1
Introduction

1.1
General Considerations

In this study the focus is on the ingredients in personal care products such as polycyclic musk fragrances, household bactericides, and organophosphate flame-retardants and plasticizers, as well as some endocrine-disrupting agents that have been studied as compounds that are entering the aquatic environment mostly via sewage treatment plants (STPs). All of these compounds are used in the range of several thousand tons annually, most of them in applications near wastewater streams such as washing and cleaning. For the flame-retardants, one of the main issues was establishment of a link to surface water contamination, because, technically, usage and wastewater are not obviously connected. For those compounds that were found to be present in surface waters in more industrialized areas such as the Ruhr metropolis, tests were performed to determine whether degradation possibly happened in the respective river or plant. Enantioselective analysis was used in some cases for chiral compounds to identify biodegradation under the assumption that only biodegradation can result in a chiral shift, i.e., an enantiomeric excess from a racemate (see Sections 2.1.3 and 2.5.2) [5].

In this work the word “degradation” will be avoided, but “transformation” will be used if a reaction from one organic compound to another by any means whatsoever is addressed. “Mineralization” will be used when it is assumed that a compound will be transformed to carbon dioxide, water, etc. “Elimination” will be used to demonstrate that the fate of the compound is unknown but the concentration of the parent compound decreases.

For all of the compounds studied, robust methods were established, and the respective standard deviations and limits of detection are given in the respective chapters. In Chapter 3 method development is discussed in more depth, e.g., some of the flaws that may be encountered while quantifying with HPLC-MS/MS.

In several experiments it is hard to discriminate between dilution of xenobiotics in the (aquatic) environment, sorption to particles and sediments, and transformation processes. To discriminate between dilution into open waters and other processes, markers can be used as demonstrated in the sections on marine pollution. In marine ecosystems, salinity is a suitable marker, as most compounds are brought into the sea by freshwater streams.

Personal Care Compounds in the Environment: Pathways, Fate, and Methods for Determination. Kai Bester

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For risk assessment, diverse pieces of legislation are currently relevant. For limnic systems, the most important one on the European scale is the Water Framework Directive [4]. On the national scale, diverse regulations result in target values regarding surface waters. Target values for the limnic situation mostly combine emissions, concentrations, and persistence, and toxicology data are combined for a risk assessment. By this approach, basic data are obtained to define target values. For the marine ecosystems of the North Sea, the Oslo and Paris Commissions for the protection of the North Sea and the Northern Atlantic, respectively, have defined different regulations. The precautionary principle is often used because gaps in data concerning concentrations, persistence, and fundamental knowledge of the ecosystems are more common than in limnic ecosystems.

All in all, “zero emissions” with regard to the marine environment are requested by the OSPARCOM regulations [6]. The intention of this study is to present data on emissions via sewage treatment processes into the rivers, to demonstrate the persistence of some compounds, and to obtain data on the introduction of some of these compounds into the marine ecosystems. For this approach methods for trace and ultra-trace analysis were developed.

This study was performed to give new insights into elimination mechanisms of xenobiotics in sewage treatment as well as to study the persistence of organic compounds in limnic and marine ecosystems. To study elimination mechanisms of xenobiotics from wastewater, mass balances including sorption of compounds to the sludge were performed. Thus it was possible to discriminate between mineralization/transformation and pure sorption to sludge. Whenever applicable, assumed transformation processes were included in this study for holistic mass balances.

1.2
Introduction to Sewage Treatment Plant Functions

Today’s sewage treatment plants (STPs) are designed to eliminate particulate material. The major task, however, is to eliminate organic carbon such as that expressed in the parameter total organic carbon (TOC) or the more biologically defined biological oxygen demand (BOD). The target was thus to prevent the receiving waters from becoming anaerobic. Additionally, most plants have also been equipped with nitrogen and phosphorus removal processes to prevent eutrophication. They have never been designed to control the emissions of priority pollutants or other persistent organic compounds.

TOC removal is realized in most STPs by aerobic activated sludge treatment in which the dissolved organic compounds are transformed into carbon dioxide and biomass. The biomass is then separated and treated in anaerobic sludge treatment before final disposal.

Nitrogen is removed by oxidizing ammonia, which is toxic to fish, to nitrate and either including this into the biomass or reducing it to elemental (gaseous) nitrogen. For this process the medium needs to be anaerobic, which is classically performed in an upstream denitrification. However, in real-life wastewater treatment, simultaneous denitrification is used with aerated and non-aerated bands or areas in activated sludge treatment.

