

## Contamination and gas chromatographic chiral separation of bromocyclen in water and fish samples from the river Stör in the northern part of Germany and in some sewage plant's waste water

Bianca Bethan<sup>1</sup>, Kai Bester<sup>1</sup>, Heinrich Hühnerfuss<sup>1</sup>\*, Gerhard Rimkus<sup>2</sup>

1 Institut für Organische Chemie, University of Hamburg,  
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

2 Lebensmittel- und Veterinäruntersuchungsamt des Landes Schleswig-Holstein, Max-Eyth-Str. 5, D-24537 Neumünster, Germany

### Introduction

During the last years, remarkably high concentrations of the contact insecticide bromocyclen (trade-name Alugan<sup>®</sup>, Bromodan<sup>®</sup>) were determined in fish samples [1,2]. Due to its low mammalian toxicity bromocyclen is widely used against ectoparasites in Europe [3]. The origin of the bromocyclen contamination found in fish is still unknown, but it is worth noting that investigations aiming at the analyses of contaminants in sewage sludges showed the prevalence of this compound [4]. Because of its high bioaccumulating potential [5] and its persistence in the environment the production of the insecticide was discontinued in Germany in 1994.

Like several other persistent polychlorinated insecticides, bromocyclen is chiral and applied as racemate. Accordingly, enantioselective enzymatic metabolism and/or bioaccumulation of bromocyclen in fish can be investigated by means of chiral gas chromatography using modified cyclodextrins as stationary phases [2,6-10].

In the present investigation, water and fish samples from different stations at the river Stör, which is located in the northern German state Schleswig-Holstein, and waste water from various sewage plants have been analysed for bromocyclen. Furthermore, the enantiomeric ratios of bromocyclen in the water and fish samples were determined. On the basis of the results, it is suggested how bromocyclen gets into the river Stör and how enzymatic processes influence the enantiomeric ratio of bromocyclen.

### Materials and Methods

Samples of water, breams (*Ambramis brama orientalis*) and brook trouts (*salmo trutta fario*) were collected in the river Stör from May to August in 1995, a tributary of the river Elbe. The waste water samples of the sewage plants of the cities Hamburg, Itzehoe and Neumünster, respectively, were also taken during this period.

# TOXA (po)

The aqueous samples were collected with a 10 L glass-sampler as described by Gaul and Ziebarth [11], extracted with 200 mL *n*-hexane for 20 min and cleaned-up using a mini-alumina column, fractionated by high-performance liquid chromatography (HPLC) and further cleaned-up by solid-phase extraction using a 2 g C18 cartridge and a mixture of 96 % methanol/water to elute the analyte. For the chiral analysis the extract was cleaned-up with an alumina column again, this time taking a smaller fraction.

The fat of the muscle tissue of the fish samples was removed with a cold water-acetone-petrolether partition extraction [12] and further cleaned-up as described by Specht and Tillkes [13]. For the chiral analysis the samples were fractionated by HPLC and by GPC. Detection was carried out by gas chromatography/electron-capture detector (ECD) and verified by GC-MS.

## Results and discussion

Figure 1 shows the bromocyclen concentrations and enantiomeric ratios determined in the water samples from the river Stör taken at different stations. The sampling stations were selected such that they comprise two sections (W1,W2) that represent relatively uncontaminated river water close to the headwaters, then the section W3 reflecting the input by the city of Neumünster, further downstream three sections in the agricultural area between Neumünster and the city of Itzehoe (W4,W5,W6), followed by three stations that are influenced by the input of Itzehoe (W7, W8, W9) and finally the section W10 of the Stör estuary. For comparison, a station W11 in the river Elbe in the vicinity of the Stör estuary was included. Significant increases in bromocyclen concentration were found downstream of Neumünster (W3), in Itzehoe (W8) and downstream of Itzehoe (W9). The increase in concentration that was detected upstream of Itzehoe (W7) can be traced back to the same source as the increase determined in sample W8 since sample W7 was taken at high tide. The decrease in concentration from W3 to W6 (Feldhusen) in the course of the downstream movement can be explained by the dilution towards the river Elbe. The concentrations at the estuary of the Stör (W10) and in the Elbe at Glückstadt close to the influx of the Stör were relatively low, respectively. With regard to the enantiomeric ratios, no significant differences in the water were found. At all stations a value close to 1.0, i.e. a value representing a racemic mixture, was determined which indicates that bromocyclen is not or hardly transformed by biotic processes.

Since the high increases in the bromocyclen concentration in the river Stör occurred close to the influxes of sewage plants and since bromocyclen had already been reported to be a contaminant in sewage sludges [4], three samples of sewage plant's waste water were also analysed in the present study. The concentrations which varied between 3.3 (Neumünster) and 11.5 (Itzehoe) ng/L were up to 100fold higher than the bromocyclen concentration detected in the river water samples at the respective locations. Therefore, sewage plants can be safely assumed as the most prominent source of the bromocyclen contamination in the river Stör. However, the exact origin remains unknown, but since the bromocyclen concentration in the sewage plant's waste water from Hamburg was in the same range (5 ng/L) as the waste water samples from

Schleswig-Holstein, it is presumed that the contamination can be traced back to a contamination from households rather than from industry or agricultural use.

