beginning of the GC run. Attempts to apply a second slow temperature ramp instead of an isothermic run were not successful as resolution started to decrease and partial coelution was observed in the more complex part of the GC chromatogram (elution of ATII, HHCB and AHTN). For the HHCB-lactone again, an isothermic temperature run revealed the best resolution of the four diastereomers. The initial temperature turned out to be not a crucial factor for the resolution of the different PCMs except for the first eluting compound DPMI. Initial temperature was set 25 °C above the recommended temperature of 60 °C for the Rt-BDEXcst column allowing at the same time classical on-column injection. Column capacity varies with different compounds and overloading results in broad tailing peaks and reduced enantiomeric separation. By injecting increasing concentrations of PCMs, we found that peak broadening started to appear between 20 and 50 ng on-column.

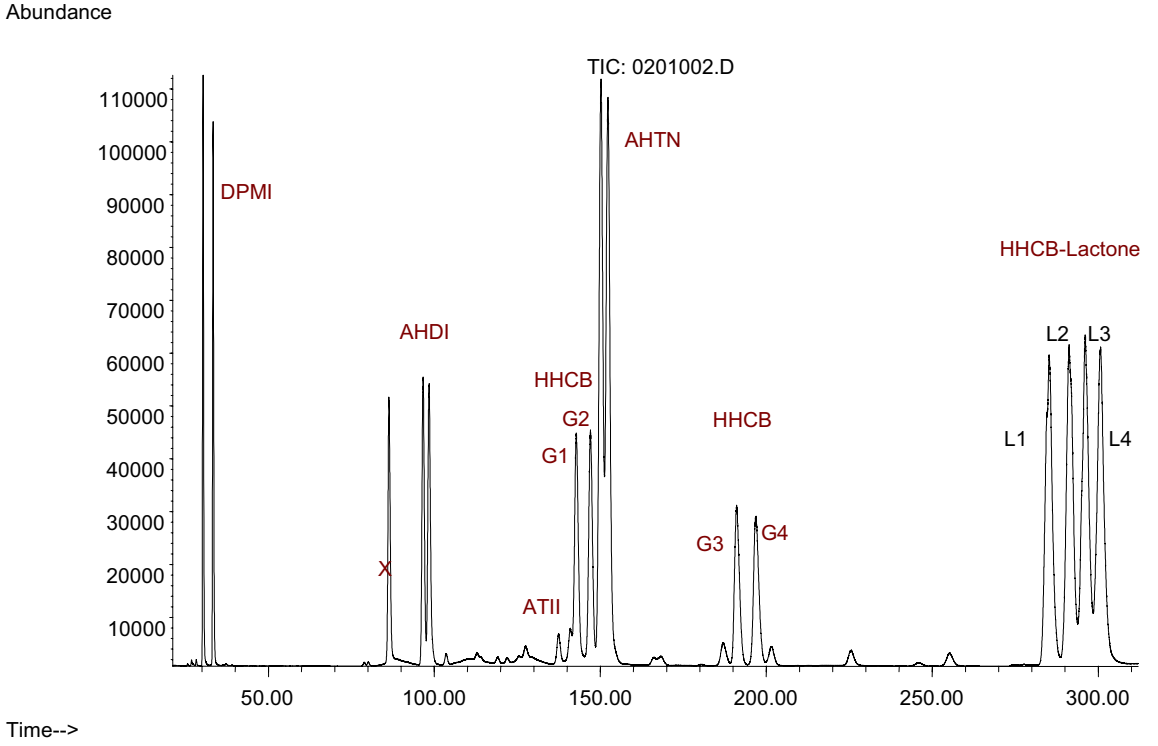

Fig. 1. Total ion chromatogram (TIC) of a commercial mixture of polycyclic musks and HHCB-lactone separated on the Rt-BDEXcst column; HHCB: G1/G4: *trans*-enantiomers and G2/G3: *cis*-enantiomers; HHCB-lactone: L1/L4: *trans*-enantiomers and L2/L3-*cis*-enantiomers.

