European Union Risk Assessment Report

1,3,4,6,7,8-HEXAHYDRO-4,6,6,7,8,8-HEXAMETHYLCYCLOPENTA-γ-2-BENZOPYRAN

(1,3,4,6,7,8-HEXAHYDRO-4,6,6,7,8,8-HEXAMETHYLIN-DENO[5,6-C]PYRAN - HHCB)

CAS No: 1222-05-5

EINECS No: 214-946-9

RISK ASSESSMENT

FINAL APPROVED VERSION

Cataloguing data can be found at the end of this publication

Luxembourg: Office for Official Publications of the European Communities, [ECB: year]
ISBN [ECB: insert number here]

Printed in Italy

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Final version, May 2008

The Netherlands

FINAL APPROVED VERSION

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Date of Last Literature Search :	**May 2007**
Review of report by MS Technical Experts finalised:	**January 2008**
Final report:	**2008**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

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1 O.J. No L 084, 05/04/199 p.0001 – 0075

2 O.J. No L 161, 29/06/1994 p. 0003 – 0011

3 Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT[4]

CAS Number: 1222-05-5
EINECS Number: 214-946-9
IUPAC Name: 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran

Environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all compartments and all scenarios.

Human health

Human health (toxicity)

Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Combined exposure

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

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4 Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Human health (physico-chemical properties)

Conclusion (**ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

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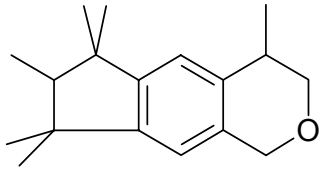
EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

1 GENERAL SUBSTANCE INFORMATION

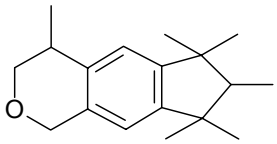
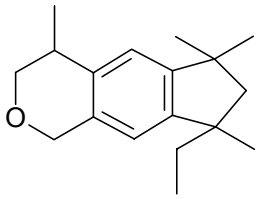
1.1 IDENTIFICATION OF THE SUBSTANCE

CAS-No.:	1222-05-5
EINECS-No.:	214-946-9
IUPAC name:	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (also CAS name)
Synonyms:	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylindeno(5,6-c)pyran (EINECS name) HHCB Abbalide Chromanolide Pearlide Galaxolide
Molecular formula:	$C_{18}H_{26}O$
Structural formula:	
Molecular weight:	258.41

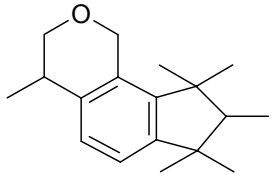
1.2 PURITY/IMPURITIES, ADDITIVES

Purity:	≥ 95% w/w (IFF 2001)
Remark:	Sum of isomers with typical composition ≥ 95%

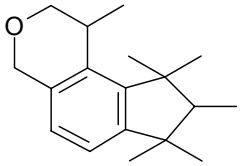
1.2.1 HHCB Isomer Structures (IFF 2001)

CAS No Main isomer	1222-05-5	74-76%	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-γ-2-benzopyran
			
CAS Nos 78448-48-3 and 78448-49-4:		6-10%	1,3,4,6,7,8-hexahydro-4,6,6,8-tetramethyl-(6 or 8)-ethylcyclopenta- γ -2-benzopyran
			

CAS No 114109-63-6:	5-8%	1,3,4,7,8,9-hexahydro-4,7,7,8,9,9-hexamethyl-cyclopenta [H]-2-benzopyran



CAS No 114109-62-5:	6-8%	1,2,4,7,8,9-hexahydro-1,7,7,8,9,9-hexamethyl-cyclopenta[F]-2-benzopyran, 6-8%



1.2.2 Impurities (by-products):

	<1%	1,1,2,3,3-pentamethyl-5-t-pentylindan
	<1%	1,1,2,3-tetramethyl-5-t-butyl-3-ethylindane
CAS No 1217-08-9, EINECS No 214-934-3	<1%	β1,1,2,3,3-hexamethylindan-5-ethanol
CAS No 66553-13-7	<1%	5-t-butyl-1,1,2,3,3-pentamethylindan
CAS No 1203-17-4, EINECS No 214-868-5	<1%	1,1,2,3,3-pentamethylindan,

Additives: none

Remark:
The manufactured purity of undiluted HHCB is >95% as a mixture of related isomers (main isomer ca 75%) Undiluted HHCB is a highly viscous liquid that is impractical to use and handle as a fragrance ingredient. In order to make it manageable for application, it is fluidised with an odour neutral diluent. These dilutions in various solvents are manufactured by blending approximately 65 weight parts of undiluted HHCB with 35 weight parts of diluent. The product name Galaxolide or Galaxolide 50 refers to HHCB diluted with diethyl phthalate (DEP), the most common form of commercial HHCB. This product is at times referred to as Galaxolide 50 DEP. In some cases, the HHCB is diluted with benzyl benzoate (BB) or isopropyl myristate (IPM). When so diluted, it is designated as Galaxolide 50 BB and Galaxolide 50 IPM. The '50' added to the name reflects the main isomer content of ca 50%. (IFF 2001).

1.3 PHYSICO-CHEMICAL PROPERTIES

In Table 1.1 the physico-chemical properties are summarised.

Table 1.1. Physico-chemical properties of HHCB

Property	Result	Comment	References
Physical state	viscous liquid		IFF, 2001
Melting point	-10 - 0 °C	determined by cooling to –30 °C and gradual warm up	IFF, 2001
Boiling point	160 °C at 4 mm Hg	recorded in the distillation of HHCB in manufacturing plant. (conform mathematical conversion into 330 °C at 760 mm Hg)	IFF, 2001
	(325 °C at 760 mm Hg)	Stein and Brown method MpBp calculated : 325 degree °C, at 760 mm Hg (162 °C at 4 hPa)	SRC MPBWIN version 1.4
Relative density	0.99 – 1.015 g/cm^3 at 20 °C	oscillating densitometer, OECD TG 109	IFF, 2001
Vapour pressure	0.0727 Pa at 25 °C (s.d. 0.0123 Pa)	gas saturation method, OECD TG 104, ^{14}C-labelled material	MacGillivray, 1966
Henry's Law Constant	36.9 Pa.m^3/mol determined at 25°C #	equilibrium partitioning in closed system and SPME	Artola-Garciana, 2002
Surface tension	39.3 dyn per centimeter	Calculated, Reid et al. 1987	IFF, 2001
Water solubility	1.75 mg/l at 25 °C (1.99 mg/l at pH 5; 1.65 mg/l at pH 7; 1.69 mg/l at pH 9) #	flask method (FDA 1987) similar to OECD TG 105, ^{14}C-labelled material	Edwards, 1996
	0.4 mg/l	calculation	SRC WsKow, version 1.27
	2.3 (±0.14) mg/l	column elution method	Artola-Garciana, 2002
Solubility in other solvents	dimethylformamide, acetone, triethylene glycol	used in aquatic toxicity studies, also apolar organic solvents	-
Partition coefficient	5.9	reversed-phase HPLC, OECD TG 117	Rudio, 1993a
n-octanol/water (log value)	6.26	calculation	SRC Kowwin, version 1.35a
	5.74	calculation	Biobyte ClogP 4.01
	5.3 #	slow stirring method	Artola-Garciana, 2002
Flash point	>100 °C	closed cup, Pensky Martens Dir. 84/449/EEC, A.9	IFF, 2001
Flammability	non flammable	not a flammable liquid.It is a combustible liquid which can burn.It has no pyrophoric properties	IFF, 2001
Autoflammability temperature	> 200 °C	Estimated, based on structure and physical data comparison.	IFF, 2001
Explosive properties	not explosive	calculated, CHETAH, v. 7.0	IFF, 2001
Oxidizing properties	not oxidizing	indication from structure and experience	IFF, 2001
Granulometry	not applicable		

: value selected for environmental risk assessment

All required physico-chemical data were submitted. The data is mainly obtained by contract laboratories or in-company laboratories and reported in test reports to the Research Institute of Fragrance Materials RIFM. The Henry's Law Constant, water solubility and log K_{ow} were also reported in a PhD thesis (Artola-Garciana, 2002).

All data are considered sufficiently reliable to fulfil the Annex VIIA requirements. No R- and S-sentences are applicable based on the physico-chemical properties.

1.4 CLASSIFICATION

1.4.1 Current classification

- Present classification according to Annex I: none
- Provisional classification by producer: N, R50/53, S61

1.4.2 Proposed classification

- Proposed classification:

  Symbols: N

  R-phrases: R50/53

  S-phrases: S60-61

- Decision of the Technical Committee for Classification and Labelling (TC-C&L), November 2005: no classification for Human Health warranted.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

The entire production of HHCB is at one plant in Europe, with a production volume in 2000 between 1000 and 5000 ton/y (HHCB undiluted). Early 2001 one smaller company stopped production. Circa 63% of the production volume (HHCB undiluted) is exported outside the EU (that is the EU-15, and including also Norway and Switzerland), of which 25% (HHCB undiluted) in form as undiluted and 37.5% (HHCB undiluted) after dilution. This is explained later in more detail, see **Figure 2.2**. To simplify handling nearly all of the HHCB produced is diluted in organic solvent to a 65% by weight pourable but still highly viscous liquid. This dilution is carried out at another plant in Europe. A relatively small portion of HHCB, about <500 ton is also diluted at the plant where it is produced.

2.1.1 Production and dilution processes

The production process includes the following steps:

- Reaction between α-methyl styrene with tertiary amylene and sulphuric acid as a catalyst to form pentamethyl indane;
- Purification by vacuum distillation;
- Friedel-Crafts reaction with propylene oxide to form HHCB-alcohol and purification by vacuum distillation;
- Cyclisation with paraformaldehyde to form HHCB (undiluted);
- Purification by vacuum distillation;
- Dilution to a viscous, pourable liquid.

2.1.2 Production capacity

Use volumes in Europe are according to RIFM (Research Institute for Fragrance Materials) and IFRA (International Fragrance Industry Association) based on surveys carried out between 1993 and 2006. The figures refer to the countries belonging to EU-15 plus the two associated countries Norway and Switzerland. This group will be noted as EU-15+2.

Table 2.1. Use volume in Europe (HHCB undiluted)

Year	Ton/year	Reference
1992	2400	RIVM 1997
1995	1482	RIVM 1997
1998	1473	Letter EFFA to BUWAL, Switzerland of July 8, 1999
2000	1427	Letter IFRA to IFF, 22 November 2001
2003	indicative: 1441	Letter EFFA/IFRA April 2005
2004	1307	Letter IFRA to IFF, August 1, 2006

The volume diluted in Europe in 2000 was circa 75% of the manufactured volume. However this volume includes export outside the EU-15+2 (37.5%). Based on surveys for 2003 and 2004, changes in the use volume between the year 2000 and 2004 are considered to be minor (IFRA/EFFA 2005, IFRA 2006). For the calculations the data for the year 2000 were used.

2.2 USES

2.2.1 Introduction

The pourable liquid is used as an ingredient in fragrance oils (by IFRA definition described as fragrance compounds [IFRA Code of Practice for the Fragrance Industry]; sometimes in literature also referred to as fragrances, fragrance composition, perfume oil or perfume compositions). HHCB is the largest volume product of the fragrance materials known collectively as polycyclic musks. Fragrance oils are complex mixtures, prepared by blending many fragrance ingredients in varying concentrations. Most of these ingredients are liquids, in which HHCB is mixed. Applications of the fragrance oils are in consumer products such as perfumes, cosmetics, soaps, shampoos, detergents, fabric conditioners, household cleaning products, air fresheners etc. The distribution over the various categories is shown in **Figure 2.1**.

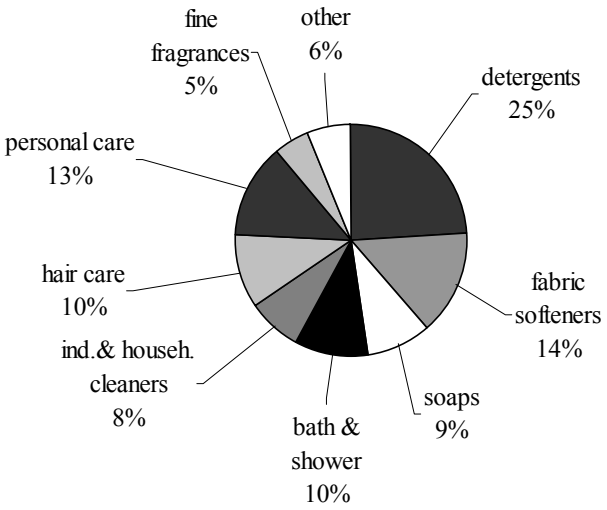


Figure 2.1. The use of fragrance oils in the EU-15 [figure taken from Balk et al. 2001, ACS Symposium Series 791, p. 171]

The use in compounding and formulation can be classified as 'non-dispersive, industrial controlled use', whereas the use by consumers can be classified as 'wide-dispersive use'.

Therefore the use is classified as follows:

- Main category: wide dispersive use
- Industrial category: category 5: personal/domestic use and/or
  category 6: public domain;
- Use category: category 9: cleaning/washing agents and additives and/or;
  category 15: cosmetics and/or
  category 36: odour agents.

2.2.2 Scenarios

2.2.2.1 Compounding

HHCB is transported to fragrance oil compounders in diluted form in tanker trucks or in containers of 200 liter or more. In general two to three thousand fragrance ingredients are used in the production of fragrances by mixing these ingredients according to various recipes These ingredients are mixed to prepare several thousand different fragrance oils. Many fragrance oils contain HHCB and, when present, at an average concentration of 4%. Fragrance oils are produced in batch volumes varying between 1 and 20,000 kg. Batches may vary in size between 1 and 20,000 kg.

In Europe there are approximately 39 compounding sites of circa 29 larger and medium sized companies excluding the use of bulk products by detergent industry. The volumes used for the preparation of fragrance oils at 6 large compounding sites that were visited are given in **Table 2.2**.

Table 2.2. Use volumes of HHCB per major compounding site (2000) (Reported during site visits) (Volumes refer to undiluted HHCB).

Site	Use volume
site 1	143,000 kg
site 2	319 000 kg
site 3	223,569 kg
site 4	107,315 kg
site 5	43,914 kg
site 6	187,000 kg
TOTAL in compounding	1,024,000 kg

This is 72% of total volume of 1427 ton reported for the EU-15+2 by IFRA. It was reported (see section 2.2.2) that circa 14% of the use volume is used for formulation by the major producers of detergents and cosmetics, leaving circa 1227 ton for compounding. This implies that the volume handled by the 6 major compounding sites is circa 84% of the volume used for compounding.

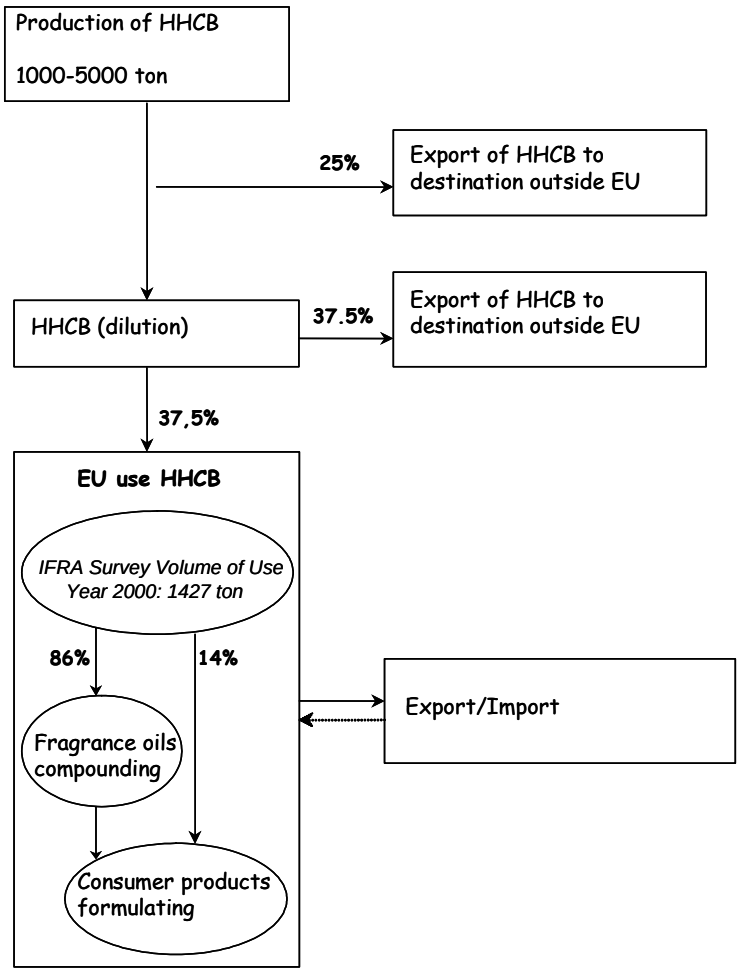


Figure 2.2. Material flow of HHCB based on Production/Sales history, Industry data 2000.

The mass flow for HHCB in Europe is illustrated in **Figure 2.2**. For the exposure calculations for the life-cycle part "end product formulation" and "private use" the volume reported by IFRA for 2000 will be used. This might be an overestimation of the real use as export of fragrance oils and of formulated products to non-EU countries is included. Industry sources estimate that 20-30% of their fragrance oils production is exported outside the EU-15+2 as finished fragrance oils or in consumer products (letter IFF, 1998).

2.2.2.2 Formulation

The fragrance oils are used in the formulation of a large variety of consumer products. A fraction of the production is directly used in bulk formulation of consumer products, such as the preparation of detergents by the larger producers. The fraction directly used is estimated at 14%.

2.2.2.3 Private use

Regional variation

For the exposure calculations for the life-cycle part 'private use' the volume reported by IFRA for 2000 of 1427 ton will be used. HHCB is applied in consumer products, mainly in cleaning agents, but also in cosmetics. An analysis was made of the regional differences of the use throughout the EU-15 member states. Data on the consumption of detergents and cosmetics in each EU-15 member state are presented in **Figure 2.3** and **Figure 2.4**. The first figure shows that the use of detergents per inhabitant is lower in some northern European countries than in southern Europe, with a maximum difference between Italy and Finland of a factor of 3.3. However, the highest per capita use (Italy, 12.6 kg per year) is above the EU average (10.1 kg) only by a factor of 1.25 (AISE 2001). The use of cosmetics (expressed in monetary units) is lowest in some southern countries. Yet the highest consumption in the EU-15, in France (€ 174), is above the EU average (€ 147) by a factor of 1.18 only. **Figure 2.4** shows that for rinse-off cosmetics only, the maximum (Denmark, € 88) is above the EU average (€ 73) by a factor of 1.21 (COLIPA 2004).

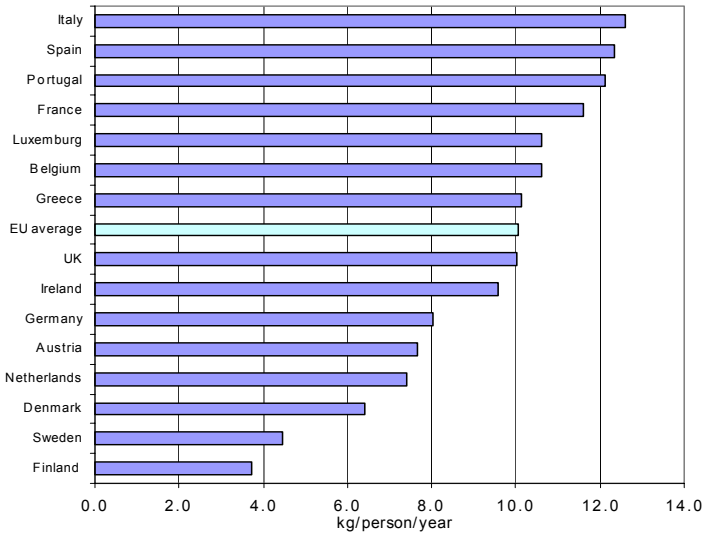


Figure 2.3. 1998 per capita detergent consumption by country. EU average is 10.1 kg (AISE, 2001).

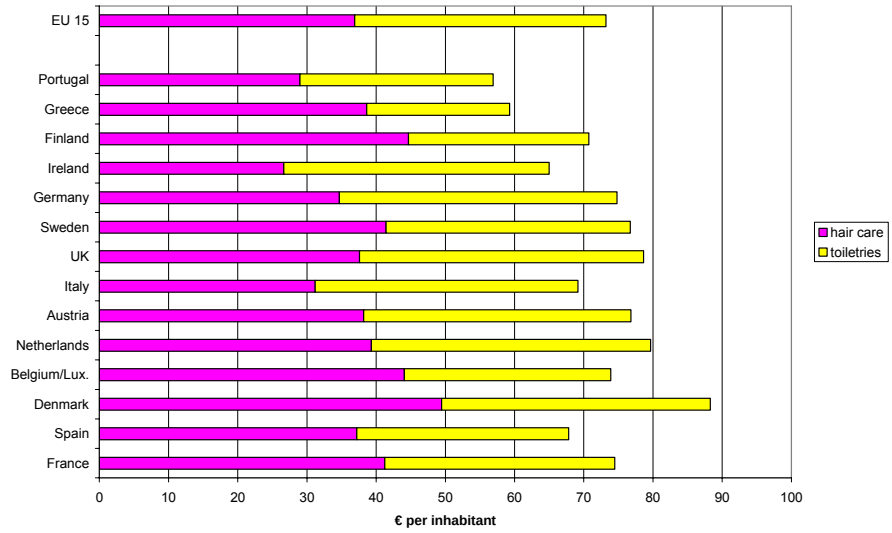


Figure 2.4. Per capita consumption of cosmetics with potential disposal to the sewer in the EU-15 in € per inhabitant, at retail prices for 2003. Weighted mean is € 73 per inhabitant (COLIPA 2004)

The use of polycyclic musk ingredients for fragrances in cosmetic and household cleaning products for the European market has significantly decreased during the second half of the nineties. Due to the publicity of these musk ingredients, major producers of personal care and detergent products with European wide brands asked their fragrance suppliers to abstain from using polycyclic musks. As a result fragrances for such European wide brands were in many instances modified and no longer contain polycyclic musks. This trend was followed by many producers of locally marketed cosmetics and household cleaning products in the Northern European countries such as Germany, The Netherlands, 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 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 a gradual replacement in North 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.

In conclusion, there are two factors that may cause an uneven distribution of the use volume of HHCB *per capita* in Europe. A 'cultural' factor of different use volumes of detergents may cause a higher use of detergents per capita by a factor of 1.25 in southern EU countries (Italy, Spain, Portugal, France, 166 million inhabitants), whereas an average use volume is found in Belgium/Luxembourg, Greece, UK and Ireland, with 84.6 million inhabitants. In the Northern countries (Germany, Austria, Netherlands, Denmark, Sweden and Finland) with 125.5 million inhabitants, the detergent use is below average by a factor of 0.7.

The second factor is the market development factor, where since 1995 polycyclic musks are gradually being replaced by other fragrance ingredients. 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 of HHCB. As both factors are independent, the combination gives a factor $1.25 \cdot 1.5 = 1.88$ above the average use in a 'worst case regional scenario for southern Europe' for the year 2000.

Environmental monitoring data between 1996 and 2000 in northern countries show a downward trend in the concentrations in the environmental compartments by a factor of circa 3 (e.g. in Hessen, HLUG 2001). Data after 2000 show a stabilisation of the concentrations as well as for the use volume. Therefore initially the risk assessment is based on the use volume for the year 2000.

For 2000 an evenly distributed use would mean 1427 ton/370 million inhabitants (3.86 g *per capita* per year) and to cover the uneven use in a realistic worst case scenario this would be $1.88 \cdot 3.86 = 7.23$ g *per capita* per year. In the northern countries the minimum use volume would be the maximum/3.3 = 7.23/3.3 = 2.2 g *per capita* per year, whereas there the highest level would theoretically be $1.0 \cdot$ EU-average = 3.86 g *per capita* per year.

According to the TGD (EC 1996, 2003), the regional use is 10% of the total use. This is used by 20 million inhabitants in the region, resulting in a *per capita* use of 7.14 g per year. Thus

the TGD regional approach equals the worst case scenario based on an extreme interpretation of the data for 2000. The various scenarios are summarised in **Table 2.3**.

