



Human & Environmental Risk Assessment on
ingredients of Household Cleaning Products

Polycyclic musks
AHTN (CAS 1506-02-1) and
HHCB (CAS 1222-05-05)

Environmental Section

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1. Executive Summary

The polycyclic musks AHTN and HHCB are used as fragrance ingredients in consumer products like cosmetics and detergents and cleaning agents. They are important ingredients in fragrances because of their typical musky scent and their fixative properties.

Over the last decade synthetic musks have attracted the attention of environmental researchers due to their presence in environmental samples and human mother's milk. As a reaction various risk assessments were carried out showing the risks were generally of low concern.

In the past decade the use volume of these substances in detergents and cleaning agents is declining, from circa 3300 tons in 1992 to 1800 tons in 2000. In household cleaning products, complex perfume-mixtures containing polycyclic musks are often purchased as such from the fragrance compounders and are used in the different formulations. Therefore, HERA was not able to come to representative volume data on use by the formulator companies.

The current risk assessment is made along the lines set out by the HERA methodology. The standard level risk assessment triggered a higher-tier approach to refine the risk assessment for the soil and sediment compartments. Using the more realistic monitoring data instead of the modelling approach, the environmental risk assessment for AHTN and HHCB shows that (1) sufficient data are available to assess the environmental risks; (2) the assessment can be based on measured concentrations in the northern region of the EU; (3) risk ratios are generally below 1; (4) however, for sediment organisms living in areas contaminated with a high effluent load, the risk ratios may be above 1. However, it should be remarked that the uncertainty around the toxicity to sediment organisms is high which is incorporated as an additional factor in the risk ratios.

Monitoring data are available for the northern region of the EU, but it is not known whether they are also representative for the southern European countries. An analysis was made of the regional variation of the use of AHTN and HHCB and of the trends in time. For this risk assessment, as a worst case, it was assumed, as a 'worst case', that the consumer use in southern European areas was 5 to 7 times above the use in northern Europe. The Berlin area was recognised as an area where sediment organisms may be at risk due to the combination of high loaded effluents and an extremely low dilution factor.

The report includes an analysis of the uncertainties in the risk characterisation. As a follow-up, a large-scale programme was launched recently to sample sewage treatment plants and sediment in a number of southern European countries and in Berlin. Moreover, toxicity studies with sediment organisms are carried out to refine the risk assessments for these organisms.

A human risk assessment document was also developed within the scope of the HERA project. The preparation of these HERA risk assessments ran in parallel to the risk assessments for AHTN and HHCB in the context of the EU Existing Chemicals Programme.

Contents

1. Executive Summary	1
Contents	2
2. Introduction	4
3. Substance Characterisation	5
3.1 CAS no and Grouping information.....	5
3.2 Physical Chemical Properties	6
3.3 Manufacturing Route and Production/Volume Statistics	7
3.4 Use applications summary	8
3.4.1 Exposure Pathway and Detergent Scenario	9
3.4.2 Regional variation and trends in time	9
4. Environmental Assessment	12
4.1 Environmental Exposure Assessment.....	12
4.1.1 Monitoring data	12
4.1.1.1 Influent and effluent of sewage treatment plants	12
4.1.1.2 Sludge	14
4.1.1.3 Surface water	16
4.1.1.4 Suspended matter and sediment	18
4.1.1.5 Concentrations in biota	21
4.1.1.6 Trends in time	25
4.1.1.7 Monitoring conclusions.....	27
4.1.2 Environmental Fate.....	29
4.1.2.1 Degradability.....	29
4.1.2.2 Removal in a sewage treatment plant.....	32
4.1.2.3 Accumulation	39
4.1.3 PEC calculations.....	41
4.1.3.2 PECsoil	45
4.1.3.3 PECpredator	46
4.2 Environmental Effects Assessment	48
4.2.1 Toxicity.....	48
4.2.1.1 Ecotoxicity – Aquatic test results.....	48
4.2.1.2 Ecotoxicity – Terrestrial test results.....	49
4.2.2 PNEC Calculations.....	50
4.2.2.1 PNEC _{STP}	50
4.2.2.2 PNEC water	50
4.2.2.3 PNEC sediment	51
4.2.2.4 PNEC soil.....	51
4.2.2.5 PNEC oral, predator	52
4.2.3 Endocrine disruption	52
4.3 Risk Characterisation.....	53
4.3.1 Aquatic compartment	53
4.3.1.1 STP.....	53
4.3.1.2 Aquatic organisms.....	54
4.3.1.3 Sediment organisms	55
4.3.2 Terrestrial compartment	57
4.3.3 Secondary poisoning.....	58
4.4 Sensitivity analysis	59
4.4.1 Regional use	59

4.4.2	Release to the STP	59
4.4.3	Predicted concentrations	60
4.4.4	Measured concentrations	61
4.4.5	Risk characterisation.....	61
4.5	Conclusions.....	63
5.	<i>Human Health Assessment</i>	65
6.	<i>References.....</i>	66
7.	<i>Contributors to this Risk Assessment.....</i>	71
Appendix 1.	<i>Removal during sewage treatment</i>	72
	Mass balance calculations	72
	Theoretical considerations	73
Appendix 2.	<i>Data for EUSES assessment.....</i>	80

2. Introduction

Polycyclic musks are important ingredients in fragrances for consumer products because of their typical musky scent and their fixative properties. The substances are applied in consumer products such as perfumes, cosmetics, soaps, shampoo, detergents, fabric conditioners, cleaning products, air fresheners, etc. The HERA risk assessment focuses on household detergent and cleaning products marketed by AISE member companies, including laundry detergents, fabric softeners, household cleaning products and soaps. Generally, the assessment focuses on the consumer use of those products. For the environmental assessment the consumer use of these substances represents the major route of entry into the environment. However, the total annual use volume in the EU including use in other applications like personal care products is used as the basis for this environmental assessment.

The polycyclic musks AHTN and HHCB have attracted attention since the nineties of the 20th century and as a consequence various risk assessments were produced (RIVM 1997, Balk and Ford 1999a,b). Both substances are included on the OSPAR list of Chemicals for Priority Action. The OSPAR Action Plan 1998-2003 stipulates that musk compounds belong to the category of diffuse sources and groups of substances to be considered for action. A background document was produced by Switzerland (OSPAR 2000). In the EU, the substances are included in the fourth priority list within the context of the Existing Chemical Programme (Council Regulation EEC 793/93). These assessments showed that an initial modelling approach based on the Technical Guidance Documents (EC 1996, 2003) triggered a more refined assessment of the risks for the soil and sediment compartments. This was confirmed by the HERA standard level approach. Therefore the current HERA environmental risk assessment does not repeat the discussions of the standard level modelling approaches but continues from there with a higher-tier assessment. Since concentrations in the compartments of concern, soil and sediment, are determined by the concentrations in the sludge and in the effluent of the sewage treatment plant, respectively, the implication is that the processes in the sewage treatment plant needed to be clarified. Monitoring data play an important role in this assessment. In the higher-tier assessment attention is also paid to the detection of the substances in fish and potential food chain effects.

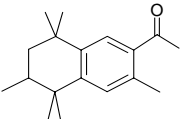
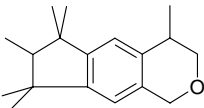
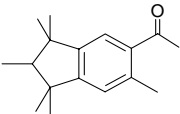
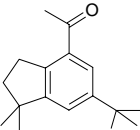
A complicating aspect is the observed decrease in the use volume of these substances over the years, requiring that monitoring data should be assigned to predictions based on the correct use volume. It becomes even more complicated when it is stated that in Northern European countries the use of these substances has been greatly reduced. With this range of uncertainties, modelling results become very uncertain. For these reasons the monitoring data deserve a prominent place in the exposure assessment.

3. Substance Characterisation

3.1 CAS no and Grouping information

Polycyclic musks are substituted indanes and tetralins. AHTN and HHCB (see Table 1) are the two largest volume products in this group, representing about 95% of the EU market and 90% of the US market for all polycyclic musks. Other members of this group are ADBI, AHMI and AITI. The chemical structure, CAS numbers and chemical names are given in Table 1. This current HERA risk assessment is focused on HHCB and AHTN.

Table 1. Identification of various polycyclic musks

Identification	
AHTN Tonalide [®] , Fixolide [®] 	CAS: 21145-77-7/1506-02-1 $C_{18}H_{26}O$, 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene or 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one or 6-acetyl-1,1,2,4,4,7-hexamethyltetraline
HHCB Galaxolide 50 [®] , Abbalide [®] 	CAS: 1222-05-5 $C_{18}H_{26}O$, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexa-methylcyclopenta-[γ]-2-benzopyran
AHMI Phantolid [®] 	CAS: 15323-35-0 $C_{17}H_{24}O$, 5-acetyl-1,1,2,3,3,6-hexamethylindan
ADBI Celestolide [®] , Crysolide [®] 	CAS: 13171-00-1 $C_{17}H_{24}O$, 4-acetyl-6-tert. butyl-1,1-dimethylindan

3.2 Physical Chemical Properties

The environmental behaviour of a substance is determined by the physical chemical properties. These include the solubility in water, vapour pressure and the octanol/water partition coefficient or other partition coefficients such as those between water and environmental matrices like soil or the organic material in sewage sludge. Empirical and/or estimated values for the physical chemical properties of the polycyclic musks are included in Table 2. Various properties were estimated by so-called QSARs, based on molecular fragments. These estimates may be improved if empirical data for closely related substances are introduced. For the estimation of log K_{ow}, the data for AHTN were used as additional input to estimate these properties for AHMI, AITI and ADBI. This affects not only the results of log K_{ow}, but also of the estimates for the solubility in water.

Common characteristics of these polycyclic musks are the hydrophobic behaviour and poor water solubility. Therefore the substances are expected to sorb onto organic matter and lipids.

Table 2. Properties of various polycyclic musks

	AHTN	HHCB	AHMI	AITI	ADBI
Physical state	solid	viscous liquid	solid	-	-
Melting point (°C)	> 54	-10 – 0	> 58	103 ^S	96 ^S
Boiling Point (°C)	180 (15 hPa)	325	318 ^S	330 ^S	319 ^S
Water solubility (mg/l)	1.25 ^M 0.36 ^S	1.75 ^M 0.19 ^S	0.9 ^S	0.3 ^S	0.22 ^S
Vapour pressure (Pa) at 25 °C	0.0608 ^M 0.0074 ^S	0.0727 ^M 0.0117 ^S	0.132 ^K 0.196 ^S	0.009 ^S	0.019 ^S
Henry's Law Constant (Pa. m ³ . mol ⁻¹)	37.1 ^N	36.9 ^N			
Log K _{ow}	5.7 ^M ; 5.4 ^N	5.9 ^M ; 5.3 ^N	4.90 ^{S,T}	5.36 ^{S,T}	5.4 ^S
Log K _{oc} in sludge	4.80 ^M	4.86 ^M	-	-	-
in sludge	3.8 ^N	3.8 ^N			
in sediment	4.0 ^P	3.85 ^P			

M : measured (reported in Balk and Ford 1999a)

N : measured (Artola 2002)

K : measured (PFW)

P : measured (Fooker 2002)

S : estimation (SRC 1999 LOGKOW v.1.63, WSKOW v1.33, MPBP v1.30)

T : estimation using experimental values^N adjusting with data for AHTN (SRC 1999 LOGKOW v.1.63)

3.3 Manufacturing Route and Production/Volume Statistics

HHCB is produced in a reaction between α -methyl styrene with tertiary amylene to form pentamethyl indane. In a following reaction with propylene oxide, HHCB-alcohol is formed. HHCB is formed after cyclisation with paraformaldehyde. This is a viscous syrup-like liquid (paste). It is fluidised with diluents to make it pourable in a proportion of HHCB to diluent of 65 to 35% by weight. Suitable solvents include dipropylene glycol (DPG), diethylphthalate (DEP), and isopropylmyristate (IPM). These dilutions are often referred to as HHCB (or a brand name) 50%. In toxicity reports or publications the purity of 'HHCB undiluted' is taken as 100% (actually defined as > 95% based on isomeric mixture).

AHTN is produced by a two step synthesis. The first step is a cyclo-alkylation. The intermediate is isolated by distillation and stored. In the next reaction step acetylation takes place at room temperature. The product mixture is washed twice with water and the washed product is distilled. The distilled product is stored in liquid form at 60 °C. Finally it is crystallised and packed in fibre drums.

The production in Europe of both AHTN and HHCB is concentrated in one plant for each substance. A significant part of this production is exported as the 'pure substance' outside the EU. Also a fraction of the compounds is exported outside the EU and in addition, a part of the formulated products (personal care products as well as cleaning agents) will be exported. Industry sources estimated that 20-30% of their production is exported outside Europe as finished fragrance compounds or in consumer products (Letter IFF 1998). These sources also declare that import volumes are expected to be far below export volumes.

The substance AHTN or HHCB (diluted) is mixed with other fragrance ingredients into fragrance oils or 'compounds'. A compound may consist of as many as 50 ingredients and a compounder may produce a large number of specified recipes out of the 2000 – 3000 different fragrance ingredients. These fragrance compounds are used in formulating products such as cosmetics, detergents etc. Many fragrance oils or compounds contain HHCB or AHTN which are the most important representative polycyclic musks; when present, at a concentration of 2 to 4% in the compounds. The concentration of fragrances in detergents and soap ranges from 0.2 to 1%.

The volumes of AHTN and HHCB in Europe are based on surveys of the volumes used in compounding, carried out by the RIFM (Research Institute of Fragrance Materials) in 1993, 1996, 1999, and by IFRA for 2000, see Table 3. According to RIFM the 1995 use volumes account for approximately 90% of the total use as 32 companies involved in fragrance compounding responded to the survey which included all of the major fragrance producers world wide. The total use volume for 2000 of AHMI, AITI and ADBI together was 30 ton.

Table 3. Use volumes in Europe (RIFM and IFRA surveys)

Year	HHCB [ton/year]	AHTN [ton/year]
1992	2400	885
1995	1482	585
1998	1473	385
2000	1427	358

3.4 Use applications summary

The use of fragrance oils per product category in the EU was summarised by Somogyi *et al.* (1995), see figure 1. Thus the share of the fragrance ingredients used in detergents, fabric softeners and cleaning products is almost 50% and the fraction in ‘down the drain’ applications is estimated at 77%.

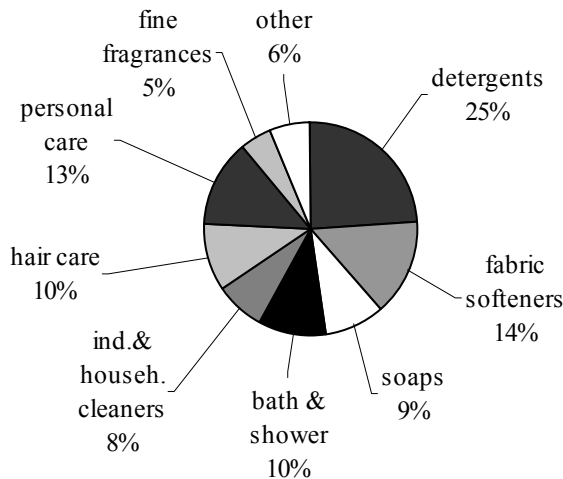


Figure 1. The use of fragrance oils in the European Union.

[figure taken from Balk *et al.* 2001, ACS Symposium Series 791, p. 171]

In household cleaning products, complex perfume-mixtures (containing polycyclic musks) are often purchased as such and used in the different formulations. Therefore, HERA was not able to come to representative volume data on polycyclic musks from the formulator companies. However, for the environmental compartment the origin of the emission is not relevant. For this reason, the data of the ECHA/IFRA survey on the volume used in compounding for the year 2000 are used in the environmental exposure assessment under the assumption that all (rather than 77%) of the use volume is discharged to the drain.

3.4.1 Exposure Pathway and Detergent Scenario

The “HERA detergent scenario” is used for the environmental exposure calculations. The entire tonnage was assumed to follow the domestic down-the-drain pathway to the environment. Production and formulation scenarios fall outside of the scope of HERA and were not explicitly considered at the local level, although both production and formulation losses are included in the regional risk assessment (Table 3).

3.4.2 Regional variation and trends in time

Data on the regional use of AHTN and HHCB are derived indirectly from the use of detergents and cosmetics. An analysis was made of the regional differences of the use throughout the EU member states. The result of this analysis is presented in figure 2 for detergents and figure 3 for cosmetics.

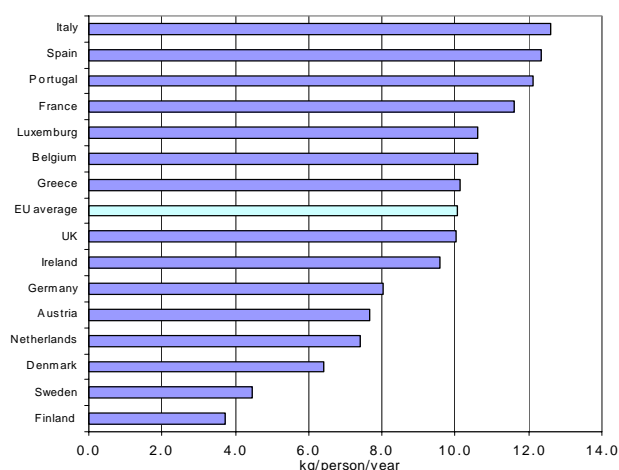


Figure 2. Detergent consumption per capita by country for 1998 (AISE 2001)

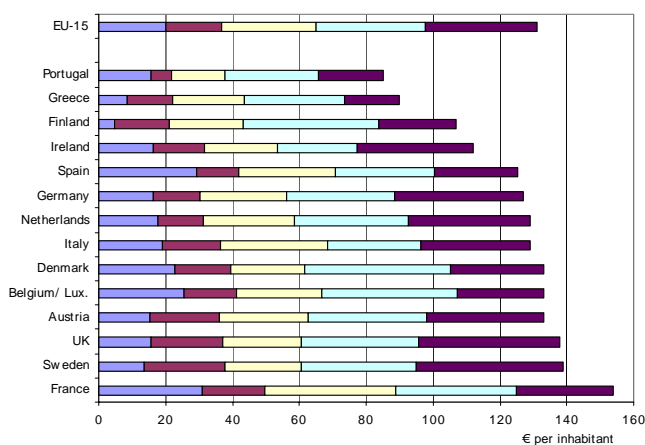


Figure 3: Cosmetics consumption per capita in the EU in € per inhabitant (retail prices for 2000. Weighted mean is €131 per inhabitant (COLIPA 2001))

Figure 2 shows that the use of detergents per inhabitant is lower in some northern European countries than in southern Europe. However, the highest per capita use (Italy, 12.6 kg per year) is above the EU average (10.1 kg) only by a factor 1.25.

The use of cosmetics (expressed in monetary units) is lowest in some southern countries. Yet the highest consumption in the EU, in France (€ 154) is above the EU average (€ 131) by a factor of 1.18 only.

The use of polycyclic musks as an ingredient of cosmetics and detergents significantly decreased during the second half of the nineties. Due to negative publicity European-wide brands abstained from polycyclic musks containing fragrances. This trend was followed by producers of locally marketed products in the Northern European countries like Germany, the Netherlands, Belgium, Switzerland, Austria and Scandinavia. In other parts of Europe locally operating producers followed this trend to a lesser extent.

Due to these market developments cosmetics and household cleaning products are probably less often fragranced with polycyclic musks in northern Europe than in southern Europe. In the extreme case that the entire European volume of use of HHCB and AHTN would be consumed only in southern Europe (i.e. France, Spain, Portugal, Greece, Italy) and the UK/Ireland with about two-thirds of the EU's population, the outcome of the risk assessment, (based on an evenly distributed use of cosmetics and detergents,) would have to be corrected by a factor of $3/2$ ($=1.5$). However, it should be noted that the factor 1.5 is rather hypothetical. It is very unlikely that the gradual replacement in northern Europe coincides with an increased use volume per capita in Southern Europe. This assumption would imply that the EU consumer's market is divided into different sections, whereas the major producers indicated that this would not be the case. A more plausible explanation might be that since 1995 the export of consumer products with polycyclic musks outside Europe has increased, whereas on the European market PM-free products are more and more replacing the PM-containing products. However, a higher export of PM-containing consumer products cannot be substantiated with adequate market data.

Recent environmental monitoring data over several years in northern countries show a downward trend by a factor of circa 3 (Hessen, HLUG 2001, see section 4.1.1.6). Assuming that this decrease reflects the reduced use volume in the northern countries, a reasonable estimate of the *per capita* use for 2000 would be $1/3$ of the 1995 use.

In conclusion, there are two factors that may cause an uneven distribution of the use volume of HHCB and AHTN per capita in Europe:

1. A 'cultural' factor of different use volumes of detergents, causing:
 - a higher use of detergents per capita by a factor of 1.25 in southern EU countries (166 million inhabitants)¹;
 - an average use in Belgium/Luxembourg, Greece, UK and Ireland (84.6 million inhabitants);

¹ This factor of 1.25 results in the HERA scenario where the regional tonnage is 7% of the total use volume.

- a use below average by a factor of 0.7 in northern countries (125.5 million inhabitants).
2. The market development factor, causing a significant decrease of use of polycyclic musks since 1995. As a maximum this would result in a higher use in the southern countries by a factor of 1.5 as compared to the average per capita use.

As both factors are independent, the combination gives a factor $1.25 * 1.5 = 1.88$ above the average use in a 'worst case regional scenario for southern Europe' for the year 2000.

For 1995 an evenly distributed use volume would mean 1482 (HHCB) or 585 (AHTN) tonne over 365 million inhabitants. As the process of replacement in the northern countries had not yet started in 1995, only a factor of 1.25 should be applied to the average *per capita* use to cover the 'cultural' factor in the southern countries. For a use below average in northern countries a factor of 0.7 should be applied.

For 2000 an evenly distributed use would mean 1427 or 385 tonne over 370 million inhabitants and to cover the uneven use in a realistic worst case scenario this would be corrected with a factor of 1.88. In the northern countries the use volume would be 1/3 of the total use *per capita* per year.

According to the EU Technical Guidance Document (TGD, EC 2003), for a regional scenario the regional use is 10 % of the total use. This total amount is used by the 20 million inhabitants in the region. Thus the TGD regional approach equals the 'worst case' scenario based on an extreme interpretation of the data for 2000.

The distinction of these different scenarios is relevant for the comparison of monitoring data with model approaches.

Table 4. Scenarios for private use (consumers)

Scenario		Derivation	Consumption in g/y <i>per capita</i>	
			HHCB	AHTN
2000	Use volume		1427 ton	359 ton
	TGD regional (10%)	10% of total use	7.14	1.79
	southern Europe,	1.25 * 1.5 * average	7.23	1.81
	worst case			
	average		3.86	0.97
	northern Europe,	1/3 * 1995 'below average'	0.95	0.37
1995	reasonable estimate			
	Use volume		1482 ton	585 ton
	southern Europe,	1.25 * average	5.07	2.0
	reasonable worst case			
	average		4.06	1.6
	northern Europe,	0.7 * average	2.84	1.12
	below average			

4. Environmental Assessment

4.1 Environmental Exposure Assessment

Since the nineties of the 20th century the presence of polycyclic musks in environmental compartments has been investigated by a number of research groups (e.g., Eschke et al. 1994, 1995, Rimkus and Wolff 1997). The investigations were carried out mainly in Germany, The Netherlands and Switzerland. In view of the fact that currently polycyclic musks are probably used to a lesser extent in these countries than in other European countries, the question of the representativeness of these observations remains open. The discussion of the different scenarios in the previous section (3.4.2) shows the variability that may be due to regional or temporal variation in the consumer use pattern. Although unlikely, this variability in the *per capita* consumption could be a factor of 5 for AHTN and for HHCB, it could be up to a factor of 7 (see table 4).

