# CHIRALITY AS INDICATIONS FOR BIODEGRADATION OF THE POLYCYCLIC MUSK FRAGRANCES AHTN AND HHCB AS WELL AS THE METABOLITE HHCB-LACTONE IN A LARGE SCALE SEWAGE PLANT AS WELL AS SURFACE WATER

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## Introduction

HHCB and AHTN are currently applied with more than 2000 t/a in Europe. Considerable amounts of these compounds have been detected in the waste water[1]. A considerable but varying elimination for diverse sewage treatment plants has been observed, but the mechanism is not been clear up to now[2]. The elimination of these compounds is of some importance, as these polycyclic musk fragrances are stable in surface and even marine waters[3]. Also both compounds have been shown to bioaccumulate[4] and to exhibit weak estrogenic effects[5]. Both compounds are chiral thus giving new options in determining elimination and degradation in a sewage plant[6]. Indeed HHCB as well as it's metabolite HHCB-lactone has two chiral centres, thus giving diastereomers while AHTN only exhibits one chiral centre.

In this study balances of HHCB and AHTN for a commercial sewage treatment plant (STP) operating on 200 000 $m^3$ wastewater per day are compared to the enantiomeric ratios of HHCB, AHTN and the metabolite HHCB-lactone of the emissions of a STP are compared to ratios and concentrations in surface waters.

## Methods and materials

Wastewater has been sampled as 24 h composite sample during a five day period. During the same time sludge samples were taken from the final dry product that is transported to dangerous waste disposal sites. Briefly the water samples were extracted with toluene, the organic phase is concentrated and analysed by GC-MS (Trace, Thermo-Finnigan). 10 g aliquots of sludge samples are lyophilised and successively extracted in a Soxhlet apparatus with 100 mL ethyl acetate for 6 h. These extracts are condensed and successively a clean up by means of size exclusion chromatography (biobeads SX3 Biorad) and silica is used. In both cases $D_{15}$ musk xylene is used as internal standard. Normal quantification is performed with a DB-5MS column, while the enantioselective separation utilising a heptakis-(2,3-di-O-methyl-6-*O*-*t*-butyldimethyl-silyl)-ß-cyclodextrin (FS-Hydrodex ß-6TBDM) was performed following[7]. Surface water samples have been obtained by means of glass bottles. These water samples have been processed the same way as the waste water samples.

## Results and discussion

The concentrations in this STP are rather low in comparison to other plants, as the domestic waste water is diluted by 50% water from a brewery (Table 1). On the other hand the elimination rates tend to be on the low end in comparison with other plants. Considering balances about 1,800 g HHCB and 540 g AHTN enter the plant with the waste water during a five days study period, about 640 (HHCB) and 200 (AHTN) leave the plant via discharge water. About 860 g HHCB and 430 g AHTN leave the plant sorbed to the sludge. Some of the missing HHCB is transformed into 130 g HHCB-lactone which leaves the plant mostly with the discharge water. This would mean about 5 % of the HHCB is transformed (Table 2).

Table 1 Concentrations of AHTN, HHCB and HHCB-lactone in inflow and effluent of a German sewage plant (average of 5 days). Additionally the day-to-day variation is given as standard deviation. Data derived from duplicate samples of five successive days each.

| | Influent [ng/L] | effluent [ng/L] | elimination rate [%] | breakthrough [%] |
|---|---|---|---|---|
| AHTN (tonalide) | 580 (+/-100) | 210 (+/-17) | 63 | 37 (+/-5,9) |
| HHCB (galaxolide) | 1900 (+/-350) | 700 (+/-58) | 63 | 37 (+/-6,6) |
| HHCB-lactone | 230 (+/-40) | 370 (+/-34) | nd | 162 (+/- 22) |

Table 2 Balance of polycyclic musk fragrance compounds HHCB and AHTN in a 5 day sampling period in a German sewage plant. +/- values are day-to-day variation calculated as standard deviations. nd: not determined.

| Compound | influent [g] | effluent [g] | sludge [g] | Balance [g] | balance [range] (%) |
|---|---|---|---|---|---|
| AHTN (tonalide) | 540 | 200 | 430 | +87 | +16 (+/-28) [-23 to 57] |
| HHCB (galaxolide) | 1800 | 640 | 860 | -290 | -16 (+/-18) [-40 to 7.4] |
| HHCB-lactone | 210 | 340 | nd | +130 | nd |

In order to study whether this is transformation of HHCB is due to chemical oxidation or enzymatic processes the enantiomeric ratios were determined. The racemic mixture enters a sewage treatment plant, while in the effluents of STPs enantiomeric ratios can be determined if enantioselective processes (such as biodegradation) take place in the STP. In this study HHCB emission to the river Ruhr were compared to background values. In these samples only the enantiomeric ratios of HHCB-lactone in the effluent were significantly different from the standard. (Fig.2). These changes in enantiomeric composition indicate that the transformation that is performed in the sewage plant (samples 57-59) is indeed biodegradation. The enantiomeric ratios are down to < 0.8 in this case. This information may be used for further optimisation of STP processes. To study whether or not further biodegradation does occur in the rivers several STP effluents were analysed in comparison to river background concentrations as well as enantiomeric ratios. Samples were taken from the spring to the source of the river Ruhr and compared with the discharges of the STP (compare figure 2). The samples from this STP have clearly elevated amounts of some HHCB metabolites in comparison as well enantiomeric ratios. While other plants (23,

24) do not show such a significant enantioselective degradation. No relevant input seems to occur further downstream station 60, thus the changes in enantiomeric ratio are either due to mixing processes in the river (compare station 56) or due to further metabolisation processes in the river. The results for enatiomeric ratios of HHCB-lactone are shown in fig. 2.