Table 2.3. Scenarios for private use

Year, tonnes	Scenario	Derivation	Consumption in g *per capita* per year	Consumption in mg *per capita* per day
2000, 1427 t	TGD regional (10%)	10% of total use	7.14	19.6
	southern Europe	1.25 · 1.5 · average	7.23	19.8
	northern Europe	maximum (7.23)/3.3	2.20	6.0
	EU average	average	3.86	10.6

2.3 TRENDS

Trends in the use of HHCB, both in time and possibly per region were discussed in the previous section.

2.4 LEGISLATIVE CONTROLS

No legislative controls are in place at the time of reporting.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Releases are discussed for each life stage: production, compounding, end-product formulation and the consumer use stage.

3.1.2 Environmental releases

3.1.2.1 Release from production

HHCB is produced on one site in the EU-15+2. A small fraction of the production is also diluted on site in a different plant. Site-specific information on the environmental emission was reported (Balk 2001a). The following waste streams are identified:

1. Concentrated process water that is treated off-site;
2. Organic by-product streams are used as fuel in a combustion process on the site or sold to a third party;
3. Secondary washes during the production process, washing water from occasional cleaning of vessels or tanks and run-off water from the contained site is treated in the site's wastewater treatment plant (WWTP).

The local release is estimated on the basis of site-specific data.

The production volume is 1000 -5000 ton per year (HHCB undiluted). The production is a continuously operated batchwise process, running 330 days per year. The effluent flow of the WWTP is around 400 – 450 m^3 per day; the average content of HHCB (total concentration) is 0.1 mg/l (maximum 0.2 mg/l). This implies that 40 – 45 g per day is lost.

The effluent stream is treated in a local STP, designed for 28,135 i.e. and with a dry weather flow of 4,500 m^3. The influent to the local STP is 45 g / 4500 m^3 = 0.010 mg/l (max. 0.020 mg/l).

The dilution factor of the effluent into the river is 3.7 (based on the 10-perc. low water flow). The sludge produced on site in connection with the treatment of waste water is collected and incinerated.

3.1.2.2 Release from formulation

3.1.2.2.1 Fragrance compounding

Emissions of HHCB in fragrance compounding facilities depend on the standard operating procedures of these facilities. Several emission routes can be distinguished:

Mixing vessels, containers and pumps may be cleaned with an organic solvent, which is collected and disposed off by incineration or recycled. Emission to waste water does not occur in this case.

1. Mixing vessels and containers may be washed with steam and/or water and HHCB present in the remaining fragrance oils is discharged to the waste water.
2. Spills may be cleaned with water and HHCB present in the spilled material is discharged to the waste water.

The total volume of HHCB used in the EU-15+2 is taken as 1427 ton/year (100%, reported IFRA volume of use in the EU-15+2). Fragrance compounding is conducted by special fragrance compounding departments of large and medium scale companies, by small enterprises and by large formulators of detergents. Their compounding use distribution is estimated by industry as 80% large/medium compounders, 6% small compounders and 14% formulators.

The volume used by six visited fragrance compounding sites is 1024 tons, see **Table 2.2**. The remainder volume of 1427-1024 tons = 403 tons is used by other large/medium compounders, small compounders and formulators. Local releases were estimated for the 6 compounding sites as presented in Table 2.2. Site-specific emission data are submitted for these sites. Furthermore the release was estimated for a generic large/medium scale site (site 7) and for a small enterprise (site 8).

Site 1

A site visit report was prepared (Balk 2001b). The total tonnage of HHCB handled on this site was 2205 ton in 2000, which is diluted to prepare the 65% solutions usually used for compounding. Circa 60% of the volume is diluted in dedicated installations, so there is no washing or rinsing, thus no loss to water. The remaining volume (882 ton undiluted) is diluted in the general compounding area and this process may contribute to emission to waste water just like the compounding of fragrance mixtures. On site, 143 tons of (already diluted) HHCB are used in compounding. The number of working days is 240 days per year. The rinsing/washing water is collected with other process water from the site and treated on site in a pretreatment facility consisting of sedimentation, oil-separation and filtration. The yearly sludge production of 80 tons (90-95% water) is disposed of in a controlled way.

A conservative estimate for the loss during compounding to the trade effluent, based on proportionality of the COD in the effluent, is 0.02%. Therefore the loss through the trade effluent from the combination of dilution and compounding would be $0.0002 \cdot (882+143)$ tonnes = 205 kg per year, or, in 240 days, 854 g per day.

The yearly trade effluent volume was 1400 m^3 in 2000. This effluent is discharged to the municipal STP (67,000 i.e., dry weather flow 18,789 m^3/d). After dilution, the concentration in the influent to the STP is 45.5 µg/l.

The STP discharges into a river with a flow of $4.3 \cdot 10^6$ m^3/d (low water flow) and so the dilution factor for the effluent into the river is 229.

Site 2

A site visit report presented the details for this site for the year 2000 (Balk 2001c). The tonnage of HHCB for fragrance compounding was 319 ton per year with 250 working days

per year. The water of the washing machines was pre-treated on site. The process included sedimentation, oil separation and pH control. The emission to waste water leaving the site was estimated from the COD of the effluent of the oil skimmer, based on proportionality of the use volume of HHCB to the total volume of compounds at 0.05% of the daily use. This implied an emission of the site of 160 kg /year.

The effluent of the pretreatment plant went to a municipal STP, with 250,000 p.e. and a mean flow of 40,605 m^3 per day. The estimated concentration in the STP influent for the year 2000 was 15.7 µg/l.

Following the risk assessment, in the year 2006 an activated carbon filter unit was installed to remove HHCB from the waste water from the fragrance compounding plant. The concentrations of HHCB measured in the final wastewater going to the STP ranged from 50-160 µg/l. The water flow was 446,613 m^3 per year, so the total loss of HHCB, based on 160 µg/l is now estimated at 71.5 kg/yr.

Other conditions also changed: the tonnage was 391 ton (2005) with 312 working days per year. The loss of HHCB was discharged into the STP with a water flow (in 2005) of $25*10^6$ m^3 water per year, so 68,493 m^3/day. Thus the concentration in the influent
$Cinfl = 71.5\ kg \cdot (312\ days \cdot 68493\ m^3 \cdot day^{-1})^{-1} = 3.4\ \mu g/l.$

The canal receiving the effluent starts at the point of discharge so there is no additional dilution with surface water. After 15 km this stream joins a larger surface water body.

Site 3

The data for this site are described in a site visit report (Balk 2001d). The tonnage of HHCB for fragrance compounding is 223.5 ton per year. There are 250 working days per year. The amount of HHCB lost per year by washings of drums, containers and filtration pumps was estimated by weightings of the residue in containers and TOC analyses of the washing water from an average batch size of 1200 kg. The loss was estimated at 0.16% of the ingredients. This implies that 358 kg is emitted with the washing water per year, or 1.43 kg per working day.

This process water is collected and treated on site in an oil skimmer. With a general efficiency for this type of operation of 90% removal, 0.143 kg HHCB will be released in the daily waste stream to the municipal STP. The STP is an activated sludge plant with a flow of 11,000 m3 per day or it treats the equivalent of 21,500 inhabitant equivalents. The concentration in the influent is 13 µg/l.

The dilution factor for the effluent into the river is 1100 (based on low-flow conditions). The TGD sets a maximum of 1000 to the dilution factor for a site-specific assessment.

Site 4

A site visit report was prepared (Balk 2001e). The tonnage per year for fragrance compounding is 107.3 ton/yr. With 250 working days per year, this is 429 kg per day. The fraction released to water is estimated by weighing the residue in mixing vessels varying in size from 3 to 1200 kg and from the scrap factor for the batches from 4 to 17 m^3. (A scrap factor is the amount that is produced in surplus to the ordered amount to take into account volumes needed for sampling, losses in various mixing vessels, volatilisation, etc. during the process. Therefore it is an overestimation of the release to water). This is converted to a loss

per day as a fraction of the total daily production, or 0.12%. So the release to wastewater would be 515 g per day. The mixing room produces 80 m^3 process water per day. This is collected with other process water of the site and first treated mechanically: oil skimming, pH control, flocculation and sedimentation, and than treated in a biological treatment plant on the site.

The efficiency of the oil skimmer with respect to HHCB is not known, but should be very high. In the oil phase, 90% of the organics consisted of toluene which will cause HHCB to partition to the oil phase and thus be effectively removed. As a very conservative value, the removal in the oil skimmer is set at 50%. The biological treatment plant on site (2700 m^3 per day, activated sludge) treats predominantly industrial waste water and 10-20% domestic waste water. The concentration in the influent to the industrial WWTP is set at 515 g/d · 0.5 / 2700 m^3 = 95 µg/l.

The actual dilution factor for the effluent of the WWTP into the river is 4481 (based on low-flow conditions). The TGD sets a maximum of 1000 to the dilution factor for a site-specific assessment.

The sludge of the industrial wastewater treatment plant is incinerated.

Site 5

The details for this site are presented in a site visit report (Balk 2001f). The tonnage for fragrance compounding is 43.9 ton/yr. With 250 working days per year, this amounts to a use of 175 kg per day. The overall scrap factor for this site is 0.2%, giving a release to water less than 350 g/d.

The water from the mixing rooms (5 m^3 per day) is pretreated in a settling tank and oil skimmer. As a worst case approach on this site, the removal during mechanical treatment is set at 0%. This water is discharged to a municipal STP, treating both domestic and industrial waste water. This is an activated sludge plant with a flow of 2.1 · 10^6 m^3 per day. The influent concentration is 0.16 µg/l.

The dilution factor for the effluent into the river is 5 (based on low-flow conditions).

Site 6

A site visit report was prepared (Balk 2002a). The tonnage for fragrance compounding is 187 ton per year. Compounding tanks are rinsed with solvent and the rinsings are recycled or disposed off by incineration. There are no emissions to water.

Site 7 – Generic scenario for a large/medium compounding site

Information from the producer indicates that in Europe there are approximately 39 compounding sites of circa 29 larger and medium sized companies. Together these 29 medium/larger companies account for 80% of the HHCB use volume in the EU-15+2. According to section 2.2, the six visited sites account for 84% of the volume used for compounding and 90% of the volume was used in the larger/medium companies. A generic scenario was developed for the large and medium scale companies that were not visited. This scenario is applied for compounding activities at remaining larger and medium size companies. It is assumed that at all these sites a high level of emission control and good housekeeping is applied in addition to some type of wastewater treatment on the site.

The emissions for the 6 sites presented above are based on site-specific information, see **Table 3.1**. Based on this data set, the realistic worst case emission factor before treatment is set at 0.2% and the emission factor after treatment is set at 0.06%.

The large and medium sites together used 1140 ton (80% of total use volume, 1427 ton). The 6 visited sites used 1024 ton, leaving 116 ton for the remaining 33 sites. Thus the average use is 116/33 = 3.5 ton. This is multiplied by a factor of 5 to obtain a realistic worst case estimate: 3.5 · 5 = 17.6 ton per year. The emission factor for these 33 sites is based on the highest emission factor after treatment observed at the six visited plants. This emission is divided over 250 working days.

Table 3.1. Emission factors of six visited plants (sites 1 to 6)

Site	Emission factor before treatment,%	Emission factor after treatment,%	Specification of treatment
1	-	0.02 **	sedimentation, oil separation, filtration
2 (year: 2006)	-	0.018 **	oil skimmer, activated carbon filter
3	0.16	0.016 – 0.048	oil skimmer
4	< 0.12 *	0.06	oil skimmer, sedimentation, biol. Oxidation
5	0.08 - 0.2 *	0.008 – 0.002	oil skimmer
6	-	0.00	no waste water incinerator

* emission factors before treatment are based on empirically verified average scrap factors for tanks and other equipment.

** the emission after treatment is based on measured COD and an assumed proportionality

Thus the generic emission for large and medium size compounders is described by:

$$17.6 \text{ ton} / 250 \text{ d} \cdot 0.06\% = 0.0421 \text{ kg/d}$$

The release to the aquatic environment for a generic site is approached by the realistic worst case scenario of the TGD, where the waste water is discharged to the sewer of a small city with a sewage treatment plant for 10,000 inhabitants, with a water flow of 2,000 m^3/d and the effluent of the STP is discharged to a relatively small size river with a dilution factor of 10.

Therefore the influent concentration is:

$$0.0421 \text{ kg. } d^{-1} / 2000 \text{ m}^3\text{. } d^{-1} = 0.021 \text{ mg/l}$$

Site 8 - Generic scenario for a small compounding site

A representative of the group of small size enterprises was not visited. The Producer information shows that about 6% of the total volume used in compounding in the EU-15+2 (85 tons) is used by a large group of small clients. The 50th-percentile of the sales in this group is 0.03% of the total volume. For a realistic worst case assessment this value is multiplied by a factor of 5, so for the generic scenario a volume of 0.15% of the total use volume is used: 0.15% of 1427 ton = 2.14 ton per year. This is a *factor of 3* above the 90th-percentile volume for this group of small clients (0.76 ton/y).

It can be assumed that the emissions are controlled less strictly and that no in-house treatment is applied. For production activities in the chemical industry the TGD gives an emission

factor to waste water of 2%. Emission scenario documents for IC-5 and IC-6 give losses to water of surfactants during production < 0.3% for batch and < 0.1% for continuous processes and for the formulation of washing powders 0.01% and liquids 0.09%. These TGD emission scenarios are not specific for the type of formulation that is conducted at compounding of fragrances. As the compounding processes in small enterprises and large companies are completely similar there is a good reason to apply the empirically verified scrap factors. At Site 5 the overall scrap factor including sampling and oversupply to customers is 0.2%. Thus, the highest emission factor to waste water before treatment is << 0.2%, and 0.2% seems a realistic worst case for small size enterprises.

HHCB is a very generally used compound and therefore it will be used daily: number of days 250.

Thus the generic emission by all small size clients is described by:

$$2.14 \text{ ton} / 250 \text{ d} \cdot 0.2\% = 0.0171 \text{ kg/d}$$

The emission is released to the standard STP with a water flow of 2,000 m^3/d and the effluent is discharged with a dilution factor of 10. The influent concentration is:

$$0.0171 \text{ kg. } d^{-1} / 2000 \text{ } m^3 \text{. } d^{-1} = 0.0086 \text{ mg/l.}$$

An overview of all relevant data from this section used for calculation of emissions for compounding sites 1-8 is given in **Table 3.2**.

Table 3.2. Summary of relevant emission data for compounding sites 1 to 8

Site	Volume of HHCB undiluted, kg	# of working days per year	Emission factor after treatment,%	Conc. in influent to STP (µg/l)
1	143,000	240	0.02	46
2 (year: 2000)	319,000	250	0.05	16
2 (year: 2006)	391,000	312	0.018	3.4
3	223,569	250	0.016 – 0.048	13
4	107,315	250	0.06	95 (WWTP)
5	43,914	250	0.008 – 0.002	0.16
6	187,000	250	0.00	0
7 – Generic scenario for a large/medium site	17,600	250	0.06 *	21 #
8 – Generic scenario for a small site	2,140	250	0.2 **	8.6 #

* Higherst release rate after treatment from the sites visited (1-6)** Highest empirically derived overall scrap factor for large/medium compounding site 5

\# TGD realistic worst case calculations

3.1.2.2.2 End product formulation

Fragrance compounding is followed by the formulation of fragrance compounds (mixtures including HHCB) in end products (cosmetics, detergents, fabric softeners etc). Extensive information was obtained from a site visit to a small formulator of cleaning agents (Balk

2002b), and additional information from questionnaires and interviews from larger companies (Communications to RIVM 2002). In the TGD an emission scenario document (ESD) "Assessment of the environmental release of soaps, fabric washing, dish cleaning and surface cleaning substances" is available. This scenario document[5] comprises Personal/domestic use (no.5) and Public domain (no 6) and use category Cleaning/washing agent (no. 9) and cosmetics (no.15).

The total volume of HHCB in end product formulation in Europe for 2000 is assumed to be 1427 tonnes (See section 2.1). According to the emission scenario document the emission factor "washing liquid"[6] for waste water is 0.0009 and air is 0.00002 (TGD Table 2). For the small formulator, the percentage lost to water was very conservatively estimated at 0.2%, whereas in larger companies it was estimated to be lower by a factor of 5 to 10. The number of sites in the EU-15+2 is estimated on the basis of the number of members of the branch organisations involved in the production of end products (soaps/detergents and cosmetics industry in the EU-15+2), which is likely to be over 2000. As a conservative estimate, 1000 sites in the EU-15+2 are assumed.

Large formulator

One of the large formulators is supplied with 3.5% of the use volume, directly from the producer. It is assumed that this volume is used on one site. The formulation at this particular site takes place during 345 working days and the specific emission factor to water is 0.017%. Therefore the daily emission to water is 1427 ton · 0.035 / 345 d · 0.00017 = 0.0246 kg/d. This is released to the municipal STP. By lack of data, the standard STP characteristics are assumed, resulting in an influent concentration of 0.0123 mg/l.

Small formulators, generic scenario

No specific information is available for deliveries by compounders to formulators. The use volume by these formulators is 1427 ton minus the 14% sold directly to the formulators, thus 1227 ton/year. Assuming 1000 formulation sites in the EU-15+2, the use of HHCB on a small formulator site is 1227 ton / 1000 = 1.227 ton per year. For the assessment of a 'reasonable worst case, this use volume is multiplied by a factor of 5: Thus, 1.227 · 5 = 6135 kg/year (or 0.4% of the total use). With the emission factor to waste water of 0.2% and 250 working days per year (Balk 2002b) for a small formulator, the loss to the STP is 6135 kg · 0.002 / 250 d = 49 g/day. Release into the standard STP results in an influent concentration of 0.0245 mg/l.

3.1.2.3 Release from industrial/professional use

Not relevant.

[5] The emission scenario document does not include air fresheners and/or odour agents.

[6] Other release factors (Table 2 of ESD in TGD) are available for regular washing powders and compact powder. The column of "washing liquid" was selected because fragrances are complex mixtures which are nearly always liquids. The category washing liquid also represents a worst case approach concerning the% emission to water (a factor of 9 higher than the other two categories).

3.1.2.4 Release from private use

After use of the fragranced consumer products (cosmetics, detergents, fabric softeners etc.) most of the materials will be emitted with the waste water of households to the sewer. Depending on the use for body care, shampoo, textile or floor cleaning, a larger or smaller fraction of the use volume will evaporate. In general, cosmetics will be emitted to waste water to a lesser extent than detergents. As a first approach for the estimation of the PECs, it is assumed that the total volume of HHCB used in compounding fragrances in Europe for 2000, i.e. 1427 tonnes is released to waste water going to a STP. **Table 3.3** presents the release per inhabitant per day as derived in Table 2.3. Since the high and low estimates differ only by a factor of 3, the estimations are first carried out according to the default TGD regional (10%) scenario.

Table 3.3. Private use

Year, tonnes	Scenario	Consumption in mg *per capita* per day
2000: 1427 t	**TGD regional (10%)**	**19.6**
	southern Europe	19.8
	EU-15 average	10.6

In Switzerland the average *per capita* use was calculated from use data as 13.4 mg per day or 4.89 g per year (cited in Buerge et al. 2003). Analysis of the concentrations in sewage sludge samples of 16 STPs in Switzerland and the sludge production allowed to determine the *per capita* input of HHCB in the areas. The mean input was 428 mg cap $^{-1}$. year^{-1} (total of HHCB and its metabolite), or 1.2 mg . cap $^{-1}$. day^{-1} (Kupper et al. 2003a, calculated from their table 10). The data of Bester (2004) in Germany showed a daily use of 1.0 mg for the year 2002. The mass balance calculations in Yang and Metcalfe (2006) for an STP in Ontario, Canada show an average *per capita* input of AHTN and HHCB of 0.64 mg per day. These values are all below the estimates given above.

3.1.2.5 Release from disposal

HHCB is used in consumer products like cosmetics and cleaning agents. The use of these consumer products is mostly associated with water that will be discharged to the sewer system. Therefore the disposal phase is already included in the use phase. The disposal of residues in empty containers is expected to be a minor volume; moreover it is expected to be disposed of as solid waste in a controlled way.

3.1.2.6 Summary of releases

In summary, HHCB may be released during the production phase, during compounding and formulation and during/after use by consumers. For the risk assessment, as a conservative approach, it is assumed that the total volume used in fragrance compounding is discharged to the sewer.

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

3.1.3.1.1 Photodegradation

The photodegradation of HHCB was studied by Aschmann et al. (2001) under laboratory conditions using blacklamps for irradiation ($\lambda > 300$ nm) at 25 °C and 740 mm Hg (0.986 bar) total pressure of purified air at ~5% relative humidity. Analysis for HHCB was by adsorption to SPME fibre with subsequent thermal desorption and GC-FID. Rate constants were measured for the gas phase reactions of OH radicals, using methylvinylketone as a reference substance: $k_1 = 2.6 \pm 0.6 \cdot 10^{-11}$ cm^3 $molecule^{-1}s^{-1}$. The reaction of OH-radicals with HHCB is predicted to proceed mainly by initial addition to the aromatic ring and by H-atom abstraction from the C-H bonds of the $–CH_2$-groups adjacent to the ether-O atom. The rate constant estimated from the structure, $3.8 \cdot 10^{-11}$ cm^3 $molecule^{-1}$ s^{-1}, agrees very well with the empirical value. Combined with estimated ambient atmospheric concentrations of OH radicals (a 12-h daylight average concentration of $2.0 \cdot 10^6$ molecule cm^{-3}), an atmospheric lifetime of 5.3 hours is calculated ($\tau½ = 3.7$ h). These data indicate that the atmospheric lifetime of HHCB is sufficiently short that it will not undergo long-range transport to any significant extent (Aschmann et al. 2001, Pers. comm. Atkinson[7]). Assuming a daylight period of 12 h and $1.5 \cdot 10^6$ OH cm^{-3}, $\tau½$ based on the empirical rate is 4.9 h or 0.41 d (Syracuse estimation programme AOP).

In a laboratory set-up HHCB at 1 µg/l was incubated in lake water from the Zürichsee (CH) or in distilled water at 20 ± 1 ºC. Aliquots of 25 ml were illuminated with actinic lamps (mercury-vapor fluorescent lamps emitting UV-radiation between 300 and 460 nm with a maximum at 365 nm, comparable to that of 24h-averaged sunlight at 50 ºN in July under clear sky conditions. The rate of photodegradation was 0.15 and 0.12 d^{-1} in lake water and distilled water, respectively (half-lives of 109 and 135 h). The minimal differences between the photolysis rate constants determined in lake water and distilled water indicate that HHCB is degraded primarily via direct photolysis and that indirect photochemical degradation by reactive oxygen species is of minor importance. Control experiments in the dark indicated that HHCB was not eliminated by other processes. The photodegradation may explain the decreased concentrations in the epilimnion of the lake in summer. The average photolysis rate for a typical winter situation, integrated over the whole depth of the Zürichsee (mean depth 50 m, attenuation 0.01 – 0.02 per cm at 315 nm) ranges from 3.3 to 6.6 * 10^{-5} d^{-1}. In summer, considering only the epilimnion, the estimate is about 2 orders of magnitude higher then in winter for the whole lake: 3.2 – 6.5 * 10^{-3} d^{-1} (Buerge et al. 2003).

The photo-induced degradation of HHCB was studied using a solid-phase micro-extraction fibre. For the UV radiation low-pressure mercury lamps (8-10 W, 254 nm) were used. The irradiation was conducted with HHCB adsorbed on the fibre, as well as solved in water. 100 µm fibres coated with polydimethylsiloxane (PDMS) were used. A 1 and 20 µg/l solution in water was heated to 100 °C with the fibre in the headspace over the sample (HSSPME) for 30

[7] Atkinson: Lifetime is the time for the initial concentration to decrease by a factor of "e". The half-life is therefore 0.693 x lifetime. Hence for an OH radical reaction, lifetime = 1/(k[OH]) and half-life = 0.693/(k[OH]) where k is the rate constant for reaction of the organic with OH radicals and [OH] is the ambient OH radical concentration.

minutes. Subsequently, the fibre was thermally desorbed in the GC injection port. The results showed that irradiation in water showed similar degradation kinetics but only slightly faster than on fibre. Photodegradation of the parent HHCB showed a half-life of about 40 minutes. After 120 minutes 90% was degraded. The tentatively identified degradation products show that HHCB is not attacked on the hexamethylcyclopenta-benzene side but at the pyran side of the molecule, either by formation of a five-ring with aldehyde group or by removal of the oxygen moiety (Sanchez-Prado et al. 2004).