In this section the available monitoring data will be presented followed by a discussion on the variability in time and regions. The monitoring data will be leading in the approach to assess the environmental exposure to polycyclic musks.

4.1.1 Monitoring data

The presence of polycyclic musks in the environment has been investigated in eight European countries. They have been detected in sewage treatment plants, surface waters, sediment and biota. The results are summarised in table 5 up to 9.

4.1.1.1 Influent and effluent of sewage treatment plants

HHCB and AHTN were detected in STP influents and effluents in different regions of Europe since the early nineties (see table 5). For each series of samples the median or mean value is presented as well as the 90th-percentile to show the variability of the individual samples in the series. Because of the regional variation of the measurements, as well as the temporal scale, the measured concentrations are to be compared to the appropriate scenarios distinguished in table 4.

Table 5. Concentrations in STP influents and effluents (µg/l)

Location	AHTN		HHCB		Reference
	Influent	Effluent	Influent	Effluent	
Germany, Ruhr <1994	mean 2.2 ¹	median 1.8 90-perc. 3.0	mean 1.5 ¹	median 1.9 90-perc. 2.3	Eschke 1994, 1995
Germany, Berlin 1996		mean 4.5 max. 5.8		mean 9.0 max. 10.8	Heberer 1999
Germany, Berlin 1996-1997		median 2.2 90-perc. 3.4 max. 4.4		median 6.7 90-perc. 10.8 max. 13.3	Fromme '00, 2001a
Germany, Hessen, 1999-2000		median 0.4 90-perc. 0.6		median 1.1 90-perc. 1.6	HLUG 2001
The Netherlands 1997-1998	median 4.0 max 8.7	median < d.l. max. 0.77	median 6.4 max 14.5	median 1.4 max 1.6	Rijs 1999
The Netherlands 1995-96		mean 0.28 max. 0.42		mean 0.36 max 0.63	Verbruggen 1999
The Netherlands 1997	0.3 – 0.4	0.3 – 0.6	0.7 – 1.1	0.4 - 1	Leonards 2000, Van Stee 2000
The Netherlands 2001	median 1.3 max 1.8	median 0.7 max. 1.2	median 3.4 max 4.3	median 1.6 max 2.2	Artola 2002
Switzerland 1998		median 1.4 90-perc. 2.0		median 2.3 90-perc. 4.7	BUWAL 1998
Switzerland 1997		median 2.0 max. 2.8		median 3 max. 3.9	Noser 2000
Switzerland 2002	mean 1.4 1.2 – 2.0 mean 1.5 0.6 (after prim. settling)	mean 0.33 0.5 – 0.2 mean 0.25 0.09	mean 4.5 2.3 – 6.9 mean 6.9 1.68 (after prim. settling)	mean 0.78 0.6 – 1.1 mean 0.86 0.26	Brändli 2002
Sweden < 1995				mean 3 range 1 - 6	Paxéus 1996
Ohio, U.S.A. 1997	mean 10.5	mean 1.3 max. 1.7	mean 12.7	mean 1.3 max. 1.6	Simonich 2000
USA 1977 - 1999	mean 10.35 7.1 – 33.9	mean 1.28 ⁽²⁾ 0.02 – 2.0	mean 12.6 8.7 – 45.5	mean 1.65 ⁽²⁾ 0.03 – 4.0	Simonich 2002
UK 1999 - 2000	3.7 – 13.2	0.6 – 2.7	9.1 – 17.6	1 – 4.6	
The Netherlands 1999	2.4 – 3.9	1.2	3.2 – 5.9	1	

¹ Unreliable results according to author; more likely 10 to 30 µg/l (Eschke 1996)² Median without the extremely low figures from lagoons

It is concluded that although influent concentrations may range considerably, all effluent concentrations are within a small range independent from the varying origins of samples and sampling times. For HHCB the median and mean effluent concentrations range from 0.36 to 3 µg/l. For AHTN this range varies from 0.23 to 2.0 µg/l. Exceptions are the concentrations measured in the Berlin area. Higher effluent levels may be related to problems with bulking sludge (as explained in more detail in section 4.1.3.4. and Annex 1). However, further details on the samples (e.g., type of sample, total suspended solids content) or sampling sites are not available. Influent data for the Berlin region are not available either and the higher effluent levels cannot be explained with current knowledge.

To illustrate the actual concentration ranges of AHTN and HHCB in effluents, for both substances frequency distributions are shown for Europe after 1996 in figure 4. From these figures the 90th-percentile values can be derived: 1.9 µg/l for AHTN and 3.8 µg/l for HHCB. The data for the Berlin area are not included here.

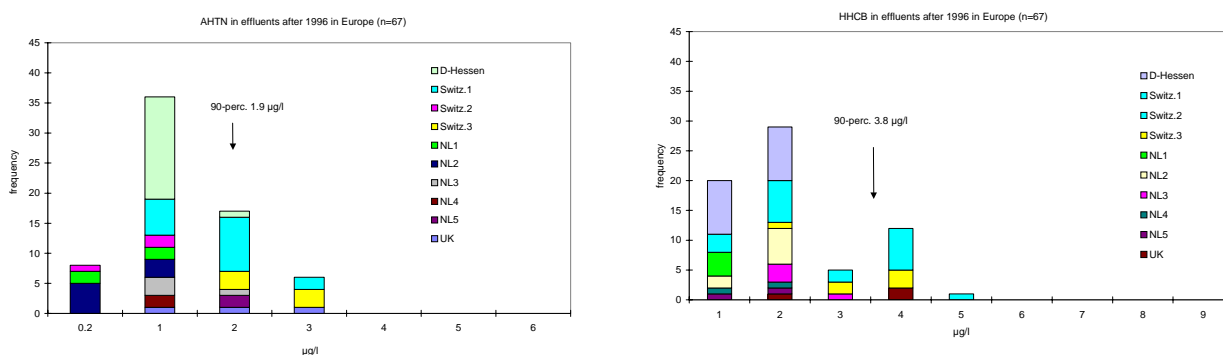


Figure 4. Frequency distributions for AHTN and HHCB in effluents after 1996 in Europe.

4.1.1.2 Sludge

As can be seen in table 6 concentrations in sludge from sewage treatment plants vary with time and location. Concentrations in activated and digested sludge were not significantly different (Blok 1998, HLUG 2001). The table shows the difference between data from 1997/1998 as compared to later samples. The concentrations measured in 1997/98 by Blok (1998) and Rijs (1998) are higher than those from a later period reported by Herren and Berset (2000) and HLUG (2001). The clear decline of the concentrations observed in Hessen from 1996 to 2000 reflects the reduction in the use of both substances over that period. On the other hand, recent data from UK indicate a high use of HHCB in that region, and not of AHTN. Contrary to the observations on effluents, the data for sludge from the Berlin area, stated as a maximum in the table, are not exceptional as compared to the other values in the list. This supports the suggestion that the high effluent concentrations are related to bulking sludge indeed.

Table 6. Concentrations in sewage sludge

Sample	AHTN [mg/kg dw]		HHCB [mg/kg dw]		Reference
The Netherlands					Blok, 1998
6 STPs 1997					
primary	mean 8.3	range 3.3 – 14	mean 13.9	range 5.4-27	
activated	mean 16.0	range 2.3 – 34	mean 27.9	range 4.4-63	
digested ¹	median 16	range 0.9 – 22	median 23	range 9.0-31	
The Netherlands					Rijs, 1998
3 STPs ‘97-’98					
primary	mean 8.2	range 3.7 - 11.7	median 13.5	range 6 –17	
activated	mean 5.3	range 0 – 13.5	median 9.7	range 4.8 – 21	
digested	median 12	range 11 – 13	mean 20	range 19 – 21	
Germany < 1997 n=2					Sauer et al., 1997
STP activated sludge	mean 8.3	range 4.0 - 12.6	mean 8.9	range 4.3 - 13.4	
Sewer slime urban/industrial area	mean 2.1	range 0.1 - 8.9	mean 1.4	range 0.1 - 5.2	
Sewer slime rural area	median 23.1	range 9.5 - 36.7	mean 15.5	range 9.1 - 21.8	
Germany, Hessen					HLUG, 2001
9 STPs					
1996, dom. act. sludge	median 14.3	range 3.5 – 20.8	median 17.1	range 11.6 – 20.4	
1996, digested sludge	median 15.6	range 14.3 – 20.1	median 18.3	range 14.1 – 21.6	
Domestic sludge ² 1996	median 15.0	range 12.0 – 20.1	median 17.1	range 11.9 – 21.6	
1997	median 12.1	range 6.4 – 17.5	median 14.1	range 7.2 - 22.3	
1998	median 9.1	range 5.8 – 18.4	median 10.3	range 6.7 - 21.9	
1999	median 6.8	range 4.5 – 8.5	median 9.1	range 5.1 - 10.8	
2000	median 4.2	range 2.9 – 6.1	median 6.7	range 4.3 - 8.6	
Switzerland 1998					Herren and Berset, 2000
12 STPs					
Digested sludge of dom. and more industrial input	median 1.3	range 0.7 – 4.2	median 3.9	range 2.3 – 12.2	
Germany, Berlin, 1997?					Heberer et al., 2002
3 municipal STPs	up to 5.1		up to 11.4		
Austria 1999					Hohenblum, 2000
1 pilot plant			range 1-2		
Activated sludge					
UK 20xx, 14 STPs	median 4.0		median 26		Stevens, 2002
Digested sludge	range 0.12 – 16		range 1.9 - 81		

1. Concentrations in digested, thickened and composted sludge

2. Surplus sludge, sometimes digested

d.l.: detection limit.

4.1.1.3. Surface water

Measured concentrations in surface water are sometimes reported as total, sometimes as dissolved concentrations. However, with a suspended solids concentration of 10 mg/l, the fraction sorbed will be less than 10%, and in general this source of variation is considered negligible. In table 7 concentrations reported in surface water are presented.

Table 7. Concentrations in surface water

Location	n	AHTN µg/l	HHCB µg/l	Reference
Germany, Ruhr <1994	30	median 0.2 ⁴ 90-perc. 0.3	median 0.4 ⁴ 90-perc. 0.5	Eschke 1994, 1995
Germany, North Sea 1990, 1995	12	median 0.00019 90-perc. 0.0009	median 0.00021 90-perc. 0.0008	Bester 1998
Germany, Elbe 1995	1	0.07	0.10	Bester 1998
Germany, Elbe 1995	2	0.09	0.07	Lagois 1996
Germany, Elbe 1996-1997	25	median 0.05 90-perc. 0.07	median 0.09 90-perc. 0.12	Winkler 1998, 1999
Germany, Berlin 1996	26	median 0.5 90-perc. 2.4 ¹	median 0.8 90-perc. 4.3 ¹	Heberer 1999
Germany, Berlin 1996-1997	34	low effluent input median 0.02 90-perc. 0.03 max. 0.06	low effluent input median 0.05 90-perc. 0.14 max. 0.32	Fromme 2000, 2001a
	40	moderate effluent input median 0.05 90-perc. 0.14 max. 0.27	moderate effluent input median 0.15 90-perc. 0.49 max. 0.81	
	28	high effluent input ³ median 0.47 90-perc. 0.91 max. 1.10	high effluent input ³ median 1.48 90-perc. 2.73 max. 3.15	
Germany, Hessen, 1999-2000	2*20	median 0.05 90-perc. 0.17	median 0.15 90-perc. 0.46	
Germany, Main 1998	29		median 0.10 ⁴ 90-prec. 0.20	
The Netherlands River Rhine, 1994-1996	32	median 0.05 90-perc. 0.10	median 0.06 90-perc. 0.16	
				Breukel 1996

Location	n	AHTN µg/l	HHCB µg/l	Reference
River Meuse, 1994-1996	35	median 0.07 90-perc. 0.11	median 0.08 90-perc. 0.19	
The Netherlands 1995-96	14	median 0.04 ² 90-perc. 0.14	median 0.027 ² 90-perc. 0.13	Verbruggen 1999
The Netherlands 1997	5	0.027 – 0.354 ²	0.006 – 0.27 ²	Leonards 2000, Van Stee 2000
Switzerland, Glatt ≤ 1995	1	0.075	0.136	Müller 1996
Switzerland 1997	8	median 0.05 max. 0.2	median 0.08 max. 0.26	Noser 2000
Switzerland 1998	20	median 0.025 90-perc. 0.045	median 0.061 90-perc. 0.097	BUWAL 1998
USA, Southwestern	3	0.027 – 0.092	0.035 – 0.152 ³	Osemwengie 2001
Japan, Tama	5		0.0007 – 0.1	Yun, 1994

1. High contribution of sewage works effluents to surface water quality due to low surface water flows and high amounts of raw sewage produced by Berlin's 3.5 million people.
2. Free (dissolved) concentrations
3. Dedicated effluent streams
4. Data points taken from graph

The data show the impact of the sampling site on the concentration level. Frequently surface water samples were taken in the vicinity of STP discharge points or in waters where effluents have a high contribution to the total water flow, as, for example, the study for the River Ruhr (Eschke et al. 1994, 1996). This is particularly clear from the areas in Berlin where a distinction between low, moderate and high effluent input areas was made. Surface water concentrations are clearly related to the distance from the STP discharge points. In order to illustrate trends in time as well as regional variations, concentrations in surface waters are presented in the frequency distributions in figure 5. The figure includes three charts, one for northern Europe before 1995, one for northern Europe after 1995 and one for the Berlin area in Germany. The 90th-percentile values are shown as well. The pictures illustrate that the concentrations before and after 1995 are not different. They also show that the levels in Berlin show the same range, but in addition, on some sites higher concentrations are observed: categories above 0.5 to 5 µg/l for AHTN and above 0.75 to 7.5 µg/l for HHCB are observed in Berlin only. The data belonging to the low and moderate effluent input areas in Berlin correspond to the range observed in other areas, whereas the true exceptions are found in the areas with high effluent input. The 90th-percentiles concentrations for the highly loaded waters are only slightly below the 90th-percentiles levels for the effluents (see figure 4). Thus one explanation for the high surface waters is the extremely low dilution in the Berlin water system, whereas another explanation is related to the high levels observed in the effluents.

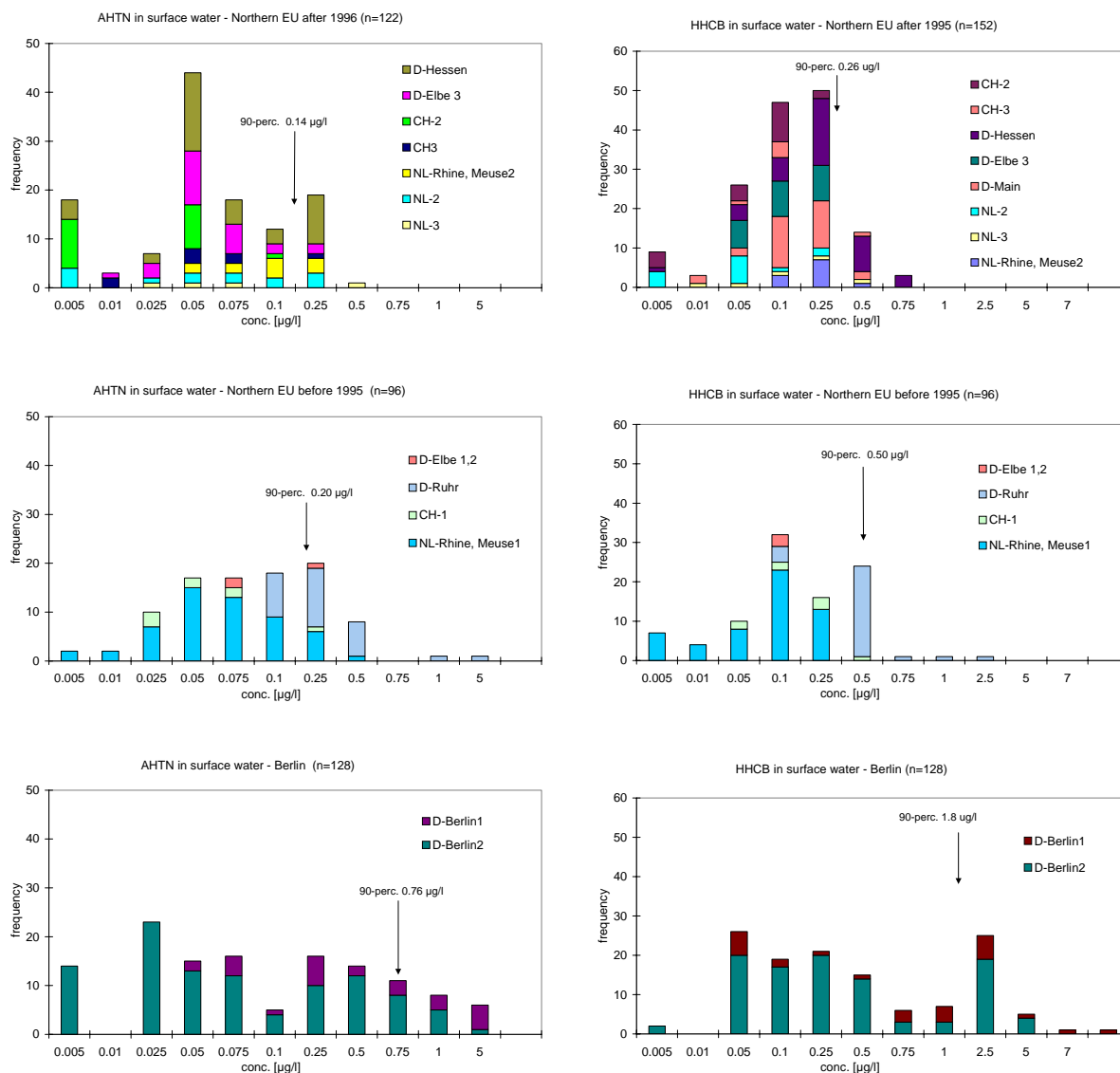


Figure 5. Frequency distributions for AHTN and HHCB in surface water: Northern EU after 1995, Northern EU before 1995 and the German Berlin area (1996-1997)

4.1.1.4 Suspended matter and sediment

After the discharge of treated sewage AHTN and HHCB will be present on the suspended solids and in dissolved form. The solids from the STP will settle within a short distance whereas the dissolved fraction will redistribute between the suspended solids in the surface water and settle after some time. At a larger distance from the outlet, photodegradation and primary biodegradation processes will lower the concentrations in the water (see section 4.1.2.1). As for the surface water, also concentrations on suspended matter and on sediment are a function of the distance from the STP and the dilution of the effluent with surface water.

Concentrations measured in suspended matter and sediment of surface waters are summarised in table 8. Some of the suspended and sediment samples were explicitly taken in contaminated brooks (Hessen) or in areas with high effluent input (Berlin) and this is reflected in the higher concentration levels. The observed high variability in the Berlin areas with high contributions of STPs was attributed to inhomogeneous sample materials.

In a 5-year programme in Hessen, Germany, in three brooks the sediment as well as suspended matter was sampled. The sediment concentrations were always considerably lower than the concentrations measured in suspended matter from the same sites.

Table 8. Concentrations in sediment and suspended solids

Location	n	AHTN (mg/kg dw)	HHCB [mg/kg dw]	Reference
Dutch borders	14	median 0.24	median 0.06	Breukel and Balk, 1996
Suspended matter Rhine 1994-1996		range 0.10-0.54	range 0.05 (d.l.)-0.16	
Suspended matter Meuse 1994-1996	14	median 0.84	median 0.20	
		range 0.06-1.2	range 0.05-0.58	
90 th percentile Rhine and Meuse 1994-1996	28	0.96	0.31	
NL surface waters 1997-1998	24	median 0.12	median 0.10	Rijs and Schäfer, 1999
Suspended matter		90 th perc. 1.0	90 th perc. 0.41	
		max. 1.7	max. 1.8	
Germany, Elbe 1996-1997	31	median 0.47	median 0.44	Winkler et al., 1998
Susp. particulate materials		range 0.19-0.77	range 0.15-0.74	
		90 th perc. 0.61	90 th perc. 0.61	
Germany, Berlin area 1996-1997	19	low effluent input	low effluent input	Fromme et al. 2000 2001a
		median 0.02	median < d.l.	
Sediment (10 cm depth)		90-perc. 0.03	90-perc. < d.l.	
	20	moderate effluent input	moderate effluent input	
		median 0.24	median 0.23	
		90-perc. 0.52	90-perc. 0.38	
	20	high effluent input	high effluent input	
		median 0.93	median 0.91	
		90-perc. 2.21	90-perc. 1.90	

Location	n	AHTN (mg/kg dw)	HHCB [mg/kg dw]	Reference
Germany, Hessen				HLUG 2001
Suspended matter				
1996	11	median 0.29 range 0.09 – 0.84	median 0.27 range 0.08 – 1.13	
1997	12	median 0.19 range 0.06 – 0.86	median 0.25 range 0.05 – 0.85	
1998	12	median 0.30 range 0.05 – 0.86	median 0.23 range 0.05 – 0.78	
1999	16	median 0.14 range 0.03 – 0.40	median 0.16 range 0.02 – 0.39	
2000	15	median 0.11 range 0.02 – 0.26	median 0.15 range 0.02 – 0.39	
Suspended matter in contaminated brooks,				
1996	11	median 3.2 range 0.54 – 12.7	median 2.7 range 0.90 – 13.7	
1997	5	median 2.7 range 1.9 – 6.6	median 1.8 range 1.7 – 5.4	
1998	5	median 2.9 range 1.6 – 7.2	median 2.5 range 1.3 – 5.2	
1999	3	range 0.4 – 2.9	range 0.4 – 2.5	
2000	2	range 0.6 – 0.97	range 0.7 – 1.1	
Sediment in contaminated brooks, 1996 - 1999	5	range 4.8 – 0.7 **	range 4.9 – 0.6 **	
Germany, Elbe, 1997 (Hamburg-Dresden)	9	mean 0.047 range 0.007 – 0.104	mean 0.101 range 0.016 – 0.180 *	Wiegel, cited in Fooken 2002
Niedersachsen, sediment 5 rivers, 1996	8	median < 0.0005 range < 0.0005 – 0.004	median 0.007 range < 0.0005 – 0.054*	Lach and Steffen, 1997 cited in Rimkus 1999

* according to Fooken (pers. comm. and Fooken 2002). These values probably need to be corrected by a factor of 0.5 due to the purity of the standard (50%).

** downward trend in time

The data were combined in frequency distributions and 90th-percentile values were derived for areas with a low or medium effluent input, see figure 6 and 7. The 90th-percentile values for suspended solids of 0.8 mg AHTN/kg and 0.5 mg HHCB/kg were clearly below the observed levels in the contaminated brooks in Hessen. There the maximum decreased from circa 13 mg/kg to 1 mg/kg (1996 to 2000) for both substances.