Figure 1 Enatioselective separations of the enantiomers of HHCB-lactone in a sewage sludge sample. Elution order following[8].

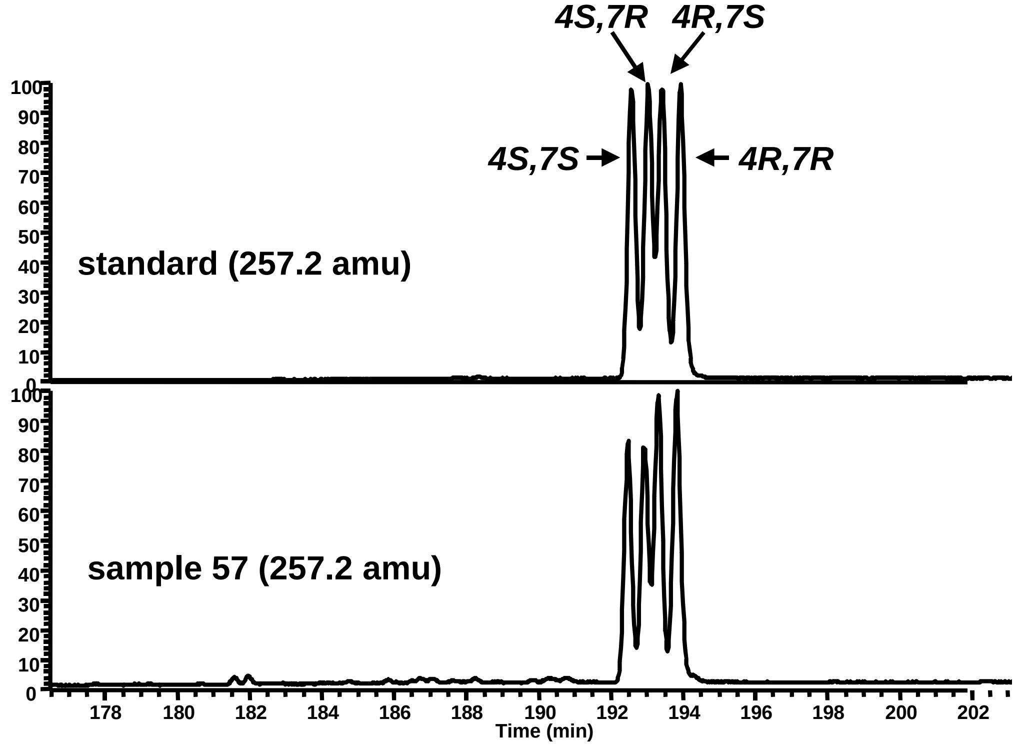
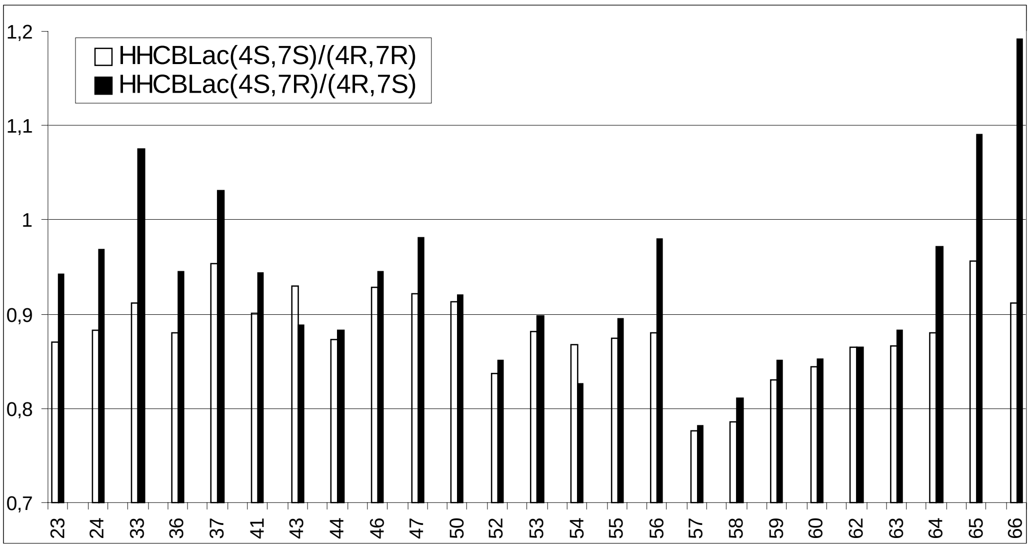



Figure 2 Enantiomeric ratios of HHCB-lactone (*4S,7S*) vs (*4R,7R*) and (*4S,7R*) vs (*4R,7S*) in river Ruhr water. The low numbers (indicate samples from near the spring while higher numbers indicate samples near the mouth of the river Ruhr. Samples 57-60 are from the discharge of a large sewage plant. Samples 63-66 are samples from the river without significant new input of sewage discharge into the river. A standard calibration with the chiral column gave ER: 0.98 with SD of 0.015 for HHCBLac (*4S,7S*)/(*4R,7R*), while for HHCBLac (*4S,7R*)/(*4R,7S*) an ER of 0.97 with an SD of 0.018 was obtained.



It may, thus, be concluded, that sorption phenomena dominate the elimination process in current sewage treatment processes. As biodegradation of HHCB only occurs in some STPs to a low extend (5-10%) this process may be optimised to give better elimination rates as well as degradation. HHCB-lactone seems to be the dead end of metabolic processes in current sewage treatment processes, though. The emissions of current STPs contain considerable amounts of HHCB-lactone which should be taken into consideration for evaluation.

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