3.1.3.1.2 Aquatic degradation (incl. sediment)

Mineralisation

The ready biodegradability of HHCB was assessed in (a) the sealed vessel headspace with TIC analysis for CO_2-evolution and an adapted inoculum (King 1994) and (b) the modified Sturm test for CO_2-evolution (Jenkins 1991). In (a), HHCB was tested as one of the commercially available qualities containing isopropyl myristate which, as a dispersant, should promote the bioavailability of the poorly water soluble HHCB. The CO_2-evolution in the test was attributed to the biodegradation of isopropylmyristate. Both tests show the absence of mineralisation under the stringent conditions of the tests for ready biodegradability. The tests are summarised in **Table 3.4**.

Primary degradation

Free (dissolved) and total concentrations of HHCB in duplicate activated sludge samples were followed in time during 2 days. The samples were not additionally spiked. Loss due to volatilisation was also determined. The initial free and total concentrations were 1.58 and 10.33 µg/l, respectively. The 'true biodegradation rate constant' based on the free concentration was 0.071 ± 0.030 h^{-1}. The rate constant based on total concentrations was 0.015 h^{-1} (Artola 2002) [8].

Table 3.4. Summary of tests for biodegradation (mineralisation)

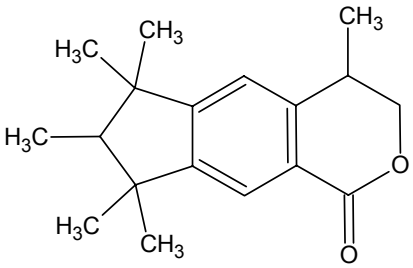
HHCB	Results
Test	**Modification of OECD 301B**, Sealed vessel TIC test acc. to Birch and Fletcher, 1991 (King 1994)
Inoculum	Effluent from SCAS after 8 weeks adaptation
Test substance	HHCB in isopropyl myristate (commercially available quality, 32.2% IPM), 10.97 mg C/l;, two identified HHCB isomers totalled 51.8% (26.4% and 25.4%), carbon 80.56% w/w
Dispersion	Injection in isopropyl myristate
Test duration	28 days
Controls	Reference substance benzyl alcohol
	No toxicity control
Detection	TIC (Total Inorganic Carbon)
Results	% CO_2 release: zero (corrected for isopropyl myristate)

8 With HHCB mainly bound to sludge, this reaction rate constant would imply that during the solids retention time (SRT) of circa 9 days a significant part (96%) would be degraded. With $Ct = Co \cdot e^{-k \cdot SRT}$, SRT = 9 d and k = 0.015 hr^{-1}, Ct/Co = 0.04 (after 9 days, 4% of the initial concentration remains). With 0.033 h^{-1} (Langworthy et al. 2000) the residue is 0.1%.

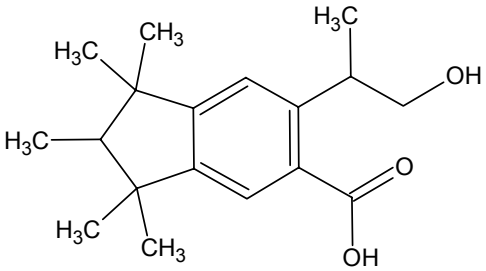
Test	**Modified Sturm test OECD 301B**, CO_2-evolution Jenkins 1991
Inoculum	sewage effluent, 1 drop/l
Test substance	HHCB, nominal 10 and 20 mg/l
Dispersion	no
Test duration	28 days
Controls	Reference substance Sodium benzoate; Toxicity control
Results	% CO_2 release: zero

^{14}C-HHCB was dosed at 25 µg/l to activated sludge collected from three different STPs, and to river water (1 µg/l) and the disappearance of the parent substance and the formation of metabolites were monitored over time by Rad-TLC (Langworthy et al. 2000). In the bioactive treatment the parent HHCB disappeared with the subsequent appearance of more polar metabolites. The half-life time for the parent in activated sludge was 21 hours (so a first order rate constant 0.033 h^{-1}) and about 85% disappeared in 150 hours. Initially a first metabolite with TLC elution time similar to lactone appeared and accounted for about 40% of the original radioactivity between day 1 and 8. Gradually a second metabolite increased, to up to 45% of the radioactivity after 650 hours. This metabolite had a similar elution time as the hydroxycarboxylic acid. The structures are shown in **Figure 3.1**. A third, highly polar metabolite made up to 15% of the radioactivity after 150 hours. The mass balance accounted for 99% of the radioactivity and no significant volatilisation was detected. In river water the half-life of the parent HHCB was 33 hours. After 300 hours about 40% of the radioactivity was lost by volatilisation. After 100 hours, more polar metabolites were detected and they made up to 60% of the original radioactivity after 700 hours. The mass balance accounted for 92% of the radioactivity (Langworthy et al. 2000). In a more recent presentation reporting on the same or more extended experiments, the figures were slightly adapted: the first order rates for the loss of HHCB in activated sludge were 0.010 and 0.021 h^{-1} with 5 and 25 µg HHCB/l, respectively (or t½ 69 and 33 hours). With 0.5 µg HHCB/l in river water, the rate constant for the disappearance of the parent substance was 0.016h^{-1} or t½ is 43 h (Federle et al. 2002).

The RP-HPLC analysis revealed that the metabolites in the activated sludge test co-eluting with the lactone (log K_{ow} 4.0) and hydroxycarboxylic acid (log K_{ow} 0.5) standards had a lower K_{ow} than the standards: from 3.1 to below 0.1, respectively. It was suggested that further oxidation of these products had occurred. The capacity to metabolise HHCB was observed in all three STPs included in the study of Langworthy (et al. 2000).



HHCB-lactone or Galaxolidone



Hydroxy acid

Figure 3.1. Structures of some transformation products

Two die-away studies were carried out with radio labelled HHCB (Schaefer 2005). To simulate the activated sludge process in a static test radio labelled ^{14}C-HHCB was added to a nominal concentration of 17.4 µg/l in activated sludge from an STP treating primarily domestic sewage. The disappearance of parent material and the formation of metabolites were assessed with SPE-extraction and thin layer chromatography (Rad-TLC) and quantified by LSC. The sludge was adjusted to 2.5 g/l TSS and the temperature was maintained at 20 $\pm$ 3 ºC. The die-away in river water was tested as a concentration of 5 µg/l in a sample of river water with activated sludge added at a level of 10 mg AS/l. The two test systems differ in the initial concentration of the test substance and in the ratio between test substance and suspended solids (17.4 µg HHCB/2500 mg TSS and 5 µg HHCB/10 mg TSS). Mass balances were made for the components in the test medium, see **Table 3.5**.

Table 3.5. Mass balance for die-away experiments with radio labelled HHCB in activated sludge (Schaefer 2005)

Time	3hr	day 5	day 14	day 28
^{14}C-HHCB, 17.4 µg/l , activated sludge 2.5 g SS/l				
% recovered in extracts with solvent				
As parent	90.6	19.2	33.4	6.3
As metabolite	0.0	42.2	39.1	70.1
% recovered in aqueous	0.5	2.3	3	2.9
% not extractable from solids	0.8	15.5	1.3	4.9
% volatilised	n.a.	0.1	0.1	0.13
Total recovery%	92	80	79	85.6

Time	5hr	day 2	day 7	day 28	Abiotic day 28
^{14}C-HHCB , 5 µg/l, river water + 10 mg AS/l					
% recovered in extracts with solvent					
As parent	91.2	63	27.7	7.8	43.4
As metabolite	0.0	25	56.1	61.6	17.5
% recovered in aqueous	0.46	1.96	0.60	1.16	0.74
% not extractable from solids	0.48	0.46	0.51	0.38	0.68
% volatilised	n.a.	1.6	7.39	15.9	16.5
Total recovery%	92.2	91.9	92.3	86.9	78.9

For both tests it is clear that the parent substance disappears relatively fast, i.e. with a half-life of 10 to 15 hours in the sludge test and with a half-life of 100 hours in the river die-away test. A variety of metabolites is formed at different rates and with increasing polarity in time. The total radioactivity of metabolites in the activated sludge test was around 40% in the period between 7 and 14 days and then increased to 70% at day 28. In the river die-away test the total radioactivity of metabolites rose from 25 to 56% between day 2 and 7 and to nearly 62% at day 28. Only in the river die away test a significant fraction of up to 16% after 28 days volatilised. The TLC showed that the parent was at 120 – 150 mm. In the test with sludge a first peak of metabolites at 120 – 140 mm of the frontline of TLC plates showed up between 5 hrs and 2 days incubation. After 3 days a large group of peaks appeared at a distance 10 – 45 mm and the fraction of total radioactivity for this range of peaks gradually increased from 37% till 78% at day 28. In the river die away test after two days various peaks appeared in the range of 95 – 115 mm with up to 22% of the total radioactivity. After 5 days various new

peaks appeared at the region 30 – 70 mm with about 30% of total radioactivity. Around day 21 many new peaks appeared in the region 10 – 80 mm (Schaefer 2005). When the results are corrected for the amount volatilised and the non-recovered fraction as well as for the abiotic transformation, the primary biodegradation on day 28 was circa 60%. According to the kinetic analysis in Schaefer (2005) the overall half-life of the parent is 100 hours.

In a study by Brändli (2002) it is shown that Galaxolidone appears during the transport in the sewer and the sewage treatment. Galaxolidone is an oxidation product with an additional oxygen on the (benzene)pyran ring. Bester (2003) showed that this structure is produced during sewage treatment.

3.1.3.1.3 Degradation in soil

A variety of 64 soil samples (sand, clay, peat and loam) from various locations in The Netherlands was screened for the presence of microorganisms able to transform HHCB into metabolites with a more polar behaviour as indicated by the lower retention factor (Rf)-value on TLC plates. This screening culminated in several pure cultures of fungus *Phanerochaete chrysosporium* being incubated with HHCB. The ethyl acetate extracts of the cultures were then analysed by GC-MS (PFW 1997).

Approximately 40% of the 64 soil samples showed a positive degrading potential towards one or both of the polycyclic musks HHCB and AHTN (17% for HHCB). In pure cultures of the white rot fungus *P. chrysosporium* (ATCC 32629) HHCB disappeared within 3 days.

HHCB radio labelled uniformly in the aromatic ring was added to various fungal species growing in mats on growth medium in closed vials at 30 °C at pH 4.5. The fungi were pure cultures of *P. chrysosporium* (two strains, ATCC 42538 and ATCC 46235) and two unknown species isolated from spontaneously air-infected cultures. Nominal concentrations of HHCB were 50 - 100 mg/l. In a second experiment the most active of these strains were incubated with nutrients and spiked with HHCB. In an additional experiment the fungal medium was adjusted to pH 7 and amended with additional nutrients and an inoculum from mixed soil and sludge samples after 4 weeks of incubation. The mineralisation and formation of metabolites was followed over time. Metabolites were extracted in ethyl acetate and separated on TLC with hexane:ethanol 80:20 solvent mixture. Volatile organic metabolites and $^{14}CO_2$ were trapped in scintillation fluids (Envirogen 1997).

After 6 weeks of incubation, no radio labelled volatile organics or carbon dioxide were produced. For the four strains, however, significant amounts of radioactivity were associated with non-volatile metabolites (5-30-50-77%). The mass balance for the radioactivity was good (recovery in extract 98 - 105%, from TLC 90 - 103%). According to the Rf-values, the metabolites were more polar than HHCB (Rf 0.89): e.g., HHCB-lactone (Rf 0.67) and polarity further increased during 6 weeks (from Rf 0.57 to Rf 0.38).

In the additional experiment (pH adjustment, additional nutrients and inoculum) the most active strain transformed 81 and 95% of the HHCB after 1 and 4 weeks, respectively. After 4 weeks, HHCB-lactone accounted for 19% of the radioactivity and 75% consisted of other, uncharacterised but more polar products. With increased incubation time, larger quantities of polar metabolites were formed. In the least active of the strains, 42% of HHCB had been transformed and 23% of the radioactivity was converted into metabolites other than HHCB-lactone in 12 weeks (Envirogen 1997).

This work shows that several pure cultures of fungi have the capacity of primary biodegradation (loss of parent structure) of HHCB without mineralisation. The most active strain was identified as *Cladosporium cladosporiodes*, a common fungus that is found in several environmental compartments including leaf litter and soils (Kuthubutheen and Pugh, 1979, Wookey et al. 1991). In the experiment with the pH-adjusted soil and sludge amended inoculum, mineralisation to $^{14}CO_2$ 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 $^{14}CO_2$. 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 according to protocols described in documents from the U.S. Food and Drug Administration of 1987. Samples were taken from an oak forest soil, an agricultural soil and the sediment of the Delaware River in central New Jersey, and from a farm with routine sludge applications from a domestic STP in southern New Jersey. Sealed flasks with soil spiked with 10 µg HHCB/g soil (10 mg/kg) were incubated at laboratory ambient temperature for one year. Periodically the headspace was flushed for oxygen replenishment and the effluent gas was drawn through a train of scintillation fluids to capture volatile organics and CO_2. After incubation, flasks were sacrificed and exhaustively extracted with ethyl acetate and/or acetone/hexane. An aliquot of the solvent was used for thin layer chromatography. Marked spots were cut out and placed into scintillation fluid for radioactivity measurement (Envirogen 1998).

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. The TLC results showed that 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. For the four different soil types an average of 14% HHCB remained after one year (Envirogen 1998). Estimated rate constants and half-lives were 0.0066 d^{-1} and 105 days for sludge amended soil, 0.0073 d^{-1} and 95 days for forest soil, 0.0029 d^{-1} and 239 days for agricultural soil ([9]) and 0.0088 d^{-1} and 79 days for river sediment. Starting from the average (in the four soils) of 14% remaining after one year, the average half-life in the four soils is 128 days.

The dissipation of fragrance materials in sludge-amended soils was studied in a 1-year die-away experiment with four different soils, with and without spiking of the test materials. The four different soils were characterised as: sandy agricultural soil from Georgetown, DE, organic matter content (OM) 1.55%, silty Midwestern agricultural soil, ILL, OM 2.63%, clayey soil from Newark, DE, OM 7.01%, highly weathered oxide rich soil from Aiken, SC,

[9] The performance of the agricultural soil in the test deviated from the other soil types. Contrary to the other three systems, a large fraction of the radioactivity was lost from this test system and the calculated biodegradation rate was lower. The lower microbial activity might also be explained by the condition of the agricultural soil (if barren, unprotected by crop, the microclimate may have caused a soil dried/sterilised by the sun & high temperatures)

OM 0.61%. Anaerobically digested and dewatered sludge was obtained from two activated sludge plants: Georgetown DE (100% domestic, 10% solids) and Wilmington DE (70% domestic, 17% solids). The concentration of HHCB in the digested sludge of Georgetown was 86 and 38 mg/kg dwt in the years 2000 and 2002, respectively. For Wilmington the concentrations were 43 and 22 mg/kg dwt, respectively. The test was carried out in trays containing 24 liter mixed with 1 liter of sludge, simulating sludge applications of 7000 wet gallons per acre and a 15 cm plow depth (or 0.6 to 1.1 kg sludge per m^2). Each of the four soils was incubated with digested sludge, unspiked as well as spiked with a mixture of fragrance ingredients. The spiking of sludge was performed by the wall coating method and the soil and sludge were mixed during 30 minutes in a cement mixer to ensure uniform mixing. Four combinations were duplicated, so a total of 20 trays were set up outdoors. Leachate samples were collected at the end of each rain event. Soil samples were extracted by accelerated solvent extraction, the leachate was extracted using C-18 speed disks. All extracts were analysed by GC/MS. The initial concentrations in spiked soil were 6 and 13 mg/kg soil, whereas the levels in unspiked soil, simulating real practice were between 0.1 and 0.27 mg/kg. In all soils the concentrations rapidly decreased. After one month the concentrations in the different soils were 30 to 90% of the initial concentration and after 90 days they ranged from 8 to 60%. During the next three months period the soil was frozen and the concentrations of all test materials remained stable. After a year the residues ranged from below 10 to 14% of the initial concentration. The rate of dissipation was higher in the soils with lower content of organic material. Loss processes may include volatilisation, leaching and biotransformation. The half-life derived based on 240 days unfrozen conditions was 141 and 144 days in the spiked and unspiked sludge amended soils, respectively. The leachate was collected during 3 to 5 months. The leached amount was 0.03 to 0.18% of the initial amount in the spiked soils. A relation with the organic material content was absent. In the leachate from the unspiked soils HHCB was not detected (DiFrancesco et al 2004).

The degradation of HHCB was studied in three soil types: sand, clay and humic. Dehydrated sludge was ground and sieved over 2 mm. Soil stored during more than 6 months in the freezer was thawed, dried, sieved over 2 mm and dried during 3 weeks. The water content was increased to 50% of the water capacity of the soil. 5 g of sludge (sampled in January) was weighed into a 80 ml centrifuge tube and spiked with HHCB. After evaporation of the solvent (acetone) 10 g of soil was added. The nominal concentration in the soil-mixture was 10 mg/kg. The actual concentrations were around 14 mg/kg. The soil-sludge combination was mixed by turning and lightly shaking of the closed tube. The samples were incubated in the dark at 20+1 ºC in a humid environment. At different time intervals the total content of the tube was extracted and analysed. The concentrations in soil showed a slow but steady decrease. After 18 weeks 59, 64 and 66% of the added amount of HHCB was detected in the sand, clay and humic soil, respectively. The report states that after 32 weeks 50 and 53% had disappeared from the sand and clay soils and 47% had disappeared from the humic soil after 37 weeks. In view of the low activity, additional tests were carried out. After 24 weeks freshly collected soil was sampled and 5 g was added to the remaining tubes and mixed. The degradation seemed to go on slowly as before. It was concluded that the fresh soil did not supply the inoculum needed for the degradation. In another experiment the sandy and clay soils were incubated as before, but with freshly collected sludge and a sterile control included. In the active control and in the sterile control no degradation was found after 8 weeks, but the variability in the results was very high. It was suggested that the fresh sludge (sampled in June) was less active than the previous sample to explain the observed lack of biodegradation (Müller et al. 2003). Some general remarks can be made regarding this test. The test was started with soil that had been frozen and dried to air, so the microbial activity was far from optimal. The test was carried out in a small volume of 15 g soil in a centrifuging tube. It is

questionable whether the mixing by 'turning and light shaking' could ensure a homogeneous mixing of (1) the stock solution with the sludge and (2) the sludge with the soil and (3) the soil mixture and the freshly collected soil in the extended experiment. All tests showed a high variability in the results over the weeks. The total lack of degradation in the non-sterile replicate of the experiment including the control vessel of the study with the sterile control showed that the results of the first tests could not be repeated. A positive control to check the quality of the inoculum (as required in the tests for ready biodegradation), was not included here. Another remark refers to the actual concentration levels present in the system, which were relatively high as compared to the levels predicted in soil (14 mg/kg as compared to PECsoil around 0.05 mg/kg dwt). These substances are highly sorptive and only a small fraction would have been in the water phase and available biodegradation by microorganisms.

In the state of Baden-Württemberg, Germany, 13 study locations were selected comprising a sewage sludge field with known history of sludge applications and a reference field. Over the years, different quantities of sludge had been applied in different periods. Total quantities of sludge applied to the fields ranged from 3.2 ton/ha to 31.5 ton/ha. A total load of 85 and 510 ton/ha was brought on the two experimental plots during 18 years, between 1972 and 1989. Soil concentrations were expressed as the sum of AHTN and HHCB. The concentrations measured in 2002 in the experimental fields were 1.1 and 5.3 μg/kg dwt. The concentrations fields amended with sludge on a regular basis between 1993 and 2001 were below 1 μg/kg dwt except on one field (2.1 μg/kg) where the concentration in the reference field was also elevated. Also the concentrations in the reference plots of the experimental areas were elevated: 0.54 μg/kg dwt (LfU-BW 2003). Using the data in the report on the quantities of sludge applied and the number of years after the last sludge application, and assuming a relatively low load of 15 mg AHTN + HHCB per kg dwt sludge, a conservative estimate was made of the estimated accumulated concentrations in soil. In the experimental plot with the highest sludge load, a total of 31.5 ton/ha was dosed over the years. This was estimated to give a concentration of 2550 μg/kg dwt, whereas the residue measured 13 years after the last application was 5.3 μg/kg, which is a maximal of 0.2% of the applied dose. (With likely higher sludge concentrations in the past, the residue will be lower.) Similarly, in the six fields amended with sludge on a regular basis the residues were <0.3% three years after the last application, 1.8% after 2 y, 0.5% after 7 y, 1.3% after 1 y, 1.9% after 3 y and <0.4% after 4 years, respectively.

Biosolids from an STP in Ontario, Canada with 470 μg HHCB per kg dw were applied on an agricultural field in October 2003. Soil samples were collected to a depth of 10 cm before and after application. No musks were detected pre-application. The concentration on day 1 was 2.0 μg/kg, after 2 week 2.8 μg/kg. After 4 weeks the level had dropped below the limit of quantitation (LOQ 0.2-2 μg/kg wet weight). After 6 months HHCB was still detectable below the LOQ (Yang and Metcalfe 2006).

3.1.3.1.4 Summary of environmental degradation

Under atmospheric conditions the half-life is 3.7 hours (Aschmann et al. 2001).

It can be concluded that a primary biodegradation to a series of more polar metabolites takes place, with HHCB-lactone and hydroxycarboxylic acid as likely intermediates. These substances still contain the same amount of organic carbon and only a small fraction of the theoretical oxygen demand has been incorporated. Thus this metabolism is in agreement with the observed low degree of mineralisation.

From the studies with activated sludge spiked with radio labelled HHCB in batch experiments (Langworthy et al. 2000, Federle et al. 2000, Schaefer 2005) it is concluded that the parent substance was transformed to a series of polar metabolites. In general the radio labelled parent HHCB disappeared with half-life values of 21 hours (Langworthy et al. 2000) and even within 10 – 15 hours (Schaefer 2005). In the river die-away test with 0.5 µg/l the parent HHCB disappeared with a half-life of 43 hours (Federle et al. 2002) to 100 hours (4 days) at 5 µg/l (Schaefer 2005).

Field measurements on sludge amended soil indicate HHCB disappeared almost completely from soil within one year. The half-life based on unfrozen conditions in sludge amended soil studies was around 140 - 145 days (DiFrancesco et al. 2004). The residues in soil after one year ranged from below 10% to 14% of the initial concentrations. The half-life of 105 days in the sludge amended soil test is of course most relevant for the fate of HHCB in soil in the EUSES model, whereas 79 days was noted for the sediment (Envirogen 1998).

Subsequently, for the environmental risk assessment, HHCB may be considered as 'inherently biodegradable, not fulfilling criteria' (terminology of the EU-TGD, EC 2003). For surface water, sediment and soil, the PECs will be calculated using conservative biodegradation rate constants expressed as half-life times: 60 d in surface water (20 ºC) and 150 d in the soil and sediment compartments (12 ºC).

3.1.3.2 Distribution

3.1.3.2.1 Adsorption

In a critical review on synthetic musks in sludge, sediment and suspended matter, partition coefficients were summarised for these solid matter matrices (Fooken 2004). Together with the Koc and Kd determined from empirical data in more recent studies there is a broad database on the sorptive behaviour of HHCB, see **Table 3.6**. By definition, the partitioning coefficicient Kd is the ratio between the concentration in the solid matrix versus the concentration in water. When degradation as well as partitioning may influence this ratio, for example in sludge and effluent, the resulting Kd is not a 'true Kd', but it is an 'apparent Kd'. This is indicated in **Table 3.6**. It is clear that the distinction between the true and the apparent Kd does not explain the wide range in the variation of Kd in sludge.