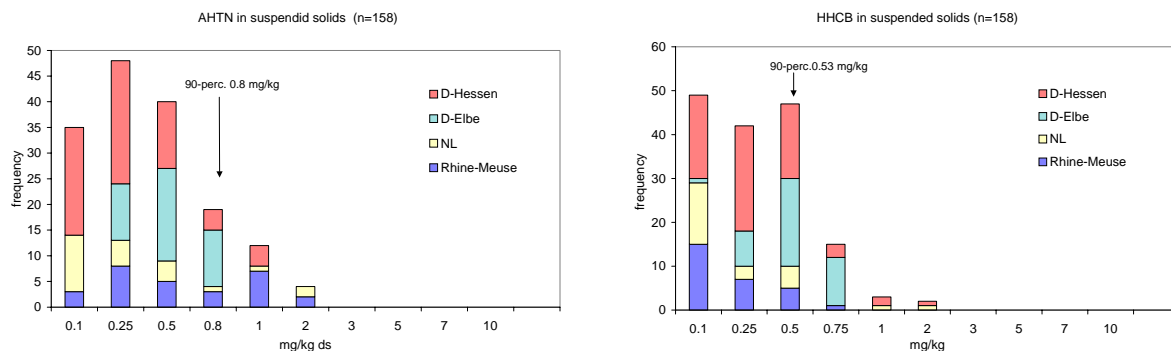


Figure 6. Concentrations in suspended matter (data from known high effluent input areas are not included)

The 90th-percentile for the sediment in areas with low or medium effluent input was 0.45 mg AHTN/kg and 0.35 mg HHCB/kg. The maximum levels observed in contaminated brooks in Hessen in 1996 were higher by an order of magnitude, but decreased to the same level in 2000. The 90th-percentiles from the high effluent input areas in Berlin were above the low/medium 90th-percentile by a factor of 5. This factor is in line with the higher concentrations observed in surface water samples from the high effluent input area in Berlin.

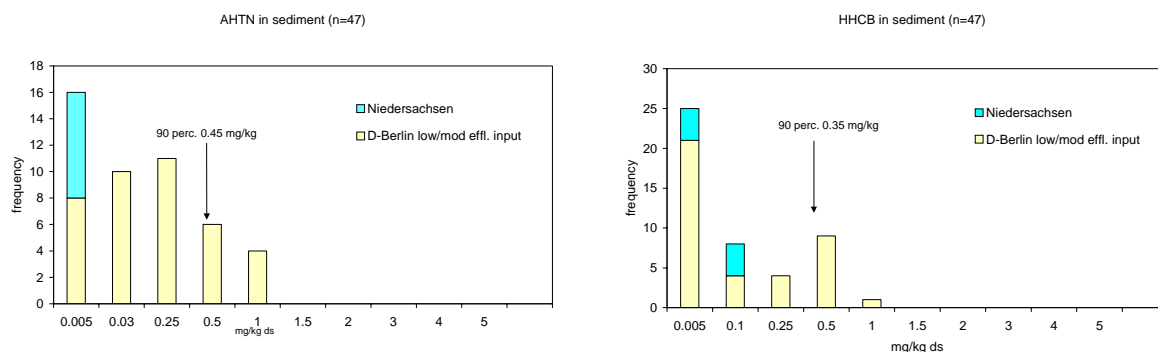


Figure 7. Concentrations in sediment (Data from known high effluent input areas are not included)

4.1.1.5 Concentrations in biota

Monitoring data are available for fish in various regions of Europe, see table 9. Concentrations may be determined in edible parts (muscle), in adipose tissue or liver and the results may be expressed based on fresh weight or on lipid contents. For the evaluation of the risk for secondary poisoning, the fish predator is assumed to eat complete fish and therefore the concentrations based on fresh weight are used.

The bioconcentration in fish depends greatly on the lipids content of the fish. As the lipid contents of eel is considerably higher (up to 25%) than for other fish (< 1 to 5%, reported by

Eschke et al. 1994; Fromme et al. 2001), a distinction was made between eel and other species. Even though the concentrations of AHTN or HHCB in lipids of the eel sometimes seem to be lower than for other species, due to its higher lipid contents the total body burden of the eel based on fresh weight is always higher.

Table 9. Concentrations in fish

Sample	AHTN		HHCB	AHTN		HHCB	Reference
	n	mg/kg lipids	mg/kg lipids	mg/kg fw	mg/kg fw		
Germany, Ruhr Non-eel	7	median 3.5 max 7.1	median 2.8 max 3.8	median 0.029 max 0.034	median 0.018 max 0.045	Eschke et al. 1995	
Eel	2	mean 0.6 max 0.7	mean 0.5 max 0.6	mean 0.13 max 0.15	mean 0.11 max 0.13		
Non-eel fish effluent pond	8	median 15.3 max 37.2	median 8.0 max 19.8	median 0.29 max 0.65	median 0.15 max 0.34		
Eel from effluent pond	5	mean 36 max 57.9	mean 35 max 63.6	mean 10 max 17.5	mean 10 max19.2		
Denmark fish pond Rainbow trout	4	mean 0.36	mean 0.36	mean 0.011	mean 0.011	Rimkus 1997	
Germany, Elbe Eel	5	mean 0.056	mean 0.048	mean 0.016	mean 0.012		
Other fish	4	mean 0.58	mean 2.31	mean 0.004	mean 0.012		
Germany river Stör near STP outfall, fish	3	mean 14.0	mean 14.3	mean 0.28	mean 0.34		
East sea Herring	1	0.53	0.75	0.046	0.065		
Denmark Herring	1	0.07	0.12	0.005	0.008		
Ireland Herring	1	<0.01	<0.01	<0.0008	<0.0008		
The Netherlands Eel 1998	6			median 0.03 max. 0.10	median 0.03 max 0.10	Rijs and Schäfer 1998	
Germany, Berlin 1996-1997							
Eel	54	median < d.l. 90-perc. < d.l.	median 0.198 90-perc. 0.81	median < d.l. 90-perc. < d.l.	median 0.050 90-perc. 0.079	Fromme et al. 2001b	
Low effl. input							
Mod.effl.input	53	median 0.186 90-perc. 0.545	median 0.426 90-perc. 1.405	median 0.032 90-perc. 0.112	median 0.077 90-perc. 0.210		
High effl.input	58	median 2.833 90-perc. 5.170	median 5.830 90-perc.11.483	median 0.668 90-perc. 1.380	median 1.473 90-perc. 2.812		
Perch							
Low/med. effl. input	19	median < d.l. max < d.l.	median < d.l. max 0.122	median < d.l. max < d.l.	median < d.l. max 0.122		
High effl. input	9	median 7.1 max 43.7	median 33.3 max 159.9	median 0.047 max 0.332	median 0.200 max 1.215		

Sample	AHTN		HHCB	AHTN	HHCB	Reference
	n	mg/kg lipids	mg/kg lipids	mg/kg fw	mg/kg fw	
Common Bream						
Low/med. effl. input	37	median < d.l. max 2.7	median 3.3 max 15.3	median < d.l. max 0.042	median 0.040 max 0.260	
High effl. input	10	median 18.4 max 35.3	median 90.1 max 143.3	median 0.324 max 0.851	median 1.571 max 3.426	
Roach						
Low/med. effl. input	48	median < d.l. max 2.0	median <d.l. max 11.8	median < d.l. max 0.050	median < d.l. max 0.260	
High effl. input	6	median 4.5 max 18.4	median 13.0 max 55.3	median 0.064 max 0.339	median 0.168 max 1.018	
Pike						
Low/med. effl. input	12	'all –1' < d.l max 2.2	median <d.l. max 14.2	low 'all –1' < d.l max 0.021	median = d.l. max 0.098	
High effl. input	2	8.0 – 10.0	61.5 - 66.5	0.044 – 0.060	0.366 – 0.370	
Pike perch						
Low/med. effl. input	25	median < d.l. max < d.l.	median <d.l. max 113.0	median < d.l. max < d.l.	median < d.l. max 0.113	
High effl. input	8	median 10.0 max 88.3	median 47.3 max 383.9	median 0.037 max 0.362	median 0.190 max 1.574	
Italy 8 rivers and lakes	28			median 0.004 90-perc. 0.026	median 0.005 90-perc. 0.034	Draisici et al. 1998
Czech Republic three rivers, 1997 – 2000						Hajslova et al. 1998, Hajslova 2002
Chub	302	median ranges 0.6 – 2.4	median ranges 0.4 – 2.7			
Bream	164	median ranges 0.9 – 3.5	median ranges 1.2 – 8.4			
Barbel	50	median ranges 0.5 – 11.4	median ranges 0.3 – 10.8			
Perch	156	median ranges 0.4 – 3.7	median ranges 0.4 – 5.8			
Trout	117	median ranges 0.3 – 3.1	median ranges 0.3 – 2.2			

Sample	AHTN		HHCB		Reference
	n	mg/kg lipids	mg/kg lipids	mg/kg fw	
Norway, high effl. input 1997, 1998					Kallenborn et al. 2001
Thornback ray filet	1	0.089	0.071	0.0008	
Haddock filet	2	mean 0.254	mean 0.343	mean 0.0014	
Atlantic cod filet	3	mean 0.008 max 0.010	mean 0.035 max 0.043	mean 0.002 max 0.003	
Saithe filet	1	0.093	0.225	0.002	
Thornback ray liver	1	0.003	0.021	0.001	
Haddock liver	3	mean 0.024 max 0.034	mean 0.243 max 0.37	mean 0.0162 max 0.023	
Atlantic cod liver	13	mean 0.096 max 0.38	mean 0.271 max 1.51	mean 0.035 max 0.13	
Saithe liver	1	0.001	0.007	0.0004	

Concentrations measured in fish are reported from areas with high effluent input and from more remote regions, in Germany, The Netherlands, Italy, the Czech Republic, Norway and the North Sea. The relation with the discharge of STP effluents is evident from the data of fish caught in effluent ponds (Eschke et al. 1995) and in the high effluent input areas as compared to the medium or low areas in Berlin (Fromme et al. 2001b). The data from the Czech Republic are collected from 1997 to 2000. The time trend observed in samples from various compartments in northern Europe is not detected in this series of fish samples taken outside the EU. By far the highest concentrations in surface waters were observed in the areas classified as 'high effluent input' areas in Berlin, Germany. These levels are of the order of magnitude of those found in effluent ponds by Rimkus (1999) and Eschke et al. (1995). The overall median of 396 fish in Germany (including Berlin), The Netherlands and Italy is at the level of the detection limits for some studies, < 0.01 mg/kg fw and the 90th-percentile is 0.52 mg/kg fw for AHTN and 1.19 mg/kg fw for HHCB. For the presentation of the distribution frequency of the concentrations in these 396 fish samples a distinction is made between the data from Berlin and from other regions, in view of the extreme concentrations observed in some surface waters in the Berlin area, see figures 8 and 9.

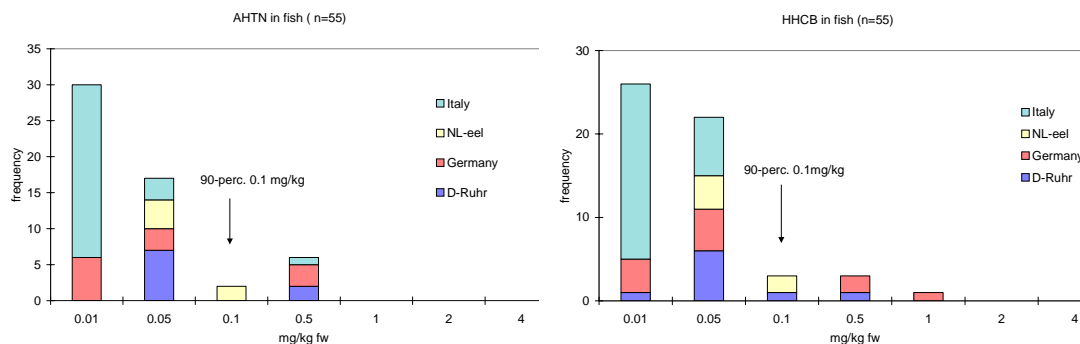


Figure 8. Concentrations in fish reported from Germany (Eschke et al. 1995, Rimkus 1997), Italy (Draisici et al. 1998) and The Netherlands (Rijs and Schäfer 1998)

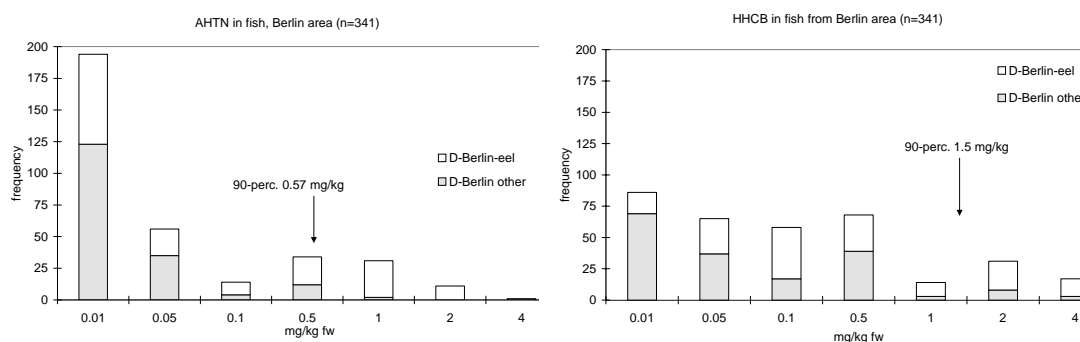


Figure 9. Concentrations in fish reported from the Berlin area in Germany (Fromme et al. 2001b)

4.1.1.6 Trends in time

In Hessen, Germany, in a 5-year programme samples were taken once per year of sludge in 9 STPs from 1996 to 2000. During this period also samples were taken of suspended matter on 17 sites along the various rivers and in some particularly contaminated brooks. Effluents of the 9 STPs and surface water samples (20 sites) were taken once in 1999 and in 2000. This large scale project yielded information on the trend of the environmental concentrations during this period. The results show a general decrease in time. The concentrations in sludge and suspended matter decreased by a factor of 3 to 4 for AHTN and 2 to 3 for HHCB, see figure 10 (note the logarithmic scale). Concentrations in effluent and surface water were available only during the latter two years, but decreased to the same extent. This decrease coincides with the decrease in use volume since 1995.

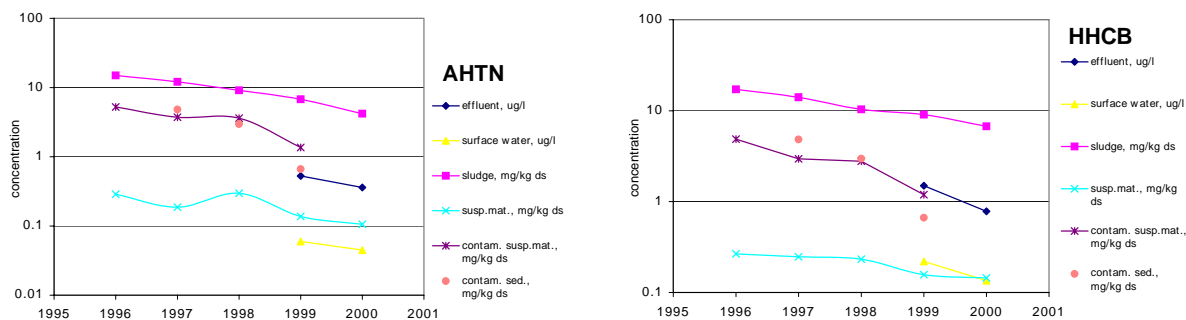


Figure 10. Trend in the median concentrations of AHTN and HHCB in various environmental compartments, Hessen, Germany (based on data from HUG 2001) (Remark: Contaminated suspended matter: mean for 3 sites; contaminated sediment: only one site. Logarithmic scale)

Concentrations on sludge are directly reflecting the decreasing input of AHTN and HHCB to the sewer systems over the years. The concentrations in the suspended matter of 17 sites in the Hessian rivers also generally decreased during the five years. Figures 11 and 12 show the individual observations for these compartments. It is remarkable that not only concentrations in suspended matter and surface water follow the downward trend but this trend was also directly observed in the sediment of contaminated brooks. For one of those brooks, data were available for three consecutive years, showing that the concentrations in sediment closely followed the drop in the suspended matter concentrations (see isolated data points in figure 10). Generally, sediment samples include materials settled during a series of years and thus include the history of the spot. Thus for non-degrading substances a reduction of the use volume would go unnoticed for some years. In the case the observed concentration drop suggests that degradation processes take place in the sediment without delay.

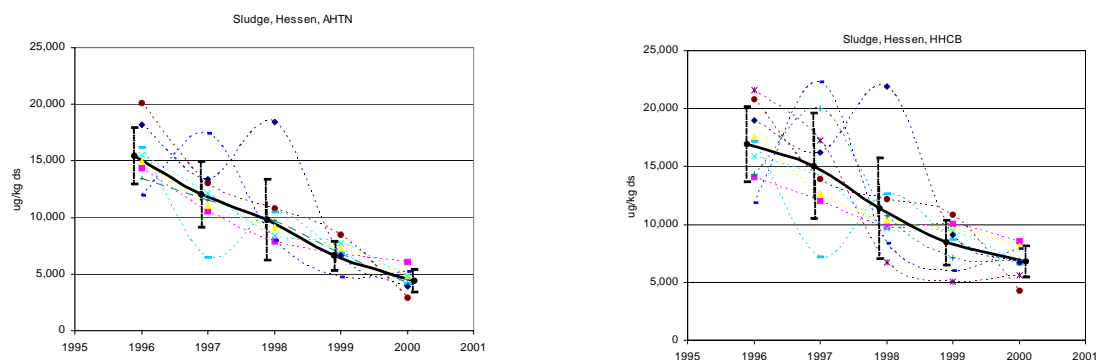


Figure 11. Trend in the concentrations of AHTN and HHCB in wasted sludge from 9 domestic STPs, Hessen. The solid line connects the mean per year. Standard deviations are included. Please note that each data point is one observation per STP per year. They are connected with a dotted line to show the individual STP not to suggest a trend. The difference between the years (e.g., 1996-2000) was statistically significant (t -test, $P < 0.01$).

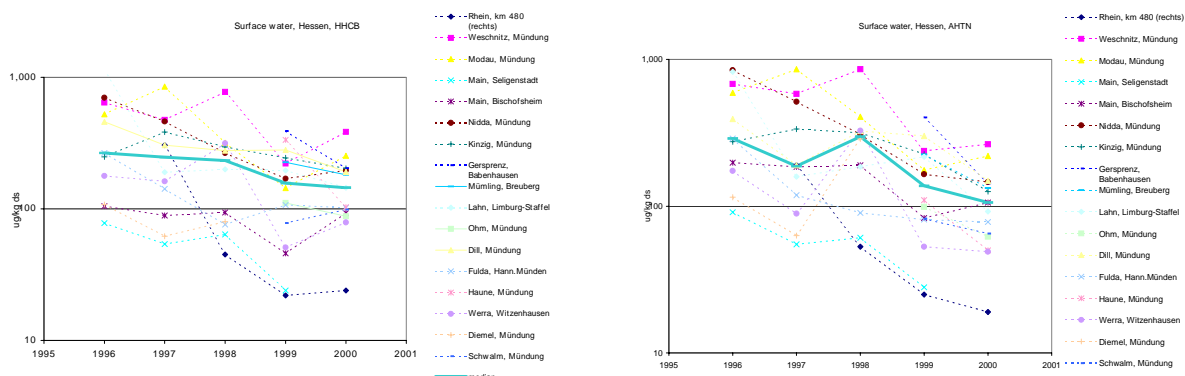


Figure 12. Concentrations of HHCB and AHTN in suspended material in water systems in Hessen from 1996 – 2000 (based on data from HUG 2001). Please note that the points represent one sample per year. Per river the points are connected by dotted lines; these are not suggesting the trend between the sampling periods (Logarithmic scale).

This decrease is also observed, but not documented as completely, in other regions of North-western Europe. Therefore the year of sampling is an important piece of information of a sample. In particular if measured data are to be compared to predictions, the proper use scenario should be known (see table 4). The downward trend was not observed in the fish samples taken from 1997 to 2000 in the Czech Republic.

4.1.1.7 Monitoring conclusions

The evaluation of the monitoring data for HHCB and AHTN leads to the following conclusions:

1. Measured concentrations vary on a spatial scale, where higher concentrations are clearly related to the discharge of STP effluents into surface water. In water systems with low effluent input, concentrations are clearly lower than in high effluent input areas, as demonstrated by the data from Berlin.
2. A monitoring programme in Hessen, Germany, between 1996 and 2000 showed a decrease in environmental concentrations, as a reflection of the decrease in use volume since 1995. This decrease is also observed but less well documented, in other regions of Europe. Therefore the year of observation is an important piece of information for a sample.
3. Environmental data are highly variable. Simply taking the highest value does not reflect a representative exposure. Therefore the data were summarised in frequency distributions where the source of the data and periods of sampling were taken into account. For understanding the behaviour of the substances the median values (per region) should be compared to model predictions for the specific region (or user scenario). For an environmental risk assessment a conservative approach should be taken where the 90th-percentile of the data (per region) is compared to the identified no effect levels (PNEC).

4. The reports for Berlin show a wide range of concentrations, extending from the low levels also observed in other regions to levels far beyond those in other areas. The Berlin samples were taken in 1996/1997. It is expected that the downward trend observed elsewhere in Northern Europe has also occurred in this area but no data are available.

The measured environmental concentrations should be assigned to an appropriate use scenario (table 4) to enable a proper comparison. The median values in table 10 are used for comparison with predictions for the various use scenarios, whereas the 90th-percentiles are used for the risk assessment.

Table 10. Summary of concentrations in the environmental compartments. Median, 90th-percentile values or maximum

Compartment	Representative scenario	Reference	AHTN		HHCB	
			median	90 th -perc.	median	90 th -perc.
Effluent (µg/l)	2000, low	figure 4	0.6	1.9	1.4	3.8
	Berlin 1996-97	table 5: Heberer et al. 1999; Fromme et al. 2001a	2.2	3.9	6.8	10.8
Sludge (mg/kg dw)	2000, low:	Hessen Germany 2000 (HLUG, 2001)	4.2	6.1 mx	6.7	8.6 mx
	2000, average	UK 200? (Stevens et al. 2002)	4	16 mx	26	81 mx
	1995, low:	NL 1997 (Blok 1998)	16	22	23	31
	Berlin 1997?	Heberer et al. 2002 referred to in Heberer et al. 2001		5.1 mx		11.4 mx
Surface water (µg/l)	2000, low	figure 5	0.045	0.14	0.095	0.26
	1995, low	figure 5	0.075	0.20	0.099	0.50
	Berlin 1996/97 high effluent input area	Heberer et al. 1999; Fromme et al. 2001a.	0.44	1.0	1.3	2.8
Suspended matter (mg/kg dw)	2000, low	figure 6; high effluent input areas excluded	0.23	0.8	0.19	0.53
Sediment (mg/kg dw)	2000, low	figure 7; high effluent input areas excluded	0.03	0.45	0.003	0.35
	1996, low	contaminated brooks Hessen (HLUG, 2001)		4.8 mx		4.9 mx
	1996-97	high effluent input area Berlin (Fromme et al. 2001a)	0.93	2.21	0.91	1.90
Aquatic biota (mg/kg ww)		Old and recent measurements except Berlin (figure 8)	0.008	0.1	0.01	0.1
		Berlin 1996/97 (figure 9: Fromme et al. 2001b)	< 0.01	0.57	0.06	1.5

4.1.2 Environmental Fate

4.1.2.1 Degradability

Abiotic degradation

The abiotic degradation of HHCB and three other fragrance molecules with a structure similar to AHTN was studied (Aschmann et al. 2001). Rate constants were measured for the gas phase reactions of OH radicals. For HHCB, $k = 2.6 \pm 0.6 \times 10^{-11} \text{ cm}^3 \cdot \text{molecule}^{-1} \cdot \text{sec}^{-1}$. The rate constants estimated for the four substances (SRC programme AOP) were within a factor of 2 (1.3 – 1.5) of the empirical values for these 4 substances. This implies that the rate constant estimated for AHTN ($k = 1.7 \times 10^{-11} \text{ cm}^3 \cdot \text{molecule}^{-1} \cdot \text{sec}^{-1}$) will also be relatively accurate. Assuming a daylight period of 12 h and a concentration of OH radicals of $1.5 \times 10^6 \text{ cm}^{-3}$, the calculated atmospheric half-life is 7.3 h for AHTN and 3.4 h for HHCB. These data indicate that the atmospheric lifetime is sufficiently short and that they will not undergo long-range transport to any significant extent.