Table 2
Chromatographic parameters of the enantioselective GC of the PCMs

Compound	t_R (min)	R	AF	EIC (m/z)
DPMI	30.31/33.34	2.03	1.25/1.33	191
AHDI	96.64/98.44	0.77	1.28/1.05	229
ATII	137.43/140.89	1.10	1.11/1.38	215
AHTN	150.24/152.28	0.68	1.05/1.21	243
HHCB G1/G4	142.70/196.86	12.75	1.25/1.41	243
HHCB G2/G3	147.02/191.05	11.28	1.19/1.23	243
HHCB-lactone L1/L4	285.22/300.75	2.38	1.00/1.15	257
HHCB-lactone L2/L3	291.22/296.07	1.02	1.27/1.03	257

t_R: Retention time (min), R: Resolution $= \frac{2\times t_{R2}-t_{R1}}{w_1+w_2}$, w: peak width, AF: Asymmetric Factor $= \frac{b}{a}\frac{0.1}{0.1}$, a: distance from peak start to peak maximum at 10% of the peak height, b: distance peak maximum to peak end at 10% peak height, EIC: Extracted ion chromatogram (mass chosen in m/z).

Some characteristic parameters of the chromatogram are summarized in Table 2. Resolution factors (R) show values $\geqslant 1$, except for AHDI and AHTN ($\approx$0.7–0.8) demonstrating that corresponding peak pairs are well separated. Asymmetric factors vary between 1.0 and 1.4 and therefore prove good peak symmetry of the different PCM signals.

In conclusion, the Rt-BDEXcst column showed to be an efficient stationary phase for the separation of chiral PCMs and the HHCB-lactone. The separation capacity of the column is however counterbalanced by the long retention times due to isothermic temperature programming of the GC run.

3.1.2. Enantiomeric ratios of PCMs in influent, effluent and sewage sludge samples

A subset of influent, effluent and stabilized sewage sludge samples was submitted to enantioselective GC to get information on enantiomeric biodegradation processes due to wastewater treatment. Frequently, the enantiomeric ratio (ER) is taken as a parameter to describe enantioselective transformations. It is assumed that an ER close to racemic (ER $\approx$ 1) indicates a low transformation potential of a system whereas ER values clearly different from 1 result from enantioselective transformation reactions. In Table 3, ER for some wastewater and sewage sludge samples are summarized and compared to ER of a commercial mixture of PCMs. ER values of DPMI could not be calculated due to the low amounts present in the environmental samples. Other ER values not measured for AHDI and ATII were due to insufficient resolution of the enantiomeric pair in the extracts. Generally, it was observed that after several injections peak resolution started to decrease and the retention gap had to be cut/replaced regularly in order to restore full peak resolution capacity. Surprisingly, HHCB-lactone which elutes as a typical quartet signal at the end of the GC run (Fig. 1) was not fully resolved in the wastewater and sewage sludge samples. Reasons for this observation might include interactions of matrix components with the enantioselective stationary phase, adsorption effects in the injection system or the column, a significant higher bleeding at the elution temperature of the compound and possibly, chemical degradation of the component in the sample.

The TICs (total ion chromatograms) of an enantioselective GC of a wastewater sample is shown in Fig. 2. The signal pattern is dominated by the compounds HHCB and AHTN. For AHTN, ER values in sludge samples from WWTP Prahins and Wohlen differ significantly from the one encountered in the commercial mixture (Table 3). For HHCB one sludge sample (WWTP Prahins) exhibits a different ER with respect to G1/G4 (*trans*-isomers) and three sludge samples with respect to G2/G3 (*cis*-isomers) (WWTPs Prahins, Echallens, Wohlen). Finally, for AHDI and ATII deviations of the ER value from the one of the synthetic mixture was observed in the sewage sludge from WWTP Prahins as well as the effluent sample. It seems that WWTPs Prahins, Echallens, Wohlen show some enantiomeric preference for the dominant PCMs HHCB and AHTN and to a lesser extend for the minor compounds AHDI and ATII. Other plants however (WWTP Konolfingen) do not seem to induce biodegradation processes for the dominant PCMs. It can be concluded, that besides sorption on sludge, considered as the most important process for elimination of PCMs from wastewater, biotransformation reactions might contribute to their removal as has been suggested by Simonich et al. (2002). Which mechanisms (biodegradation, photo-oxidation, different adsorption or solubility capacities of the enantiomers/diastereomers) are responsible for the significant deviations of the ER values in some environmental samples, remain to be studied in the future.