Activated sludge and soil

The sorption to activated sludge was determined from isotherm experiments producing the Freundlich isotherm constants for the test substances. LSC analyses were used as non-specific analytical techniques. The study was carried out with 2.5 g Suspended Solids/l (19% OC) and 10, 50, 150 and 300 µg test substance/l. The test system was equilibrated for 16 hours. The total recovery was 89 to 105%. The sorption isotherm was linear. The sorption coefficient Kd was 13,600 l/kg, whereas the sorption coefficient related to organic carbon was 74,722 l/kg (log K_{oc} = 4.87) (MacGillivray 1996).

K_{oc} was determined in activated sludge. Samples with different sludge concentrations were prepared and after extraction by nd-SPME and liquid/liquid extraction for free (dissolved) and total concentrations, respectively, the concentrations were determined by GC analysis. Log K_{oc} was 3.8 (± 0.2) (Artola 2002).

Sludge-water partitioning coefficients for adsorption and desorption were studied by entering HHCB either to the water or to the sludge. Sludge was spiked with HHCB in 4L-glass jars and rolled at 10 rpm for 1 hour. The sludge was equilibrated at 5ºC in the darkness during 3 weeks and sterilised by 0.01 M HgCl. Log Kd (adsorption) = 3.12, whereas log Kd (desorption) = 3.31 (Zhang et al. 2003).

Kd was determined as the ratio between the concentration of HHCB in sludge and in effluent sampled at the same time. A total of 15 different STPs in Italy, Spain and Greece was sampled four times over a period of three months in 2004 (Blok et al. 2005). The relation between the 60 effluent and sludge concentrations can be expressed in a regression line with a slope of 45º resulting in log Kd = 3.92 ([10]). Individual values varied between 3.59 and 4.36. It was questioned whether Kd was actually independent of the concentration levels. If the partition coefficient depends in some way on the concentration of HHCB, this relation will deviate from the straight 45º line. The deviation from this line was significant ($p<0.0002$) with best fitting line

$$\log C_{effluent} = -0.367 + 0.599 \cdot \log C_{sludge} \quad \text{, so} \quad C_{effluent} = -0.430 \cdot C_{sludge}^{0.599} .$$

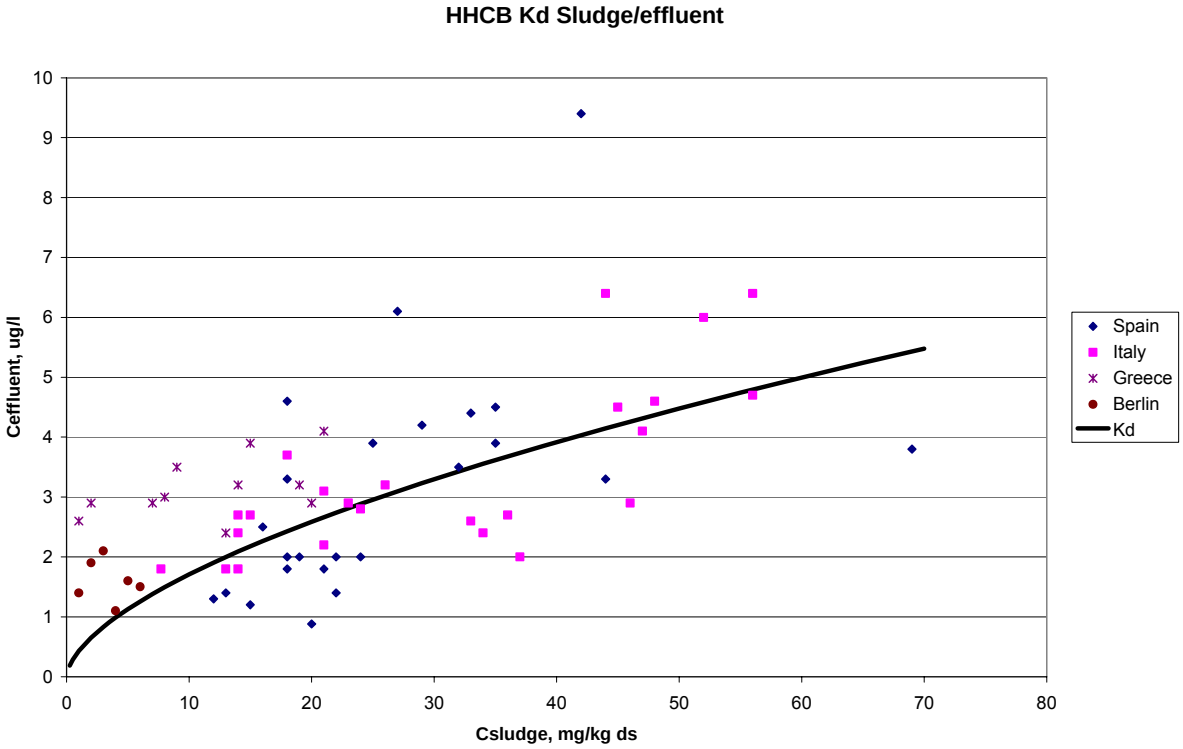


Figure 3.2. Kd (defined as Csludge/Ceffluent) based on the data of Blok et al. 2005. Kd is not linear with the concentration in sludge and effluent

The deviation from a constant partition coefficient implied that at low (overall) HHCB concentration the effluent concentration was relatively high (small Kd) and at high overall HHCB concentration the effluent concentration was relatively small (high Kd). Apparently sludges have a higher adsorptive potential at higher concentrations (Van der Hoeven 2005).

[10] With Kd independent of the concentration levels, the sorption isotherm on a non-logarithmic scale is linear. Then the log-log transformed graph is a straight line with a slope parameter of 1, thus forming an angle of 45º with the X-axis (if y = a*x, logy = log x + C). Kd is calculated as the ratio of the back-transformed values in this line.

The TOC was measured at only 9 sampling points. The organic carbon fraction (TOC) varied between 0.24 and 0.29. In these 9 samples the relation between Kd and TOC was not significant (p=0.27). Transformation of log Kd for the average OC fraction of 0.26 gives log K_{oc} = 4.5 (Blok et al. 2005), see **Figure 3.2**.

Kd was determined based on concentrations measured in effluents and sludge reported by Müller et al. (2002) for 21 STPs sampled in Germany. From their results we derived the ratio of sludge and effluent sampled on the same day. Log Kd is 4.5, ranging from 4.2 to 5.15. The same authors also determined K_{oc} using the HPLC method: log K_{oc} = 3.35. Kd values in three soil types with different organic matter content ranged from 95 to 380 l/kg. Desorption tests showed that the adsorption is not reversible: up to only 30 to 35% of the material is desorbed. Log K_{oc} values ranged from 3.6 to 3.9 (Müller et al. 2002).

For the determination of Kd, batches of primary and secondary sludge were sampled from a large STP in Germany and spiked to a nominal concentration of 70 to 80 µg HHCB/l. The sludge was stirred under argon for a variable time and the concentrations in water and sludge were determined to give the Kd. Kd in primary sludge (35% TOC per total suspended solids) was 4920 ± 2080 l/kg, in secondary sludge (34% TOC) it was 1810 ± 530 l/kg (Ternes et a. 2004).

Suspended matter and sediment

Samples of suspended particulate matter (SPM) and surface water were taken from the Elbe river in Magdeburg, Germany from June 1996 to May 1997 weekly in the first 4 months and biweekly thereafter (Winkler et al. 1998). Field derived partition coefficients varied in time: from 1676 l/kg to 14865 l/kg, average was 5430 l/kg. Normalisation for organic material reduced the variation to some extent: the mean field derived log K_{oc} for the Elbe was 4.7 (4.3 – 5.0). Apparently the sorption varies over time with the composition and the quantity of the suspended materials.

Partition coefficients may also be estimated from concentrations in suspended matter and surface water samples taken in rivers and brooks in Hessen, Germany, in 1999 and 2000 (HLUG 2001). The partition coefficients varied in time and place, from 233 to 2149 l/kg, the mean value was 845 l/kg in surface water. Normalisation for organic material was not possible.

The partition coefficient between suspended matter or sediment and water was determined in water from small streams with a relatively high input of waste water in Baden-Württemberg, Germany. The total organic carbon content in the suspended matter ranged from 9.7 and 13.1% and in the sediment from 1.0 to 2.9% (LfU-BW 2001).

The partition coefficient was reported for field samples of suspended solids and streamwater in Pennsylvania. Log Kd was determined in two samples: 4.0 and 4.4 (l/kg) (Standley et al. 2000).

Calculated

A K_{oc} value for HHCB can be estimated from the K_{ow} value of 5.3 using the QSAR outlined in chapter 4 of the Technical Guidance document. The equation recommended for predominantly hydrophobics is log K_{oc} = 0.81xlog K_{ow} + 0.10. Using this equation a log K_{oc} value of 4.39 can be estimated.

The theoretical partition coefficients derived from EUSES are compared to experimentally derived data in **Table 3.6**. It is concluded that the empirical values vary considerably but the

predictions by EUSES are within that range. Therefore the calculations were carried out with the predictions made by EUSES on the basis of log K_{ow}.

Table 3.6. Partition coefficients ('true' partition coefficients, or else determined from concentration ratios not excluding other processes like, e.g., degradation)

Partition coefficient	Estimated by EUSES	Experimental
log K_{oc} in activated sludge	4.39 *)	'true' 4.87 (MacGillivray 1996) 'true' 3.8; 'apparent' 3.3 (Artola 2002) 'apparent' 4.5 (Blok 2005) 'true' 3.8 (based on Ternes et al. 2004)
activated sludge - water	Kd: 9150 l/kg	'true' Kd: 13,600 l/kg (MacGillivray 1996) 'apparent' Kd: 8570 l/kg (Blok et al. 2005) 'apparent' Kd: 33,500 l/kg (based on data Müller et al. 2003) 'true' Kd: 1810 ± 530 l/kg (Ternes et al. 2004) 'true' Kd in primary sludge: 4,920 l/kg (Ternes et al. 2004) true' log Kd (ads) 3.12, log Kd (des) 3.31 (Zhang et al. 2003)
soil - water	Kd: 495 l/kg $K_{soil\text{-}water}$: 742 m^3/m^3	Kd: 95 – 380 l/kg (Müller et al. 2002)
log K_{oc} in soil		3.6 – 3.9 (Müller et al 2002)
suspended matter -water	Kd: 2474 l/kg $K_{susp\ water}$: 619 m^3/m^3	'app' Kd: 5430 l/kg (with high variability, from data of Winkler 1998, 1999) 'app' Kd: 845 l/kg (with high variability, HLUG 2001) 'app' Kd: 2400 – 5640 l/kg (Fooken 2004) 'app' Kd: 600 – 3700 (LfU-BW 2001)
log K_{oc} in suspended matter		'app' 4.3 (Fooken 2004) 'app' 3.76 – 4.48 (LfU-BW 2001)
sediment - water	Kd: 1273 l/kg $K_{sed.water}$: 619 m^3/m^3	Kd: 124 l/kg (Fooken 2004) Kd: 63 – 180 l/kg (LfU-BW 2001)
log K_{oc} in sediment		3.85 (Fooken 2004) 3.63 – 4.04 (LfU 2001)

*) based on log K_{ow}, used in the further calculations in EUSES

3.1.3.2.2 Precipitation

No information available.

3.1.3.2.3 Volatilisation

Using a vapour pressure of 0.0727 Pa and a water solubility of 1.75 mg/l a Henry's Law Constant of 10.7 $Pa.m^3/mol$ is calculated. The Henry's Law Constant was empirically determined by Artola (2002) at 36.9 $Pa.m^3/mol$. The latter was used in the PEC calculations.

Losses from the waterbody of the Zürichsee in Switzerland were estimated by an approximation using the fraction of HHCB solved in water (Fw), the transfer rate in water (Vw) and the depth of the water body: Kwater-air = (Fw · Vw) / DEPTH. In winter during circulation, Kwater-air values thus range from ≈ 1.5 to 3.0 $\cdot 10^{-3}$ d^{-1} with the lower limit expected to be closer to real conditions. In summer, considering only the upper layer (epilimnion), the rate constant is estimated ≈ 1.4 to 2.9 $\cdot 10^{-2}$ d^{-1} with the upper limit expected to be more realistic. In winter HHCB is eliminated primarily by outflowing water and due to losses to the atmosphere. Sorption and photolysis are minor processes. In summer, all processes in the epilimnion are faster by at least a factor of 10 than those for the whole lake in winter (Buerge et al. 2003).

Volatilisation rates were obtained by purging head-space of a spiked soil-sludge mixture (24:1) with nitrogen and trapping HHCB vapours with polyurethane foam. The initial volatility rate strongly related with the vapour pressure for a range of fragrance ingredients, including HHCB. Details are not available (Zhang et al. 2003).

3.1.3.3 Fate and behaviour in wastewater treatment plants

The behaviour of HHCB in a sewage treatment plant is discussed based on the model SimpleTreat in EUSES, the removal in actual sewage treatment plants and a mass balance approach.

3.1.3.3.1 Predictions in EUSES

According to the SimpleTreat model, HHCB entering an STP partitions between the sludge, water and air. The partitioning is predicted on the basis of K_{oc}, water solubility and vapour pressure. Then the fraction in the water phase is degraded according to the rate constant assigned to inherently degradable substances (TGD: 0.1 h^{-1} or 0 h^{-1}). In EUSES the volume of domestic waste water is set at 200 l/d *per capita*, the solids production from the STP is 79 g/d *per capita*, and the concentration of suspended solids in the effluent is 30 mg/l. With log K_{ow} = 5.3 and k_{biodeg} = 0, the fate of HHCB predicted by EUSES is presented in **Table 3.7**.

Table 3.7 Distribution of HHCB in an STP (%) predicted by EUSES

Fate in STP	%
Air	10.4
Water	22.4
Sludge	67.2

3.1.3.3.2 Removal percentages

The levels of fragrance materials were measured in the influent and effluent of two STPs in the USA. During three days, daily composite flow-proportional samples were taken from the influent, primary effluent and final effluent of an activated sludge and a trickling filter STP. The total concentration of fragrance materials in the influent showed a considerable diurnal variation around the mean (± 60%), whereas the effluent concentration was very stable. The measurements in influents and effluents showed that on average 91.5% of HHCB is removed after treatment in an activated sludge plant (Simonich et al. 2000).

In Austria the behaviour of HHCB was studied in a small municipal STP in 2002 (Clara et al. 2004). The STP includes an activated sludge process, nitrate removal by intermittent aeration and simultaneous sludge stabilization and has a relatively high Solid Retention Time (SRT). The process was compared to a pilot Membrane Bioreactor plant operated in parallel with the same wastewater. Sludge separation was performed by a membrane module. The partitioning of polycyclic musks by effluent and sludge was estimated from the picture (no data are given). The removal ranged from 80 to 90%. The highest removal was achieved at the highest temperature of 27 ºC. At 22 ºC a difference of SRT between 275 days and 11 days gave the same removal (Clara et al. 2004).

In a rural area in the most eastern part of Austria, at 2-months intervals between 2000 and 2001, seven times a 24 hours composite sample was taken at different stages of wastewater treatment and post treatment of an STP, as well as in the groundwater. The system has no primary settling but low loaded aeration with a long hydraulic retention time of 10 days and full nitrification and denitrification and iron precipitation of phosphorous. After settling the effluent is treated in a polishing lagoon, in a gravel filter and is lead into an infiltration pond to the groundwater. In the STP the reduction of the HHCB concentration was 80%: from 2.0 μg/l in the influent to 0.4 μg/l in the effluent. After the polishing lagoon the concentration is 7% and after the infiltration pond only 3% of the influent concentration. In the groundwater no further decrease of the concentration was observed (Kreuzinger et al. 2004).

The mean removal in a Swiss STP, 2001, serving 210 inhabitants (60 m^3 per day) in a sequencing batch reactor was 87% (Berset et al. 2004). The difference between the minimum and maximum concentrations in the influent and effluent as well as the sludge was within a factor of 2 (Berset et al. 2004).

Samples were taken in 21 activated sludge plants in Germany in summer 2000 and in January 2001. The concentrations were measured in influents and effluents. The mean removal percentage was 76%, varying between 46 and 99% (Müller et al. 2002). The higher removal percentages were related to the higher influent concentrations (3 – 5 μg/l), the lower removal percentages were observed for the lowest influent levels (<1 μg/l).

Samples were taken in 4 activated sludge plants in the Netherlands. The total influent and total effluent concentrations were used to estimate the removal (Artola 2002). The resulting removal was low as compared to the other studies, but this may be explained by the relatively low influent levels.

In UK samples were taken of influents and effluents in 6 different types of STP in 2001. The removal ranged from 74 to 90%. Here the influent concentrations were between 2.2 and 8.1 μg/l (Kanda et al. 2003).

The removal of HHCB was studied in the STP in Dortmund, Germany (Bester 2004). The plant received 184,000 m^3 water per day from 350,000 inhabitants and industries. The system includes primary settling, activated sludge treatment, secondary settling and anaerobic digestion. The Hydraulic Retention Time was 8 hours; the Solids Retention Time was 8-10 days in the aerator and 20 days in the digester. Time proportional composite samples were taken during 5 days in April 2002. The mean concentration in the influent was 1.9 μg HHCB/l and 0.23 μg HHCB-lactone/l, in the effluent it was 0.70 μg HHCB/l and 0.37 μg HHCB-lactone/l, a removal of 64% for HHCB. The concentration in digested sludge was 3.1 mg/kg. An attempt was made for a mass balance over the STP but it varied between -40% and +7% and it did not include the formation of HHCB-lactone (Bester 2004). Thus it seems that the basic data are not sufficiently accurate for this calculation (e.g., incomplete recovery in

analytical procedure, time proportional sampling rather than flow-proportional, non-representative sampling scheme of the digested sludge and estimate of sludge production).

A pilot plant for ozonisation received effluent from an STP to test the removal of HHCB. The effluent had pH 7.2, COD 30 mg/l, BOD 2.8 mg/l and 4.5 mg suspended solids per liter. The water was treated by ozone, 5, 10 and 15 mg/l during 18 minutes. With the lowest ozone dose the concentration in the effluent was reduced from 0.73 μg/l to 0.09 μg/l (88% removal) and at the higher dose it was below the limit of quantitation (< 0.05 μg/l, >93% removal) (Ternes et al. 2003).

An analysis of the results of a number of these studies carried out in the USA, UK, The Netherlands, Germany and Switzerland (n=85 in 39 different STPs) generally showed removal percentages of 80 – 90%, see **Figure 3.3**. However, also higher and considerably lower removal percentages were observed, see the lines in the upper parts of graph B in **Figure 3.3**. In the study of Simonich et al. (2002) the removal percentage correlated well with the removal of Total Suspended Solids (TSS) in the system (graph B). The TSS removal is a characteristic of the STP that is determined by the treatment process, the plant design and the settling properties of the sludge and is not related to HHCB. The high correlation of the removal of AHTN and HHCB in the STPs supports this thesis.

It is concluded that a fixed removal percentage (according to a fixed first order rate constant dependant on the concentration in water) is not the best model for the removal of HHCB in an STP. The observation of the non-linearity of Kd (see **Figure 3.2**) supports this observation. The removal percentage is lower at lower influent concentrations and increases at higher levels, see also **Figure 3.3**. A key in the explanation of this phenomenon may be that biodegradation is not taken into account in the modelling.

3.1.3.3.3 Mass balance in STP

In a study by Brändli (2002) it is shown that Galaxolidone appears during the transport in the sewer and the sewage treatment. Galaxolidone is an oxidation product with an additional oxygen on the (benzene)pyran ring (HHCB-lactone). Galaxolidone was already present in the influents at 0.1, 6 and 16% respectively, of the HHCB level in three different STPs in Switzerland. In the effluents Galaxolidone was present at 0.1, 13 and 20% of the HHCB level in the influent. The increase during treatment was largest in the treatment plant with extended aeration. According to Berset et al. (2004) HHCB-lactone was present at the same level as HHCB in the effluent, whereas its concentration in the activated sludge was 1.42 mg/kg or 33% of the concentration of HHCB on sludge.

A mass balance over a week was established for primary and biological water treatment and the sludge line in a conventional activated sludge sewage treatment plant in Switzerland serving a population of 23,000 inhabitants. The degradation of the polycyclic musks including HHCB in the activated sludge tank was 40-50%, whereas more than 50% was sorbed to sludge. In the anaerobic digester the polycyclic musks decreased by 50% suggesting that they are degraded anaerobically (Kupper et al. 2006).

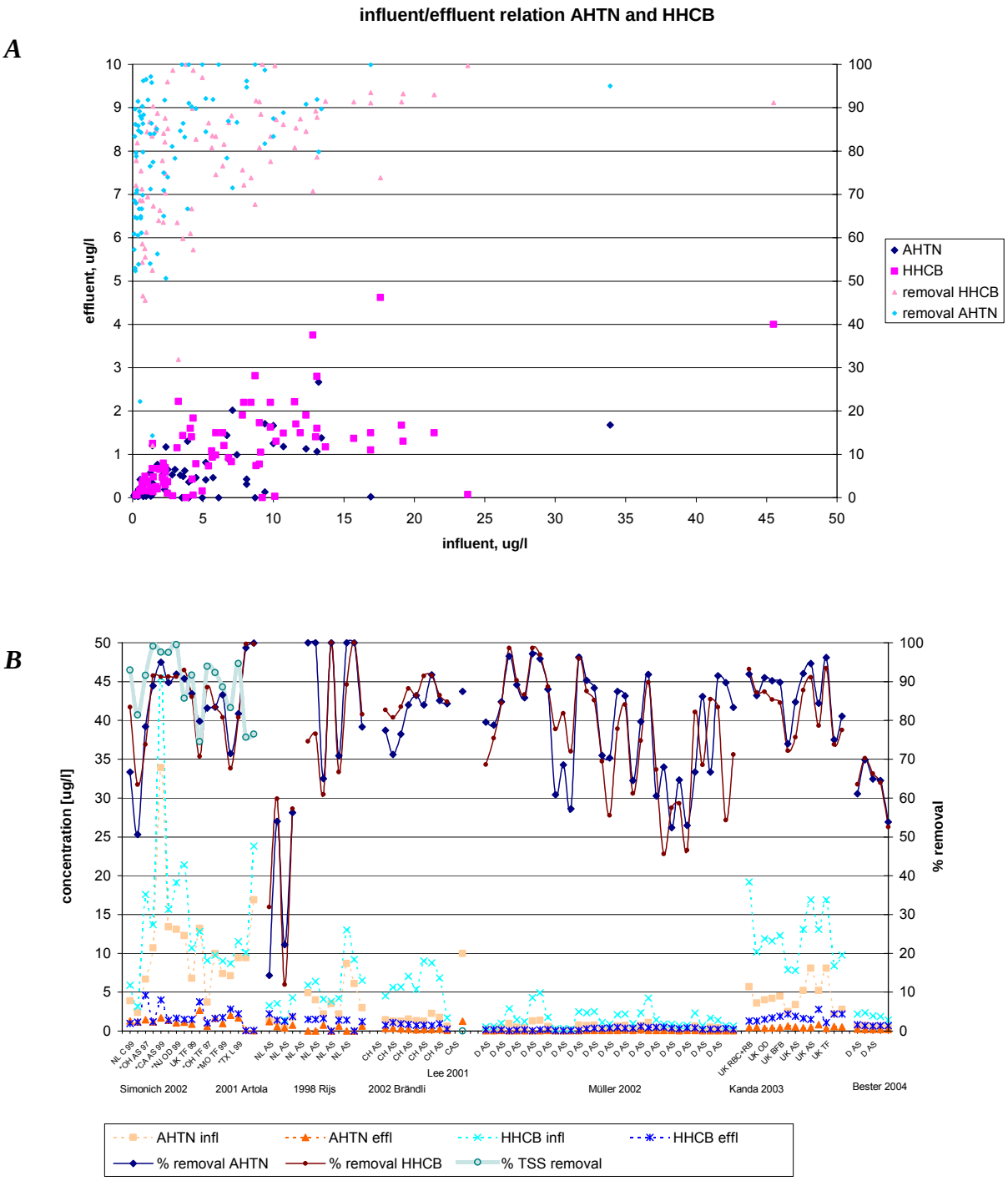


Figure 3.3. Removal of AHTN and HHCB in various STPs.
A. The picture shows the concentrations in 91 paired samples of influent and effluent (left Y-axis) and the percentage removal in 40 different STPs (right Y-axis)
B. The picture shows the concentrations in the same paired samples (left Y-axis, dotted lines) measured in the STPs indicated on the X-axis. The removal% is given on the right Y-axis. ;
NL, UK, D, CH etc: European countries and (with *) USA states;
C: Caroussel, AS: Activated sludge plant, OD: oxidation ditch, TF: trickling filter, RBC: Rotating Biological Contactor, BFB: Biological Filter Bed, L: Lagoon, CAS: Continuous Activated Sludge (test) System.
Median, 90th perc. and maximum effluent concentrations for HHCB (n=85): 0.8, 2.2 and 4.6 µg/l
Sources: Simonich et al. 2002, Artola et al. 2002, Rijs 1998, Brändli 2002, Eschke et al. 1994, Lee et al. 2001, Müller et al 2002, Kanda et al. 2003, Bester 2004.