The photochemical degradation of AHTN was demonstrated using a Mercury high pressure lamp at 20 °C. The degradation half-life $t_{1/2}$ was 1.25 minutes. In the absence of oxygen the rate was strongly reduced ($t_{1/2} = 20$ minutes). No stable metabolites were detectable. This experiment shows the potential for photochemical degradation, although the conditions are not relevant from an environmental point of view (Willenborg and Butte 1998).

Biological mineralisation

The ultimate degradability of both HHCB and AHTN was assessed in the tests for ready biodegradation: the modified Sturm test for CO₂-evolution OECD 301B (Jenkins, 1991a,b) and a modification of OECD 301B: the sealed vessel headspace with TIC analysis for CO₂-evolution and an adapted inoculum (King, 1994a,b). For AHTN, OECD 302C, the modified MITI II test was also carried out (Rudio 1993). These tests showed the absence of mineralisation under the conditions of the standard tests. In the two-phase closed bottle test (ISO 10708) AHTN showed 21% oxidation after 3 weeks and after repetitive additions of AHTN a marginal 12% after 7 weeks (Boersma and Hagens, 1991).

Primary biodegradation in the aquatic compartment

In batch experiments reported by Langworthy et al. (2000) and Federle et al. (2002) freshly collected activated sludge from three different sewage treatment plants was spiked with radiolabeled HHCB (5 and 25 µg/l ¹⁴C-HHCB). In all three activated sludge samples HHCB was transformed into more polar metabolites. The initial metabolites had TLC-elution times similar to HHCB-lactone and hydroxycarboxylic acid, later much more polar metabolites appeared. The first order rate constants for the loss of parent HHCB on the sludge were 0.0101 and 0.021 h⁻¹ with 5 and 25 µg HHCB/l, respectively (or $t_{1/2}$ 69 and 33 hours). In effluent diluted in river water with 1 and 0.5 µg radiolabeled HHCB/l, the half-lives were 33 and 43 h, respectively.

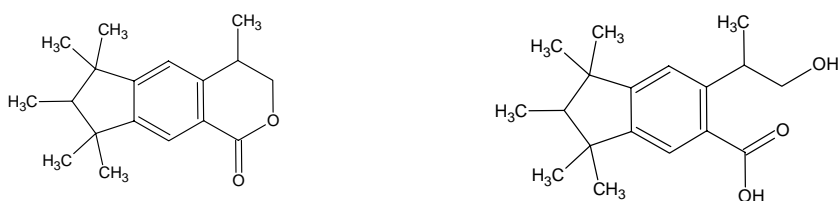


Figure 13. HHCB-lactone or Galaxolidone and Hydroxy acid

An extensive study was performed on the biotransformation of ^{14}C -AHTN in activated sludge at 5 to 50 μg AHTN/l. The half-life of the parent AHTN was 12-24h. The related first-order reaction rate constant was $0.029 - 0.057 \text{ h}^{-1}$. In a Continuous Activated Sludge (CAS) test with ^{14}C -labelled AHTN at 10 $\mu\text{g/l}$ and realistic STP operation conditions (addition of wastewater, sludge retention time 10d, hydraulic retention time 6h) a complete mass balance was drawn up. The study showed that in the total removal of the parent AHTN of 87.5%, half (42.5%) was caused by biotransformation and half by sorption (44.3%), whereas volatilisation played a minor role (3.3%) (Lee et al., 2001).

The concentration decrease of AHTN and HHCB was followed during two days in duplicate activated sludge samples (slurries) that were not additionally spiked. Loss due to volatilisation was also determined. The 'free' (dissolved) and total concentrations were 1.15 and 5.25 $\mu\text{g/l}$ for AHTN and 1.58 and 10.33 $\mu\text{g/l}$ for HHCB. The degradation rate constant based on total concentrations were 0.0075 h^{-1} for AHTN and 0.015 h^{-1} for HHCB (Artola 2002).

The degradation product HHCB-lactone or Galaxolidone was shown to appear during transport in the sewer and the STP. It was already present in the influents 0.1, 6 and 16% of the HHCB level in three STPs. In the effluents it was present at 0.1, 13 and 20% of the respective HHCB influent levels (Brändli 2002).

Primary biodegradation in soil

In the Netherlands a variety of 64 soil samples from various locations were screened for the presence of micro-organisms able to transform AHTN and HHCB into metabolites with a more polar behaviour as indicated by the lower retention factor (Rf)-value on TLC plates. Approximately 40% of the 64 samples showed a positive degrading potential towards one or both of the polycyclic musks: 17% for HHCB and 28% for AHTN. Several pure cultures of fungi, e.g., *Aureobasidium pullulans* and *Phanerochaete chrysosporium* have the capacity of primary biodegradation of AHTN and HHCB into a series of more polar metabolites (PFW 1996, 1997).

Extensive studies showed that several pure cultures of fungi have the capacity of primary biodegradation of HHCB. The most active strain was identified as *Cladosporium cladosporioides*, a common fungus that is found in several environmental compartments including leaf litter and soils. In experiments with a soil and sludge amended inoculum, mineralisation of radiolabeled HHCB to $^{14}\text{CO}_2$ reached a plateau after 100 days. After 200 days 51% of the total radioactivity was recovered from the organic extract while 31% remained in the aqueous slurry and 18% was recovered as $^{14}\text{CO}_2$. Five % of the radioactivity

was attributed to HHCB and HHCB-lactone. Results show that the degradation pathway may be a two-stage fungal/bacterial process where fungi convert HHCB to more polar metabolites that are rapidly degradable by other common soil organisms (bacteria or fungi) (Envirogen 1997).

The fate of ^{14}C -HHCB in soil or sediment was studied in microcosms started from samples of an oak forest soil, an agricultural soil and the sediment of the Delaware River, and from a farm with routine sludge applications from a domestic STP (all samples of New Jersey, USA). Sealed flasks with soil spiked with 10 mg HHCB/kg soil were incubated at laboratory ambient temperature. After one year significant amounts of polar metabolites were found. Only 4, 7 and 9% of the initial HHCB concentration remained in the river sediment, the forest soil and the sludge amended soil, respectively, and 35% remained in the agricultural soil. HHCB was degraded to various more polar fractions. The total recovery of radiolabel from the solvent extraction was 80% for the river sediment, 104% for the forest soil, 73% for the sludge amended soil and 52% for the agricultural soil. For the sludge-amended soil it was shown that an additional 20% of the radiolabel was recovered in an aqueous extract after an alkaline hydrolysis. It is hypothesised that the majority of unrecovered radiolabel becomes covalently bound to soil organic compounds (i.e. immobilised by humification). This has been observed with numerous organic compounds that are degraded to more polar, oxidised products (Envirogen 1998). Observed rate constants were 0.0066 d^{-1} for sludge amended soil, 0.0073 d^{-1} for forest soil, 0.0029 d^{-1} for agricultural soil and 0.0088 d^{-1} for river sediment. The estimated half-lives were 105, 95, 239 and 79 days, respectively.

Summary of environmental biodegradation

Both for HHCB and AHTN direct mineralisation is observed on a limited scale: for AHTN mineralisation started in the two-phase closed bottle test (12-21% oxidation), and for HHCB in a sludge and soil amended inoculum 18% was finally recovered as $^{14}\text{CO}_2$. The observed rates of biodegradation in similar test systems seem to be of the same order of magnitude: in an activated sludge die-away test the (pseudo-first order) rate constant for AHTN was $0.029 - 0.057\text{ h}^{-1}$ or $t_{1/2}$ is 12-24 h (Lee et al. 2001) whereas the rate constant for HHCB was reported as $0.033, 0.010$ and 0.021 h^{-1} or $t_{1/2}$ is 21, 69 or 33 h (Langworthy et al. 2000, Federle et al. 2002). The biodegradation patterns are similar in a way that more polar metabolites are being produced as time progresses.

In view of the observed degradability, for the environmental risk assessment, AHTN and HHCB may be considered as inherently biodegradable. For surface water, sediment and soil, the PECs will be calculated according to the TGD, using very conservative biodegradation rate constants expressed as half-life times: 150 d in surface water, and 180 d in the sediment and soil compartments.

The short calculated atmospheric half-lives indicate that the substances will not undergo long-range transport to any significant extent.

4.1.2.2 Removal in a sewage treatment plant

EUSES SimpleTreat calculation

According to the SimpleTreat model in EUSES, HHCB and AHTN entering an STP partition between the sludge, water and air. Although the substances are inherently biodegradable, they do not fulfil specific criteria in the TGD (table 6) and thus $K_{\text{biodeg}} = 0$. Using $\log K_{\text{ow}} = 5.4$ for AHTN and 5.3 for HHCB, the fate of HHCB and AHTN as predicted by EUSES is presented in table 11.

Table 11. Distribution of AHTN and HHCB in STP as predicted by EUSES

fate in STP	prediction by EUSES		observed in CAS test
	HHCB	AHTN	AHTN
to air, %	10.4	9.2	3.3
to water, %	22.4	20.6	12.5
to sludge, %	67.2	70.2	44.3
degraded, %	0	0	42.5

Removal percentages

In a number of studies both influents and effluents of an STP were sampled and analysed for AHTN and HHCB. From pair-wise comparison of the concentrations in the effluent and influent, removal percentages were derived. Usually it is assumed that this is a substance-specific characteristic that can be transposed to other STPs. This is the basis of the SimpleTreat model in EUSES. The concentrations as well as the removal percentages determined in 33 STPs in Germany, Switzerland, The Netherlands, UK and USA are shown in figure 14.

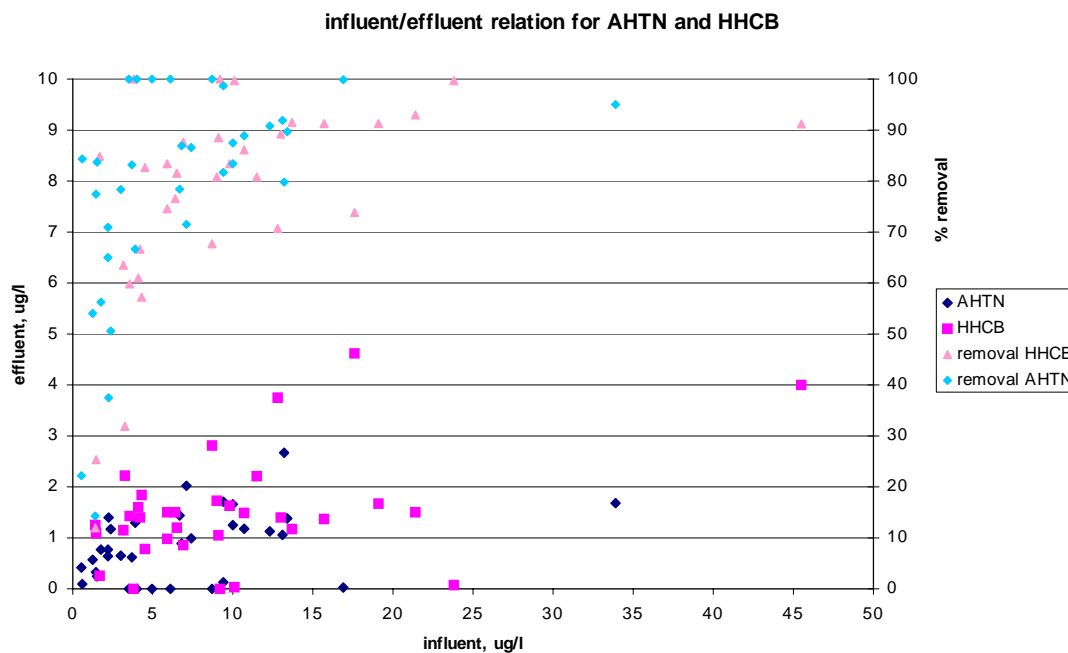
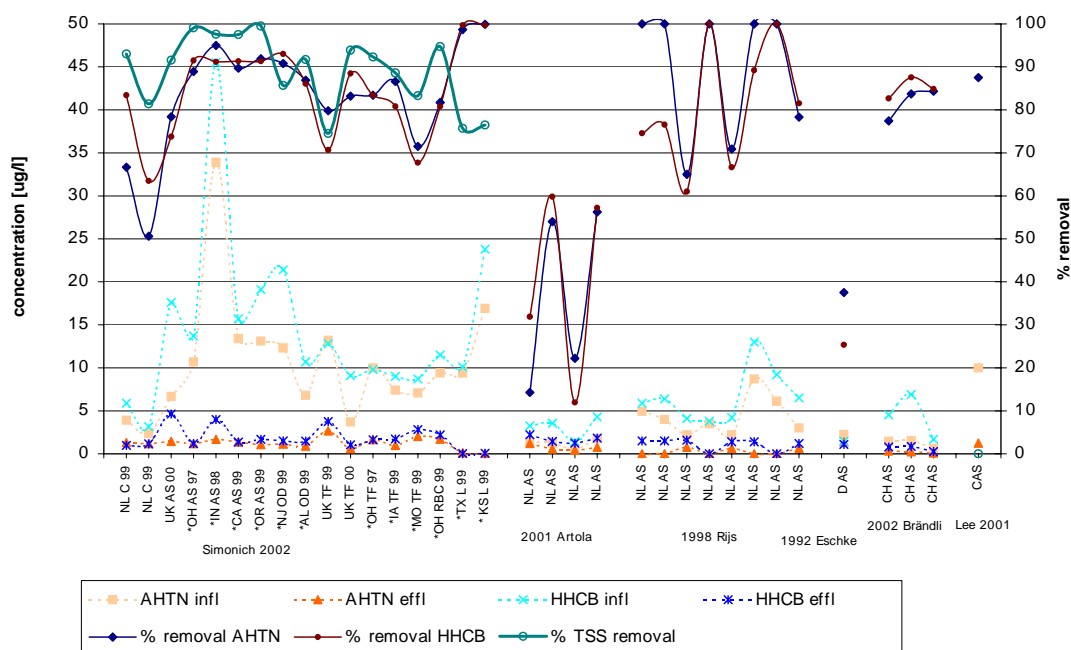
A**B**

Figure 14. Removal of HHCB and AHTN in 33 STPs.

A. Relation between the concentration in the influent and the effluent (left Y-axis) and percentage removal (right Y-axis);

B. Influent and effluent concentrations (left Y-axis, dotted lines) measured in the STPs indicated on the X-axis. Removal % is given on the right Y-axis.

Median, 90th perc. and max. effluent concentrations (n=33) for AHTN: 0.77, 1.7, 2.7 µg/l, for HHCB: 1.4, 2.7, 4.6 µg/l.

NL (Netherlands), UK(United Kingdom), CH (Switzerland), D (Germany), USA states (with *), CAS (simulation test); C: Caroussel, AS: Activated sludge plant, OD: oxidation ditch, TF: trickling filter, RBC: Rotating Biological Contactor, L: Lagoon, CAS: Continuous Activated Sludge (test) System.

Sources: Simonich et al. 2002, Artola et al. 2002, Rijs and Schäfer 1998, Brändli 2002, Eschke et al. 1994, Lee et al. 2001.

At higher influent levels, the removal percentages are generally above 80-90%. At lower concentrations, the removal efficiency is highly variable, between 50 and 85%. The variation in the removal percentages is caused by an apparent stability of the effluent concentrations. The effluent concentrations are at a level around 1.4 µg/l for HHCB and 1 µg/l for AHTN, regardless of the influent concentrations.

In the study of Simonich et al. (2002) the removal percentage correlated well with the removal of Total Suspended Solids in the system (figure 8B). The TSS removal is a characteristic of the STP which is determined by the treatment process the plant design and the settling properties of the sludge and is not related to AHTN or HHCB. From these observations it is concluded that a fixed removal percentage (according to a fixed first order rate constant dependant on the concentration in water) can not be used to model the removal of AHTN or HHCB in an STP.

Mass balance calculations

In this section a summary is presented of more detailed considerations presented in Appendix 1. In a CAS test (Lee et al. 2001, see section 4.1.2.2) AHTN was dosed in the influent at 10 µg/l. A complete mass balance was established including the distribution to the various compartments and the loss due to primary degradation. The results are included in table 11. The results show a discrepancy with the EUSES prediction for AHTN, in particular for the fraction on sludge and the fraction degraded.

From the study of Langworthy et al. (2000), with freshly collected activated sludge from three different plants in the USA spiked with ¹⁴C-HHCB for a batch experiment, the radiolabelled parent HHCB disappeared almost linear in time. Between time zero and 24 hours 30 % disappeared on average and after 150 hours 85 % of the parent had disappeared. As the experiments were conducted with freshly collected sludge samples, this rate of disappearance should hold for all of the HHCB on the sludge and not only for the spiked radiolabelled HHCB. An indicative calculation was made assuming that the concentration of HHCB on the activated sludge is similar as on average in Europe, see Annex 1. The estimated degradation capacity would imply about 36 – 72 % degradation of the daily load of HHCB.

The experiments of Artola (2002) yielded degradation rate constants for HHCB and AHTN in sludge. To put these rate constant into a proper perspective they should be converted to a degradation capacity per kg sludge and per day that can be compared to the average load per kg sludge per day, see Annex 1. The capacity for the degradation of HHCB was 84 – 118 % of the daily load. Likewise for AHTN the capacity was 47 to 82% of the daily load of AHTN.

Based on the above considerations it is estimated that between 40 and 100% of the daily load to the STP may be degraded.

Theoretical considerations

Although it is common use to apply a fixed removal percentage or a fixed first order concentration dependant rate constant, this concept is in sharp contradiction with the observed variability of removal percentages. Figure 14 shows that the effluent concentrations are below

a level of circa 3 µg/l regardless of the varying influent concentration. These data cover observations from 33 STPs (of 6 different types) in various countries in the EU and states in the USA, sampled between 1993 to 2002.

In the predictions according to EUSES of the distribution over air, sludge and effluent, the main removal process is adsorption and for biodegradation the rate constant is set to zero. The data on primary degradation, however, indicate that a significant part may be degraded and this has implications for the model approach to estimate the concentration in sludge and effluents.

Removal by adsorption implies that the total effluent content consists of a dissolved fraction in partition equilibrium with a concentration on the solids. This bound fraction depends on the amount of suspended solids in the effluent and the concentration of the substance on the solids, which is directly related to the amount in the influent. The derivation of the following equations is presented in Annex 1.

$$C_{sludge} = C_{infl} \cdot SORB \cdot 2.53 \quad [C_{sludge} \text{ in mg/kg, } C_{infl} \text{ in } \mu\text{g/l}]$$

$$C_{effl.total} = C_{infl} \cdot (SUSP + 1000 / K_p) \cdot SORB \cdot 2.53$$

According to EUSES, $SUSP = 0.03$ g/l, and

$$\begin{array}{llllll} \text{for} & \text{AHTN} & K_p & = & 11000 & \text{and} & SORB & = & 0.702: \\ & C_{effl.total} & = & 0.22 \cdot C_{infl} \end{array}$$

$$\begin{array}{llllll} \text{for} & \text{HHCB} & K_p & = & 9150 & \text{and} & SORB & = & 0.672: \\ & C_{effl.total} & = & 0.24 \cdot C_{infl} \end{array}$$

In practice the efficiency of solids removal in the secondary settler is highly variable, both from day to day and between different plants. A realistic range for the suspended solids concentration (SUSP) in the effluent is between 10 and 100 mg/l. The correlation between solids removal and the removal of AHTN or HHCB is indeed clearly illustrated by the data of Simonich et al. (2002) in figure 14.

Furthermore the total amount of sludge production is variable. Variability exists between summer and winter and between different plants due to the design. The more surplus and primary sludge is produced, the lower the concentration of the substance on the sludge will be and vice versa. As a result of this variability the relation should be considered with its upper and lower limits. The extreme cases are presented in figure 15 according to the following the equations:

$$C_{effl} = C_{infl} \cdot (0.1 + 1000 / K_p) / 0.3$$

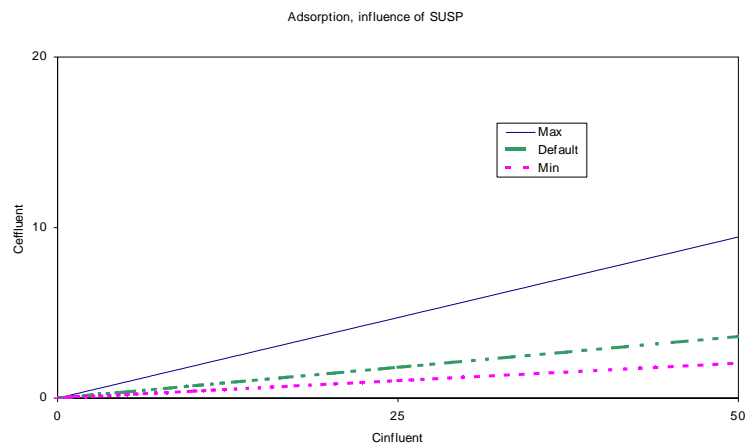
and

$$\text{for AHTN: } C_{effl} = C_{infl} \cdot (0.01 + 1000 / K_p) / 0.56$$

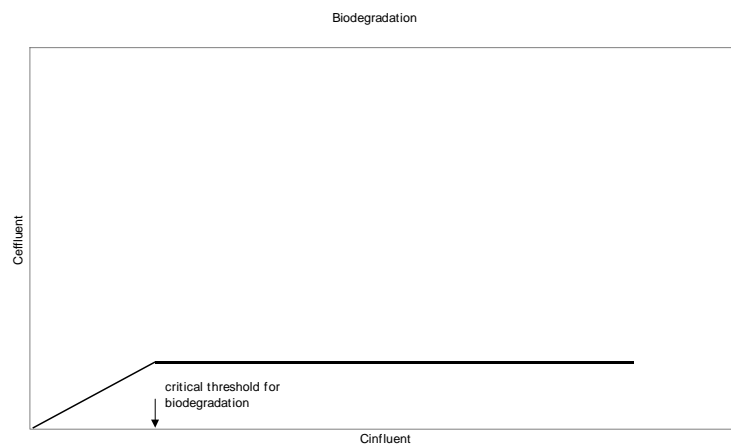
$$\text{for HHCB: } C_{effl} = C_{infl} \cdot (0.01 + 1000 / K_p) / 0.59$$

In other words, for strongly adsorbing substances the removal percentage is strongly influenced by the system properties and the operational conditions of the system.