3.2. Analysis and fate of PCMs on WWTP Chevilly (part 2)

For HHCB and AHTN, mean concentrations of 6900 and 1520 ng/l, respectively, were found in the influent. Mean contents of the other PCMs were $\leqslant$200 ng/l (Table 4). These data reflect the consumption of these

Table 3
Enantiomeric ratios (ER) of PCMs in a technical mixture, sewage sludge and wastewater samples; ER = peak area Signal 1/peak area Signal 2; ER (*trans*-HHCB) = G1/G4, ER (*cis*-HHCB) = G2/G3 (see Fig. 1); areas obtained from EIC: HHCB/AHTN: *m/z* 243, DPMI: 191, AHDI: 229, ATII: 215, HHCB-lactone: 257

Matrix/ compound	WWTP	HHCB *Trans* G1/G4	HHCB *Cis* G2/G3	AHTN	AHDI	ATII	DPMI	HHCB-lactone L1/L4	HHCB-lactone L2/L3
Techn. mixture	–	0.97	1.01	1.01	0.96	0.91	0.99	0.98	0.99
Influent	Prahins	1.00	0.97	0.94	0.97	0.86	n.d.	n.d.	n.d.
Effluent	Prahins	0.81	1.00	0.96	1.19	2.94	n.d.	n.d.	n.d.
Aerobically stabilized sewage sludge	Prahins	0.93	1.08	1.17	1.16	0.92	n.d.	n.d.	n.d.
Anaerobically	Echallens	1.01	1.09	0.98	1.00	n.m.	n.d.	n.d.	n.d.
stabilized	Wohlen	1.06	1.09	0.91	n.m.	n.m.	n.d.	n.d.	n.d.
sewage sludge	Konolfingen	0.98	0.99	0.99	0.95	0.79	n.d.	n.d.	n.d.

n.d.: not determined, n.m.: not measured.