The removal of HHCB was studied in the STP in Dortmund, Germany. An attempt was made for a mass balance over the STP but it varied between -40% and +7% and it did not include the formation of HHCB-lactone (Bester 2004). Thus it seems that the basic data are not sufficiently accurate for this calculation (e.g., incomplete recovery in analytical procedure, time proportional sampling rather than flow-proportional, non-representative sampling scheme of the digested sludge and estimate of sludge production).

In two large STPs in Düsseldorf and Cologne in Germany, both serving > 10^6 inhabitant equivalents (i.e.), mass balances were made including sampling of influent, effluents and surplus sludge as well as digested sludge. In the first plant the total elimination was 85% including 30% that was biodegraded. In the second plant 80% was eliminated partly due to a high biological degradation of 53% (Fahlenkamp et al. 2005).

In conclusion, the available studies are not conclusive on the quantitation of the biodegradation of HHCB in an STP.

As no suitable data are available to predict biodegradation in the STP, the EUSES model is used for local industrial scenarios (**Table 3.7**) whereas the estimation of PEClocal for consumer use is based on the concentrations measured in effluent and sludge in recent monitoring programs (see section 3.1.4.1.3).

3.1.3.4 Accumulation and metabolism

3.1.3.4.1 Accumulation and metabolism in fish

The bioconcentration in fish was studied in various experiments. A GLP-study was carried out according to OECD Guideline 305E. Bluegill sunfish (*Lepomis macrochirus*) were exposed in a flow-through system to two concentrations of radio labelled HHCB (radiochemically pure, three isomer groups) (Van Dijk 1996). A solubiliser (DMF, Tween 80) was used to prepare a solution (concentration 0.001% (w/v)). Identification of the parent compound in water and fish was performed by TLC/HPLC. Nominal exposure concentrations were 1 and 10 µg /l. The fish were exposed for 28 days; the elimination period was also 28 days. The bioconcentration factor (BCF) was derived from actual concentrations of parent compound in exposure water Cw and the plateau level in fish Cf (days 21 and 28): BCF = Cf /Cw. The elimination rate constant (k2) was estimated from the elimination curve (first order kinetics). The uptake rate constant (k1) was calculated by: $k1 = BCF \cdot k2$.

Results of the bioconcentration tests are presented in **Table 3.8**.. The concentration of HHCB in the fish reached plateau levels after 3-7 days of exposure. An uptake rate constant (k1) could not be directly calculated from the increase of concentrations in fish due to rapid attainment of the final plateau level. Elimination followed first order kinetics with a half-life of 2 - 3 days for HHCB, allowing calculation of the rate constant for elimination (k2). Based on the concentration of parent material, the BCF for the whole fish was 1584.

Metabolites

In this bioconcentration test with HHCB, at least one polar radioactive fraction was found in water accounting for 10 to 19% of the total radioactivity in water. A polar metabolite fraction was found in the organic extracts of fish edibles and non-edibles that accounted for 9 to 24% of the total radioactivity in tissue. The polar fraction of the metabolite in tissue proved to be

identical to the one in water based on TLC and with HPLC retention times. Based on Rf values on TLC and elution times on HPLC, the parent compound occurred in three slightly different subfractions identical to the isomers in the unpurified sample of the stock solution (Van Dijk 1996).

For the same experimental results, a mass balance was made for the dynamic flow-through system containing water plus fish. For the mass balance of the dynamic system, the amounts of HHCB and metabolites in the fish and leaving the fish body (expressed in mg/kg bw and day) should be compared to the amounts measured in water that passes through the system with a certain flow rate (expressed in µg. $l^{-1}.d^{-1}$) These two compartments, fish and water, are related by the so-called "fish to water loading rate" (of 0.4 g. $l^{-1}.d^{-1}$) as mentioned in the study reports. This expression is in fact the reciprocal of the water flow per unit of fish weight. During the exposure period of 28 days a steady state is reached between the uptake and the depuration to give a plateau level for radioactivity in the body. In this calculation the loss of total radioactivity directly after day 28 is used. This loss of total radioactivity from fish is then compared to the measured radioactivity in metabolites in the water leaving the system, to see if the depuration can be explained quantitatively by the metabolite. After termination of the dosing of HHCB on day 28, the radioactivity in the fish tissue decreased with a rate of 0.33 mg/kg/d from a level of 1.5 mg/kg (22% per day) and with 3 mg/kg/d from a level of 14 mg/kg (21%) at the two dose levels. Multiplication of this 'loss' by the average fish-to-water loading rate (0.4 g/l/d) gives the nominal concentration of 'lost' radioactivity in water leaving the system: 0.132 µg/l in the low dose level and 1.2 µg/l in the high dose level. These concentrations are compared to the average measured concentrations of metabolites in the water: 0.097 for the low dose level and 1.03 µg/l for the high dose levels. Here the estimated loss of radioactivity from the tissue exceeds the loss based on measured concentrations of polar metabolite in water.

Table 3.8. Bioconcentration of HHCB in bluegill sunfish *Lepomis macrochirus* in a flow-through system (Van Dijk 1996).

Parameter	**Results**		
Initial fish weight [g]	1.2-1.4		
Low dose [µg/l]	0.91 ± 0.10		
High dose [µg/l]	8.84 ± 0.89		
	edible	non-edible	whole fish
Plateau level low dose [mg/kg]	0.45	1.98	1.49
Depuration residue low [mg/kg]	0.015	0.031	0.023
Plateau level high dose [mg/kg]	4.82	22.16	14.26
Depuration residue high [mg/kg]	0.109	0.221	0.105
Elimination rate constant k_2 [d^{-1}]	low: 0.215, r^2=0.93; high: 0.261, r^2=0.99		
Uptake rate constant k_1 [l/kg/d]	low: 352 high: 421		
Bioconcentration factor (whole fish, wet weight) [l/kg]	parent substance : 1584 total radioactivity: 1624		
Partitioning of radioactivity in: - water - fish edibles - fish non-edibles	 HHCB 81-90%, metabolites 10-19% HHCB 79-88%, metabolites 9-16% HHCB 72-87%, metabolites 11-24%		

Water soluble metabolites became apparent in the water from day 3 of the exposure period. During the period between day 3 and day 28 the daily amount of water soluble metabolite was 38 – 50% of the average level of radioactivity present in the fish tissue.

Thus it can be concluded that during exposure and during depuration the parent compound is metabolised and the metabolite is excreted with a turnover rate of about 38 – 50% per day (Van de Plassche and Balk, 1997, Balk and Ford, 1999a).

An accumulation study was performed with zebrafish (*Brachydanio rerio*), aged 4-6 months with a length of 2.5-3 cm and a weight of 0.15-0.4 g. The exposure concentration of HHCB was 7.3 µg /L in a flow-through study according to former OECD guidline 305E. Tap water with a pH around 8 was used. Methanol (0.05%) was used as co-solvent. The concentrations in fish and water were analysed by GC-MS/MS. No information is given on the number of fish, the test volume, the number of replicates, the test temperature, and feeding regime. The exposure during the uptake phase was 14 days. The concentration in fish reached its plateau level after less than 5 days. The elimination period lasted 26 days with a depuration half-life of less than three days. The concentrations in fish and water were analysed by GC-MS/MS. The uptake rate (k_1) was 560 d^{-1} and the clearance rate was 0.75 d^{-1}. The bioconcentration factor was 624 L/kg (fresh weight) or 33200 based on the lipid content of the fish (Butte and Ewald 1999).

In another study with the same species, juvenile transgenic zebrafish of 4 to 5 weeks were exposed in 200 ml tap water at 26-27 ºC for 96 h to concentration of 25.8 and 258 µg/l. Fish were fed once daily with live *Artemia salinas* and half of the medium was renewed daily. After 96 h the concentrations in water without fish were 25-40% below nominal. In the presence of fish the concentrations were 2.9 and 15 µg/l (6-11% of nominal). This decrease in concentrations was observed after 24 h as well as after the next intervals. Also the internal concentrations observed just before the renewals were roughly the same during the 4-day exposure period: 18.9 and 135 mg/kg wwt in the low and high concentrations, respectively (Schreurs et al. 2004). The decrease in exposure concentrations can at least partly be explained by sorption by the fish and subsequent metabolism. Assuming an exponential decrease in concentration between the renewal periods the average concentrations in the low and high exposure concentrations would be in the order of 5 and 60 µg/L. With the corresponding internal concentrations, this would imply BCF values of 2400-2500 L/kg.

The latter value is substantially higher than the BCF value from the BCF study with the same species performed according to OECD guideline 305E. The study by Schreurs et al. (2004) was not intended as a bioaccumulation study. According to the test guidelines for fish bioaccumulation testing (OECD TG 305, EU C.13), the exposure of the fish is solely through the aquatic phase where a constant concentration is maintained and care is taken to minimise oral exposure by removing excessive food and faeces from the test containers within 30 minutes to an hour after feeding. In the test by Schreurs et al. (2004) it is not clear whether feeding with live brine shrimp took place before or after renewal. Consequently, part of the decrease in aqueous concentration might be caused by sorption of test substance to the brine shrimp. Since the medium was only partly renewed, also half of the *Artemia* that had not yet been consumed as well as faeces continued to be present. In this way, more of the test substance would sorb causing an increasing oral exposure next to exposure through the aquatic phase.

Further, the number of fish is relatively small. Only 5 or 6 fish per group were tested, whereas 50 fish per group and at least two groups are used according to the OECD305. The recommended weight of the fish is 0.2 to 0.4 g, which corresponds to zebrafish of several

months old. In the study by Schreurs et al. the tested fish were only 4-5 weeks old. Although the weight of the fish is not given it is likely that the maximum loading of fish of 0.8 g/L is exceeded due to the small water volume of 200 mL. According to the OECD guideline the test concentration may not deviate more than 20% from the mean measured concentration in the uptake phase. It is evident that the decrease in concentrations caused the exposure concentration to fluctuate widely. Further, the highest concentration must be at most $1/100^{th}$ of the acute 96-h EC50, or the 96-h EC50 divided by a proper acute-to-chronic ratio. In the study by Schreurs, the highest concentration is on average somewhat higher than the NOEC for the same species reported in section 3.2, with inititial concentrations clearly exceeding this value.

Given the uncertainties in especially exposure concentration and contribution of additional oral exposure, bioconcentration factors derived from the study by Schreurs are prone to deviations. The bioconcentration study with the same species performed according to the OECD guideline showed similar results as the bioconcentration study with bluegill sunfish. For this reason, it is concluded that the bioconcentration factor of HHCB in different fish species is in the order of 600-1600 L/kg.

Table 3.9. Comparison of field derived ratios of C_{fish} and C_{water} to experimentally determined bioconcentration factors for fish

BCF	Chemical analysis	Source
Bluegill sunfish (28+28)d-BCF_{wwt} = 1584 *	^{14}C, LC/HPLC	Balk and Ford 1999a
Zebrafish (14+26)d-BCF_{wwt} = 624	GC/MS	Butte and Ewald 1999
Zebrafish (4d exp)-BCF_{wwt} = 600 [a]; 2400-2500 [b]	GC/MS	Schreurs et al 2004
environmental samples: Eel BCF_{wwt} = 150 to 600 non-eel BCF_{wwt} = 49 to 188	GC/MS/MS	Balk and Ford 1999a from data in Eschke et al. 1995a, Rijs and Schäfer 1998, Rimkus 1997
Environmental samples: Eel BCF_{wwt} = 862 (range 201 – 1561)	GC/MS	Fromme et al. 2001b
Eel BCF_{wwt} = 995		Heberer 2002 from data of Fromme et al. 2001b
Environmental samples [c] : Rudd BCF_{wwt} = 20 Tench BCF_{wwt} = 510 Crucian carp BCF_{wwt} = 580 Eel BCF_{wwt} = 290 Zebra mussel BCF_{wwt} = 620	GC/MS	Gatermann et al. 2002a

[a] Based on initial concentrations, see text

[b] Based on estimated average concentrations

[c] Species differences related to fat content

* Used in EUSES calculations

As an indication of the bioconcentration under natural conditions, ratios were calculated for the concentration in fish and in surface water, see **Table 3.9**. Data are presented in the section on monitoring data for fish (section 3.1.7.2). The resulting field ratios seem to be lower than the ones determined in the laboratory. An explanation may be that wildlife fish tend to have a lower lipid content than laboratory specimen.

Fromme et al. (2001b) determined the bioconcentration factor in 165 eel samples in the Berlin area. The BCF (eel) ranged from 201 to 1561. Based on the same data set Heberer (2002) uses the median surface water concentration of 1.48 µg/l, median content (5.83 mg/kg lipids or 1.473 mg/kg wwt) to estimate the BCF_{lipids} (eel) = 3939 and BCF_{fw} (eel) = 995.

Gatermann et al. (2002a) published concentrations in fish measured in an effluent pond (1997). They suggest that bioconcentration factor C_f/C_w is species dependant and thus they postulate a species dependant metabolism. However, a closer look at their data reveals that this dependency is completely explained by the lipid content of the fish. A different metabolism may be postulated only for eel, which accumulates HHCB to lower concentrations than expected on the basis of its lipid content.

For substances with log K_{ow} < 6 the BCF can be estimated using the following QSAR according to the TGD (EC 2003): log BCF(wet weight) = 0.85 · log K_{ow} - 0.70. With log K_{ow} = 5.3, the estimated BCF is 6,383 l/kg. This theoretical value exceeds the measured BCF value (1584) by more than a factor 4. A more recently developed relation included in the SRC estimation programme BCFWin (SRC, 1999), taking also the molecular structures into account, predicts a lower bioconcentration factor: 2404. This prediction exceeds the experimental value by a factor of 1.5.

In conclusion, for the environmental risk assessment for the fish to predator chain the experimental BCF value of 1584 l/kg, derived from the study of Van Dijk (1996) will be used.

3.1.3.4.2 Accumulation in benthic organisms

The bioconcentration of HHCB in two benthic organisms was described by Artola (2002). Fourth instar midge larvae (*Chironomus riparius*) and the worm *Lumbriculus variegatus* were exposed in a 9 liter-flow-through system with a flow of circa 2 liter per hour at a loading of 600 mg midge larvae and 2600 mg of worm. The organisms were not fed during the 12d-exposure period. Samples of water and organisms were extracted in cyclohexane and analysed by GC/MS. The lipid content of *L. variegatus* was determined gravimetrically. 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).

The aqueous concentrations in the test with *C. riparius* were stable at 9.8 ± 1.4 µg/l. The concentrations in the organisms increased to a maximum level between day 1 and 3 and then the level rapidly decreased to a new steady state. With log BCF given as 1.93 and 2.14, BCF = 85 – 138. These values are lower than predicted based on a correlation for chlorobenzenes (Roghair et al, 1992: BCF 3890) by a factor of 40. With the addition of PBO, BCF was higher, 525 and Artola (2002) concludes that the low BCF values are likely caused by a relatively fast biotransformation of HHCB in *C. riparius*. The uptake curve showed that the enzyme activity responsible for the transformation of HHCB may be induced after a short lag time.

The concentration in water in the experiment with *L. variegatus* was 4.6 ± 0.6 µg/l. The uptake of HHCB in worms reached a plateau level after 3 days. Log BCF was given as 3.43. This is at the same level as the predicted BCF based on K_{ow} and lipid content (McCarty et al. 1991), indicating that in this organism biotransformation does not take place.

3.1.3.4.3 Accumulation in earthworms

The bioconcentration in earthworms is assumed to be proportional to the soil pore water concentration. Bioconcentration can be described as a hydrophobic partitioning between the pore water and the phases inside the organism and can be modelled according to the following equation as described by Jager (1998)

$BCFworm = (0.84 + 0.012 \cdot K_{ow})/RHOearthworm$

For $RHO_{earthworm}$ (bulk density of worm) by default a value of 1 ($kgwwt.l^{-1}$) can be assumed.

This leads to a BCFworm of 2395 $l.\ kg^{-1}$.

3.1.3.4.4 Accumulation in plants

Transfer coefficients were determined in lettuce and carrots growing on sludge amended soil samples. Dried sludge was spiked with HHCB to a level of 300 mg/kg dwt and mixed with three different soils, sand, loam and a humic soil to reach a soil concentration of 30 mg/kg soil dwt. The test design required such high concentrations in soil to allow precise measurements. After a week young salad seedlings were planted and carrots seeds were sown in the treated soil. The plants were harvested and analysed after 12 weeks. Parallel to the laboratory tests, similar tests were also carried out in the field. With the extremely high soil concentrations the growth of the plants was inhibited. The concentrations detected in salad leaves in the replicate tests were highly variable and seemed to be independent of the type of soil. For carrots the soil type did seem to influence the levels in the leaves. The soil-plant transfer factor (ratio Cplant/Csoil) was small for the salad leaves (0.003 – 0.006) as well as for the carrot leaves (0.003 – 0.036). For carrot root, the transfer ratios were 0.1 and 0.48 in two different soils. The authors conclude that in spite of the high dosage no relevant accumulation in leaves is observed and that even for the carrot root the ratio is still below the 'critical level of 1'. They suggest that the higher ratio in carrot roots is related to a direct contact between the musks in the soil and the oil deposits in the carrot roots that allows a direct partitioning. The low concentrations in the above ground parts of the carrot plant show that there is no transport within the plant (Müller et al. 2002). Thus it is concluded under normal conditions that transfer of HHCB from the soil to plants is not relevant.

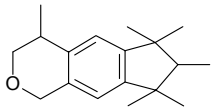
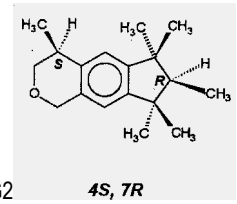
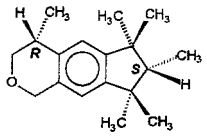
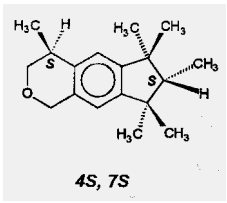
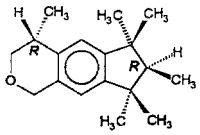
3.1.3.5 Isomers of HHCB

The manufactured, undiluted HHCB has a purity of >95%. This technical HHCB consists of a mixture of structural isomers, with a main isomer of ca 75% and several other isomers (see section 1.2.1). The gas chromatogram of the undiluted technical HHCB shows a characteristic profile with typical peak ratios for the isomers.

The main isomer consists of two diastereoisomers, which are not separated on the commonly used methylsilicone (= non-polar phase) capillary columns. Only on specific capillary columns, such as with polyethylenglycol (= polar) stationary phase, the diastereoisomers are separated on the GC in a ratio of 1:1.

Each diastereoisomer consists of a pair of enantiomerical isomers. Only very special analytical columns using modified cyclodextrins as chiral stationary phase are able to separate the enantiomeric isomers. HHCB has two asymmetric carbon atoms present and thus four enantiomers as shown in **Table 3.10**.

Table 3.10 Isomers and enatiomers of technical HHCB

main isomer 75%			15-20% other isomers, section 1.2.1
Two diastereoisomers:	each with two enantiomers:		
cis- isomers	4S,7R and 4R,7S	 G2 **4S, 7R**  G3 **4R, 7S**	
trans- isomers	4S,7S and 4R,7R	 G1 **4S, 7S**  G4 **4R, 7R**	
		musky odour ↑ (mainly 4S,7R)	

Concentrations in the environment (summarised in various tables in the next sections) were carried out on common capillary columns and with quantitative determinations according to standard analytical principles using HHCB technical as a reference. After extraction, HHCB is analysed by the combination of (common) capillary gas chromatography and mass spectrometry, GC-MS using SIM mode (quantifier ion 243, qualifier ion 213 and 258). The most common practice for quantitation is by measuring the main isomer signal response in the investigated sample in relation to the main isomer signal response in the HHCB substance reference sample. In the calculations, the appropriate dilutions and the weights of investigated sample and the weight and purity of the reference sample are taken into account.

Where the HHCB isomer ratio in sample and reference were shown to be the same, the result of the analysis of the environmental/biota sample is correctly reported as the concentration of the HHCB (as technical mixture).

Analysis of isomers

Escke et al. (1995) detected next to the main HHCB isomer some other isomers [second row in **Table 3.10**]. Quantification was based on the main isomer. For their analyses of fish samples they state that no change of the isomer ratios was detected (as compared to the HHCB sample) and moreover, the other isomers formed only a small portion of the total amount.

Műller et al. (1996, their figure 1) showed that the ratio between the main diastereomers [*cis* and *trans* isomers] and the main and other isomers [second row] in adipose tissue remained similar to that in the HHCB substance sample. The same was observed by Draisci et al. (1998, their figure 1, 3, 4) in fish. They noted that the ion intensity ratio of the two isomers was the same in the reference and in their fish samples.

Franke et al. (1999) separated the 4 enantiomers [G2, G3 and G1, G4] in technical HHCB showing an approximately 1:1 mixture of both diastereomers with a slight excess of the *cis* isomer (G2+G3). They quantified the enantiomers in 5 (shell) fish species and detected that

the enatiomeric ratios deviated from 1 for some species. G4 was least affected. In particular in the Crucian carp *Carassius carassius*, G3 was slightly reduced as compared to G4, whereas the G2 and in particular G1 were more affected (0.1 * G4). The ratios in the aquatic medium deviated only slightly from 1 (0.8 to 0.9 * G4) as determined by SPMD (Franke et al. 1999, p. 800).

Reporting on the same study, according to Gatermann et al. (2002b) no significant changes in the *cis/trans* ratios are found in water, sediment, sewage sludge and SPMD samples and therefore the physicochemical properties of the isomers are expected to be relatively similar. The deviation of the ratios in *C. carassius* indicate that in particular the G1 and G2 isomers seem to be selectively transformed. In other species the ratios were close to 1 and calculations support that no selective metabolism had occurred (Gatermann et al. 2002b, p. 451-452).

Hühnerfuss et al. (2001) report on the concentrations of the main HHCB metabolite HHCB-lactone or galaxidone in the same environmental samples as studied by Franke et al. (1999) and Gatermann et al. (2002a,b). As for HHCB, no pronounced enantiomeric shifts were found in the water both for *trans*- and *cis*-HHCB-lactone. Similar to the findings for HHCB, a deviation on the enantiomeric ratio was found for the lactone in *C. carassius,* although less pronounced. It is noted that an enantioselective transformation of the 4S enantiomers (G1 and G2) of the diastereomeric pairs was observed: the HHCB isomers that are preferentially transformed are being subject to preferential further transformation (see Gatermann et al. 2002a, p. 443).

Berset et al. (2003, 2004) reported on the enantiomer ratios of HHCB in sewage water, activated sludge and effluents of sewage treatment plants. They observe that the ratios (G1/G4 as well as G2/G3) do not significantly deviate from the technical mixture. A slight deviation may be observed for *cis*-HHCB (G1/G4 = 0.81) in a treated effluent, showing some enantioselective transformation of G1. This could be due to differential metabolism as well as to differential adsorption or water solubility.

Bester (2003) determined the enantiomer ratios (ER) of HHCB and HHCB-lactone in STPs and in the River Ruhr, Germany. He found that the ER for HHCB-lactone varied between 1.2 and 0.8 for the G2/G3 lactone and between 0.8 and 0.95 for the G1/G4 lactone. For HHCB no deviations from the standard were found. On the other hand, Bester (2002) and Bester and Spiteller (2002) report 'significant differences' for ER for HHCB in sludge, as compared to the standard HHCB: 0.87 as compared to 0.92 and 0.89 as compared to 1.0. Also for the lacton a slight deviation was found as compared to the standard.