A



B



C

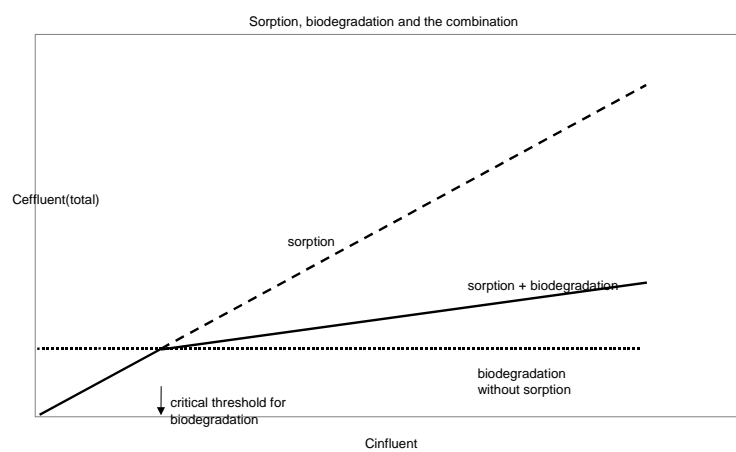


Figure 15. Relation between influent and effluent in different model concepts.

- A. Removal by sorption, with extreme values for the concentration of suspended solids in effluent (SUSP).
 B. Removal by biodegradation without sorption.
 C. Removal by combination of sorption and adaptive biodegradation.

Removal by degradation can be described by the theory of adaptation: over a long term the micro-flora in the activated sludge will adapt to the average supply (Blok 2001). A higher average supply results eventually in a higher specific metabolic activity in the sludge and thus in a higher degradation rate constant. If the rate constant for biodegradation is modified in proportion to the average supply, after adaptation this will result in a constant value in the effluent (dissolved). There is no constant and universal removal percentage, nor a universal rate constant for degradation, but the dissolved concentration in the effluent of various treatment plants will be rather similar and independent of the influent concentration as long as the concentration is sufficiently high to guarantee the maintenance of adapted microflora in the system. Below a certain threshold there will be no biodegradation, see figure 15b.

Above the critical threshold, the algorithm for $C_{effl}(diss.)$ is:

$$C_{effl}(diss) = Constant$$

Below the critical threshold, adsorption is the only removal mechanism.

The effect of the combination of adsorption and biodegradation is presented in figure 15c. Due to the biodegradation that is assumed to take place in the water phase, the concentration in the water will be kept constant. When this concentration comes below the equilibrium concentration, the sludge will partly be desorbed. This will result in lower concentrations on the sludge than without biodegradation.

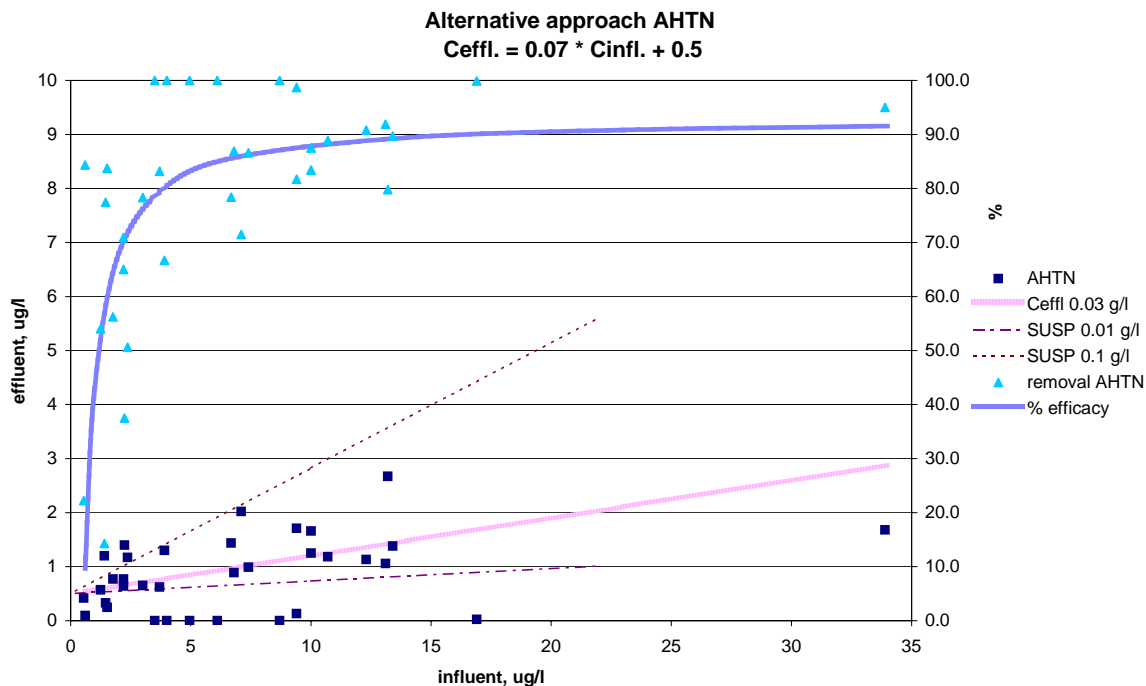
Based on empirical data for the sludge and influent concentrations, and using the default operation parameters in the standard STP, the following relations are derived (see Annex 1):

$$\text{for AHTN: } C_{effl}(total) = C_{sludge} \cdot 0.03 + 0.5$$

$$\text{for HHCB: } C_{effl}(total) = C_{sludge} \cdot 0.03 + 1$$

These relations for AHTN and HHCB are included in figure 16. The observed points have not been used to derive the lines. Thus, the observations are considered as a validation of the theoretical considerations. The apparent outliers of effluent data are within the range of the variability related to the higher or lower concentrations of suspended matter in the effluent. Thus the relation between influent concentration and removal percentage is explained.

A



B

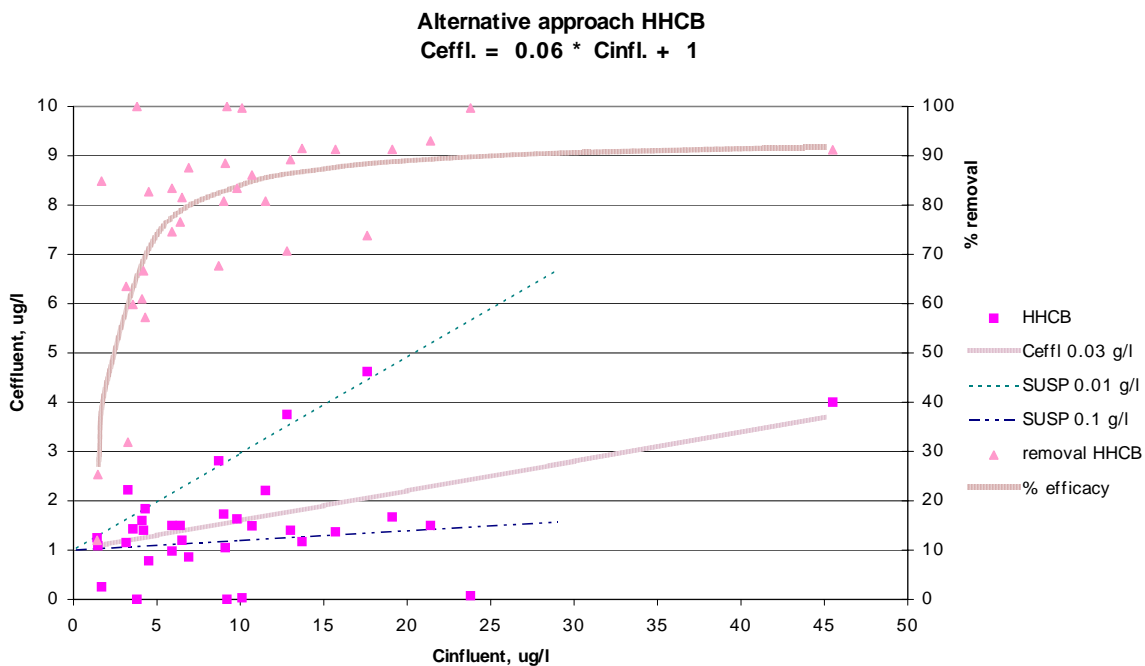


Figure 16. Observed (data points) and proposed (lines) influent-effluent relation and removal for A: AHTN and B: HHCB. The observed points have not been used to derive the lines. Thus, the observations are considered as a validation of the theoretical considerations.

4.1.2.3 Accumulation

Fish

The bioconcentration of AHTN and HHCB in bluegill sunfish (*Lepomis macrochirus*) was studied according to OECD Guideline 305E. The fish were exposed in a flow-through system to levels of 1 and 10 µg/l of radiolabelled material, during 28 days. The elimination period was also 28 days. The concentrations of AHTN and HHCB reached plateau levels after 3 to 7 days of exposure. The uptake rate constants k_1 were 442 – 765 l. kg⁻¹. d⁻¹ for AHTN and 352 – 421 l. kg⁻¹. d⁻¹ for HHCB. Elimination followed first-order kinetics with $k_2 = 0.34 – 0.58$ d⁻¹ or $t_{1/2} = 0.8 – 2.1$ d for AHTN and $k_2 = 0.22 – 0.26$ d⁻¹ or $t_{1/2} = 2 – 3$ d for HHCB. Based on concentrations of the parent material, the BCF for the whole fish was 597 for AHTN and 1584 for HHCB (Balk and Ford 1999a). Mass balance calculations based on the polar fractions observed in water and in fish showed that during exposure and during depuration the parent compounds are metabolised and the metabolites are excreted from the fish with a turnover rate of circa 38 to 50% per day.

Table 12. Comparison of field derived ratios of C_{fish} and C_{water} to experimentally determined bioconcentration factors for fish

BCF	AHTN	HHCB	Chemical analysis	Source
Laboratory: Bluegill sunfish (28+28)d BCF _{fw}	597 *	1584 *	¹⁴ C, LC/HPLC	Balk and Ford 1999a
Laboratory: Zebrafish (14+26)d BCF _{fw}	600	624	GC/MS	Butte and Ewald 1999
Environmental samples: Eel BCF _{fw} non-eel BCF _{fw}	200 to 650 50 to 145	150 to 600 49 to 188	GC/MS/MS	Balk and Ford 1999a from data in Eschke et al. 1995
Environmental samples: Eel BCF _{fw}	1069 (range 250 – 1791)	862 (range 201 – 1561)	GC/MS	Fromme et al. 2001b
Environmental samples ^a Rudd BCF _{fw} Tench BCF _{fw} Crucian carp BCF _{fw} Eel BCF _{fw} Zebra mussel BCF _{fw}	40 280 670 400 570	20 510 580 290 620	GC/MS	Gatermann et al. 2002

^a Species differences related to fat content

* Used in calculations

As an indication of the bioconcentration under natural conditions, ratios were calculated for the concentration in fish and in surface water, see table 12. The resulting field ratios seem to

be lower than the ones determined in the laboratory, suggesting that in the field some form of adaptation might have occurred. Another explanation might be that wildlife fish tend to have a lower lipid content than laboratory specimen.

Benthic organisms

The bioconcentration of AHTN and HHCB was studied in fourth instar midge larvae (*Chironomus riparius*) and in the worm *Lumbriculus variegatus*. The organisms were exposed in a flow-through system. They were not fed during the 12-day exposure period. In parallel to the bioconcentration experiment, a similar experiment was run with the addition of 5 mg/l of the cytochrome P-450 inhibitor piperonyl butoxide (PBO) to inhibit biotransformation.

The aqueous concentrations in the test with *C. riparius* were stable at 9.8 µg HHCB/l and 5.8 µg AHTN/l. The concentrations in the organisms increased to a maximum level of circa 750 µg AHTN/kg or 2500 µg HHCB/kg and then the level rapidly decreased to a new steady state of circa 290 µg AHTN/kg or 750 µg HHCB/kg. For AHTN the BCF was 50 to 112, whereas for HHCB the BCF was 85 – 138. These values are lower than predicted on the basis of a correlation for stable organic chemicals (BCF 5623 and 3890 for AHTN and HHCB, respectively) by a factor of 40. With the addition of PBO, BCF was higher, 7943 for AHTN and 525 for HHCB, confirming that the low BCF values are caused by biotransformation of HHCB in *C. riparius*.

The concentrations in water in the experiment with *L. variegatus* were circa 4 µg/l. The uptake in worms reached a plateau level of 20 mg HHCB/kg after 3 days. BCF was 2692. For AHTN a plateau level of 11 mg/kg was reached after 3 days (BCF ≈ 3000), but after 8 days a new but highly variable level seemed to be reached (BCF = 6918). These levels are at the same level as the predicted BCF based on Kow and lipid content, indicating that biotransformation does not take place in this organism.

Earthworms

Empirical BCF-values are not available. Thus the bioconcentration in earthworms is estimated according to the EU TGD (EC 2003), assuming proportionality to the soil pore water concentration: $BCF_{worm} = (0.84 + 0.012 \cdot Kow) / RHO_{earthworm}$

This leads to a BCF_{worm} of 3015 l. kg⁻¹ for AHTN and 2395 l. kg⁻¹ for HHCB.

4.1.3 PEC calculations

4.1.3.1. PEC for the aquatic compartment

After use of fragranced consumer products most of the materials will be emitted with the households waste water to the sewer. For the calculation of the environmental concentrations it is assumed that the total use volume of AHTN and HHCB is discharged down the drain, regardless whether the application is in detergents or in cosmetics. This is a 'worst case' approach as a fraction used in detergents and down-the-drain cosmetics will remain on the substrate whereas another fraction of the consumer products has an application that is not washed-off. These fractions will never reach the sewer systems. For the scenarios for private use the consumption per inhabitant per year is estimated in Table 4. According to the EU-TGD the amount per inhabitant is discharged along with 200 l of domestic waste water per inhabitant per day. In the STP the sludge production is 79 g per inhabitant per day and the concentration of suspended materials in the effluent is 30 mg/l. The distribution over air, sludge and effluent according to EUSES is given in Table 11. Calculations with EUSES for the 2000 scenario (with a 10% regional use or the 'worst case') yielded the concentrations in influent, effluent, sludge and the surface water system. For the other use scenarios the concentrations are proportional to the use volume, see Table 13.

Table 13. Predictions according to the EUSES distribution fractions in the STP for the various use scenarios. Regional scenario with 80% emission to STP. (NB: factor for discontinuity = 1, see explanation in text)

Scenario		Local, Cinfluent µg/l	Local, Ceffluent* µg/l	Local, Csludge mg/kg	PEC _{regional} water µg/l	PEC _{local} water µg/l	PEC _{sediment} mg/kg ww
2000 – TGD regional (10%), 'worst case'	AHTN	24.5	5.1	43.6	0.0355	0.52	0.34
	HHCB	98	21.9	166	0.148	2.26	1.22
2000 – average	AHTN	13.3	2.7	23.6	0.019	0.28	0.18
	HHCB	53	11.8	90	0.080	1.22	0.66
2000 – northern Europe, reasonable estimate	AHTN	5.1	1.0	9.0	0.007	0.11	0.07
	HHCB	13	2.9	22	0.020	0.30	0.16
1995 – southern Europe, reasonable worst case	AHTN	27.4	5.6	48.7	0.040	0.58	0.38
	HHCB	69	15.6	118	0.105	1.61	0.87
1995 – average	AHTN	21.9	4.5	39.0	0.032	0.46	0.30
	HHCB	56	12.5	94	0.084	1.29	0.69
1995 – northern Europe, below average	AHTN	15.3	3.2	27.3	0.022	0.32	0.21
	HHCB	39	8.7	66	0.059	0.90	0.49

*) total effluent concentration (including the amount adsorbed to suspended solids)

According to the TGD an additional factor of 4 should be used to cover fluctuations of the release in a realistic worst case scenario for daily discharges. HERA (2002) applies for detergents a factor of 1.5, based on observations on the temporal variation of concentrations of Boron in influents (Fox 2002). The application of this factor for the calculation of the concentration on sludge and in the effluent of a STP is not very relevant in the case of substances that are removed by adsorption. This can be illustrated for HHCB as follows:

For a normal STP about 0.5 – 1 kg of activated sludge *per capita* is present in the aeration tank. The concentration in sludge of 166 mg/kg in the TGD regional 10% scenario, means an amount of 83 to 166 mg HHCB *per capita* (166 mg/500 g or 166 mg/kg) is present in the aeration tank. The daily release of 20 mg *per capita* (see table 4: 7.14 g/y = 19.6 mg/d) is only a small fraction of this amount of 83-166 mg. A factor 1.5 to this 20 mg would mean a maximum extra amount of 10 mg, which would mean an increase of only 6-12% of the absorbed amount present in the system.

There are many measured concentrations for the STP. Of these data, concentrations on sludge are the most stable and most distinguishing, more than influent and effluent concentrations (see considerations in section 4.1.2.2). Data are available for the scenarios ‘2000 – reasonable estimate’ and ‘1995 – below average’, and it is clear that the EUSES calculations are conservative even without an additional factor of 1.5 to account for fluctuations in the release. As shown in table 14, the predicted concentrations in sludge are above the observed levels by a factor of 2 to 6 for AHTN and by a factor of 3 to 4 for HHCB. The predicted influent and effluent concentrations are overestimated to the same extent.

Table 14. Comparison of predicted (EUSES) and observed concentrations

Consumption, mg /d per capita	Local, Cinfluent µg/l	Observed	Local, Ceffluent µg/l	Observed	Local, Csludge mg/kg	Observed	Overestim ation ratio calc./obs.
AHTN							
2000 – TGD regional (10%), ‘worst case’	24.5		5.1		43.6		
2000 – average	13.3	^{a)} range 4- 13 av. 7.9	2.7	^{a)} range 0.6-2.7 av. 1.5	23.6	^{g)} med. 4.0	infl: 2 effl: 2 slu: 6
2000 – northern Europe, reasonable estimate	5.1	^{b)} range 0.5- 3.9 av. 1.8	1.0	^{b)} range 0.1-1.3 av. 0.75	9.0	^{c)} med. 4.2	infl: 3 effl: 1 slu: 2
1995 – reasonable worst case	27.4		5.6		48.7		
1995 – average	21.9		4.5		39.0		
1995 – below average	15.3		3.2	^{d)} 2 – 6 av. 4.5	27.3	median ^{d)} 16 ^{e)} 15	effl:1 slu: 2

Consumption, mg /d per capita	Local, Cinfluent µg/l	Observed	Local, Ceffluent µg/l	Observed	Local, Csludge mg/kg	Observed	Overestim ation ratio calc./obs.
HHCB							
2000 – TGD regional (10%), 'worst case'	98		23		166		
2000 – average	53	^{a)} range 9-18 av. 13.2	12	^{a)} range 1- 4.6	90	^{g)} med. 26	infl: 4 effl: 4 slu: 3.5
2000 – northern Europe, reasonable estimate	13	^{b)} range 1-7 av. 4.7	2.9	^{b)} range 0.2-2.2	22	^{c)} median 7	infl: 3 effl: 3 slu: 3
1995 – reasonable worst case	69		16		118		
1995 – average	56		12		95		
1995 – below average	39		8.7	^{f)} 6-11	66	^{d)} median 28 ^{e)} 12-20	effl: 1 slu: 2.4 ; 4

^{a)} UK 1999-2000 (Simonich et al. 2002)

^{b)} NL 1999 (Simonich et al. 2002); NL 2001 (Artola 2002), CH 2001/2002 (Brändli 2002)

^{c)} Hessen, D 2000 (HLUG 2001)

^{d)} NL 1997 (Blok 1997)

^{e)} Hessen, D 1996 (HLUG 2001)

^{f)} Berlin, D 1996 (Heberer 1999)

^{g)} UK 2000 (Stevens et al. 200XX)

In view of the conservatism in the EUSES calculations and because of the incorrect estimate of the removal in the STP by a concentration-independent removal percentage, an alternative approach has been added. In the alternative approach the measured concentrations on sludge have been used to scale the sludge concentrations for various use scenarios (see figure 17). This will enable a sensible comparison of model calculations with measured data and thus enable a better estimate of the concentration levels in regions where no measurements have been performed.

Figure 17 shows how the available measured concentrations have been used to develop an alternative approach to improve the predictions by EUSES. The alternative approach is based on the combined concept of sorption and biodegradation (see Annex 1). Concentrations measured in sludge were available for the 'average' and 'northern Europe reasonable estimate' scenarios. The overestimation ratios for sludge (see table 14, indicated as (1) in figure 17) were used to calibrate the sludge concentrations predicted by EUSES ((2) in figure 17) for all user scenarios. As a next step the concentrations on sludge are translated into effluent concentrations (*Annex 1, eq.16*). This is indicated in figure 17 as (3). The results of the calculations after calibration are presented in table 15. The concentrations in surface water are calculated with a dilution factor of 1:10 for effluent to water and the concentration on sediment is derived from equilibrium partitioning as described in the EU-TGD.

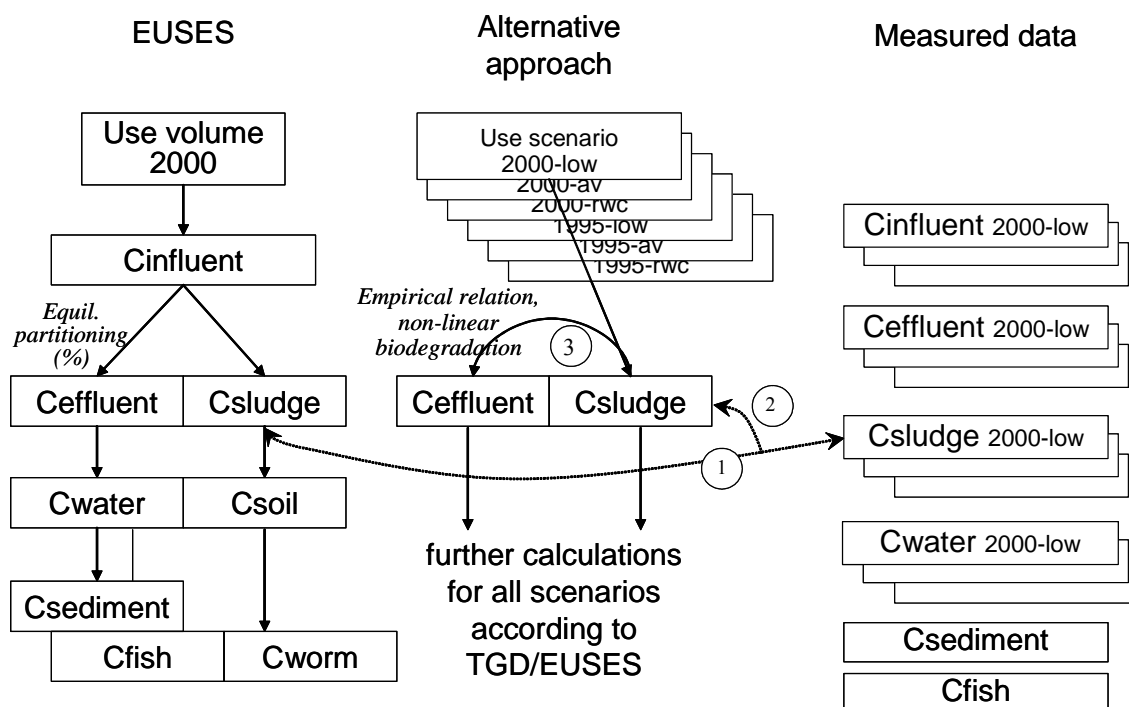


Figure 17. Relation between the three approaches to estimated the exposure

A comparison of a model to the real world can only be based on realistic or median levels for the measured concentrations as well as for the model. The purpose of this action is to understand the behaviour of the substances in the STP environment. This is necessary (1) to compare measured concentrations to the predictions of the respective use scenarios and (2) to make sensible estimates of the concentrations in the regions where no measurements have been carried out ('southern Europe'). For the risk assessment the 90th-percentiles will be used to take a realistic worst case into account and the uncertainties need to be considered.