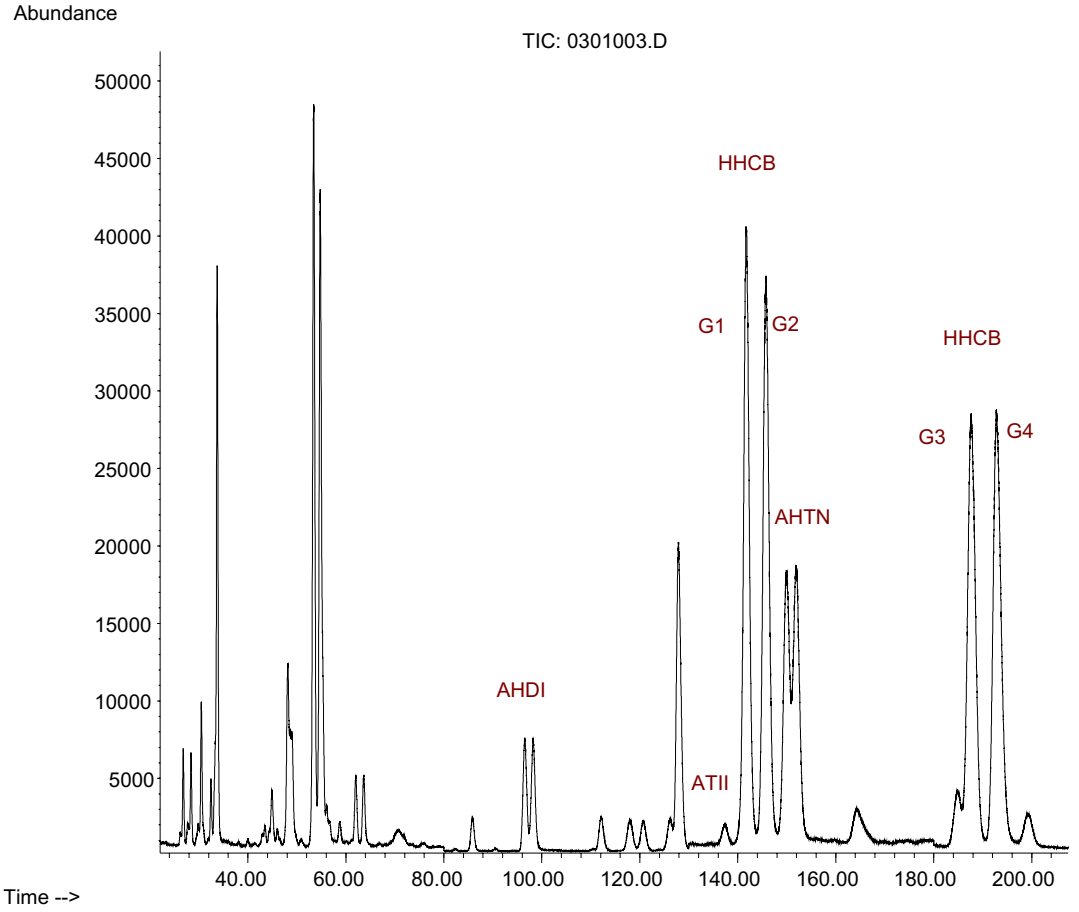

Fig. 2. TIC of an enantioselective GC of an influent sample (WWTP Prahins); peak assignment according to Fig. 1.

compounds which is highest for HHCB and AHTN but lower for the other compounds by one to two orders (OSPAR Commission, 2000). It has to be noted that enantiomers of PCMs were not determined in the samples of WWTP Chevilly because the results of part 1 of this study did not reveal enantioselective biodegradation being a process of predominant significance during wastewater treatment (see Section 3.1.2). Values between

1420 and 4300 ng/l were detected for HHCB by Artola-Garicano et al. (2003). Simonich et al. (2002) report higher concentrations of this compound in the influent of five WWTPs in Europe and 12 WWTPs in USA of 9710 and 16 600 ng/l, respectively. Contents of AHTN in the influent of WWTP Chevilly are comparable to values reported by Artola-Garicano et al. (2003). Simonich et al. (2002) found higher values of 5970 and 12 500 ng/l, respectively. This is probably due to a different consumption profile in Switzerland (Kupper et al., 2004). Only few data about the other PCMs are reported in the literature. ADBI was measured in the range between 40 and 140 ng/l (Eschke et al., 1994). The OSPAR Commission (2000) reports maximum values for ADBI, AHDI and ATII of 290, 700 and 590 ng/l, respectively, which are higher than the contents measured in this study.

HHCB-lactone was found in the influent at relatively high concentrations (mean: 430 ng/l). This might be due to degradation of HHCB in the sewer system indicating that degradation is induced immediately after discharging of products containing PCMs considering a residence time in the collector system of less than one hour. During our studies, a series of commercial products (shampoos, soaps and eau de toilette) were analyzed for HHCB-lactone (unpublished data). As this compound was only detected in one sample (eau de toilette) at non-quantifiable amounts we consider direct input of HHCB-lactone originating from fragrance containing products as negligible.

The highest daily loads in influent were observed for HHCB, AHTN and HHCB-lactone. The loads were highest during weekend and on Monday. This can be explained by a higher use of products for personal care during weekend and textile washing on Monday.

Mean values of HHCB and AHTN in the effluent were 860 and 250 ng/l, respectively (Table 4). Concentrations of the other PCMs were considerably lower. In comparison, contents found in other studies are generally higher (range: 1000–13 300 ng/l for HHCB and 420–4300 ng/l for AHTN; Paxeus, 1996; Heberer et al., 1999; Fromme et al., 2001b; Simonich et al., 2002; Artola-Garicano et al., 2003). Results obtained recently in effluents from 8 Canadian and Swedish WWTPs (Ricking et al., 2003) revealed values similar as in the current study (157–1300 and 42–520 ng/l for HHCB and AHTN, respectively). Concentrations of HHCB and AHTN reported by Ternes et al. (2003) are in the same range. It has to be considered, that these samples were filtered before extraction and solid phase extraction was used in the two studies mentioned before. According to results of Artola-Garicano et al. (2003) concentrations in effluents obtained by liquid/liquid extraction are generally higher for AHTN than those obtained by solid-phase microextraction but no significant difference was observed for HHCB.