Conclusion

Most concentration measurements are based on analysis of the main isomer and its calibration to a reference sample (HHCB technical). Available studies indicate that the ratio of the main isomer versus other isomers in environmental samples is the same as in the reference HHCB technical sample.

The main isomer of HHCB consists of two diastereomers each with 2 enantiomers. In general the 4 enantiomers occur in the environmental samples in the same ratio as in the technical HHCB mixture. Selective transformation of the G1 enantiomer was observed in one fish species (an order of magnitude difference). The selectivity towards G2 and G3 was lower. In 4 other fish species and in zebramussels minor to no selective transformation was observed.

Toxicity and ecotoxicity studies have been carried out with HHCB technical. As the ratios in the environment are generally the same as in the HHCB technical, no recalculation or

correction for other isomer ratios is needed for the risk assessment. The values can be directly compared.

3.1.4 Aquatic compartment (incl. sediment)

3.1.4.1 Calculation of predicted environmental concentrations (PEC_{local})

For HHCB a large database is available with concentrations measured in the environmental compartments in a large number of countries within and outside the EU-15+2. In addition, the fate and behaviour of the substance is also well known. However, the quantification of the material actually being discharged to the sewer is rather complicated. This implies that modelling starting from the volume used in compounding should be given less weight than the results from monitoring campaigns.

Modelling by EUSES serves different purposes. When no data are available, it helps to identify the compartments at risk. However, with the large database available, EUSES may be used as a tool to better understand and evaluate the environmental exposure of HHCB. For that purpose the predictions should be refined as well as possible. Therefore a refinement of the predicted concentrations was introduced using the 90th-percentiles of the available data.

3.1.4.1.1 Calculation of PEClocal for production

The influent concentration was estimated in section 3.1.2.1. The fraction of input to the STP to air is 0.10, to water 0.22 and to sludge 0.67 (see section 3.1.3.3.1). Further calculations were performed according to the TGD using EUSES. For PECregional see section 3.1.4.1.4.

Table 3.11. Calculations for PEC_{STP}, $PEClocal_{water}$ and $PEC_{sediment}$ for production

Site	Influent STP, µg/l	Effluent STP, µg/l	Csludge, mg/kg dwt	Dilution factor to surface water	$Clocal_{water}$ µg/l	PEC regional = xxx µg/l → $PEClocal_{water}$	$PEClocal_{sediment}$ (equil. part.) mg/kg wwt
Production	10	2.3	15.1	1 : 3.7	0.63	+ 0.032 → 0.66	0.358

3.1.4.1.2 Calculation of PEC_{local} for compounding and formulation

The influent concentration was estimated in section 3.1.2.2. The concentration in effluent of the local STP is estimated based on partitioning between air, water and sludge from SimpleTreat (EUSES). Further calculations were performed according to the TGD. For site 2, the calculations are presented for the year 2000 as well as for 2006. The result for 2006 is used for the risk characterisation.

Table 3.12. Calculations for PEC_{STP}, $PEClocal_{water}$ and $PEC_{sediment}$ for compounding and formulation

Site	Influent STP, µg/l	Effluent STP, µg/l	Csludge, mg/kg dwt	Dilution factor to surface water, µg/l	$Clocal_{water}$ µg/l	PEC regional = xxx µg/l → $PEClocal_{water}$	$PEClocal_{sediment}$ (equil. part.) mg/kg wwt
Compounding Site 1	45.5	10.6	68.5	229	0.045	+0.032 →0.078	0.042
Compounding Site 2 year 2000	*15.7*	*3.7*	*23.7*	*1*	*3.7*	*+ 0.004 → 3.68*	*1.98*
Compounding Site 2 year 2006	3.4	0.761	5.78	1	0.73	+0.004 → 0.74	0.397
Compounding Site 3	13	3.0	19.6	1100, default 1000	0.003	+ 0.004 → 0.007	0.004
Compounding Site 4	95.4	22.3 (WWTP)	<143.7>	4481 default 1000	0.022	+ 0.004 → 0.026	0.014
Compounding Site 5	0.17	0.039	0.25	5	0.008	+ 0.032→ 0.040	0.021
Compounding Site 6	-				0	+ 0.032 → 0.032	0.017
Compounding Site 7 (Large-medium generic)	21.1	4.9	31.8	default 10	0.49	+ 0.032 → 0.526	0.28
Compounding Site 8 (Small generic)	8.6	2.0	12.9	default 10	0.20	+ 0.032 → 0.232	0.13
Formulation Large company	12.3	2.9	18.5	default 10	0.29	+ 0.032 → 0.32	0.17
Formulation generic scenario	24.5	5.7	37.0	default 10	0.57	+ 0.032 → 0.61	0.33

3.1.4.1.3 Calculation of PEC_{local} for private use

According to EUSES the predicted distribution of HHCB over air, sludge and effluent in the generic STP is 0.104, 0.672 and 0.224, respectively. The volume of domestic waste water is 200 l/d *per capita*, the amount of surplus solids from the STP is 79 g/d *per capita*, and the concentration of suspended solids in the effluent is 30 mg/l. The concentrations in influent, effluent and sludge are calculated with the standard settings in EUSES (TGD regional 10%). The results are given in **Table 3.13**. The results predicted by EUSES are compared to the median and 90th-percentiles of the concentrations measured in effluents and sludge in the northern and southern regions of the EU-15+2.

An extensive monitoring campaign was carried out in southern Europe to generate details on the concentrations in STPs in those regions. Samples were taken in three countries, Greece, Spain and Italy (15 STPs, 60 samples) in 2004. More details are given in 3.1.4.3.3. The results showed that the concentrations (overall 90th-percentiles) are below the EUSES predictions by a factor of 16, see **Table 3.13** and **Table 3.14**. For the northern European countries, in

particular Germany, a large database is available with concentrations measured recently in the STPs (84 samples in total). These concentrations are below the EUSES predictions for a generic region by a factor of 60. The distance between the actual levels in the two regions is larger than the difference between the assumed daily consumer use in the northern and southern countries (10.6 and 6.0 mg per inhabitant).

Table 3.13. Concentrations predicted by EUSES and recently measured concentrations

Scenario	Predicted Cinfluent µg/l	Predicted Ceffluent* µg/l	Predicted Csludge mg/kg dwt	Ceffluent measured * µg/l	Csludge measured mg/kg dwt
TGD regional (10%)	391	87.5	665	southern Europe median: 2.9 90-perc.: 4.7, ratio: 18.6 northern Europe median: 0.49 90-perc.: 1.4, ratio: 62.5	southern Europe median: 22.5 90-perc.: 46.9, ratio: 14.2 northern Europe median: 6.9 90-perc.: 11.5, ratio: 29.5

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

The large deviation of the observations from the predictions may be explained by a number of factors as illustrated in the diagram in **Figure 3.4**.

As already indicated in section 2.2.2.1 part of the volume used in compounding is exported as fragrance oil (estimated at 15 to 30% of the volume used in compounding) or in fragranced consumer products (10-20% of the remaining 70 to 85% that was not exported). Import of these materials is considered to be of minor importance. HHCB is used for detergents and cosmetics. Part of the products will be in applications of products that are not discharged with water to the sewer (estimated at 10 to 30% of all material in consumer products). During use a fraction of the HHCB in detergents and cleaning agents will stick to fibres and surfaces and will eventually evaporate. The remaining fraction, an estimated 40% of the initial 100% used in compounding, will be discharged to the sewer. In the STP the material will biodegrade and partition to air, sludge and effluent. The fraction finally going to effluent is estimated at 5% of the initial 100% used in compounding (40% to STP * 60% not degraded * 20% to effluent), and similarly the fraction to sludge is 17% (40% to STP * 60% not degraded * 70% to sludge). The figures in the diagram are based on expert judgement; they are rounded to serve as an illustration of possible pathways other than to the sewer. They are not used for the further calculations.

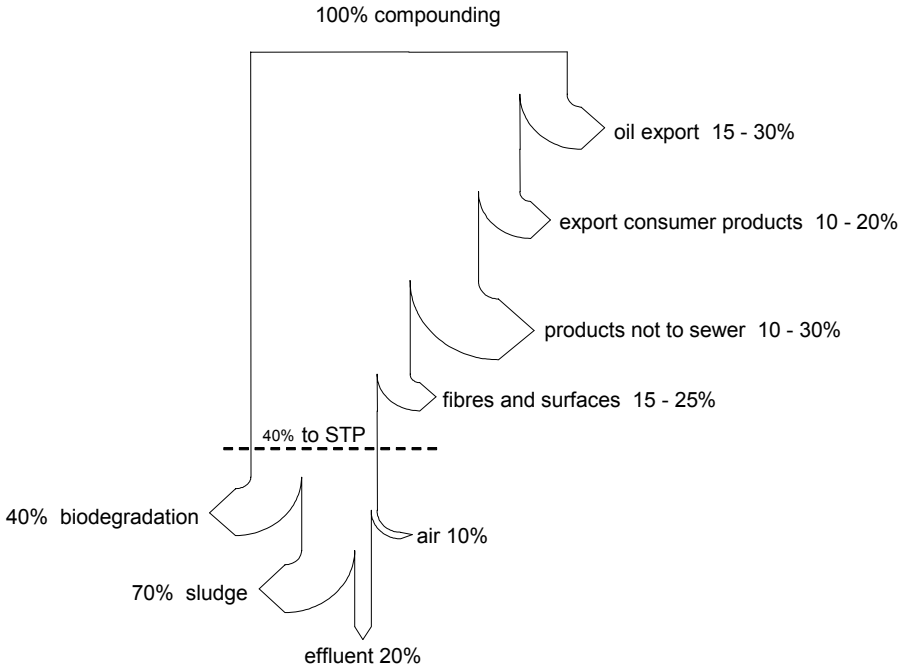


Figure 3.4. Pathways to the environmental compartments, tentative quantification of losses for HHCB. The percentages quantifying the 'arrows out' refer to the amount present at that level, see further explanation in the text

In contrast, with the approach in EUSES, the total use volume is released to the sewer (release factor = 1). Next the use volume is multiplied by a factor of 4 to account for both temporal and spatial variability in use within a region. In the model the rate of biodegradation is zero and the fractions to sludge and effluent are 67% and 22% of the initial volume. The combination of these deviations: (1) a factor of 4 for the variability, and (2) the amounts in effluents (22% vs 5%), in sludge (67% vs 17%) and biodegraded (0% vs 40%), explains the difference between the predictions by the TGD regional (10%) scenario and the observed concentrations in the southern EU-15 countries. This theory is supported by the observation of Buerge et al. (2003) who stated that the effluent load to the environment per capita is 0.80 $\pm$ 0.22 mg p.p.p.d., corresponding to circa 6% of the daily use cited as 13.4 mg p.p.p.d. (in Switzerland).

Modelling by EUSES serves different purposes. When no data are available, it helps to identify the compartments at risk. However, for HHCB a large database is available and then EUSES may be used as a tool to better understand and evaluate the environmental exposure of HHCB. For that purpose the predictions should be refined as well as possible. Therefore a refinement of the predicted concentrations was introduced using the 90^{th}-percentiles of the available data for effluent and sludge. This refinement was used for the estimation of the concentrations in the compartments following the environmental pathways of the effluent and sludge, see **Table 3.14**.

Table 3.14. Local concentrations predicted by EUSES and based on measurements (90th-percentiles) in effluent and sludge

Scenario	Influent STP, µg/l	Effluent STP, µg/l	Csludge, mg/kg	$Clocal_{water}$ µg/l	PECregional [1)]	$PEClocal_{water}$ µg/l	$PEClocal_{sediment}$ mg/kg wwt
TGD regional (10%)	391	87.5	665	8.44	0.348	8.70	4.73
southern EU-15 measurements		90th-percentile: 4.7	90th-percentile: 46.9				
ratio TGD regional (10%) / southern EU-15		*ratio* 18.6	*ratio* 14.2				
southern EU-15 predictions	25.4	EUSES: 5.69	EUSES: 43.2	0.453 *	0.0318	0.485 *	0.261 *
northern EU-15 measurements		90th-percentile: 1.4	90th-percentile: 11.5				
ratio TGD regional (10%) / northern EU-15		*ratio* 62.5	*ratio* 58				
northern EU-15 predictions	6.65	EUSES: 1.49	EUSES: 11.3	0.135 *	0.00417	0.139 *	0.0749 *

1) PECregional, see explanation in 3.1.4.1.4.

* Prediction based on concentrations measured in effluent and sludge (90th-percentiles)

3.1.4.1.4 Regional concentrations

For calculating the PECs at the regional scale of 200 · 200 km^2, only the emissions due to private use are taken into account. At such scale emissions from compounding sites are negligible as compared to those from private use. According to the 10% rule, the regional emission is 10% of the EU-15+2 volume or 142.7 ton. Assuming a split up of 20% discharge directly to surface water and 80% to a STP (EU-TGD, EC 2003), EUSES calculates a PECregional of 0.362 µg/l for surface water (total) and 0.311 mg/kg wwt for sediment.

However, as shown in **Table 3.13**, the initial prediction of $Csludge_{local}$ and $Ceffluent_{local}$ were highly conservative for a number of reasons and therefore, for the further calculations they are replaced by measured values. This implies that also the estimates of the regional concentrations will need to be reduced proportionally. In EUSES this can be achieved by adapting the fractions of the tonnage released to wastewater which is originally set at 1 [11]. The implication of the diagram in **Figure 3.4** is that only a relatively small fraction will be released to wastewater whereas at the same time an equal fraction will be released to air (the fractions 'cosmetics not to sewer' and 'fibres and substrates'). The effluent and sludge concentrations in southern European countries are predicted well when the release factor to water and air is set at 0.065 (=1/15; this value is derived from the ratios in **Table 3.13**: 1/18.6 and 1/14.2), whereas an acceptable prediction for northern European countries is achieved with a release factor of 0.017 (=1/59,see **Table 3.14**: ratios 1/62.5 and 1/58). In this way PECregional is set for these scenarios. The calculations of $PEClocal_{water}$, $PEC_{sediment}$, PEC_{soil},

[11] Under the entry in EUSES: Release estimation/Intermediate Results/Use pattern1/Private Use/Release Fractions

etc. are carried out using the 90th-percentile values of the actually measured effluent and sludge concentrations.

In the generic approach 80% of the population is connected to a sewage treatment plant. When different scenarios are used for northern and southern Europe, the connection rates should be differentiated as well. According to EUROSTAT data, the current connection rate in Germany (origin of effluent and sludge data, representative of Northern EU country) is > 95%, whereas according to experts in the three southern EU countries Greece, Spain and Italy, the lowest connection rate is 60%. These connection rates were used in the calculation of PECregional. The results of the calculations are shown in **Table 3.15**.

Table 3.15. Regional concentrations for the various scenarios

Consumption, mg /d *per capita*	**PECregional $_{water}$, µg/l (dissolved)**	**PECregional $_{sediment}$, mg/kg wwt**
2000 – TGD regional (10%)	0.348	0.311
southern EU	0.0318	0.0284
northern EU	0.00417	0.00372

3.1.4.2 Measured levels

The presence of polycyclic musks in the environment has been investigated in several European countries. They were detected in sewage treatment plants, surface waters, sediment and in fish and shell fish. The results are summarised in **Table 3.16**, **Table 3.17** and **Table 3.18**. The results are often summarised as a minimum and a maximum, with occasionally a mean, median or 90th-percentile value. For the risk assessment, the 90th-percentile is the preferred value, whereas for an understanding of processes and trends, the median value is more relevant.

Most used extraction techniques are by solid phase micro-extraction of the aqueous phase and solvent extraction of the solid phase. Identification and quantification is mainly by GC/MS. Total concentrations are presented unless explicitly stated that free (dissolved) concentrations are given.

3.1.4.2.1 Concentrations in sewage treatment plants

Total influent and effluent concentrations were determined for three STPs of the German Ruhrverband during one week. According to the author (pers. comm., Eschke 1996) figures on the influent in Eschke et al. (1994) are not representative, but more likely in the range of 10 – 30 µg HHCB/l. Effluent samples were 24-h time-proportional samples. The median concentration in the effluent was 1.2 µg/l for HHCB (Eschke et al. 1994, 1995a).

Flow-proportional influent and effluent samples and samples of primary, activated and digested sludge were taken from three STPs in The Netherlands in 1997-1998. The median influent concentration of HHCB was 6.4 µg/l/, whereas the concentration in effluent was 1.4 µg/l (Rijs and Schäfer 1998).

Flow-proportional daily composite samples were taken in effluents from the three largest STPs in Sweden and analysed for the presence of a large number of organic pollutants

including HHCB in December 1993 and January 1994. The concentrations of HHCB ranged between 1 and 6 μg/l (Paxéus, 1996).

"Free dissolved concentrations" were estimated in effluents of four STPs in The Netherlands (1995-96) from amounts accumulated on Empore disks. The mean of the concentrations, which according to the author is accurate within a factor of 2, was 0.36 μg/l (Verbruggen et al. 1999). In 1997 concentrations determined with the same technique were obtained from Leonards et al. (2000) showing influent concentrations of 0.7 and 1.1 μg/l and effluent concentrations of 0.4 and 1.1 μg/l.

Time proportional daily composite samples were collected over a 3-day period of influent, primary effluent and final effluent of an activated sludge and a trickling filter wastewater treatment plant in Ohio (USA) in 1997. The mean concentration in the influent was 13.7 and 9.8 μg/l, and 1.2 and 1.6 μg/l in the effluent of the activated sludge plant and in the trickling filter system, respectively. Whereas influent concentrations showed considerable diurnal fluctuation (a factor of 4), the effluent concentration remained relatively constant throughout the day (Simonich *et al.* 2000). In a more extensive project on fragrance ingredients the concentrations of HHCB were measured in the influent, primary effluent and effluent of 17 STPs in the USA, the UK and The Netherlands. The treatment plants were representative of a diversity of systems: with and without primary gravitational settling, activated sludge, carousel, oxidation ditch, trickling filter, rotating biological contactor and lagoon types were included. Industrial contribution to the STPs was in all cases less than 20%. Apart from the two STPs in Ohio (1997) mentioned above, samples were taken from 1998 to 2000. Hourly samples were collected over several days with an autosampler. Flow proportional daily composite samples were produced (Simonich *et al*. 2002). Concentrations in influent in 2 STPs in the Netherlands ranged from 3 to 6 μg/l, with effluents at 1 μg/l. In the 3 STPs in the UK the influents were 3.7 to 12.8 μg/l with effluents from 1 to 4.6 μg/l. In the 12 STPs in the USA the influents ranged from 7 to 34 μg/l with effluents between 1.2 and 4 μg/l. There was a clear relation between removal of TSS (total suspended solids) and concentration in the effluent (see also section 3.1.3.3.2). The purification process in lagoons deviates from the other treatment plants. Concentrations in the effluent of lagoons were clearly very low: < 0.1 μg/l (Data Simonich pers. comm. 2002).

In Switzerland effluent samples (grab or mixed) were taken from 17 STPs in March 1998. The median concentration was 4.7 μg/l (BUWAL, 1998).

In Switzerland in the Kanton of Basel effluent samples were taken in 6 STPs in March 1997. The median concentration as determined after SPME was 3 μg/l (Noser et al. 2000).

In a screening programme of the Berlin surface waters in 1996, samples were taken from three STP discharge points. The mean concentration of HHCB (SPME), was 9.0 μg/l (Heberer et al. 1999). In a more extensive study of the same area, 30 proportional daily composite samples were collected from five municipal treatment facilities in 1996. These samples were prepared by simultaneous steam distillation/solvent extraction. The median concentration was 6.7 μg/l. The effluent concentrations in one STP throughout the year varied by a factor of 2, with the highest value 13.3 μg/l observed in winter (Fromme et al. 2000, 2001a, Heberer et al. 2001). Fromme et al. (2000) indicate that their measurements probably show a worst case scenario, because of the high degree of urbanisation, little producing industry and for this sampling series, they were taken in a dry weather period. Moreover, the surface water system of Berlin has a very high fraction of effluent input (> 90%) and low natural surface water flow rates. (In addition, he states that the total drinking water use in Germany is only 127 liter per i.e.).

In a pilot plant in preparation of an extension of the main sewage treatment plant of Vienna (Austria), concentrations of HHCB in the influent were below the detection limit (0.1 µg/l). Concentrations in the activated sludge were between 1 and 2 mg/kg dwt (Hohenblum et al. 2000).

In a research programme in the state Hessen, Germany, in 9 STPs one effluent sample was taken in 1999 and one in 2000 (HLUG 2001). The median concentration for the two years was 1.1 µg/l. The median concentration in 2000 was lower than in 1999.

In Germany, the concentrations were measured in the effluent of 3 STPs along tributaries to the River Elbe in the region of Leipzig in 1999. The concentrations ranged from 2.5 to 5.7 µg/l (ARGE 2000). In Sachsen-Anhalt the effluent concentrations of 5 STPs ranged from 3.1 to 8.1 µg/l (cited in ARGE 2000).

The concentrations were measured in the STP in Dortmund, Germany in 2002. The plant received 184,000 m^3 water per day from 350,000 inhabitants and industries. The mean concentration in the influent was 1.9 µg HHCB/l and 0.23 µg HHCB-lactone/l, in the effluent it was 0.70 µg HHCB/l and 0.37 µg HHCB-lactone/l, a removal of 64% for HHCB. Large STPs in Köln and Düsseldorf (1,000,000 i.e.) were sampled in 2003 resulting in similar levels (Fahlenkamp et al. 2004).

As part of an investigation in the Lippe River, tributary to the River Rhine, in North-Rhine/Westphalia, Germany, the concentrations of influent and effluent of an STP in a city of 182,000 inhabitants were measured. They were at the level of 1 µg/l (Dsikowitzky et al. 2002).

In North-Rhine/Westphalia, Germany an overall study was completed on the presence of organic pollutants in sewage sludge. A one-year analysis in 156 municipal STPs was sampled (representing 80% of the state's annual sewage sludge volume); in 17 STPs the analyses included the polycyclic musks. The median concentration of HHCB was 4.7 mg/kg dwt, the 90^{th}-percentile was 11.8 mg/kg dwt. A correlation between the HHCB levels in sludge and characteristics of the region served by the STP as well as the treatment method was low. However, it was clear that aerobic sludge stabilisation resulted in all lower levels in sludge than when the sludge was not treated in this way (Friedrich et al. 2004). When sludge is stabilised, the dry solids content is decreasing and as a result the concentration of a persistent substance on the dry solids would increase. Thus if the HHCB level on the sludge decreased (to 40%), HHCB was degraded during the aerobic stabilisation as well.

Samples were taken from 21 STPs in Germany in summer 2000 and in January 2001. The concentrations were measured in influents, effluents and activated and digested sludge. The median concentration in the influent was 1.3 µg/l (range from 0.25 to 4.94 µg/l), whereas the median concentration in the effluents was 0.27 µg/l (0.04 to 0.59 µg/l). The influent concentrations seem to be higher in summer then in winter (factor 1.6), whereas the effluent concentrations were lower in summer (factor 0.5). The variation, expressed as the ratio of the 90^{th}-percentile/median in the influent concentrations was 2.4. For the effluents this ratio was 2.1 in summer and 1.3 in winter (Müller et al. 2002).

Samples were taken in all compartments of four STPs (activated sludge plants) in The Netherlands in 2001 and both free (dissolved) and total concentrations were determined. The free concentration was constant in all matrices (influent, primary settler, primary sludge, aeration tank, effluent, waste sludge): 1.0 – 2.0 µg/l, whereas the total concentrations ranged between 1 and 250 µg/l (Artola 2002). The median (total) influent concentration was 3.4 µg/l, the median (total) effluent concentration was 1.6 µg/l. In this study the diurnal variation found

by two-hourly sampling was circa 19% for the total influent concentration (sum of AHTN and HHCB) but it was not even 10% for the free concentrations in the influent (Artola 2002).

In Switzerland a larger monitoring network was initiated where the various compartments in a wastewater treatment plant were sampled. Flow proportional samples were taken daily during a week in April 2002. After extraction with hexane and clean-up by GPC, the quantification of the substances was by GC-MS. Mean concentrations in influent ranged from 1.7 to 7 μg/l, in effluent from 0.3 to 0.9 μg/l (Brändli 2002). In the same programme in Switzerland daily fluctuations in the wastewater concentrations were measured in a small STP (210 i.e.) in a week in April 2001. The mean influent concentration was 6.9 μg/l and ranged from 5.4 to 9.0 μg/l, whereas the effluent concentration ranged from 0.7 to 1.1 μg/l, mean 0.9 μg/l. Overall the variation within a week is ± 25% (Kupper et al. 2003b).

