DETERMINATION OF PHENOLIC XENOESTROGENS IN SEDIMENTS AND SEWAGE SLUDGES BY HRGC/LRMS

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Introduction

Recent discoveries of an increase of plasma vitellogenin levels in male fish in rivers downstream of municipal sewage plants indicate the occurrence of estrogenic active substances in the aquatic environment [1 - 3], suggesting sewage plant effluents to be the major source for the release of these compounds. Once released via waste water treatment systems into the aquatic environment, a partition from the aqueous phase to particulate matter can take place. The adsorption of a chemical to sediments or sewage sludge depends on their content of organic matter and mainly on the physical properties of the substance. Phenolic compounds like p-nonylphenol with a high octanol/water partition coefficient (log K_{OW} > 4 [4]) occur in sediments in concentrations which are generally one to three orders of magnitude higher than those in the aqueous phase [5].
In vitro and partly in vivo tests have shown an estrogenic activity of the eight structurally different phenolic compounds analyzed in this work. We already established a GC/MS method for the simultaneous quantitative determination of these chemicals in sewage and surface water [6, 7]. The determination in sediments and sewage sludge completes their analysis in the aquatic environment, thereby providing information on the distribution between aqueous and particulate phase, persistence and biodegradibility of the eight phenolic xenoestrogens.

Material and Methods

Chemicals: The reference standards were dissolved in methanol: 4-t-octylphenol >90% purity, techn. 4-nonylphenol with ~85% content of p-isomers, bisphenol A ~97%, 4-hydroxybiphenyl >98%, 2-hydroxybiphenyl >98% (all obtained from Fluka, Buchs, Switzerland), 4-chloro-3-methylphenol 99%, 4-chloro-2-methylphenol 97%, and 2-t-butyl-4-methylphenol 99% (all purchased from Aldrich, Steinheim, Germany). As internal standard for quantification D_{10}-biphenyl (Cambridge Isotope Laboratories, purchased from Promochem, Wesel, Germany) was used. Solvents (Promochem, Wesel, Germany) were of nanograde purity; NaCl (analytical grade) was treated at 400 °C for 4 hours; HCl conc. and NaOH (p.a.) were purchased from Merck, Darmstadt, Germany.

Sample Treatment: Sewage sludge samples were taken from the municipal sewage plant Steinhäule, Ulm, Germany. Sediments were collected from two small streams and the Lake Constance in South Germany. The samples were freeze-dried and pulverized. 3 - 5 g of the sample were transferred into the pre-extracted paper thimble of a soxhlet apparatus and extracted with a mixture of hexane : acetone : diethylether (5 : 5 : 1) and 1 % (v/v) HCl conc. for 12 hours. The extract, evaporated to 3 - 5 ml and refilled to 50 ml with hexane, was shaken with 3 x 20 ml of a 2 M NaOH solution. NaCl was added, if necessary, for better phase separation. The combined aqueous phases were acidified with HCl conc. to pH 2 - 3 and enriched on 200 mg of the

polystyrene copolymer phase ENV+ (6 ml, ICT, Bad Homburg, Germany). After drying of the column under nitrogen, the elution was performed with 2 x 2.5 ml acetone. The extract was evaporated to a final volume of 0.5 ml. The organic phase was evaporated to a volume of 5 ml. 50 µl of both extract phases were separately methylated and, after addition of D_{10}-biphenyl as internal standard for quantification, analyzed by HRGC/LRMS. Quantification was performed according to the procedure reported recently [6, 7].

Reproducibility and Recoveries: Three aliquots of 50 ml hexane were spiked with a defined amount (about 1 µg each) of the eight phenolic compounds and the recoveries determined after performance of the whole clean up procedure.
To demonstrate the reproducibility of the extraction and the following clean up method for a complex matrix, three aliquots of a sewage sludge sample, collected at March 12, 1998, were extracted, purified, and analyzed.

GC/MS Parameters: HRGC/LRMS analysis of the eight phenolic xenoestrogens was carried out using a HP 4890 Series II gas chromatograph directly coupled to HP 5972 A mass selective detector. A 15 m DB-XLB fused silica capillary column with 0.25 mm inner diameter and 0.25 µm film thickness (J&W Scientific Products, Köln, Germany) was used with helium as carrier gas (flow rate 1.16 ml/min). The methylether of the phenolic compounds were detected in the selected ion monitoring mode. Splittless injection (1 µl) with the injection port at 240 °C was performed by a HP autosampler. The temperature of the GC/MS transfer line was 290 °C. The temperature of the GC oven was programmed from 80 °C (1 min hold) to 180 °C at 7 °C/min, then to 240 °C at 12 °C/min and to 300 °C at 20 °C/min (3 min hold).