Table 4
Concentrations of PCMs in influent, effluent, activated sludge and mean removal rates of WWTP Chevilly

	HHCB	AHTN	ADBI	AHDI	ATII	DPMI	HHCB-lactone
Influent (ng/l), n = 7							
Mean	6900	1520	80	200	170	30	430
Median	6830	1330	80	180	150	30	420
Min	5390	1240	50	100	90	20	390
Max	9020	2280	110	340	320	50	500
SD	1500	380	20	80	80	10	40
Effluent (ng/l), n = 7							
Mean	860	250	20 (LOQ)	70	20	<LOQ	900
Median	830	250	20	60	20	<LOQ	750
Min	730	180	<LOQ	50	15 (LOQ)	<LOQ	680
Max	1080	370	20	90	30	<LOQ	1190
SD	130	70	–	10	–	–	220
Activated sludge (μg/kg d.m.), n = 7							
Mean	4300	1720	60	210	170	n.d.	1410
Median	4300	1680	60	210	160	n.d.	1420
Min	3170	1250	50	170	120	n.d.	1280
Max	5270	2060	70	240	210	n.d.	1570
SD	640	260	10	30	30	n.d.	90
Removal rate (%)							
Mean	87	83	80	61	87	77	75

n.d.: not detected.

Values of the other PCMs reported in the literature are higher than in the current study (ADBI: 40–430 ng/l, Eschke et al., 1994; Heberer et al., 1999; OSPAR Commission, 2000; Fromme et al., 2001b; AHDI: 100–580 ng/l, ATII: 40–700 ng/l, Fromme et al., 2001b). Ricking et al. (2003) report values of 2–19 ng/l for ADBI and AHDI and concentrations of ATII and DPMI below the limit of detection.

The concentration of HHCB-lactone was in the same order of magnitude in the effluent as its parent compound HHCB (mean 900 ng/l) and twice as high as in the influent. This indicates degradation of HHCB to HHCB-lactone during wastewater treatment.

The contents of PCMs in activated sludge are summarized in Table 4 as well. Mean values of HHCB and AHTN were 4300 and 1720 μg/kg d.m., respectively. Mean concentration of HHCB-lactone was 1410 μg/kg d.m. The other PCMs were found in concentrations between 60 and 210 μg/kg d.m. with highest values for AHDI. Concentrations of PCMs, particularly for AHTN, were generally lower compared to values of other studies (Van de Plassche and Balk, 1997). This is probably due to lower influent concentrations. Additionally, due to the high solids retention time of about 20 days degradation is expected to be enhanced which is supported by high concentrations of HHCB-lactone in activated sludge.

Mean percent removal rates for HHCB and AHTN were at 87% and 83%, respectively (Table 4). Lowest removal rate was observed for AHDI (61%). These data are in agreement with the study of Simonich et al. (2002) who found removal rates for HHCB and AHTH of 90% and 89% in a WWTP with oxidation ditch.

4. Conclusions

The Rt-BDEXcst capillary column showed to be an efficient stationary phase for the separation of chiral PCMs and HHCB-lactone. Occasionally, some enantioselective biodegradation of PCMs during wastewater treatment was observed. Processes inducing deviations of the ER values in some environmental samples remain to be studied in the future.

In general, concentrations of PCMs in influent and effluent samples as well as removal of PCMs in the WWTP found in this study correspond with published data. The observed content of HHCB-lactone in the influent indicate degradation of HHCB after disposing of fragrance containing products into the sewer and increasing HHCB-lactone concentrations during wastewater treatment a significant degradation of HHCB. For a better understanding of the degradation processes and for characterizing the importance of degradation for removal of HHCB and other PCMs during wastewater treatment a correct balancing in studies on WWTPs or laboratory experiments are necessary. Finally, research is needed to elucidate the formation of further transformation products.

Acknowledgments

The Swiss Agency for the Environment, Forest and Landscape (SAEFL) is highly acknowledged for funding this study. We are grateful to Franz X. Stadelmann and Hans-Jörg Bachmann (FAL) for their support, Jean-Michel Roch (WWTP Chevilly) for collaboration during sampling and Werner Stauffer and Oskar Fankhauser (FAL) for the dry matter determination of the sludge. The Givaudan company, Dübendorf, Switzerland is highly acknowledged for sending pure diastereomers of HHCB. Finally, we thank the NMR group of P. Bigler, Department of Chemistry and Biochemistry, University of Bern, Switzerland for the ^{13}C measurements of HHCB-lactone.

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