Table 15. Alternative approach: Derivation of local concentrations. The concentration on sludge determines the concentration in effluent

Consumption, mg /d per capita	Csludge mg/kg after calibration	Ceffluent, µg/l	PECregional water, µg/l ¹⁾	PEClocal water, µg/l	PEClocal sediment, mg/kg wwt
AHTN					
2000 – TGD regional (10%), 'worst case'	44 : 2 = 22	1.15	0.036	0.15	0.10
2000 – average	24 : 2 = 12	0.85	0.019	0.10	0.065
2000 – northern Europe, reasonable estimate	9 : 2 = 4.5	0.64	0.007	0.07	0.045

Consumption, mg /d per capita	Csludge mg/kg after calibration	Ceffluent, µg/l	PECregional water, µg/l ¹⁾	PEClocal water, µg/l	PEClocal sediment, mg/kg wwt
1995 – reasonable worst case	49 : 2 = 24	1.23	0.040	0.16	0.10
1995 – average	39 : 2 = 19	1.09	0.032	0.14	0.09
1995 – below average	27 : 2 = 14	0.91	0.022	0.11	0.07
HHCB					
2000 – TGD regional (10%), 'worst case'	166 : 3 = 55	2.7	0.148	0.42	0.23
2000 – average	90 : 3 = 30	1.9	0.080	0.27	0.15
2000 – northern Europe, reasonable estimate	22 : 3 = 7	1.2	0.020	0.14	0.075
1995 – reasonable worst case	118 : 3 = 39	2.2	0.105	0.33	0.18
1995 – average	94 : 3 = 32	2.0	0.084	0.28	0.15
1995 – below average	66 : 3 = 22	1.7	0.059	0.23	0.12
PECregional is derived for each scenario in proportion to the respective sludge concentrations					

4.1.3.2 PECsoil

As no measured concentrations in soil are available, the predictions are limited to the 2000 – use scenarios. According to EUSES the sludge concentrations were 44 mg AHTN/kg and 166 mg HHCB/kg in the 2000 'worst case' scenario and the resulting predictions by EUSES in soil and groundwater are given in table 16 in bold. For the alternative approach, PECregional_{soil} as calculated for the private use-EUSES scenario was scaled (using the overestimation ratio in table 14) to derive PECregional_{soil} for the TGD regional (10%) – 'worst case' scenario. Next PECregional_{soil} for the lower private use scenarios was derived from this PECregional for the (10%)-'worst case' scenario on the basis of proportionality in consumer use. PECsoil was calculated for the other use scenarios based on the sludge concentrations after calibration. The concentrations in groundwater were also predicted on the basis of proportionality (that implies that no proportional correction was made for the regional groundwater concentrations).

Table 16. Local PECs for the terrestrial compartment. The calculations from sludge to soil according to EUSES were used as a basis

	Csludge mg/kg	PECsoil _{regional} mg/kg wwt	PECsoil _{local} mg/kg wwt, 180 d	PECgroundwater _{local} , µg/l
AHTN				
Private use EUSES	43.6	0.00322	0.053	0.074
2000 – TGD regional (10%), ‘worst case’	22	0.003	0.028	0.038
2000 – average	12	0.002	0.016	0.022
2000 – northern Europe, reasonable estimate	4.5	0.001	0.006	0.008
HHCB				
Private use EUSES	166	0.0123	0.202	0.337
2000 – TGD regional (10%), ‘worst case’	55	0.012	0.075	0.125
2000 – average	30	0.007	0.041	0.068
2000 – northern Europe, reasonable estimate	7	0.002	0.010	0.017

4.1.3.3 PECpredator

The concentrations in the food of predators are estimated from predictions for fish and worms. The BCF for fish is 597 l/kg for AHTN and 1584 for HHCB. For earthworms the BCF is estimated from log Kow resulting in a value of 3015 kg/kg for AHTN and 2395 for HHCB. The biomagnification factor (BMF) is based on the measured BCF value: with $BCF_{(fish)} < 2000$, the default BMF value for organic substances is 1 (EU-TGD, section 3.8.3.4, EC 2003).

PEC_{fish} is calculated as:

$$PEC_{fish} = 0.5 (PEC_{water_{local}} + PEC_{water_{regional}}) * BCF_{fish} * BMF$$

Likewise, PEC_{worm} is calculated on the basis of the concentration in porewater:

$$PEC_{worm} = 0.5 (C_{porewater_{local}} + C_{porewater_{regional}}) * BCF_{worm} * BMF$$

The results are presented in Table 17. Comparison of the predicted concentrations with concentrations measured in fish (Table 9) illustrate that the scenario for 2000'- northern Europe is a good approach to estimate the median value (within a factor of 4), whereas the direct EUSES estimates are good predictors for the 90th-percentile in fish. The latter values should be used for the risk assessment.

Table 17. PECs in fish and worm

	PEC _{water} local (µg/l)	PEC _{water} regional (µg/l)	PEC _{coral, fish} mg/kg wwt	PEC _{ground} water local, µg/l	PEC _{ground} -water regional, µg/l	C _{pore-water} µg/l	PEC _{coral, worm} mg/kg wwt
AHTN							
Private use EUSES	0.52		0.166	0.074		0.04	0.121
Private use EUSES PEC _{regional}		0.036	0.021		0.006		
2000 – TGD regional (10%)	0.15	0.036	0.055	0.038	0.006	0.022	0.066
2000 – average	0.10	0.019	0.035	0.022	0.003	0.013	0.038
2000 – northern Europe, reasonable estimate	0.07	0.007	0.023	0.008	0.001	0.005	0.014
1995 –reasonable worst case	0.16	0.040	0.061				
1995 – average	0.14	0.032	0.051				
1995 –below average	0.11	0.022	0.040				
HHCB							
Private use EUSES	2.26		1.907	0.337		0.183	0.438
Private use EUSES PEC _{regional}		0.148	0.243		0.0281		
2000 – TGD regional (10%)	0.42	0.148	0.450	0.125	0.028	0.077	0.184
2000 – average	0.27	0.08	0.277	0.068	0.015	0.042	0.101
2000 – northern Europe, reasonable estimate	0.14	0.02	0.127	0.017	0.004	0.011	0.026
1995 –reasonable worst case	0.33	0.11	0.341				
1995 – average	0.28	0.08	0.292				
1995 –below average	0.23	0.06	0.228				

4.2 Environmental Effects Assessment

4.2.1 Toxicity

4.2.1.1 Ecotoxicity – Aquatic test results

For AHTN and HHCB standard tests were carried out with algae, crustaceans and fish. Various results of prolonged toxicity tests are available. Tests are also available for other species of the class of crustaceans, insects and annelids; however, most of these data are considered not reliable or the reliability could not be assessed (e.g., no measured concentrations, no information on dose-response, variability of replicates, control survival, etc.). In the following tables the available aquatic toxicity data are summarised.

Table 18. Aquatic toxicity of AHTN and HHCB

Species	Test method	Results AHTN (mg/l)	Results HHCB (mg/l)	Reliability ¹⁾	Reference
Short term toxicity					
Algae <i>Pseudokirchneriella subcapitata</i>	72-h static, biomass production inhibition	EC50 = 0.468	EC50 = 0.723	1	Van Dijk, 1997a,b
Midge larvae <i>Chironomus riparius</i>	96-h semi-static, mortality	NOEC > 0.5	LC50 = 0.288	4 ²⁾	Artola, 2002
Marine copepod <i>Acartia tonsa</i>	48-h, marine, mortality	LC50 = 0.71 LC10=0.45	LC50 = 0.47 LC10 = 0.12	2	Wollenberger et al., 2003
Marine copepod <i>Nitorca spinipes</i>	96-h mortality	LC50 = 0.61	LC50 = 1.9	2	Breitholtz et al., 2003
Prolonged toxicity					
Algae <i>Pseudokirchneriella subcapitata</i>	72-h static, biomass production inhibition	NOEC = 0.204 NOEC = 0.374 geom. mean of 2 NOECs above = 0.28	NOEC = 0.201	1	Van Dijk, 1997a,b
<i>Daphnia magna</i>	21-d semi-static, reproduction	NOEC = 0.196 EC50 = 0.244	NOEC = 0.111 EC50 = 0.282	1	Wüthrich, 1996a,b
Bluegill sunfish <i>Lepomis</i>	21-d flow-through, growth	NOEC = 0.089 LC50 = 0.314	NOEC = 0.093 (E: clinical signs)	1	Wüthrich, 1996c,d

Species	Test method	Results AHTN (mg/l)	Results HHCB (mg/l)	Reliability ¹⁾	Reference
<i>macrochirus</i>			LC50 = 0.452		
Fathead minnow	32-d post hatch,	NOEC = 0.035	NOEC = 0.068	1	Croudace, 1997a,b
<i>Pimephales</i>	36-d overall, larval	(E: development)	LC50 > 0.140		
<i>promelas</i>	survival and development	LC50 = 0.100			
Zebrafish	32-d intermittent	NOEC = 0.035		1	Hooftman, 1999
<i>Brachydanio rerio</i>	flow-through, development				
Worm	5-d static, immobility	EC50 = 0.397	EC50 = 0.394	4 ²⁾	Artola, 2002
<i>Lumbriculus variegatus</i>					
Marine copepod	7 – 8.5-d larval	NOEC _{ld} > 0.06	NOEC _{ld} = 0.07	3 ³⁾	Breitholtz et al., 2003
<i>Nitorca spinipes</i>	development rate population growth	NOEC _{pg} > 0.06	NOEC _{pg} > 0.2		
Marine copepod	5-d larval	NOEC = 0.01	NOEC = 0.02	3 ⁴⁾	Wollenberger et al., 2003
<i>Acartia tonsa</i>	development rate	EC50 = 0.026	EC50 = 0.059		

¹⁾ Klimisch 1997

²⁾ Publication does not specify details

³⁾ Analysis showed dosing and maintenance of test concentrations to be problematic; levels highly variable and down to below 10% of nominal); no details on time-effects relation neither on relevance of slight larval development rate differences on population level. No effects at population level at range tested

⁴⁾ No analysis of concentrations (methodology similar to previous, see ³⁾); no details on time-effects relation neither on relevance of observed effect

For micro-organisms no specific toxicity tests have been carried out. In the biodegradation tests, no inhibition was observed for both substances. Therefore the NOEC for AHTN is above 30 mg/l and for HHCB above 20 mg/l.

The acute toxicity of AHTN and HHCB was tested on the South African clawed frog larvae (*Xenopus laevis*) in a procedure analogous to ASTM guideline E 1439-91. For AHTN the 96h-LC50 for embryo-adult was > 2.0 mg/l, the 96h-EC50 was > 1.0 mg/l for embryo growth and > 4.0 mg/l for embryo malformation. The 96h-LC50 for HHCB for embryo-adult was also > 2.0 mg/l, the 96h-EC50 was > 2.0 mg/l for embryo growth and > 4.0 mg/l for embryo malformation (reliability class 3: no details available, Dietrich and Chou 2001).

4.2.1.2 Ecotoxicity – Terrestrial test results

For terrestrial toxicity two tests were carried out for both substances, with springtails and earthworms. The results and details are listed in table 19.

Table 19. Terrestrial toxicity of AHTN and HHCB

Species	Test method	Effect	Results AHTN (mg/kg dw)	Results HHCB (mg/kg dw)	Reliability	Reference
Earthworm	4 weeks	growth	NOEC > 250	NOEC = 105	1	Gossmann, 1997a,b
<i>Eisenia foetida</i>	10% o.m., 5% o.c.	mortality	NOEC > 250	NOEC = 250		
	8 weeks	reproduction and food consumption	NOEC = 105	NOEC = 45		
Springtail	4 weeks, mortality and reproduction		NOEC = 45	NOEC = 45	1	Klepka, 1997a,b
<i>Folsomia candida</i>						

4.2.2 PNEC Calculations

4.2.2.1 PNEC_{STP}

For micro-organisms no specific toxicity tests have been carried out. In the biodegradation tests the NOEC for AHTN is above 30 mg/l. With an assessment factor of 10, the PNEC_{STP} would be > 3 mg/l. This PNEC is above the water solubility of AHTN of 1.25 mg/l.

For HHCB the NOEC is above 20 mg/l. With an assessment factor of 10 this results in a PNEC_{STP} > 2 mg/l. Also this PNEC is above the water solubility of HHCB of 1.75 mg/l.

4.2.2.2 PNEC water

For the derivation of a PNEC for aquatic organisms, NOECs are available from prolonged studies with algae, daphnia and fish with reliability score from 1 to 4. Only data with score 1 and 2 are taken into account. The aquatic PNEC is determined with an application factor (AF) of 10 with the lowest chronic NOEC, see table 20.

For AHTN the lowest NOEC is 0.035 mg/l in an early life stage test with fathead minnow (*Pimephales promelas*), resulting in a PNEC_{water} = 3.5 µg/l.

For HHCB the lowest NOEC of 0.067 mg/l is also obtained for fathead minnow. This results in a PNEC_{water} = 6.8 µg/l.

4.2.2.3 PNEC sediment

No toxicity data are available on the toxicity of AHTN and HHCB in sediment. Therefore the $PNEC_{\text{sediment}}$ is derived from the $PNEC_{\text{water}}$ according to the using equilibrium partitioning theory:

$$PNEC_{\text{sed}} = \frac{K_{\text{sed.-water}}}{RHO_{\text{sediment}}} \cdot PNEC_{\text{water}}$$

$K_{\text{sed.-water}}$: sediment-water partition coefficient
(for AHTN: 746 m³/m³; for HHCB: 619 m³/m³)
 RHO_{sediment} : bulk density of suspended matter (1300 kg_{wwt}/m³)

The resulting $PNEC_{\text{sed}}$ is given in mg per kg wet weight. This is converted to dry weight by multiplication with a factor of 2.6. The results are included in table 20.

Table 20. Summary of PNECs (AF: assessment factor)

	AF	AHTN	HHCB
$PNEC_{\text{STP}}$ (µg/l)	10	> 3000	>2000
$PNEC_{\text{aquatic}}$ (µg/l)	10	3.5	6.8
$PNEC_{\text{sediment}}$ (mg/kg wwt)		2	3.24
$PNEC_{\text{sediment}}$ (mg/kg dw)	(eq.part.)	5.2	8.4
$PNEC_{\text{soil}}$ (mg/kg wwt)		0.28	0.28
$PNEC_{\text{soil}}$ (mg/kg dw)	50	0.31	0.31
$PNEC_{\text{oral}}$ (mg/kg wwt)	90, 300	1	3.3

4.2.2.4 PNEC soil

Data are available from two prolonged studies with soil organisms, which gives an assessment factor of 50 to the lowest NOEC to derive $PNEC_{\text{soil}}$. The lowest NOECs are 45 mg/kg for AHTN as well for HHCB. However, first this NOEC is to be normalised from the standard OECD soil with 10% organic material to the standard soil of the TGD, containing 3.4% of organic material, resulting in $NOEC = 45 * (0.034/0.1) = 15.3$ mg/kg. The resulting $PNEC_{\text{soil}}$ are for both substances:

$PNEC_{\text{soil}} = 0.28$ mg/kg wwt, or with a wet to dry weight conversion factor of 1.13,

$$\text{PNEC}_{\text{soil}} = 0.31 \text{ mg/kg dw}$$

If $\text{PNEC}_{\text{soil}}$ were derived from $\text{PNEC}_{\text{aqua}}$ by equilibrium partitioning, $\text{PNEC}_{\text{soil, equil}} = 1.84 \text{ mg/kg ww}$ for AHTN and 3.0 mg/kg ww for HHCB.

4.2.2.5 PNEC oral, predator

No specific toxicological data are available on predatory wildlife. The PNEC for secondary poisoning will therefore be based on mammalian toxicity data described in the Human Health part of the HERA risk assessment.

For AHTN, a NOAEL of 5 mg/kg bw/d is derived from a 90-day oral study with rats. As toxicity is based on the P-generation (rats > 6 weeks) for the conversion to a dose in food, a conversion factor of 20 has to be used resulting in a NOEC of 100 mg/kg food (e.g., in fish). For the derivation of $\text{PNEC}_{\text{oral}}$, the test duration of 90 days implies an assessment factor of 90, giving $\text{PNEC}_{\text{oral}} = 1.1 \text{ mg/kg food}$.

For HHCB, in the same study the NOAEL is 150 mg/kg bw/d , resulting in a NOEC of 3000 mg/kg food and with AF 90, the $\text{PNEC}_{\text{oral}}$ is 33.3 mg/kg .

In a 21-day reproduction and development toxicity study with AHTN, the NOAEL is 15 mg/kg/d . Using the same conversion as above, the NOEC in food is 300 mg/kg . With an assessment factor of 300 for a 28d-test a $\text{PNEC}_{\text{oral}}$ of 1 mg/kg food is derived. However, a lower assessment factor of 90 could be justified, as this test is on reproduction toxicity (short term). In that case PNEC would be 3.3 mg/kg food . For AHTN, $\text{PNEC}_{\text{oral}} = 1 \text{ mg/kg food}$ is used.

In the 21-day reproduction and development toxicity study for HHCB, the NOAEL is 50 mg/kg/d and the NOEC in food is 1000 mg/kg . With an AF of 300, the resulting PNEC is 3.3 mg/kg food . However, as for AHTN, a lower assessment factor of 90 could be justified ($\text{PNEC} = 33.3 \text{ mg/kg food}$). For HHCB, $\text{PNEC}_{\text{oral}} = 3.33 \text{ mg/kg food}$ will be used.

4.2.3 Endocrine disruption

In an E-screen assay using an estrogen receptor-positive human mammary carcinoma cell line, the estrogenic activity was receptor mediated but the potency was low and substances should be classified as partial agonists. The relative potency as compared to 17β -estradiol was low (5 orders of magnitude) (Bitch et al. 2002).

The potential estrogenic effects of AHTN and HHCB were assessed *in vitro* in ER α - and ER β -dependent gene transcription assays with Human Embryonal Kidney 293 cells. A weak response was observed in one of the cell lines but the estrogenic activity of 17β -estradiol and the two test substances differed by six orders of magnitude. In the mouse uterine assay (*in*

vivo) no uterotrophic activity was noted. No *in vitro* estrogen-dependent vitellogenin production was found in carp hepatocytes (Seinen et al. 1999).

The selective binding to the estrogen receptor was studied in various cell lines using sensitive *in vitro* reporter gene assays. Weak estrogenic effects were observed at relatively high concentration (10 μ M) and anti-estrogenic effects were observed in various cell lines starting at 0.1 g/ μ M. In comparison with the well-known selective estrogen receptor modulator 4-hydroxytamoxifen, both AHTN and HHCB have a much lower potency in suppressing estradiol-induced transactivation (Schreurs et al. 2002).

These results are probably of little environmental importance as the absence of *in vivo* activity in the mouse and *in vitro* carp hepatocytes showed. It was concluded that the estrogenic potency of the substances is too low to induce estrogenic effects in wildlife species or humans at current levels of exposure (Seinen et al. 1999).

4.3 Risk Characterisation

For the risk assessment the actually measured or predicted concentrations (PEC) in each compartment are compared to the predicted no effect concentrations (PNEC) in a PEC/PNEC ratio. Where the risk ratio is below 1, the risk is considered low.

The risk assessment is presented as a tiered approach: first the risk ratios are presented according to the EUSES calculations. Where the risk ratio is above 1 and a refinement seems to be necessary, the measured concentrations are taken into account.

4.3.1 Aquatic compartment

4.3.1.1 STP

The risks for the sewage treatment are expressed as the risk for the micro-organisms in the STP. The calculations by EUSES refer to the total effluent concentration (solved + sorbed). Strictly speaking, for comparison with the PNEC only the solved fraction should be taken into account. However, with the current extremely low risk levels, this distinction does not need to be made. The risk ratios for the micro-organisms in the STP are far below 1. As all other calculated and all measured concentrations are below the EUSES prediction, AHTN and HHCB pose no concern for the STP.

Table 21. Risk ratios for the sewage treatment plant

Predicted or measured concentration	AHTN		AHTN risk ratio	HHCB		HHCB risk ratio
STP: Effluent (µg/l)	PEC			PEC		
PNEC (AHTN) > 3000, (HHCB) > 2000						
EUSES PEC _{local}	5.1		<17*10 ⁻⁴	21.9		<11*10 ⁻³
Alternative predictions:						
2000 – TGD regional (10%), ‘worst case’	1.15		<3.8*10 ⁻⁴	2.7		<1.4*10 ⁻³
2000 – average	0.85		<2.8*10 ⁻⁴	1.9		<1.0*10 ⁻³
2000 – northern Europe, reasonable estimate	0.64		<2.1*10 ⁻⁴	1.2		<0.6*10 ⁻³
1995 – reasonable worst case	1.23		<4.1*10 ⁻⁴	2.2		<1.1*10 ⁻³
1995 – average	1.09		<3.6*10 ⁻⁴	2.0		<1.0*10 ⁻³
1995 – below average	0.91		<3.0*10 ⁻⁴	1.7		<0.9*10 ⁻³
Measured:	median	rwc or 90 th -perc.	risk ratio for 90 th -perc.	median	rwc or 90 th -perc.	risk ratio for 90 th -perc.
2000, low (figure 4)	0.6	1.9	<6.3*10 ⁻⁴	1.4	3.8	<1.5*10 ⁻³
Berlin 1996-97 (table 5: Heberer et al. 1999; Fromme et al. 2001a)	2.2	3.9	<13*10 ⁻⁴	6.8	10.8	<5.4*10 ⁻³

4.3.1.2 Aquatic organisms

The risks for the aquatic compartment are expressed as the risk for aquatic organisms. The risk ratios predicted by EUSES are below 1. All other calculated concentrations are below the EUSES predictions. The 90th-percentiles of the measured concentrations are within a factor of 2 of the predictions for the respective use scenarios. The situation in Berlin is an exception in the series: in the area with high input of effluents, the 90th-percentiles are above the predictions by an order of magnitude. Yet also for these areas in Berlin the risk ratios are below 1 and thus there is no concern.