Phosphorus removal is normally performed as precipitation with iron salt solutions. In most cases simultaneous phosphorus elimination is performed, and thus the iron salt solutions are added to the main treatment basin.

During the passage of the wastewater, the water experiences different ecological situations and predominant bacterial communities; thus, it is hard to predict in which part of the sewage treatment which processes might be relevant for a given anthropogenic and possibly unwanted compound.

A schematic sketch of STP functioning is given in Fig. 1.1.

Typical elimination pathways include:

1. Sorption to sludge (biomass). Primary sludge should normally contain higher concentrations than excess sludge.
2. Oxidative transformation, especially in the aerated parts of the activated sludge treatment. Ideally, the final products of this process should be carbon dioxide, etc.
3. Reductive transformation, especially in the non-aerated parts of the activated sludge treatment. This might be especially relevant for dehalogenation processes. However, these processes are normally too slow to be performed within a few hours residence time of the activated sludge treatment.

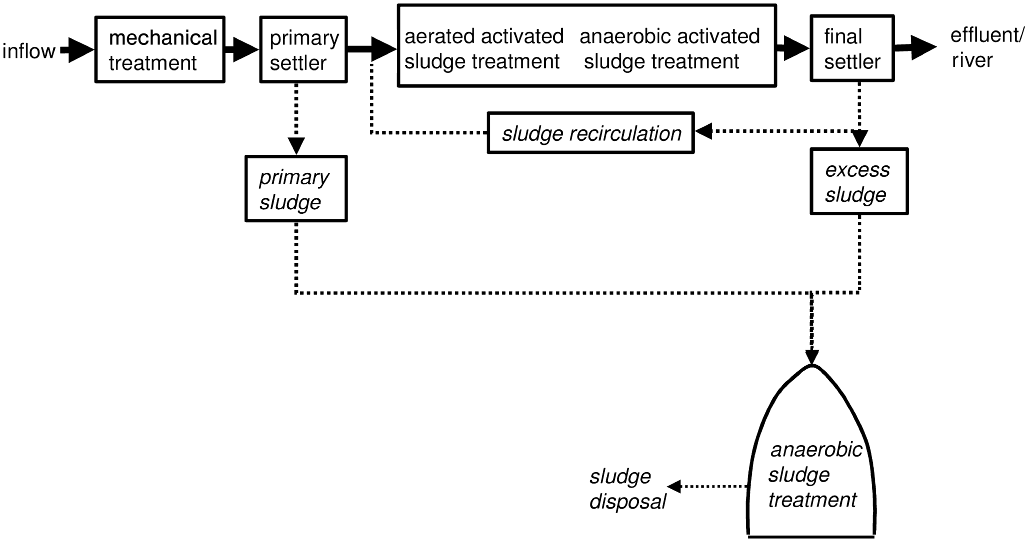

Fig. 1.1 Basic functionalities in a sewage treatment plant.

1.3
Enantioselective Analysis in Environmental Research

Several xenobiotic compounds – including pesticides such as chlordane, toxaphene, and metalaxyl; pesticide impurities such as α-HCH; and synthetic fragrances – are chiral compounds (see Fig. 2.1) [5]. Most of these compounds are supposed to interact with chiral biological receptors because of their desired biological effects. It has been shown that biotransformation reactions of such compounds in vertebrates, as well as in sediment–water systems (as a result of microorganisms), often are enantioselective processes, as a multitude of enzymes that take part in these transformation processes perform the respective reactions with considerable enantioselectivity [7–12] .

Considering instrumental analysis, it should be kept in mind that in some cases apparent racemates do not give peak ratios 1 : 1 at all concentrations. Enantioselective calibration is thus essential for all enantioselective chromatographic systems.

A very good overview on enantioselective separation systems is given by Ward [13], who also indicated that enantioselective gas chromatography (GC) is still a more dynamic research area in comparison to enantioselective high-performance liquid chromatography (HPLC) systems or other separation methods. A good overview on enantioselective analysis for environmental issues is given in Ref. [8].

1.3.1
Enantioselective Gas Chromatography Techniques

Enantioselective GC phases are based mostly on cyclodextrins, which have become more and more available and stable in the last few years. Long retention times of about 30–90 min still need to be taken into account, as the solvation enthalpy differences of the respective enantiomers often are small. These long retention times thus have relatively broad peaks (about 20 s), while in conventional capillary GC, peak widths of 4 s are experienced. These broader peaks consequently give lower detection limits. On the other hand, the precision of the determination is limited only to chromatographic overlaps and the precision of the integrating system. This is due to the fact that the enantiomers behave physically absolutely identical. This means that recovery rates, evaporation, and sorptive losses, etc., are identical for both enantiomers, as long as no chiral materials are used in the sample preparation scheme. Standard deviations of 1% and less are regularly obtained with established separation systems.