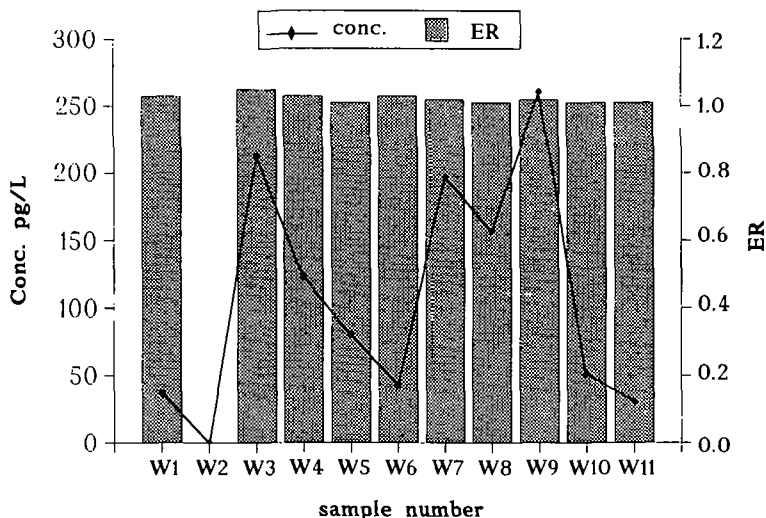


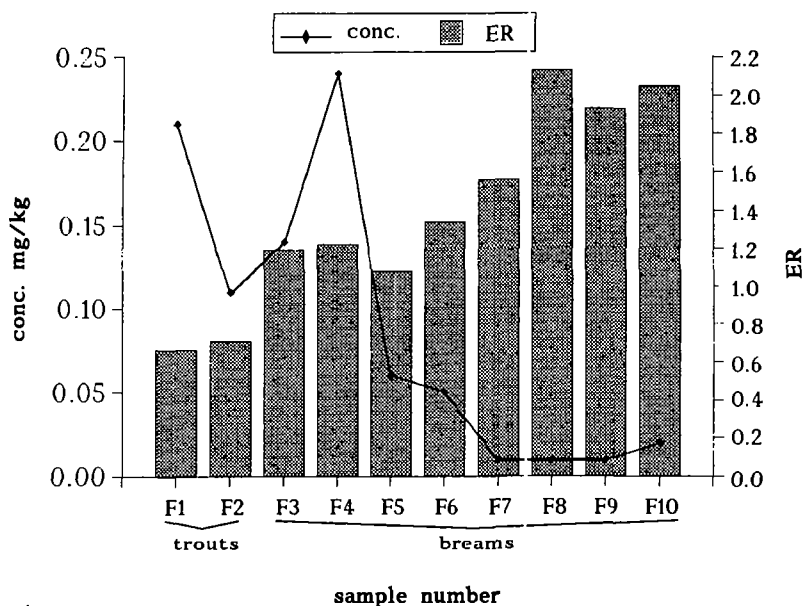
Figure 1

Concentrations and enantiomeric ratios of bromocyclen in water samples from the river Stör; limit of quantification: 1.5 pg/L; recovery rate: 60 %; average error of the ER values: +/- 0.04

In addition to the water samples, the muscle tissue of ten fishes (eight breams and two trouts) were analysed to gain deeper insight into potential enzymatic degradation processes. For the chiral analysis the same column as used in a previous work [2] was applied. The concentrations and enantiomeric ratios as determined in the fish samples are shown in Figure 2. The ER values determined in the bream samples turned out to be higher than 1 (F3-F10), which indicates a preferential degradation of the second eluting (+)-enantiomer, while the ones of the two trouts were significantly lower than 1 (F1,F2). The basically different ER characteristics found in breams and trouts cannot be explained by now. The assumption of a different enzymatic process in trouts in comparison with breams is quite improbable and could not yet be confirmed by further investigations with rainbow trouts.

With regard to the ER values that were detected in the breams, a correlation between the enantiomeric ratios and the concentrations can be inferred. Though this result is based on a small data set, a first indication is obtained that higher concentrations may result in weaker decomposition of the (+)-enantiomer, alternatively, an increasing degradation of the (-)-enantiomer. However, it should be noted, that for a safe significant correlation the present values have to be complemented by further investigations. Besides, nothing is known about the toxicity of the single enantiomers of bromocyclen until now, which would be useful for a further interpretation of the present data set.

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**Figure 2**

Concentrations and enantiomeric ratios of bromocyclen in fish samples

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