Table 22. Risk ratios for aquatic organisms

Predicted or measured concentration	AHTN		AHTN risk ratio	HHCB		HHCB risk ratio
Surface water (µg/l)	PEC			PEC		
PNEC (AHTN)= 3.5; (HHCB) = 6.8						
EUSES PEC _{local}	0.52		0.15	2.26		0.33
EUSES PEC _{regional}	0.036		0.01	0.148		0.02
Alternative predictions:						
2000 – TGD regional (10%), ‘worst case’	0.15		0.04	0.42		0.06
2000 – average	0.10		0.03	0.27		0.04
2000 – northern Europe, reasonable estimate	0.07		0.02	0.14		0.02
1995 – reasonable worst case	0.16		0.05	0.33		0.05
1995 – average	0.14		0.04	0.28		0.04
1995 – below average	0.11		0.03	0.23		0.03
Measured:	median	90 th -perc.	risk ratio for 90 th -perc.	median	90 th -perc.	risk ratio for 90 th -perc.
2000, low (figure 5)	0.045	0.14	0.04	0.095	0.26	0.04
1995, low (figure 5)	0.075	0.20	0.06	0.099	0.50	0.07
Berlin 1996/97 high effluent input area (Heberer et al. 1999, Fromme et al. 2001a).	0.44	1.0	0.29	1.3	28	0.41

4.3.1.3 Sediment organisms

For the sediment, the calculations in EUSES are expressed as concentrations wet weight, whereas the measured concentrations are based on dry weight. The concentrations are to be compared to a PNEC with the same basis. The PNEC_{sediment} is derived by equilibrium partitioning from PNEC_{aquea} and for substances with logK_{ow} above 5, this approach is associated with the application of a factor of 10 to the exposure level to take intake of the test substance by ingestion of food into account. Thus the PEC/PNEC ratios for sediment based on the calculated PEC_{sediment} are proportional to those for surface water (times circa 10). The risk ratios calculated by EUSES are above 1. The calculations for the various use scenarios according to the alternative approach, including the 2000 – ‘worst case’ scenario, show risk ratios below 1.

Measured concentrations are also available. These have been taken in relatively unpolluted areas as well as in contaminated sites. The risk ratios for the 90th-percentile of the recent

sediment concentrations are below 1. For the Berlin area with high effluent input the risk ratio based on 1996/97 concentrations was above 1. The same is true for the contaminated brook in Hessen in 1996. An elaborate monitoring programme in this area showed that the environmental concentrations in this region decreased considerably and so in the same brook in 2000 the risk ratio was greatly reduced and shifted to below 1 for HHCB.

Table 23. Risk ratios for benthic organisms

Predicted or measured concentration	AHTN		AHTN risk ratio * 10	HHCB		HHCB risk ratio * 10
	PEC			PEC		
Sediment (mg/kg wwt) PNEC (AHTN) =2.0 ; PNEC (HHCB) = 3,2						
EUSES PEClocal (wwt)	0.34		1.7	1.22		3.8
EUSES PECregional (wwt)	0.035		0.18	0.12		0.37
2000 – TGD regional (10%), ‘worst case’ (wwt)	0.10		0.50	0.23		0.71
2000 – average (wwt)	0.065		0.33	0.15		0.46
2000 – northern Europe, reasonable estimate (wwt)	0.045		0.23	0.075		0.23
1995 – reasonable worst case (wwt)	0.10		0.50	0.18		0.56
1995 – average	0.09		0.45	0.15		0.46
1995 – below average	0.07		0.35	0.12		0.37
Sediment (mg/kg dw) PNEC (AHTN) =5.2 ; PNEC (HHCB) = 8.4	median	90th-perc.	risk ratio for 90th-perc. * 10	median	90th-perc.	risk ratio for 90th-perc. * 10
2000, low (figure 7; high effluent input areas excluded) (mg/kg dw)	0.03	0.45	0.85	0.003	0.35	0.4
1996, low Hessen, contaminated brook		4.8 max	9.2		4.9 max	5.8
2000, low Hessen, same contaminated brook		0.7	1.3		0.6	0.71
1996-97 Berlin, high effluent input (table 8)	0.93	2.21	4.25 ¹⁾	0.91	1.9	2.25 ¹⁾

1) Ratios are 1.9 for AHTN and 1 for HHCB with PNEC corrected for the actual organic carbon content in the sediment

In the application of equilibrium partitioning, PNEC is standardised for sediment containing 5% organic carbon. For the risk characterisation based on the monitoring data, a correction should be made for the organic content in the samples. The samples from contaminated areas are most probably related to bulking sludge problems: some of the effluent concentrations in the Berlin area are also extreme, and this can only be explained by higher concentrations of suspended solids in the effluent (see section 4.1.1.2). With the occurrence of bulking sludge, it is anticipated that the organic carbon content in the sediment will be considerably higher than 5%. This implies that these values should be compared to a PNEC derived for the higher organic carbon content (thus a higher PNEC), giving a lower RCR for the monitoring data. The organic matter content in the sediment in the Teltow Canal in Berlin, for example, were 11 to 12% in 1993/1994 (Ricking et al. 2003), which is above the standard by a factor of 2.2 to 2.4. Thus the PNEC for the Teltow Canal is increased by the same factor, resulting in reduced risk ratios of 1.9 for AHTN and 1.0 for HHCB.

No measured data are available for other regions with potentially higher use. Therefore no general conclusion can be drawn.

4.3.2 Terrestrial compartment

For the risk assessment on soil organisms no measured soil concentrations are available, so the risk can only be assessed on the basis of a model approach. However, data are available on concentrations on sludge, showing that the concentration levels estimated by EUSES are above actual levels by a level of at least a factor of 7. Therefore the model approach was applied using the measured concentrations on sludge in the various use scenarios.

The risk ratios based on EUSES are below 1, albeit that for HHCB the ratio seems to be relatively high. Using realistic concentrations on sludge, however, the risk ratios are all well below 1, indicating that there is no concern for the terrestrial compartment.

Table 24. Risk ratios for soil organisms(PECsoil, 180 days after application)

Predicted or measured concentration	AHTN PEC	AHTN risk ratio	HHCB PEC	HHCB risk ratio
Soil (mg/kg wwt)				
PNEC (AHTN) = 0.28				
PNEC (HHCB) = 0.28				
EUSES PEC _{local} (wwt)	0.053	0.19	0.202	0.72
EUSES PEC _{regional} (wwt)	0.003	0.01	0.012	0.044
2000 – TGD regional (10%), ‘worst case’ (wwt)	0.028	0.10	0.075	0.27
2000 – average (wwt)	0.016	0.06	0.041	0.15
2000 – northern Europe, reasonable estimate (wwt)	0.006	0.02	0.010	0.04

4.3.3 Secondary poisoning

For the assessment of secondary poisoning, the concentration in fish or earthworms is compared to the PNEC_{oral} derived for a predator. The risk ratios according to EUSES are below 1. The concentrations calculated according to the various other use scenarios are all well below 1. Based on measured concentrations, the risk ratios, including for the fish caught in the high effluent input areas, are also below 1. This indicates that there is no concern for secondary poisoning through the aquatic food chain.

Table 25. Risk ratios for a predator in the aquatic food chain

Predicted or measured concentration	AHTN			HHCB		
	PEC			PEC		
Aquatic biota (mg/kg ww)				risk ratio		
PNEC (AHTN)= 1 (ww)						
PNEC (HHCB) = 3.33 (ww)						
EUSES PEC _{local} (ww)	0.166		0.17	1.907		0.57
EUSES PEC _{regional} (ww)	0.021		0.02	0.243		0.07
2000 – TGD regional (10%), ‘worst case’ (ww)	0.055		0.06	0.450		0.14
2000 – average (ww)	0.035		0.04	0.277		0.08
2000 – northern Europe, reasonable estimate (ww)	0.023		0.02	0.127		0.04
1995 – reasonable worst case (ww)	0.061		0.06	0.341		0.10
1995 – average	0.051		0.05	0.292		0.09
1995 – below average	0.040		0.04	0.228		0.07
<i>Measured:</i>	median	90th-perc.	risk ratio for 90th-perc.	median	90th-perc.	risk ratio for 90th-perc.
Old and recent measurements except Berlin (figure 8)	0.008	0.1	0.1	0.01	0.1	0.03
Berlin 1996/97 (figure 9: Fromme et al. 2001b)	<0.01	0.57	0.57	0.06	1.5	0.45

No measured concentrations are available for the concentrations in earthworms or other terrestrial invertebrates. Therefore only estimated concentrations can be used for a risk assessment. The risk ratios are all well below 1, suggesting that there is no concern for secondary poisoning through the terrestrial food chain.

Table 26. Risk ratios for a predator in the terrestrial food chain

Predicted or measured concentration	AHTN		HHCB	
	Terrestrial organisms (mg/kg ww)	PNEC = 1 (wwt) risk ratio	PNEC = 3.33 (wwt) risk ratio	
EUSES PEClocal (wwt)	0.161	0.16	0.587	0.18
2000 – TGD regional (10%), ‘worst case’ (wwt)	0.089	0.09	0.234	0.07
2000 – average (wwt)	0.048	0.05	0.127	0.04
2000 – northern Europe, reasonable estimate (wwt)	0.018	0.02	0.031	0.01

4.4 Sensitivity analysis

4.4.1 Regional use

An exposure assessment should preferably be based on measured concentrations. Many data are available for AHTN and HHCB, but they are mostly restricted to areas in northern Europe where the use and release is expected to be lower than might be the case in southern Europe. In order to make predictions for the southern region, the difference with the northern region should be quantified. A complicating factor is the reduction in use volume between 1995 and 2000. Therefore an effort was made to develop a number of use scenarios both in 1995 and 2000. The different use volumes were based on observed time trends in environmental concentrations in the northern region on one side and on purely arithmetic considerations on the other side. The different use scenarios reflect the variability between a region with a low use, an average use and a ‘worst case’ situation. The difference between the extremes amounts to a factor of 7 for HHCB and 5 for AHTN. However, it should be remarked that actual data on the variation of the use volume within the EU are not available.

4.4.2 Release to the STP

The release to the STP is estimated from the average use per inhabitant released to an STP of 10,000 inhabitant equivalents. According to the TGD, a factor of 4 should be applied to the estimated release to cover temporal and regional fluctuations in the release to an STP. HERA (2002) and Fox (2002) observe that a ‘reasonable worst case’ treatment plant will receive less than 1.5 times the average input calculated for that country. For theoretical reasons explained in section 4.1.4.1, for a highly lipophilic substance the additional impact of the factor of 1.5 would be negligible as compared to load already present on the activated sludge in the STP and therefore the calculations were carried out with a factor of 1.

Recent reports confirm that the spatial variation in the load of AHTN and HHCB to the STP within a region is limited indeed. In a research programme currently running in Switzerland, the load was estimated based on concentrations in sludge, inhabitant equivalents and sludge production. The results show that the load to 16 STPs may vary with a factor up to 1.6 for AHTN and up to 1.7 for HHCB (max/median) in small STPs that represent a worst case situation with little buffering (Kupper et al. 2003). The low spatial variation is confirmed by their analysis of other reported sludge concentrations that in a region vary within a factor of 1.3 to 1.5.

These factors are similar to the factor of 1.5 proposed by HERA on the basis of Boron studies. As stated above, in our current EUSES calculations no such factor was taken into account (or factor = 1).

The approach in the TGD assumes that the volume used in compounding is completely discharged by consumers to the sewer system. Influent concentrations predicted by EUSES, however, are above the observations (in the appropriate use scenario, see table 14), showing that the release is overestimated by a factor of 2 to 4. Explanations are that the use volume is not completely discharged to the sewer, as a fraction of the down-the-drain-products will remain associated to textile fibres or surfaces of treated materials and another fraction is used in (cosmetic) products that are not associated with the use of water at all. A third explanation may be that a part of the volumes used in compounding is exported as fragrance compound or in consumer products outside the EU.

The observations of Simonich et al. (2000) on the concentration of AHTN and HHCB in influents illustrate that the temporal variation during the day/night is within a factor of 2 (maximum/mean). The variation throughout the week was within a factor of 1.3 (Brändli 2002).

4.4.3 Predicted concentrations

Where the predicted influent concentration only depends on the release scenario, the predictions for the concentrations in effluent and sludge are also influenced by the model for the STP. For these substances the model in EUSES assumes removal to occur only by adsorption, whereas the available data show that primary degradation occurs as well. Therefore an alternative approach was included to better present the actual behaviour in the STP. For lower influent concentrations (in the 2000 - low use region), the difference with the EUSES approach is not relevant (within a factor of 2), but it becomes more important for the higher influent concentrations (scenario 2000 – ‘worst case’): a factor of 5 to 9. So for a proper comparison with concentrations measured in the high use regions where influent concentrations are expected to be higher, the alternative approach becomes more relevant.

The alternative approach is based on empirical data, and therefore it avoids the uncertainties in the release and biodegradation.

4.4.4 Measured concentrations

Environmental samples are preferably taken in sites where elevated concentrations are expected to be found and therefore also the statistics (median, 90th-percentiles are biased towards the higher concentrations. In EUSES, predicted concentrations are labelled as local or regional, reflecting the situation in the vicinity of an effluent discharge point and in a remote area. For the comparison between predictions and measured concentrations, this difference should be accounted for. However, sampling points do not correspond to the virtual points used in the predictions. Although it is expected that most samples refer to 'local situations', a fraction of the samples will be of more remote areas as well. Combining these two observations, it is still reasonable to assume that the 90th-percentile values give a 'realistic worst case' for the local concentrations.

For the comparison of predictions to measured concentrations, the median of the measured concentrations is the most appropriate statistic, as the extremes are more vulnerable to variability. Therefore it was also important to have predictions of the '*median*' concentration levels without too many incorporated uncertainties. The uncertainties may be quantified afterwards. The risk assessment is based on the 'realistic worst case' or the 90th-percentiles of the measured concentrations.

4.4.5 Risk characterisation

The section on risk characterisation presents the risk ratios for the EUSES calculations, for the alternative approach differentiated into use scenarios for three regions and for the 90th-percentile of the measured concentrations, also differentiated according to regions. Except for the sediment, all risk ratios are below 1 and thus the risk of AHTN and HHCB for those protection targets is low.

Risk ratios for the sediment are above 1 in the EUSES calculations, but not in the alternative approach. The 90th-percentile of a series of measured concentrations that are not particularly related to areas with high effluent input are below the PNEC, but data from the areas in Berlin with high effluent input show a risk ratio above 1, and this is also the case in Hessen. Although concentrations in Hessen were reduced between 1996 and 2000, the risks are not yet at a low level. The high concentrations were observed in Berlin in 1996/1997 so it may be assumed that also in Berlin current levels are being reduced due to the reduced use volume. Assuming that the reduction parallels the reduction observed in Hessen (generally a factor of 3), the present day risks will also be lower, but not at really low level. The elevated surface water concentrations in high effluent input areas may be explained by higher influent concentrations due to high consumer use or, more likely, by suboptimal removal in the STP (e.g., bulking sludge) and/or by a low dilution factor of the effluent in the receiving surface water. This raises the question of the wider occurrence in all of Europe of surface water where high effluent loads are combined with low dilution. In this HERA risk assessment, the definition of the different consumer use scenarios took account of the high consumer use situations, whereas the default surface water dilution factor of 1:10 is intended to present an EU-wide 'reasonable worst case situation'. A Europe-wide survey of STP-surface water

dilution factors should identify the potential worst cases (dilution factor below 1:10) in other EU areas.

For the derivation of the $PNEC_{\text{sediment}}$, no data on the toxicity to sediment organisms are available and the PNEC was derived by equilibrium partitioning and a factor of 10 on the PEC is associated with this. The factor of 10 reflects the uncertainty in the $PNEC_{\text{sediment, equil.}}$ due to the contribution of oral uptake in the exposure of sediment organisms. This uncertainty cannot be quantified. The factor of 10 can only be omitted when sufficient toxicity tests with sediment organisms are available for the derivation of $PNEC_{\text{sediment}}$. Another uncertainty in $PNEC_{\text{sediment}}$ consists of the standardisation of the values based on the organic carbon content of 5% for a standard sediment. The content of organic matter in the samples of Berlin is unknown, but as they are from areas with a high effluent input with a suspicion of bulking sludge problems, the OC contents in the sediment are expected to have been elevated. This could reduce the risk ratios possible by a factor of 3, but no data are available.

Since the discussion on the discontinuity factor (1, 1.5 or 4) relates only to the influent and is bypassed by the alternative approach to estimate the effluent concentration, it does not influence the outcome of the risk characterisation. An aspect of the alternative approach that does need some further consideration is the use of median values instead of the 90th-percentile to calibrate the active sludge concentrations (table 15). This would imply a difference of a factor of 1.5 to 2. This would not affect the conclusions of the risk assessment.

A summary of these observations is presented in table 27.

Table 27. Summary of uncertainties

Uncertainty		Remarks
Use volume	A. not corrected for export	
Regional use	B. AHTN: 'worst case' = 5 * low use	expected to be highly exaggerated.
	HHCB: 'worst case' = 7.5 * low use	remedy: <i>measured</i> data to provide actual information
Release to STP	C. Load may vary by factor of 1.6 to 1.7, not by the default factor of 4 in the TGD	currently not incorporated (factor =1)
Predicted concentrations in effluent	D. Difference EUSES and alternative approach in 'worst case' scenarios a factor of 5 to 9 as compared to empirical data	both approaches were applied
		with the alternative approach, uncertainties in use volume and degradation are circumvented
	E. Use of median sludge concentrations instead of 90 th -percentile for the alternative approach. Generally a factor of 1.5 to 2	no change from PEC/PNEC < 1 to > 1 for the defined use scenarios in the alternative approach if factor of 2 (representing 90 th -perc.) would be applied

	Uncertainty	Remarks
Measured concentrations	F. Distinction between 'local' and 'regional' data is difficult	current risk assessment differentiates between areas with very high effluent input and other areas
	G. Risk assessment is based on 90 th -percentile levels of series including bias towards elevated concentrations but also including samples of more remote sites	90 th -percentile is probably still the 'realistic worst case'
Risk characterisation	H. PNEC _{sediment} , equilibrium entails a factor of 10.	sediment toxicity data might reduce the risk ratios
	I. Risks for Berlin and Hessen based on historic samples (1996/1997)	concentrations may be reduced by a factor of 3 due to reduced use volumes
	J. EU-wide frequency of occurrence of high effluent input/low surface water dilution situations	
	K. Risks are based on standard sediment, whereas higher OC content is expected or reported	difference may be up to a factor of 3
	L. Use of median instead of 90 th -perc. conc. in activated sludge, factor of 1.5 to 2 for calibration in alternative approach	conclusions remain the same

4.5 Conclusions

The environmental risk assessment for AHTN and HHCB shows that:

- Sufficient data are available to assess the environmental risks;
- The assessment can be based on measured concentrations in the northern region of the EU, representing average and below average use scenarios.
- Generally a decrease in measured concentrations is observed when data from years around 1996 are compared to recent results from the same areas.
- Risk ratios are below 1. In specific areas characterised by high effluent input, risk ratios for sediment organisms may be above 1. This is the case for historic data in Berlin (1996/97) and Hessen (1996). There is a need for more recent and more detailed data on concentration levels in Berlin. The uncertainty of the PNEC may be reduced by carrying out toxicity studies with sediment organisms.
- For the 'worst case' use scenario no measured concentrations are available. There is a need for information on concentration levels in the southern European region.

As a follow up to the environmental risk assessments a large-scale research programme was launched recently to sample sludge and effluents in sewage treatment plants in Greece, Italy and Spain. In the Berlin area samples are taken from the sewage treatment plants as well as from sediments to establish the current situation. Moreover, toxicity studies with sediment organisms are carried out to refine the risk assessment for these organisms.

5. Human Health Assessment

[CURRENTLY NOT INCLUDED]

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7. Contributors to this Risk Assessment

This risk assessment was developed on behalf of the European Flavour & Fragrance Association EFFA

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Additional input was given by the HERA environmental Task Force.

Appendix 1. Removal during sewage treatment

Mass balance calculations

From the study of Langworthy et al. (2000), with freshly collected activated sludge from three different plants in the USA spiked with ^{14}C -HHCB for a batch experiment, the radiolabelled parent HHCB disappeared almost linear in time. Between time zero and 24 hours 30 % disappeared on average and after 150 hours 85 % of the parent had disappeared. As the experiments were conducted with freshly collected sludge samples, this rate of disappearance should hold for all of the HHCB on the sludge and not only for the spiked radiolabelled HHCB. Unfortunately, neither the total concentrations of HHCB on the sludge samples nor the concentrations of sludge in the mixed liquor are given in the paper. An indicative calculation can be made assuming that the concentration of HHCB on the activated sludge is similar as on average in Europe. For data of Germany and The Netherlands in 1996 an average of 18 mg/kg can be used. Thus, a disappearance of 30 % in 24 hours would mean that in each kg of sludge about 5.4 mg HHCB is degraded per day in addition to the disappearance of the radiolabelled compound. The labelled compound had a concentration of 25 $\mu\text{g/l}$ and assuming that in the mixed liquor 3 g/l of suspended solids is present, this would represent another 8.3 mg/kg of which 2.5 mg was degraded. Thus altogether 7.9 mg/kg was degraded per kg sludge. This capacity can be compared to the daily load in a dynamic situation. With a daily load per kg sludge of 10.9 mg in the average use scenario of 1995 (table 4: 4 g/y = 10.9 mg/d) and a plant design with 0.5 - 1 kg activated sludge in the system *per capita*, this degradation capacity would imply about 36 - 72 % degradation of the daily load of HHCB.

In the experiments of Artola (2002) a rate constant of 0.015 h^{-1} was derived for HHCB in sludge and 0.0075 h^{-1} for AHTN. To put these rate constant into a proper perspective they should be converted to a degradation capacity per kg sludge and per day that can be compared to the average load per kg sludge per day. Unfortunately Artola reports the concentrations of AHTN and HHCB on the activated sludge in mg/l and not in mg/kg dw. Assuming an average solids content in the aeration tank of 2.5 g SS/l, the sorbed fraction can be divided by 2.5 g SS/l g to convert the concentration to mg/kg. Thus the concentration in sludge is 2.3 mg AHTN/kg dw and 5.0 mg HHCB/kg dw (table 2, page 33 in Artola 2002). These value are below the average of 4 and 7 mg/kg, respectively, observed elsewhere in NW Europe around 2000. A rate constant of 0.015 h^{-1} or 0.36 per day for HHCB implies a degradation capacity of $0.36 \cdot 5 = 1.8\text{ mg. kg dw}^{-1} \cdot \text{d}^{-1}$ (or $0.36 \cdot 7 = 2.5\text{ mg. kg dw}^{-1} \cdot \text{d}^{-1}$ elsewhere). Likewise for AHTN, the rate constant of 0.0075 h^{-1} or 0.18 per day gives a degradation capacity of $0.18 \cdot 2.3 = 0.41\text{ mg. kg dw}^{-1} \cdot \text{d}^{-1}$ (or $0.18 \cdot 4 = 0.72\text{ mg. kg dw}^{-1} \cdot \text{d}^{-1}$ elsewhere).