No chemically bonded phase is commercially available nowadays. Thus, temperature stability of the GC phase is gained by mixing the enantioselective discriminator, such as the cyclodextrin derivative, with other phases, such as OV-1701. These phases can operate at limits of up to 230 °C, which is an enormous improvement considering the situation five years ago. This limit still inhibits classical baking of the enantioselective columns in comparison to, e.g., DB-5 columns, which exhibit temperature limits of up to 400 °C. Thus, for enantioselective analysis more-selective cleanup procedures than in conventional analysis are needed. Enantioselective GC equipped with mass spectrometric (MS) [9] or electron capture detection (ECD) [7] has been used to determine enantioselective degradation of organochlorine pesticides in vertebrates, thus giving good evidence for biodegradation or biotransformation of such compounds in, e.g., marine mammals. Typically, two-step cleanups, e.g., consisting of size-exclusion and silica-sorption chromatography, are used for enantioselective determinations. Especially for ECD analysis, additional normal-phase HPLC fractioning for sample preparation was necessary in some cases [14]. Fractionation is especially important for the enantioselective analysis of toxaphenes, as the original toxaphene pattern is extremely complex. Thus, enantioselective separation has to be performed in combination with a classical separation of some hundred congeners [14]. It must be taken into consideration that there is no such thing as "the enantioselective GC phase." Some columns separate several compounds easily but fail on very similar substances. An overview on separations of relevant chiral pollutants that have already been separated is given in Table 1.1.

Table 1.1 GC phases for separation environmentally relevant enantiomers.

GC phase	Trade name	Analytes separated
Heptakis(3-O-butyryl-2,6-di-O-pentyl)-β-cyclodextrine	–	α-HCH, PCCHs [8]
Heptakis(2,3,6-tri-O-n-pentyl)-β-cyclodextrine in 50% OV1701	Lipodex C	α-HCH, β-PCCH [8]
Heptakis(2-O-methyl-3,6-dipentyl)-β-cyclodextrine	–	Oxychlordane, *cis*-heptachlor epoxide [8
Heptakis(6-O-*tert*-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrinin in 20–50% OV1701	Hydrodex; BGB172	Bromocyclene [10], PCB 88, PCB149, PCB183, PCB171, PCB 174 [8], oxychlordane, *trans*-heptachlor epoxide, allethrin, bioallethrin, methamidophos, acephate, trichlofon, bromacil, PCB 45, 95, 91, 136, 131, 176, 175 [16] dimethenamid, metalaxyl, metolachlor [11] HHCB, AHTN [35] ATII, AHDI [12], o,p′-DDT [17], methylated mecoprop [18]
Octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrine in 50% OV1701	Lipodex E	α-HCH, *trans*-chlordane, PCB95, PCB136 [19]
Octakis(2,6-methyl-3-pentyl)-γ-cyclodextrin (in 80% OV1701)	–	α-HCH, *cis*-chlordane, *trans*-chlordane [16]
Heptakis(2,6-methyl-3-pentyl)-β-cyclodextrin (in 80% OV1701)	–	Heptachlor, *cis*-heptachlor epoxide [16]
Heptakis(2,3,6-trimethyl)-β-cyclodextrin with some *tert*-butyldimethyl substituents	–	Toxaphenes [9, 14]
Heptakis(2,3,6-tri-O-*tert*-butyldimethylsilyl)-β-cyclodextrin coupled to RTX 2330	–	*cis*- and *trans*-chlordane, *trans*-nonachlor [20]

1.3.1.1 Applications of Enantioselective Gas Chromatography

Determination of Biodegradation

Enantioselective GC has been used extensively to determine whether or not biodegradation is relevant in selective media such as vertebrate tissue, surface water, sediment, tissue, sewage sludge, soil, etc. [7–12, 14, 15]. These experiments work well under the assumption that only enzymes perform enantioselective reactions in the environment. Thus, if an enantiomeric excess is determined, a biodegradation is highly probable.