In Switzerland 24h-flow-proportional samples were obtained from 5 STPs in the region of Zürich in 2001. The samples were taken of the effluent of activated sludge plants after nitrification, phosphate precipitation and sand filtration. The capacity of the plants ranged from 5000 to 36,000 i.e. The concentrations ranged from 0.72 to 1.95 μg/l (Buerge et al. 2003).

Samples were taken in a rural area in Austria at two month intervals between 2000 and 2001. The composite samples were taken 7 times over a 24 hourperiod at different stages of wastewater treatment and post treatment of an STP, as well as in the groundwater. The system has no primary settling but low loaded aeration with a long hydraulic retention time of 10 days and full nitrification and denitrification and iron precipitation of phosphorous. The mean concentration was 2.0 μg/l in the influent and 0.39 μg/l in the effluent (Kreuzinger et al. 2004).

In Galicia, NW Spain, the behaviour of HHCB was studied in an STP serving 100,000 inhabitants. The plant consists of three sections including pre-treatment with coarse screens and grit and fat removal, primary treatment by sedimentation and secondary treatment in an activated sludge process. In 2001 - 2002 three campaigns were carried out. Samples were taken every hour during 24 hours and mixed. The samples were extracted by SPE and analysed by GC/MS as well as by a complementary technique where an SPME fibre was exposed to the headspace over the sample and analysed by GC/MS. The concentration in the raw influent was in the range of 2.1 to 3.2 μg/l, after the filters and primary settling it was 1.4 to 1.8 μg/l and after secondary settling (effluent) it was 0.5 to 0.6 μg/l. The average removal efficiency was 83% (71 – 85%) (Carballa et al. 2004). In an earlier report on this SPME fibre technique the HHCB concentration in effluent was 0.5 μg/l (García-Jares et al. 2002).

In Girona, Spain, the concentration in the effluent of an STP serving 32,000 to 60,000 inhabitants (winter and summer) was measured between 1999 and 2001. The concentration was 2.34 μg/l. Further treatment (infiltration/percolation) reduced the concentration to 0.12 μg/l. The samples were extracted on a closed loop stripping on an activated carbon filter that was extracted by CS_2 followed by GC/MS (Romero et al. 2003).

During the development of a new analytical technique using closed-loop stripping analysis followed by GC/MS, samples of influent and effluents in STPs in Germany, Austria, Belgium and Spain and surface waters in Germany, Spain and France were analysed. Samples were taken in May 2003. The concentrations in Germany ranged from 0.7 to 1.4 μg/l in the 4 influents, and from 0.2 to 0.6 in the effluents (removal 50 – 70%). The effluent concentration in the sample of Austria was of the same level. The influent samples from Belgium and Spain

(each one sample) were slightly higher (< factor of 2), whereas the effluents were between 0.5 and 1.4 μg/l, showing a low removal (Mitjans and Ventura 2004).

A large campaign was carried out in Italy, Spain, Greece and Berlin, Germany to sample the effluents and activated sludge in 2004. In both Italy and Spain, samples were taken in 6 STPs, whereas in Greece three STPs were sampled. A total of 4 samples were collected with intervals of 2 to 4 weeks in Italy, Spain and Greece. The selected plants treated mainly municipal sewage for more then 10,000 inhabitants. The process and design characteristics were collected from the operators. Sampling was conducted under normal dry weather conditions between May and October 2005. The activated sludge was centrifuged and freeze dried. The effluent was left to settle for 0.5 hour and the supernatant was extracted by SPE (Speed Disk). After elution with solvent and concentration of volume, the extract was analysed. Sludge samples (freeze-dried) were solvent extracted with dichloromethane (DCM) by Accelerated Solvent Extraction equipment (ASE) and cleaned-up over a silicagel chromatographic column. The analyses were performed by GC/MS in SIM mode. Control samples, standard additions and analytical controls were included to check the loss of substances and the recovery of the system (Blok et al. 2005,). A detailed statistical analysis was made of the results (Van der Hoeven 2005). The median concentration in effluent in Italy was 2.8 μg/l, in Spain 2.9 μg/l, and in Greece 3.0 μg/l. As an indication of the variation in these countries and in time, the ratio between the median and the 90th-percentile concentration was established. The ratio was 1.6. The median concentrations in sludge were 29.3 mg/kg dwt in Italy, 22.0 mg/kg dwt in Spain and 19.5 mg/kg dwt in Greece. For sludge the ratio between median and 90th-percentile was 2.1. The overall 90th-percentile for these southern European countries was 4.7 μg/l in the effluent and 46.9 mg/kg dwt in sludge (Blok et al. 2005).

In the same campaign the concentrations were measured in Berlin, Germany in 3 STPs. The samples were collected twice with an interval of 6 months. Two of the three STPs had been included in the study carried out by Heberer in 2000. In 2004 the mean concentration in sludge was 10.2 mg/kg dwt, which was at the same level as in the year 2000. However, also the mean concentration in the effluent was low, 1.6 μg/l. In 1996/1997 these concentrations had been relatively high and no reasonable explanation was found at that time (Heberer et al. 1999, Fromme et al. 2001a). The observations in 2004 are in line with other recent observations in Germany (see **Table 3.16**).

In the UK samples were taken of influent and effluent in 6 STPs (Kanda et al. 2003). The STPs included a variety of processes and systems: a rotating biological contactor and reed beds, an oxidation ditch, biological filter beds, activated sludge processes, and trickling filters. A system with a submerged aerated filter was not taken into consideration as was not fully matured at the time samples were taken. The influent concentrations ranged from 7.8 and 19.2 μg/l and the effluent concentrations ranged from 1.1 to 2.8 μg/l (Kanda et al. 2003). It is remarkable that these overall minimum and maximum concentrations in the effluent were observed on two consecutive days in one of the 6 STPs.

In The Netherlands flow proportional samples were taken of the effluent of 5 STPs along the River Meuse and its tributaries. The samples were collected in three periods in 2002/2003 with intervals of two months and screened for a large number of substances. The concentrations ranged from 0.01 to 10.4 μg/l (Berbee et al. 2004). The concentrations were extremely variable in time (a factor of 400) as well as in place and the AHTN/HHCB ratios were not always consistent (in contrast to the majority of results from other studies). The analytical procedure followed enables a correct identification of the substance. However, the authors (Berbee et al. 2004) acknowledge that the quantification was not reliable as it is not known whether the response in a GC/MS for the substance is the same as for the standard

used (1-chlorodecane). Also the used calculation programmes gave considerably different results due to differences in background levels. Therefore the results are only indicative for the presence of HHCB.

In 5 STPs in Sweden composite effluent samples were taken by means of SPE extraction in June-December 1999. All STPs received mixed household/industry effluents and serve populations ranging from 2600 to 80,000 inhabitants. The wastewater treatment includes an activated sludge process supplemented by varying additions to facilitate sludge settling. The concentrations ranged from 0.16 to 0.42 µg/l. The same authors report on samples taken in 3 STPs in Nova Scotia and New Brunswick, Canada in January 2002. The STPs treat mixed household/industry effluents of cities with 20,000 to 350,000 inhabitants. The concentrations ranged from 0.25 to 1.3 µg/l (Ricking et al. 2003b).

An on site sampling technique was developed in southwestern USA where 60 l water samples were extracted on solid phase. Samples were from two dedicated effluent receiving streams (only effluent and run-off). Concentrations varied in time, between 0.035 and 0.152 µg/l (Osemwengie and Steinberg, 2001). In 2001 concentrations were measured in the confluence of effluent streams from 3 Nevada STPs, twice per month. The concentrations ranged from 0.03 to 0.1 µg/l (Osemwengie and Gersenberger 2004).

Table 3.16. Concentrations of HHCB in influents, effluents and surface water (µg/l). Mean, median, maximum and 90th-percentile of the data

Location	n	influent	n	effluent	n	surface water	reference
Germany, Ruhr <1994	7	settled infl. [mean 1.5] 10-30(1)	21	median 1.9 90-perc. 2.3	30	median 0.4 90-perc. 0.5	Eschke '94, '95a
Germany, North Sea 1990, 1995 Germany, Elbe 1995					12 1	median 0.00021 90-perc. 0.0008 0.10	Bester '98
Germany, Elbe 1995					2	0.07	Lagois '96
Germany, Elbe 1996-1997					25	median 0.09 90-perc. 0.12	Winkler '98
Germany, Elbe 1998-1999 Elbe Czech border to North Sea 1996-1997 Tributaries region Leipzig 1999 Germany, Sachsen-Anhalt, 5 STPs <2000			3 5	mean 3.6 max. 5.7 3.1 – 8.1	 41 11 	 median 0.12 0.03 – 0.41 median 0.380 0.15 – 0.61 	ARGE 2000 cited in ARGE 2000
Germany, Berlin 1996			3	mean 9.0 max. 10.8	27	median 0.8 90-perc. 4.3 (2)	Heberer '99

Location	n	influent	n	effluent	n	surface water	reference
Germany, Berlin 1996 (5 STPs)			30	median 6.7 90-perc. 10.8 max. 13.3	34 40 28	*low effluent input*: median 0.05 90-perc. 0.14 max. 0.32 *moderate effluent input:* median 0.15 90-perc. 0.49 max. 0.81 *high effluent input:* median 1.48 90-perc. 2.73 max. 3.15	Fromme '01a
Germany, Hessen, 1999-2000			2 · 9	median 1.1 90-perc. 1.6	2 · 20	median 0.15 90-perc. 0.46	HLUG 2001
Germany, Dortmund 2002	5	mean 1.94 1.41 – 2.32	5	mean 0.70 0.65 – 0.80			Bester 2004
Germany, Düsseldorf, Köln 2003	2	2.5 – 4.6	2	0.95 – 1.3			Fahlenkamp 2004
Germany, Main [4)] 1998					29	median 0.10 90-prec. 0.20	Klasmeier 2001
Germany, Lippe 1999	1	1.0	1	1.4	76	median 0.08 90-perc. 0.17	Dsikowitzky 2002
Germany, 2000 - 2001	2 · 21	summer median 1.44 90th perc. 3.72 winter median 0.98 90th perc. 2.31 0.25 – 4.94	2 · 21	summer median 0.15 90th perc. 0.31 winter median 0.38 90th perc. 0.48 0.04 – 0.59			Müller 2002
The Netherlands River Rhine, '94-'96 River Meuse, '94-'96					32 35	median 0.06 90-perc. 0.16 median 0.08 90-perc. 0.19	Breukel '96
The Netherlands 1997-1998	8	median 6.4 max 14.5	8	median 1.4 max 1.6			Rijs 1998
The Netherlands 1995-96			4	mean 0.36 [(5)] max 0.63	14	median 0.027 [(5)] 90-perc. 0.13	Verbruggen '99
The Netherlands 1997	2	0.7 – 1.1 [(5)]	2	0.4 – 1 [(5)]	5	0.006 – 0.27 [(5)]	Leonards 2000, Van Stee 2000

Location	n	influent	n	effluent	n	surface water	reference
The Netherlands 2001	4	median 3.4 max 4.3	4	median 1.6 max 2.2			Artola 2002
The Netherlands 2002-03			15	median1.81 (6) 90-perc. 7.35 (6)			Berbee 2004
The Netherlands River Rhine 2001					8	median 0.009 (5,6) 90-perc. 0.015	Geerdink 2004
The Netherlands River Meuse 2002/3					circa 270	90-perc 0.01 (5,6,7)	Jeuken 2004, Kalf 2005
Switzerland, Glatt ≤ 1995					1	0.136	Müller'96
Switzerland 1998			17	median 2.3 90-perc. 4.7	20	median 0.061 90-perc. 0.097	BUWAL 1998
Switzerland 1997			6	median 3 max. 3.9	8	median 0.08 max. 0.26	Noser 2000
Switzerland 2002	7 7 1	mean 4.5 2.3 – 6.9 mean 6.9 1.68 (after prim. settling)	7 7 1	mean 0.78 0.3 – 1.1 mean 0.86 0.26			Brändli 2002
Switzerland 2001			5	0.72 – 1.95	28	contam. rivers mean 0.24 max 0.564 lakes median 0.015 range < 0.02 – 0.05	Buerge 2003
Austria 2000-2001	7	mean 2.0 0.14 – 3.2	7	mean 0.39 0.27 – 0.53			Kreuzinger 2004
Sweden < 1995			3	range 1 - 6			Paxéus '96
Sweden 1999 Canada 2002			5 3	0.16 – 0.42 0.25 – 1.30			Ricking 2003b
Spain, Galicia 2004	3	2.1 – 3.4	3	0.5 – 0.6			Carballa 2004
Germany 2003 Austria Belgium Spain France	4 1 1	0.7 – 1.4 1.3 2.2	4 1 1 2	0.2 – 0.6 0.2 1.1 0.47 – 1.4	2 1 1	0.04 – 0.06 0.39 0.052	Mitjans 2004
Germany 2004			2 - 3	mean 1.6 1.1 - 2.1			Blok 2005

Location	n	influent	n	effluent	n	surface water	reference
Spain 2004			4 · 6	median 2.9			
				90-perc 5.3			
				0.9 – 9.4			
Italy 2004			4 · 6	median 2.8			
				90-perc 6.2			
				1.8 – 6.4			
Greece 2004			4 · 3	median 3.0			
				90-perc 4.0			
				2.4 – 4.1			
Overall SEU			60	90-perc 4.7			
UK, 6 STPs 2001	13	mean 11.7	13	mean 1.8			Kanda 2003
		7.8 – 19.2		1.1 – 2.8			
USA, Ohio 1997	2 · 3	mean 12.7	2 · 3	mean 1.3			Simonich '00
USA 1977 - 1999	12	mean 12.6	12	mean 1.65 [3]			Simonich 2002
		8.7 – 45.5		0.03 – 4.0			
UK 1999 - 2000	3	9.1 – 17.6	3	1 – 4.6			
The Netherlands 1999	2	3.2 – 5.9	2	1			
USA, Southwestern			3	0.035 – 0.152			Osemwengie 2001
USA, Nevada 2001			9 · 2	median 0.044	7 · 2	median 0.0002	Osemwengie 2004
				0.033 – 0.098		0.00006 – 0.001	
USA, Lake Michigan 1999 - 2000					13	95% confid. interval: 0.0047 ± 0.0025	Peck 2004
USA, Iowa 2001					30	low-flow: max 0.26	Kolpin 2004
					23	normal-flow: n.d.	
					23	high-flow: max 0.056	
USA, San Francisco Bay, 1999 – 2000					2 · 13	0.003 – 0.131	Oros 2003
Japan, Tama					5	range 0.0007 - 0.1	Yun '94

[1] Unreliable results according to author; more likely 10 to 30 µg/l (Eschke 1996, pers. comm.)

[2] 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 population

[3] Median without the extremely low figures from lagoons

[4] Data points read from graph

[5] 'Free concentrations'

[6] Quantification not reliable

[7] Detected only on one of 6 locations

Samples of primary, activated and tertiary sludge were taken from 6 STPs in The Netherlands during two sampling periods in 1997. In addition, the input and output of a compost facility were sampled three times. This facility treats digested activated sludge from several STPs. The median concentration in digested sludge was 23 mg/kg dwt. HHCB (Blok 1998). One year later in a similar study, concentrations were slightly lower (Rijs and Schäfer 1998).

For the analysis of sewer slime, 17 samples were taken from sewer systems collecting waste water of domestic or industrial origin in Germany. The mean concentration in the industrial area was low, 2 mg/kg dwt, whereas in the domestic area the mean concentration was 16 mg/kg dwt. Furthermore two activated sludge samples were taken from domestic sewage treatment plants. The mean was 9 mg/kg dwt (Sauer et al. 1997).

In Hessen, Germany, the concentrations in activated sludge and in digested sludge of 6 domestic STPs were compared in 1996. There was no systematic relation between the concentrations in digested sludge and in activated sludge. From 1996 to 2000, the wasted sludge of 9 STPs was sampled once per year. Seven out of the 9 treatment plants have a sludge digestion step, but this is not reflected in the levels in the sludge. The median concentrations showed a steady downward trend: from 17.1 mg/kg in 1996 to 6.7 mg/kg in 2000 (HLUG 2001).

Sludge was sampled in the STP in Dortmund, Germany in 2002. The plant received 184,000 m^3 water per day from 350,000 inhabitants and industries. The system includes primary settling, activated sludge treatment, secondary settling and anaerobic digestion. The Solids Retention Time was 8-10 days in the aerator and 20 days in the digester. The concentration in digested sludge was 3.1 mg/kg (Bester 2004).

In Switzerland, surplus sludge from different catchment areas was sampled in 1998. Seven samples were from systems mainly fed by domestic sewage; three were obtained from systems receiving a mixture of domestic sewage, storm water runoff and low amounts of industrial water, and two samples were from systems with a higher input of industrial waste water. The median concentration was 3.7 mg/kg dwt.. The concentrations in the area with a higher industrial contribution to waste water seemed to be lower. The median in the sludge of mainly domestic origin was 5.4 mg/kg dwt (Herren and Berset 2000). The maximum concentrations here were below the median concentrations in The Netherlands in 1997 and 1998 and in Hessen (1996-2000), Germany.

Samples were taken from 21 STPs in Germany in summer 2000 and in January 2001. The concentrations were measured in activated and digested sludge. The median concentration in activated sludge was 7.25 mg/kg dwt (range 2.69 – 14.4 mg/kg dwt). The median concentrations were similar in summer and winter but in winter the maximum was higher. In 9 STPs the concentrations in digested sludge were measured as well. These concentrations were 1.5 to almost twice as high as those in activated sludge. For an evaluation of the anaerobic degradability, however, the loss of organic matter during the digestion process should be taken into account (Müller et al. 2002).

In three municipal STPs in Berlin, Germany, 14 days-collective samples were taken of the sewage sludge. Two samples were taken in February 2000. The average concentration was 8.3 mg/kg dwt, ranging from 6.0 to 11.5 mg/kg dwt (Heberer 2002).

In a recent campaign in the UK the concentration on sludge was measured in the digested sludge of 14 wastewater treatment plants. The treatment plants varied largely in water flow as well as the origin of the influent, and also the levels of HHCB were extremely variable

between different plants, ranging from 1.9 to 81 mg/kg dwt. The median was 26 mg/kg dwt (Stevens et al. 2003).

In Switzerland samples were taken of the activated sludge in 16 STPs in 2001. The average concentration was 20.3 (range 11.6 to 31.4) mg/kg. A second sample in five of these plants was within 25% of the first value. These plants were generally small with 210 to 17140 inhabitants connected. The concentration of HHCB-lacton was 1.8 (0.7 – 3.3) mg/kg (Kupper et al. 2003a). In another study of this Swiss programme the weekly fluctuation in the concentrations on the activated sludge in a small STP (210 i.e.) were determined in a week in April 2001. The mean concentration was 4.3 mg/kg dwt, ranging from 3.2 to 5.3 mg/kg.dwt, so a variation in time of ± 25% (Kupper et al. 2003b).

In Galicia, NW Spain in 2001 – 2002, concentrations were measured on sludge from an STP serving 100,000 inhabitants. The plant consists of three sections including pre-treatment, primary treatment by sedimentation and secondary treatment in an activated sludge process. The concentration on sludge was determined by headspace SPME extraction and GC/MS analysis. The average concentration on the activated sludge as derived from the concentration in the outlet of the biological reactor and the total suspended solids was 18.1 (13.5 – 20.6) mg/kg dwt (Carballa et al. 2004). During the development of their analytical methods, the concentration in secondary sludge was 3 mg/kg dwt (converted from fresh weight, Llompart et al. 2003).

In a joint monitoring campaign in the Nordic countries a total of 27 STPs was sampled in Denmark, Sweden, Finland, Norway and Iceland in 2002. The type of sludge was not further specified. Highest concentrations were found in Denmark and Sweden (median 14.1 and 13.9 mg/kg dwt), intermediate in Norway and Finland (median 7.7 and 5.4 mg/kg dwt), whereas the levels were low in Iceland (0.5 mg/kg dwt) (Mogensen et al. 2004).

Table 3.17. Concentrations in sludge in STP

Sample	n	HHCB, [mg/kg dwt]		Reference
NL 1997				Blok 1998
primary sludge	11	mean 13.9	range 5.4-27	
activated sludge	12	mean 27.9	range 4.4-63	
digested sludge [1]	13	median 23	range 9.0-31	
NL 1997-1998				Rijs and Schäfer 1998
primary sludge	8	median 13.5	range 6 –17	
activated sludge	7	median 9.7	range 4.8 – 21	
digested sludge	2	mean 20	range 19 – 21	
Germany ≤ 1997				Sauer *et al*. 1997
activated sludge	2	mean 8.9	range 4.3 - 13.4	
Sewer slime, urban/industrial area	17	mean 1.4	range 0.1 - 5.2	
Sewer slime, rural area	2	mean 15.5	range 9.1 - 21.8	

Sample	n	HHCB, [mg/kg dwt]		Reference
Germany, Hessen 1996				HLUG 2001
domestic waste water				
activated sludge	6	median 17.1	range 11.6 – 20.4	
digested sludge	6	median 18.3	range 14.1 – 21.6	
sludge (wasted, mostly digested)				
1996	9	median 17.1	range 11.9 – 21.6	
1997	9	median 14.1	range 7.2 - 22.3	
1998	9	median 10.3	range 6.7 - 21.9	
1999	9	median 9.1	range 5.1 - 10.8	
2000	9	median 6.7	range 4.3 - 8.6	
Germany, Dortmund 2002	5	mean 3.1	range 2.7 – 3.3	Bester 2004
Digested sludge				
Germany, Nordrhein-Westfalen >2000	19	median 4.7	range 1.2 - 15	Friedrich 2004
Germany, 21 STPs, 2000 - 2001	21	summer: median 6.41	range 2.9 – 10.4	Müller et al. 2002
activated sludge		winter: median 7.5	range 2.7 – 14.4	
dewatered/digested		winter: median 12.0	range 6.0 - 23	
Germany, Berlin 2000	3 · 2	mean 8.3	range 6.0 – 11.5	Heberer 2002
3 municipal STPs				
Switzerland 1998				Herren and Berset 2000
Wasted sludge (both domestic and with more industrial input)	12	median 3.9	range 2.3 – 12.2	
Switzerland 2001				Kupper et al. 2003a
Wasted sludge both domestic and with more industrial input	16	mean 20.4	range 11.6 – 31.4	
Switzerland 2001	1	mean 4.3	range 3.2 – 5.3	Kupper et al. 2003b
Small STP (210 i.e.)				
Austria 1999				Hohenblum 2000
Activated sludge	2	range 1-2		
Nordic countries, 2002				Mogensen 2004
Denmark	5	median 14.1	range 11.4 – 26.5	
Sweden	8	median 13.9	range 7.8 – 24.0	
Norway	5	median 7.7	range 0.25 – 22.4	
Finland	5	median 6.0	range 0.5 – 8.75	
Iceland	4	median 0.52	range 0.41 – 2.16	
UK (year ?) 14 WWTPs	14	median 26	range 1.9 - 81	Stevens et al. 2003
Digested sludge				

Sample	n	HHCB, [mg/kg dwt]		Reference
Spain 2004 Activated sludge STP (100,000 i.e.)	3	mean 18.1	range 13.5 – 20.6	Carballa et al. 2004
Germany 2004	3 · 2	mean 10.2	range 6.5 - 12.0	Blok et al. 2005
Italy 2004	4 · 6	median 29.3	range 7.7 – 56.0 90th-perc 54.0	
Spain 2004	4 · 6	median 22.0	range 12.0 – 69.0 90th-perc 43.0	
Greece 2004	4 · 3	median 19.5	range 12.0 – 39.0 90th-perc 37.1	
Overal SEU	60		90th-perc 46.9	

[1] Concentrations in digested, thickened and composted sludge
Median and 90th-percentile include samples with concentrations below d.l.

3.1.4.2.2 Concentrations in surface water, suspended matter and sediment

A summary of the concentrations in surface water is given in **Table 3.16** and **Table 3.18**.