The average load of HHCB per kg dw sludge can be derived indirectly from the organic load of $0.15\text{ kg BOD. kg dw}^{-1} \cdot \text{d}^{-1}$ and the default values for the load and flow *per i.e.* being 54 g BOD. d^{-1} *per i.e.* and 200 l.d^{-1} *per i.e.* respectively (EU-TGD, EC 2003). Thus the load is 2.7 *i.e.*/kg dw or 540 l/kg dw and with an average concentration in the water after the primary settler of 3.98 $\mu\text{g HHCB/l}$ (table 2, page 33 in Artola 2002), this means $2.14\text{ mg. kg}^{-1} \cdot \text{day}^{-1}$. This is to be compared to the estimated degradation capacity between 1.8 and $2.5\text{ mg. kg}^{-1} \cdot \text{d}^{-1}$. Therefore the capacity for HHCB is 84 – 118 % of the daily load. Likewise for AHTN, with an average concentration in the water after the primary settler of 1.64 $\mu\text{g/l}$ (Artola 2002), the average load is $0.88\text{ mg. kg}^{-1} \cdot \text{d}^{-1}$. This is to be compared to the estimated degradation

capacity of 0.41 to 0.72 mg. kg⁻¹. d⁻¹, Thus the capacity is 47 to 82% of the daily load of AHTN.

Based on the above considerations it is estimated that between 40 and 100% of the daily load to the STP may be degraded.

Theoretical considerations

The removal by adsorption (without biodegradation) implies that the total content in the effluent consists of a dissolved fraction in partition equilibrium with a concentration on the solids. This bound fraction depends on the amount of suspended solids in the effluent (SUSP) and the concentration of the substance on the solids. In EUSES the default for the suspended solids concentration in the effluent is 0.03 g SS/l. The concentration of the substance on the solids follows directly from the amount in the influent, the volume of the influent (200 l/capita per day), the percentage that is sorbed (SORB: HHCB: 67.2%, AHTN: 70.2%, see table 11) and the amount of sludge production, set to 79 g/capita per day (SimpleTreat):

$$C_{sludge} = SORB \cdot C_{infl} \cdot 200 / 79$$

$$\text{or } C_{sludge} = C_{infl} \cdot SORB \cdot 2.53 \quad [eq. 1]$$

$$[C_{sludge} \text{ in mg/kg, } C_{infl} \text{ in } \mu\text{g/l}]$$

$$\text{for AHTN: } C_{infl}/C_{sludge} = 0.56, \text{ for HHCB: } C_{infl}/C_{sludge} = 0.59$$

Assuming equilibrium partitioning between the dissolved fraction in the effluent and the sorbed fraction on sludge,

$$K_p = C_{sludge} / C_{effl(diss.)} \cdot 1000 \text{ [l/kg]}, \text{ so}$$

$$C_{effl(diss.)} = C_{sludge} \cdot 1000 / K_p \quad [eq. 2]$$

Substitution of C_{sludge} [eq.1] in [eq.2] results in

$$C_{effl(diss.)} = C_{infl} \cdot SORB \cdot 2.53 \cdot 1000 / K_p \quad [eq. 3]$$

The concentration in the effluent bound to solids is

$$C_{effl(sorbed)} = SUSP \cdot C_{sludge} \quad [eq. 4]$$

Substitution of C_{sludge} [eq. 1] in [eq. 4] gives

$$C_{effl(sorbed)} = SUSP \cdot C_{infl} \cdot SORB \cdot 2.53 \quad [eq. 5]$$

The combination of [eq. 3] and [eq. 5] gives the total effluent concentration:

$$C_{effl.total} = C_{effl(sorbed)} + C_{effl(diss.)} \quad [eq. 6]$$

$$\begin{aligned} &= SUSP \cdot C_{infl} \cdot SORB \cdot 2.53 + C_{infl} \cdot SORB \cdot 2.53 \cdot 1000 / K_p \\ &= C_{infl} \cdot (SUSP + 1000 / K_p) \cdot SORB \cdot 2.53 \end{aligned}$$

According to EUSES, $SUSP = 0.03$ g/l, and

$$\text{for AHTN } K_p = 11000 \text{ and } SORB = 0.702: \quad C_{effl.total} = 0.22 \cdot C_{infl} \quad [eq. 7a]$$

$$\text{for HHCB } K_p = 9150 \text{ and } SORB = 0.672: \quad C_{effl.total} = 0.24 \cdot C_{infl} \quad [eq. 7b]$$

In practice the efficiency of solids removal in the secondary settler is highly variable, both from day to day and between different plants. A realistic range for the suspended solids concentration (SUSP) in the effluent is between 10 and 100 mg/l. The correlation between solids removal and the removal of AHTN or HHCB is indeed clearly illustrated by the data of Simonich et al. (2002) in figure A2.

Furthermore the total amount of sludge production is variable. Variability exists between summer and winter and between different plants due to the design. The more surplus and primary sludge is produced, the lower the concentration of the substance on the sludge will be and vice versa. Adsorption will occur partly in the primary settler where primary sludge is removed without recycling and also in the aeration tank where activated sludge is accumulated by recycling. Because of this recycling, the mass flux for removal from the aeration tank is determined by the net sludge removal rate from the aeration tank. That is normally between 5 and 20% of the total amount present in the aeration tank per day (usually expressed as the sludge age, which is the reciprocal of the removal rate). With a high sludge age a larger fraction of the solids is mineralised and the amount of surplus is lower. STPs are designed with or without a primary settler and for different sludge ages. As a result the total removal of solids per day and *per capita* may vary between 35 and 75 g. The default value of 79 g/d in EUSES (SimpleTreat) is at the high side of this range.

As a result of this variability [eq. 6] should be considered with its upper and lower limits. With the sludge production lowered by a factor of 2, for both substances the relation $C_{infl}/C_{sludge} = 0.3$ (derived [from eq. 1]). In the extreme cases the equations will be:

$$C_{effl} = C_{infl} \cdot (0.1 + 1000 / K_p) / 0.3 \quad [\text{eq. 8}]$$

and

$$\text{for AHTN: } C_{effl} = C_{infl} \cdot (0.01 + 1000 / K_p) / 0.56$$

$$\text{for HHCB: } C_{effl} = C_{infl} \cdot (0.01 + 1000 / K_p) / 0.59 \quad [\text{eq. 9}]$$

This relationship is presented in the figure A1.

Finally the volume of influent, default set at 200 l, is variable. Variability is caused by climate, the design of the sewer system, the contribution of water by industries and the consumers water use volume. Extremes are 100 and 500 l/d.

In other words, for strongly adsorbing substances the removal percentage is strongly influenced by the system properties and the operational conditions of the system.

The great impact of STP design and operation on the concentrations in sludge is illustrated by highly differing concentrations in plants in one region with the same use pattern, e.g., in The Netherlands in 1996 concentrations of HHCB on sludge ranged from 4.4 to 63 mg /kg (see table 6).

The removal by degradation (without adsorption) can be described by the theory of adaptation. A mechanism explaining this adaptation is that over a long term the micro-flora in the activated sludge will adapt to the average supply (Blok 2001). Another mechanism is the induction of enzymes for cometabolic degradation by the average supply level. According to this theory a higher average supply results eventually in a higher specific metabolic activity in the sludge and thus in a higher degradation rate constant. If the rate constant for

biodegradation is modified in proportion to the average supply, after adaptation this will result in a constant value in the effluent (dissolved). In this case there is no constant and universal removal percentage, neither a universal rate constant (or half-life value) for the degradation, but the dissolved concentration in the effluent of various treatment plants will be rather similar and independent of the influent concentration as long as the concentration is sufficiently high to guarantee the maintenance of adapted microflora in the system.

This level of the dissolved concentration in the effluent is mainly caused by the system properties and can not be seen as a universal substance property. Below a certain threshold level, the adapted fraction may not succeed to meet the dynamics of sludge in the system. As a consequence, below a certain threshold there will be no biodegradation, see figure A1b. A system with a high production of surplus sludge will show a higher threshold because the adapted fraction requires a higher metabolic rate to maintain itself.

Above the critical threshold, the algorithm for $C_{effl}(diss.)$ is:

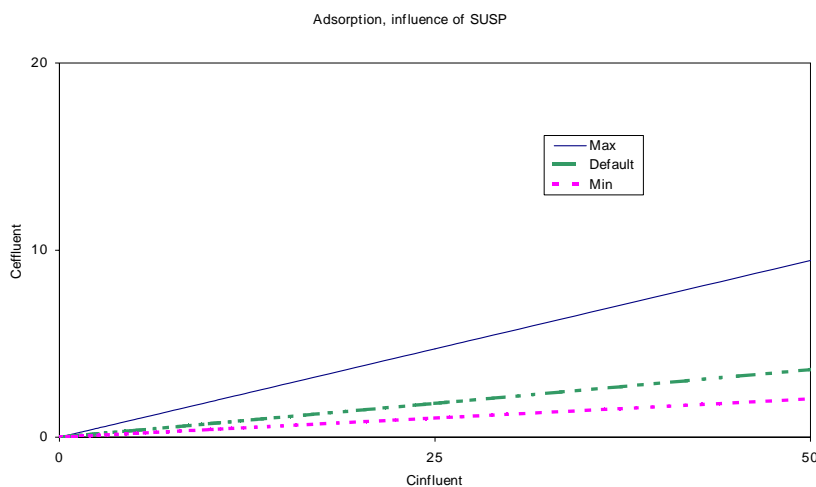
$$C_{effl}(diss) = Constant \quad [eq. 10]$$

Below the critical threshold, [eq. 7] holds:

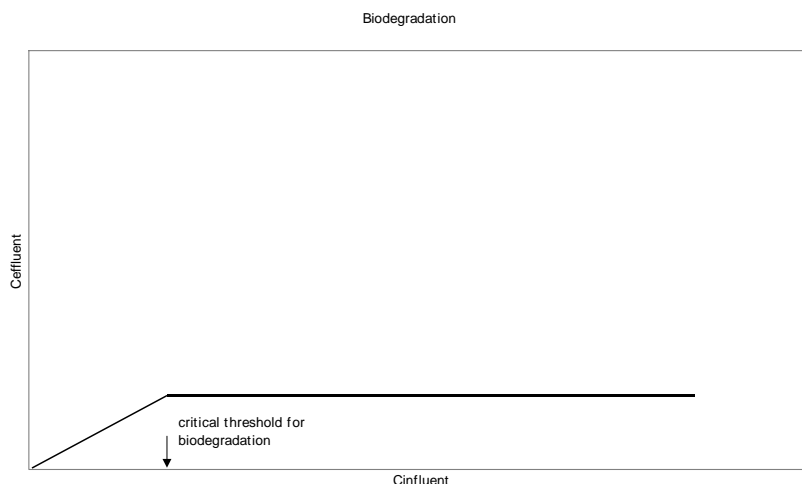
For AHTN: $C_{effl.total} = 0.22 \cdot C_{infl}$

For HHCB: $C_{effl.total} = 0.24 \cdot C_{infl}$

A



B



C

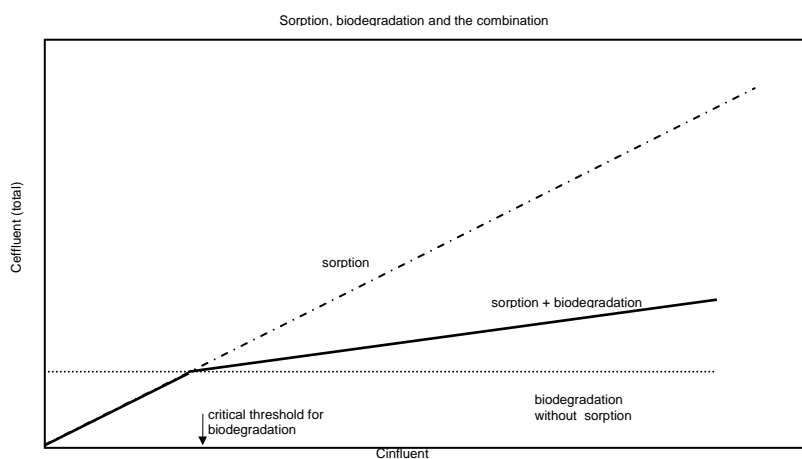


Figure A1. Relation between influent and effluent in different model concepts. A. Removal by sorption, with extreme values for the concentration of suspended solids in effluent (SUSP). B. Removal by biodegradation without sorption. C. Removal by combination of sorption and adaptive biodegradation.

In 33 STPs from different regions and use patterns, the concentrations of HHCB and AHTN in the effluent were within a narrow range indicating that the above described concept of biodegradation is applicable. This is confirmed by data for Hessen, Germany. These show that within the year's sampling (1999 and 2000), the total effluent concentrations in 9 STPs ranged from 0.9 to 1.9 $\mu\text{g/l}$ (1999) and from 0.5 to 1.3 $\mu\text{g/l}$ (2000) for HHCB and from 0.23 to 1.5 $\mu\text{g/l}$ for AHTN (see table 5). The observations of Artola (2002) and Verbruggen et al. (1999) confirm that the free concentrations of HHCB in effluent ranged from 0.16 to 1.97, on average circa 1.0 $\mu\text{g/l}$ and of AHTN from 0.1 to 0.55 (average circa 0.4 $\mu\text{g/l}$).

The effect of the combination of adsorption and biodegradation is presented in figure 15c. Due to the biodegradation that is assumed to take place in the water phase, the concentration in the water will be kept constant. When this concentration comes below the equilibrium

concentration, the sludge will partly be desorbed. This will result in lower concentrations on the sludge than without biodegradation. This implies also that the adsorption process is not at equilibrium but “oversaturated”, or in other words, the concentration in water is below equilibrium distribution. As the differences are small and may occur as a gradient between the surface of the sludge flocs and the inner parts of the flocs it may be very difficult to measure the deviation of the Koc.

A concentration on the sludge lower than predicted, due to biodegradation may also be difficult to observe because of the high variability between plants, summer and winter conditions. If for example the sludge production is 40 g. i.e.⁻¹. d⁻¹ and approximately 50% of the substance is degraded, this will give the same concentration on sludge as with 80 g. i.e.⁻¹. d⁻¹ without degradation.

For both substances, measured concentrations are available for sludge and influent that are related to the same use scenarios, see table 12.

Table A1: Measured concentrations to establish [eq. 1] with empirical data

Scenario		Measured concentrations on act. sludge (mg/kg)		Measured concentrations in influent (µg/l)		Country
		HHCB	AHTN	HHCB	AHTN	
2000	northern Europe, reasonable estimate	6.7	4.2	4.7	1.8	Hessen, Germany
2000	average	26	4	13.2	7.9	UK
1998	northern Europe	9.7	5.3	6.4	4.0	NL

Empirical ratios C_{infl} / C_{sludge} [in g/l] can be made for AHTN:

$$(UK) \quad C_{infl} / C_{sludge} = 7.9 / 4.0 = 2$$

$$(Hessen) \quad C_{infl} / C_{sludge} = 1.8 / 4.2 = 0.43 \quad [eq. 12]$$

$$(NL) \quad C_{infl} / C_{sludge} = 4.0 / 5.3 = 0.75$$

and for HHCB:

$$(UK) \quad C_{infl} / C_{sludge} = 13.2 / 26 = 0.51 \quad [eq. 13]$$

$$(Hessen) \quad C_{infl} / C_{sludge} = 4.7 / 6.7 = 0.70$$

$$(NL) \quad C_{infl} / C_{sludge} = 6.4 / 9.7 = 0.66$$

The ranges of the empirical ratio C_{infl} / C_{sludge} include the ratios according to EUSES (0.56 and 0.59, respectively). As a conservative approach, the lower ratios are used [eq. 12 and 13].

Combination of the mechanisms of sorption and adaptive biodegradation is derived from [eq. 12 or 13] and [eq. 10]:

Below threshold it is assumed that removal occurs only by partitioning according to [eq. 6]:

$$\text{for AHTN: } C_{\text{effl}}(\text{total}) = C_{\text{infl}} \cdot (\text{SUSP} + 1000 / K_p) / 0.56$$

$$\text{for HHCB: } C_{\text{effl}}(\text{total}) = C_{\text{infl}} \cdot (\text{SUSP} + 1000 / K_p) / 0.59$$

Above threshold:

$$C_{\text{effl}}(\text{total}) = C_{\text{effl}}(\text{sorbed}) + C_{\text{effl}}(\text{diss.}) = C_{\text{sludge}} \cdot \text{SUSP} + C_{\text{effl}}(\text{diss.}) \quad [\text{eq. 14}]$$

or with [eq. 12 or 13] selected as a conservative approach:

$$\text{for AHTN: } C_{\text{effl}}(\text{total}) = C_{\text{infl}} / 0.43 \cdot \text{SUSP} + C_{\text{effl}}(\text{diss.}) \quad [\text{eq. 15}]$$

$$\text{for HHCB: } C_{\text{effl}}(\text{total}) = C_{\text{infl}} / 0.51 \cdot \text{SUSP} + C_{\text{effl}}(\text{diss.}) \quad [\text{eq. 16}]$$

The concentration of suspended solids (SUSP) may deviate from the default value of 0.03 g/l within a range between 0.1 and 0.01 g/l.

For a relatively wide range of influent concentrations, $C_{\text{effl}}(\text{diss.}) = 0.5 \mu\text{g/l}$ (AHTN) and

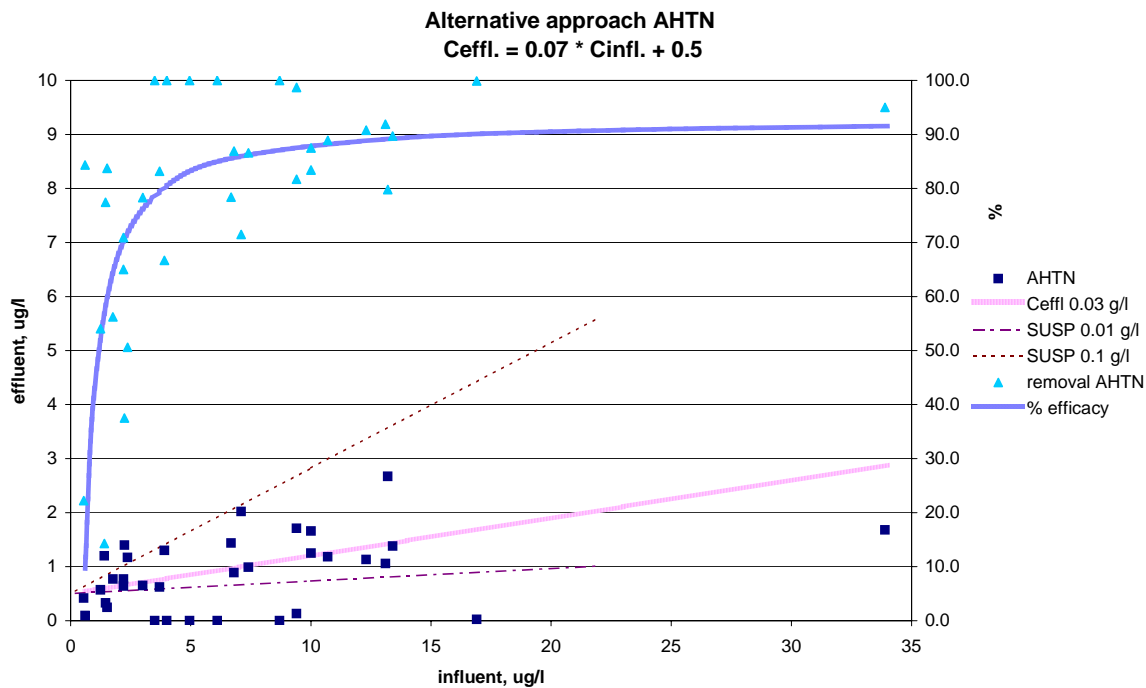
$1 \mu\text{g/l}$ (HHCB) may be used. For the standard STP with $\text{SUSP} = 0.03 \text{ g/l}$, the relation becomes

$$\text{for AHTN: } C_{\text{effl}}(\text{total}) = C_{\text{sludge}} \cdot 0.03 + 0.5 \quad [\text{eq. 17}]$$

$$\text{for HHCB: } C_{\text{effl}}(\text{total}) = C_{\text{sludge}} \cdot 0.03 + 1 \quad [\text{eq. 18}]$$

These relations for AHTN and HHCB are included in figure 16. The apparent outliers of effluent data are within the range of variability related to the higher or lower SUSP in effluent.

A



B

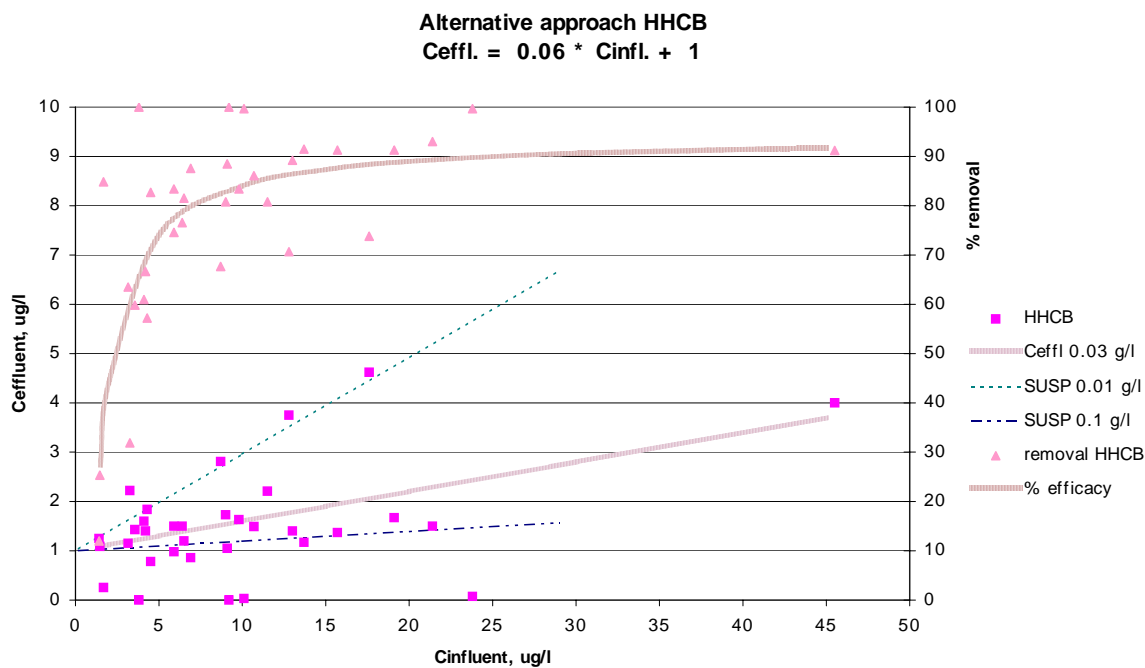


Figure A2. Observed (data points) and proposed (lines) influent-effluent relation and removal for A: AHTN and B: HHCB. The observed points have ***not*** been used to derive the lines. Thus, the observations are considered as a validation of the theoretical considerations.

Appendix 2. Data for EUSES assessment

Parameter	AHTN	HHCB
Use Volume [ton/year]	358	1427
Mol weight	258.4	258.4
Log Kow	5.4	5.3
Vp [Pa]	0.0608	0.0727
Water solubility [mg/l]	1.25	1.75
Regional use volume	10% of total EU volume	10% of total EU volume
Fraction of the main local source	0.0005 (i.e. factor of 1 instead of 4)	0.0005
BCF fish	597	1584
BCF worm	3015	2395
Koc	29800	24700
t _{1/2} in water [d]	150	150
t _{1/2} in aerated sediment [d]	180	180
t _{1/2} in soil [d]	150	150