Results and Discussion

The recoveries of the phenolic xenoestrogens in spiked aliquots of hexane, determined after performance of the clean up procedure, were as follows: Bisphenol A (98 %), 4-hydroxybiphenyl (123 %), 2-hydoxybiphenyl (98 %), 4-chloro-3-methylphenol (97 %), and 4-chloro-2-methylphenol (92 %) were found quantitatively in the solid phase extract of the aqueous phase. Techn. 4-t-nonylphenol, 4-t-octylphenol, and 2-t-butyl-4-methylphenol were determined with recoveries of 87 %, 75 % and 88 % in the hexane phase. The lipophilic character and the resulting poor phenate formation of the latter three substances may explain their whereabouts in the organic phase. The lipophilic character is supported for 4-t-nonylphenol and 4-t-octylphenol by the high log $K_{OW} > 4$ [4] and for 2-t-butyl-4-methylphenol by the possible shielding of the phenolic group by the bulky t-butyl group in the ortho position. The standard deviations of the triple analysis were below 10 % for all eight compounds.
Figure 1 shows the quantitative results of three independent analyses of the phenolic xenoestrogens in a sewage sludge sample after performance of the whole extraction and clean up method. The compounds are ordered from left to right with decreasing concentrations. The results of the concentrations of the five quantified xenoestrogens proved the reproducibility of the method. The standard deviations ranged between 2 and 22 % except for 4-t-octylphenol (38 %), which may be explained by low levels close to the limit of quantification.

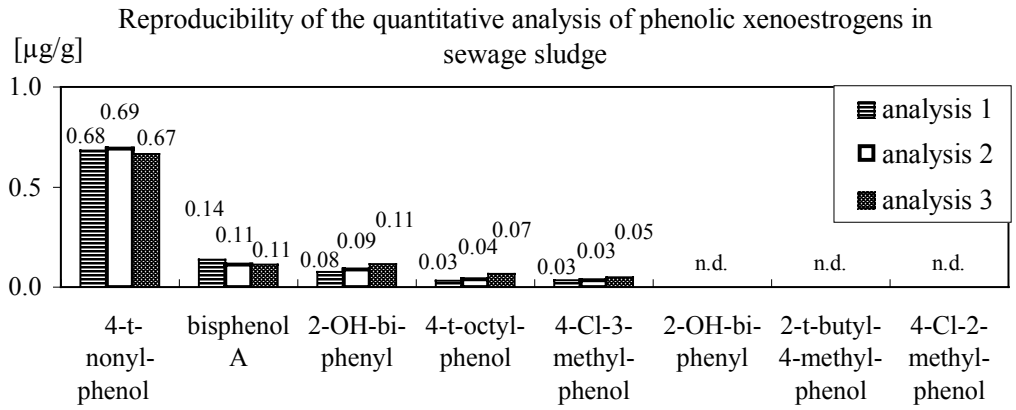

Figure 1: Independent analyses of three aliquots of a sewage sludge sample (municipal sewage plant Steinhäule, Ulm, Germany, collected at March 12, 1998) after performance of the whole extraction and clean up method. The concentrations are given in µg/g dry weight.

The concentrations of the eight phenolic xenoestrogens in sediments of two small streams and of the Lake Constance, located in South Germany, and in sludge of the municipal sewage plant Steinhäule (Ulm, Germany) are summarized in table 1. The levels ranged from not detectable (n.d.) to 0.7 µg/g dry weight. Both, sediments and sewage sludges showed for 4-t-nonylphenol the highest concentrations of the eight compounds. 4-Cl-2-methylphenol and 2-t-butyl-4-methylphenol were detec-ted neither in the sediment nor in the sewage sludge.

Table 1: Concentrations [µg/g dry weight] of the eight phenolic xenoestrogens in sediments of two small streams and the Lake Constance (located in South Germany) and in sluge of a municipal sewage plant (Steinhäule, Ulm, Germany)

[µg/g]	Körsch Sediment	Sulzbach Sediment	Lake Constance Sediment	Sludge March 2, 1998	Sludge July 8, 1998
4-t-nonylphenol	0.04	0.04	0.05	0.68	0.71
4-t-octylphenol	n.d.*	n.d.*	n.d.*	0.05	n.d.*
bisphenol A	<0.01	<0.01	<0.01	0.12	<0.01
2-OH-biphenyl	0.004	n.d.*	n.d.*	0.09	0.01
4-OH-biphenyl	0.005	<0.004	0.004	n.d.*	n.d.*
4-Cl-3-methylphenol	0.002	n.d.*	n.d.*	0.04	0.003
4-Cl-2-methylphenol	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
2-t-butyl-4-methylphenol	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*

* n.d. not detectable

The data of the sewage slugde, together with parallel analyses of raw sewage and effluent samples, give information on the fate of the eight phenolic xenoestrogens during modern waste water

treatment: Besides 4-t-nonylphenol, 4-t-octylphenol, bisphenol A and 3-t-butyl-4-hydroxyanisole, 2-hydroxybiphenyl (1.5 - 3.5 µg/l) and 4-chloro-3-methyl-phenol (0.2 - 0.5 µg/l) were detected in the raw sewage [8]. After sewage treat-ment the two compounds were no more until 0,01 µg/l detectable in the effluent sample. Therefore adsorption on the sewage sludge or biodegradation must have taken place. The results of the sludge analyses indicate a partial adsorption to the sewage sludge. To make a valid statement on persistence and biodegradibility of the eight phenolic xenoestrogens, more analytical data are necessary.

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