Determination of Phase Transfer of Pollutants

For some time it was assumed that, e.g., HCHs might evaporate from the Great Lakes in the U.S. and Canada. It is extremely difficult to prove this assumption based on Henry's law, and it is difficult to analyze these compounds at levels of nanograms per liter in the water or picograms per cubic meter in the air. A mass transfer for such huge ecosystems is thus very hard to determine. On the other hand, knowledge of such processes is essential for the assessment of transport of these organochlorine compounds into the Arctic. Ridal et al. [21] found that α-HCH exhibited a peculiar enantiomeric distribution in the water of Lake Ontario, which could be determined with extreme precision: about 1% standard deviation was found for the determination of enantiomeric ratios as sample-to-sample deviation, as well in air, rain, and surface water samples. In comparison, rainwater samples, which were taken as a measure of the enantiomeric ratio of α-HCH in the higher atmospheric layers because the droplets were formed in high altitudes, were found to contain racemic α-HCH. Additionally, the enantiomeric ratio of this compound was measured in air samples taken from sea level as well as from lake water. Because the higher levels of the atmosphere (rain) contained true racemic composition, and the enantiomeric ratios of α-HCH in the air samples in summertime were very similar to the water, it could be concluded that indeed in summertime a vaporization of α-HCH from the water occurred. However, in wintertime the situation may be different.

Determining the Dominant Sources of Pollution

Mecoprop is a chiral phenoxyalkanoic acid herbicide that is marketed as an enantiopure compound for agriculture (pesticide). Its levels in Swiss surface waters are moderate but are surprisingly high considering that it has only agricultural applications. In 1998 it was found that the same compound was used in roof materials to prevent plants from growing on top of flat roofs. In contrast to the agricultural applications, the mecoprop used for roof sealing is marketed as racemate. Thus, the surface water samples were analyzed for the enantiomeric ratios. Because the enantiomeric ratio was about 0.5 in environmental samples, while 0 for agriculture and 1 for the rooftop material, it was possible to determine that about 50% of the mecoprop in Swiss surface water originated from rooftops and not from agriculture [18].

1.3.1.2 New Developments

Currently enantioselective GC is used, e.g., to determine whether or not chiral synthetic fragrances such as polycyclic musk fragrances are possibly biodegraded or whether adsorptive processes dominate the elimination in sewage treatment plants. These compounds bioaccumulate in fish; therefore, higher elimination rates in the respective wastewater treatment processes are urgently sought after. Chirality could give an indication as to which parameter (aerated biologically activated sludge, anaerobic treatment, or sorption phenomena) in the plant should be optimized.

In this study enantioselective analysis was performed as gas chromatographic separations for the synthetic fragrances HHCB, AHTN, and HHCB-lactone, as well as for the insecticide bromocyclene to observe transformation processes in sewage treatment plants (see Sections 2.1.3 and 2.5.2).

1.3.2
Enantioselective HPLC

Enantioselective HPLC is also used in environmental studies [7], though the major applications of enantioselective HPLC separations at the moment are in the field of drug development. This is probably due to the fact that the separation power of enantioselective HPLC columns with regard to the complex environmental matrix is somewhat limited. Therefore, the risk arises that the true enantioselective separation overlaps with compounds in the matrix, thus giving unreliable results. High selectivity of the respective detectors, such as tandem mass spectrometry, and a well-known matrix, as in a controlled soil degradation experiment, are thus essential prerequisites for the application of enantioselective HPLC columns in environmental sciences. It should also be noted that a multitude of different separation mechanisms are currently utilized in HPLC. In any case, the possibilities of combining columns with eluents are fascinating.

1.3.2.1 Applications of Enantioselective HPLC

Metalaxyl, metolachlor, and alachlor are chiral pesticides that have been marketed as racemates, while only one stereoisomer gives most of the desired biological effects (herbicides and fungicides). Some of these compounds cannot readily be separated by enantioselective GC but can easily be separated by HPLC, e.g., on a Whelk-O 1 column. Nowadays these studies are used to estimate the fate of both enantiomers in diverse ecosystems, especially in soil, to determine whether or not there are differences under diverse climatic and ecological situations [11, 15].

Enantioselective analysis used to be a method that could be used only by very specialized laboratories for fancy purposes. This situation has changed in the last few years to a method that any laboratory that has some experience in chromatography and sample pretreatment can use with reasonable effort. New insights into biodegradation as well as transport phenomena can be gained from this technique. Enantioselective analysis is thus a dynamic field bridging issues from environmental sciences, bio and life sciences, metabolomics, and analytical chemistry.