Thirty surface water samples were taken along a stretch of 160 km in the river Ruhr in Germany. These samples were taken before 1994. The total concentrations were generally at a level of 0.4 μg/l, but higher levels were found where tributaries enter the main stream, up to 11.2 μg/l.. Under dry weather conditions, these tributaries are fed between 50 and 90% from effluents (Eschke et al. 1995a) and as much as 25% of the water in the Ruhr itself may be effluent water.

The influence of STP effluents is even more pronounced in the water system of the Berlin area (Heberer et al. 1999, 2001, Fromme et al. 2001a), where certain sections of the water system contain a very high proportion of effluents (60 to 97%). Samples were taken of canals, rivers and lakes upstream and downstream of sewage works outlets. The median concentration, measured after solvent extraction, in the sections with a high contribution of effluents was 1.48 μg/l HHCB (Fromme et al. 2001a). This is below the median effluent concentrations by a factor of 4. Similar data were reported by Heberer et al. (1999) for 30 representative sampling locations in the same area, despite the different methodology (solid-phase micro extraction). The median concentration in sections with low input of effluents is 0.05 μg/l (Fromme et al. 2001a).

In the River Elbe in Magdenburg, Germany, a monitoring programme was run from mid 1996 to mid 1997. Samples of river water and suspended matter were taken with one or 2 weeks intervals. The median surface water concentration was 0.09 μg/l (total) (Winkler et al. 1998). Other data reported for the Elbe by Bester et al. (1998) and Lagois (1996) show similar levels of total concentrations. Using SPME and GC/MS, the concentration of HHCB in 6 samples of the Elbe were 0.12 μg/l (Winkler et al. 2000).

The monitoring activities in the Elbe were continued and reported more extensively by ARGE (2000). Surface water samples were taken along the River Elbe starting from the Czech border going until the outflow to the sea 700 km downstream. During 1996-1997, 6 samples were taken with 2 months intervals on 7 sites along the River Elbe, including sites where two highly loaded tributaries join the river. Samples were also taken on 8 sites along the two tributaries in the region of Leipzig. Going downstream the concentrations of HHCB in the

surface water significantly increased after the entry of the two tributaries and then decreased again. The lowest concentrations were found in summer (range 0.03 – 0.21 μg/l) and were highest in winter (0.13 – 0.61 μg/l). On all sites the concentrations in winter were above the summer concentrations by a factor of 4. The concentrations in the tributaries in the Leipzig region were on the high side of this range. During 1998 and 1999, each month samples were taken of suspended matter on 10 sites along the river in a sedimentation chamber. For the analyses they were combined to two-months samples. The results along the river profile parallel the concentrations found in surface water, showing an increase from 0.03 mg/kg dwt to 0.22 mg/kg dwt decreasing again more downstream. Also here the high concentrations in the suspended matter are related to the contribution of the tributaries. The concentrations fluctuate during the year with the lower concentrations in summer. The overall range was given as 0.003 to 0.13 mg/kg dwt, median 0.06 mg/kg for the Elbe. The highest median concentration in the tributaries was 0.17 mg/kg with a maximum of 0.4 mg/kg. It was noted that the concentrations decreased in 1999 as compared to 1998 by 25 to 40% and in the tidal area the decrease was even 50 to 70%. On 4 sites in one of the tributaries (Saale) sediment samples were taken. The concentrations ranged from 0.045 – 0.21 mg/kg dwt (ARGE 2000). These studies were continued over the following years. The concentrations on suspended matter after 1998 seemed to be stable at a lower level; however, it was also remarked that the concentrations of HHCB in 1998/99 were too high due to an incorrect reference standard (Wiegel und Stachel 2003). After the flooding in September 2002 along the Elbe, 37 sediments samples were taken covering the entire river stretch. The concentrations ranged from < 0.001 to 0.160 mg/kg dwt with some peaks observed on the Czech stretch of the river (Stachel et al.2005).

In Hessen, Germany, surface water samples were taken on 20 sites in rivers in 1999 and 2000, once per year (HLUG 2001). The total concentrations ranged from 0.03 to 0.55 μg/l. The median was 0.22 μg/l for 1999 and 0.14 μg/l for 2000. From 1996 to 2000, concentrations in suspended matter were analysed once per year in 17 samples from rivers and in 12 samples from brooks with a high effluent input. The concentrations in the rivers ranged from 0.02 to 1.13mg/kg dwt, whereas the median decreased from 0.27 in 1996 to 0.15 mg/kg dwt. in 2000. The number of brooks sampled decreased from 11 in 1996 to 2 in 2000. Concentrations in the brooks containing a high fraction of waste water ranged from 0.40 to 13.7 mg/kg dwt. For three sites a series of data is available for four years. Here the concentrations decreased by a factor of 3.6 to 7. During the same period the sediment was also sampled in three brooks. These concentrations were always considerably lower than the concentrations measured in suspended matter from the same sites. In one brook samples in three consecutive years showed a decrease by a factor of 7.8. The trends observed in this monitoring programme in Hessen are illustrated in **Figure 3.5**.

In the project GREAT-ER (Geographic Referenced regional Exposure Assessment Tool for European Rivers) concentrations were measured along the Main river in Bayern, Germany, in May 1998. The median concentration was 0.1 μg/l (Klasmeier et al. 2001).

In Baden-Wüttemberg, Germany a monitoring programme was carried out along the Rivers Rhine, Neckar and Donau. Samples of suspended matter and sediment along the River Rhein (270 km) and Neckar (200 km) were analysed for HHCB in 1998 – 1999. The samples were extracted with cyclohexan and ultrasone treatment and analyses by GC/MS in SIM mode. In 27 out of 39 sediment samples (or 70%) HHCB was detected up to a level of 0.03 mg/kg dwt. The maximum sediment concentration was 0.11 mg/kg dwt. This particular sampling site showed to be an outlier in the Neckar where also the highest total organic carbon content was observed. The next lower concentration was lower by a factor of 4. In all of 117 samples of

suspended matter, HHCB was detected. The maximum concentration was 0.31 mg/kg dwt. The concentrations of organic carbon in the suspended matter ranged from 3 to 16% whereas for the sediment they ranged from 1 to 4.7%. In general the incidentally high concentrations correlate with low flow conditions in the rivers. Concentrations in 3 water samples were 0.2 – 0.4 μg/l (LfU-BW 2001). It was remarkable that the concentrations were always lower in summer than in winter as was also observed in the Elbe.

In 1999, in Nord-Rhein/Westfalen, Germany, samples were taken of river water and surface sediments on river banks on 19 sites on a longitudinal section along the River Lippe, tributary to the River Rhine. The median concentration in water was 0.1 μg/l. In general the concentrations were low (0.05 μg/l) in the upper reached of the river and they increase more downstream (0.18 μg/l) when the river reached the more densely populated area whereas in the river mouth the concentrations decreased again (0.08 μg/l). Parallel to this profile, the concentrations in the sediment were low in the upper reach of the river and increased after the river crossed the more densely populated region (range < 0.002 to 0.12 mg/kg dwt or < 1 to 15 mg/kg TOC). The maximum concentration was found at the mouth of the river, 0.19 mg/kg dwt (with 1.8% TOC converted to 10.6 mg/kg TOC), whereas on that site in a year 2000-sample the concentration on suspended solids was 0.50 mg/kg dwt (7.5% TOC), converted to 6.7 mg/kg TOC. This was not due to a local source but explained by the local favourable conditions for sedimentation due to low flow and the availability of terrestrial suspended matter for adsorption of contaminants. Based on analyses of the HHCB load (in $g.day^{-1}$) all over the river it was concluded that the decrease of the load caused by degradation and/or partitioning processes takes place on a short time scale of about 6 – 19 hours (Dsikowitzky et al. 2002).

These investigations were continued twice per year until March 2001 in a more generic screening programme in the Lippe River. The data for the concentrations in water per site were rather consistent in time (Dsikowitzky 2002). The overall median concentration was 0.08 μg/l, the 90^{th}-percentile was 0.17 μg/l. It was also observed that the load ($g.\ day^{-1}$) of HHCB was lower in summer than in winter, probably as a result of seasonally changing input or degradation (Dsikowitzky et al. 2004). In all sediment samples in the second to fourth campaign the HHCB levels were relatively low (< 0.0005 to circa 0.040 mg/kg dwt, Kronimus et al. 2004). However, when the data of Dsikowitzky et al. (2002) are combined with those of Kronimus et al. (2004), the concentrations on the sediment from the same sampling sites seem to be highly variable in time (e.g., from 191 to <0.5 μg/kg dwt within 6 months and up to 41 μg/kg dwt in 1.5 years). The levels seem to be determined more by the time of sampling than by the site. Therefore the absolute value of these analyses is to be considered as doubtful.

In the Netherlands, 67 samples of water and 28 suspended matter samples were taken from the rivers Rhine and Meuse at their point of entry into the country between 1994 and 1996. The analyses of the water samples included only truly dissolved material. Median concentrations were approximately 0.07 μg/l (Breukel and Balk 1996).

Samples of suspended solids were taken from representative main water courses in The Netherlands in 1998. The median concentration was 0.01 μg/l (recalculated from concentrations in suspended matter from Rijs and Schäfer 1998).

In a research project concentrations in 14 surface water samples in The Netherlands and Belgium were estimated from 'biomimetic extractions'. Median concentrations (dissolved) were 0.03 μg/l (Verbruggen et al., 1999).

In the period 2001-2003 concentrations were determined in the rivers Rhine and Meuse in The Netherlands. In the Rhine 8 samples were taken on three different locations. The analytical procedure was specific for synthetic musks. The median concentration was 0.009 µg/l, whereas the 90th-percentile as well as the maximum was 0.015 µg/l (Geerdink en Schrap 2004). On six locations on the Meuse, weekly samples were taken throughout 2002/2003 (circa 270 samples). HHCB was detected only at the border where the Meuse enters into the country and only at the start of the year 2003. The 90th-percentile was 0.01 µg/l, maximum 0.127 µg/l. Here the method used for quantification was not reliable for HHCB, as acknowledged by the authors (Jeukens and Barreveld 2004, Kalf and Berbee, 2005).

In Switzerland 20 samples were taken from the River Aare and its tributaries and lakes in 1998. The median total concentration was 0.06 µg/l (BUWAL 1998). Median concentrations in other Swiss rivers and a lake were at the same level (Schmid 1997 pers. comm. cited in OSPAR 2000). These levels are below the concentration found in a sample taken in the River Glatt, downstream of a STP (Müller et al. 1996).

In Switzerland in the Kanton of Basel surface water samples were taken between January and April 1997. The concentrations as determined by SPME ranged from 0.01 to 0.26 µg/l. The median concentration was 0.08 µg/l (Noser et al. 2000).

Surface water was sampled at several lakes and rivers in the Swiss midland region, a small mountain lake (Murgsee, altitude 1820 m and in the Mediterranean Sea. The samples were taken during 2000 and 2001. Following solid phase extraction, they were analysed by GC-MS or SIM. The concentrations in the lakes ranged from 3.8 to 23 ng/l. A higher level was found in one lake in the watershed area of the River Glatt, 36 to 47 ng/l. In the small mountain lake, HHCB was not detected (< 2 ng/l). Levels were higher in the rivers and streams, ranging from 5 ng/l upstream of an STP to (maximum) 560 ng/l in a small river downstream of an STP. A vertical concentration profile was produced for the Zürichsee between 1 and 130 m depth in March, May and July 2001. In March, during circulation of the lake, HHCB was more or less uniformly distributed (ca. 20 ng/l). In July significantly lower concentrations were observed in the epilimnion of the lake, showing that elimination takes place near the water surface. HHCB was not detected in the samples taken in the Mediterranean Sea at -5m and -150 m (Southern Spain; one sampling point was water primarily from the Atlantic Ocean) (Buerge et al. 2003).

The concentrations in a German lake and in the German and French river were 0.04 and 0.06 µg/l, whereas in the Spanish river the concentration was 0.4 µg/l (showing a high effluent load). HHCB was not detected in drinking water (Mitjans and Ventura 2004).

Streamwater was collected in Pennsylvania, USA and two other locations. HHCB was detected in 47% of the samples (n=19). The mean concentration ± s.d. was 5.9 ± 16 ng/l. The concentrations ranged from below d.l. to 67 ng/l (Standley et al. 2000).

Surface water was sampled in Lake Mead, Nevada, USA, in 2001 twice during 7 months. The concentrations ranged from 0.00006 to 0.001 µg/l (Osemwengie and Gerstenberger 2004). The lower concentrations were observed in summer and the higher in winter, as was also observed in the Elbe by ARGE (2000).

Surface water samples were taken in the San Francisco Estuary in the dry seasons (July) of 1999 and 2000. Whole water samples were taken at 1 meter below the water surface. The sample extract fractions of the two years were combined to get representative samples of five different regions in the Bay area. The concentrations in the water were highest in the sewage impacted South Bay area, 0.131 µg/l. In the other regions the levels were 0.003 to 0.028 µg/l,

whereas HHCB was not detected in the open ocean background site approximately 2 miles offshore (Oros et al. 2003).

The surface water of Lake Michigan, USA, was sampled during June 1999 and May 2000. HHCB was detected in 92% of the samples above the blank levels and it was found primarily, for 99%, in the dissolved phase. The concentration in the dissolved phase was 0.0047 ± 0.0025 µg/l (Peck and Hornbuckle 2004). Water samples were taken from 23 stream locations situated upstream and downstream of 10 to 14 cities in IOWA, USA. A total of 76 samples were taken during high, normal and low streamflow conditions during 2001. The maximum concentration of HHCB during low-flow conditions was 0.26 µg/l. The concentrations were lower during normal and high-flow conditions (Kolpin et al. 2004).

In California, USA, agricultural fields are irrigated with disinfected tertiary recycled wastewater or streamwater predominantly consisting of wastewater effluent. The recycled wastewater had undergone activated sludge treatment with extended aeration, sedimentation, nitrification/denitrification, sand filtration and chlorination. Six fields were sampled during the dry and wet weather over the course of two crop seasons. HHCB was identified in the water phase from two fields (0.4 – 1.3 µg/l, Pederson et al. 2003). Marine water samples were taken at seven stations in the German Bight of the North Sea from a depth of 5 m in the summer of 1990 and 1995. The concentrations decreased with the distance from the mouth of the Elbe. The highest concentration was 0.004 µg/l, the median concentration (total) was 0.00022 µg/l (Bester et al., 1998).

In Japan samples were taken along a stretch of the Tamagawa River. Upstream total concentrations were at the 1 ng/l level whereas further downstream concentrations ranged between 0.08 and 0.1 µg/l (Yun et al. 1994).

Table 3.18. Concentrations in sediment and suspended solids

Location	n	HHCB [mg/kg dwt]	Reference
Dutch borders	14	median 0.06 range 0.05 (d.l.)-0.16	Breukel and Balk, 1996
Suspended matter Rhine 1994-1996			
Suspended matter Meuse 1994-1996	14	median 0.20 range 0.05-0.58	
90th percentile Rhine and Meuse 1994-1996	28	0.31	
NL surface waters 1997-1998	24	median 0.10 90th perc. 0.41	Rijs and Schäfer 1998
Suspended matter		max. 1.8	
Germany, Elbe 1996-1997	31	median 0.44 range 0.15-0.74	Winkler *et al.,* 1998
Susp. particulate materials		90th perc. 0.61	
Germany, Elbe, suspended matter	82	median 0.06 range 0.003 – 0.22	ARGE 2000
1998 - 1999			
and tributaries	12	median 0.17 range 0.07 – 0.40	
	12	median 0.14 range 0.0.03 – 0.37	
	12	median 0.10 range 0.06 – 0.31	
Germany, Berlin area	19	*low effluent input*: median < d.l., 90-perc. < d.l.	Fromme et al., 2001a
1996-1997	20	*moderate effl input:* median 0.23, 90-perc. 0.38	
Sediment (10 cm depth)	20	*high effluent input:* median 0.91, 90-perc. 1.90	
Germany, Berlin Teltow Canal	4	range < d.l. to 0.34	Schwarzbauer et al. 2003

Location	n	HHCB [mg/kg dwt]		Reference
1998 – 1999				
and core 1985 - 1990				
Germany, Berlin Teltow Canal	9	median 0.22	range < 0.1 – 0.46	Blok et al. 2005
Dec 2003				
Sediment (0-5 cm)				
Germany, Hessen				HLUG 2001
Suspended matter 1996	11	median 0.27	range 0.08 – 1.13	
1997	12	median 0.25	range 0.05 – 0.85	
1998	12	median 0.23	range 0.05 – 0.78	
1999	16	median 0.16	range 0.02 – 0.39	
2000	15	median 0.15	range 0.02 – 0.39	
Suspended matter in contaminated brooks, 1996	11	median 2.7	range 0.90 – 13.7	
1997	5	median 1.8	range 1.7 – 5.4	
1998	5	median 2.5	range 1.3 – 5.2	
1999	3	range 0.4 – 2.5		
2000	2	range 0.7 – 1.1		
Sediment in contaminated brooks, 1996 - 1999	5	range 4.9 - 0.6 **		
Germany, Elbe, susp. matter, 1997 (Hamburg-Dresden)	9	mean 0.101	range 0.016 – 0.180 *	Wiegel, cited in Fooken 2004
Germany Niedersachsen, sediment 5 rivers, 1996	8	median 0.007	range <0.0005 – 0.054 *	(Lach and Steffen 1997 cited in Rimkus 1999)
Germany Nord-Rhein/Westfalen, sediment Lippe, 1999	19		range < 0.002 – 0.19	Dsikowitzky 2002
2000 – 2001	27	median 0.001	range <0.0005 – 0.056	Kronimus 2004
9 common points, 4 times 1999-2001	35	median < 0.002	90th-perc. 0.10	
Suspended solids,1999	1	0.50		Dsikowitzky 2002
Germany Baden-Württemberg, sediment Rhein, Neckar,Donau, 1998 - 1999	39	median 0.006	range <0.005 – 0.110	LfU-BW 2001
Suspended matter, 1998-1999	107		range <0.005 – 0.310	
Austria, Donau sediment	7		range < 0.001 – 0.005	Scharf et al. 2004
Tributary sediment	1	0.052		
Donau suspended solids	2		range 0.004 – 0.011	
Sediment				Biselli et al. 2005
German Bight 1997	3	<2, 6, 10		
North Sea 2000	3	< 3		
Baltic Sea 2001	4	n.d.		

* according to Fooken (pers. comm. and Fooken 2004). These values probably need to be corrected by a factor of 0.5 due to the purity of the standard (50%).

** downward trend

Concentrations in suspended matter and river sediment are presented in **Table 3.18**.

In 1994-1996, concentrations were determined in 28 samples of suspended matter in the Rhine and Meuse at their point of entry in The Netherlands. Median concentrations were 0.06 and 0.20 mg/kg in the Rhine and Meuse, respectively (Breukel and Balk, 1996). Levels in suspended matter in 24 samples taken from the main surface waters in The Netherlands (Rijs and Schäfer 1998) were similar to levels in the Rhine.

Samples of suspended material were taken from the Elbe near Magdeburg in Germany during one year at weekly or 2-week intervals in 1996-1997. The median concentration was 0.44 mg/kg dwt (Winkler et al. 1998) which is similar to the levels in the Meuse, but with considerably lower variability.

Following these observations on suspended matter of Winkler et al. (1998), HHCB was also included in the following investigation. In the state Saxony-Anhalt, Germany, transects were made through the floodplain of the River Elbe, from the dike to the riverbank and on 6 positions showing elevated concentrations of heavy metals, the depth distribution (to 1.5 m) of other contaminants was investigated. The concentrations of musk fragrances (in 'dry soil') were only in the range of the blank, 0.01 to 0.02 mg/kg dwt (Witter et al. 2003).

In Germany, 59 sediment grab samples were taken from the central bed of the rivers in the Berlin area. The levels were linked to the concentrations found in the surface water. Concentrations ranged from below the detection level (0.03) to 2.2 mg/kg dwt. The observed high variability in the areas with high contributions of STPs was attributed to inhomogeneous sample materials (Fromme et al. 2001a). In a total of 28 surface sediment samples from the Havel and Spree River representing the sedimenting record from 1979/1980 to 1994 was screened for the presence of a large number of organic pollutants. The median TOC content was 10.4%, the 90th-percentile was 14.7% (ranged 0.9 – 55.6%). HHCB was detected in all samples but not quantified. The sum of AHTN and HHCB in a sediment core in the Teltow Canal, representative of approximately 20 years, was 0.2 mg/kg (Ricking et al. 2003a).

The Teltow Canal in Berlin was recognised as a particular area were high levels were found in the water as well as in the sediment. Four sediment samples were obtained from three locations, including one sample core representing current time (1998-1999) and an older accumulation time between 1985 and 1990. The concentrations were determined in the extractable fraction as well as in the non-extractable fraction after a separate application of different chemical degradation procedures. The concentrations ranged from below detection level to 0.41 mg/kg dwt in the extractable fraction and from below detection level to 2.5 mg/kg in the different non-extractable fractions. The total organic content of the samples varied between 7.1 and 12.1% (Schwarzbauer et al. 2003).

In 2003 samples were taken again on seven different places in the Teltow Canal (this was included in the high effluent input area of Fromme et al. 2001a. A core was taken deep frozen so a stratified sample of the sediment was obtained. The top 5 cm-layer was analysed and samples were also taken from the 5-7 cm-layer and the 7–12 cm-layer on one site. The sediment samples were taken in the middle of the canal, 0.1 km upstream of the STP Wassmansdorf and 0.5, 1.0 and 1.5 km downstream. Other samples were taken 1 km downstream of the STP Ruhleben, 1 km downstream of the STP Stahnsdorf and at the entrance to the Havel River. The samples were freeze dried. The freeze dried samples were extracted by Accelerated Solvent Extraction (ASE) with a first Silicagel purification on line, followed by Dichloromethane extraction at 2000 psi. Extracts were treated for clean-up with an SPE column (details of extraction and GC/MS analysis in Bonnet et al. 2003 and Otten and

Meijer, 2004). There was a clear relation with the distance to the STP: the OC content as well as the concentrations of HHCB and other substances increased right after the point of discharge (from 0.26 to 1.1 mg/kg dwt) and decreased after 1 and 1.5 km (from 0.2 to < 0.1 mg/kg dwt) downstream. The content of organic carbon ranged from <1 to 14% (median 5%). Overall the concentrations in the sediment samples ranged from < 0.1 to 0.89 mg/kg dwt (Blok et al. 2005).

In the Austrian section of the River Donau between the borders with Germany and Slowakia the sediment was sampled at seven sampling points in August 2001. An additional sediment sample was taken in the confluent with a major tributary, the River Schwechat. The concentrations in the sediment samples in the Donau ranged from < 0.001 – 0.005 mg/kg dwt, whereas with 0.052 mg/kg dwt the level in the tributary was higher. In two samples of the suspended matter taken in the upper section of the Donau the concentrations were 0.004 and 0.011 mg/kg dwt (Scharf et al. 2004).

Furthermore, data were cited by Rimkus from other sources: In 1997, 9 suspended matter samples were taken from the River Elbe (Hamburg – Dresden), probably by a sedimentation chamber. The mean concentration was 0.1 mg/kg HHCB. In Niedersachsen sediment 8 samples were taken from 5 rivers in 1996: from Elbe, Weser, Ems each 2 samples, from Leine and Oker, each 1 sample. The median concentration was 0.07 mg/kg [Lach & Steffen 1997, cited in Rimkus].

A study on bio-assay directed fractionation of organic extracts of marine surface sediments from the North and Baltic Sea (Biselli et al. 2005) report HHCB concentrations of <2, 6 and 10 mg/kg dwt in sediments from the German bight (samples from 1997). In other North Sea sediment samples and Baltic Sea sediment samples HHCB levels were below <3 mg/kg dwt or below detection limit, respectively.

3.1.4.2.3 Trends in time

In Hessen, Germany, samples of sludge were taken once per year from 9 STPs over a 5-year period (1996-2000). During this period, samples of suspended matter were also taken from 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 2 to 3, see **Figure 3.5** (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.

Concentrations on sludge are directly reflecting the decreasing input of 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. **Figure 3.6** and Figure 3.7 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 3.5**). 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 this case the observed concentration drop suggests that degradation processes take place in the sediment without delay.

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 (as discussed in Section 3.1.4.1.3).

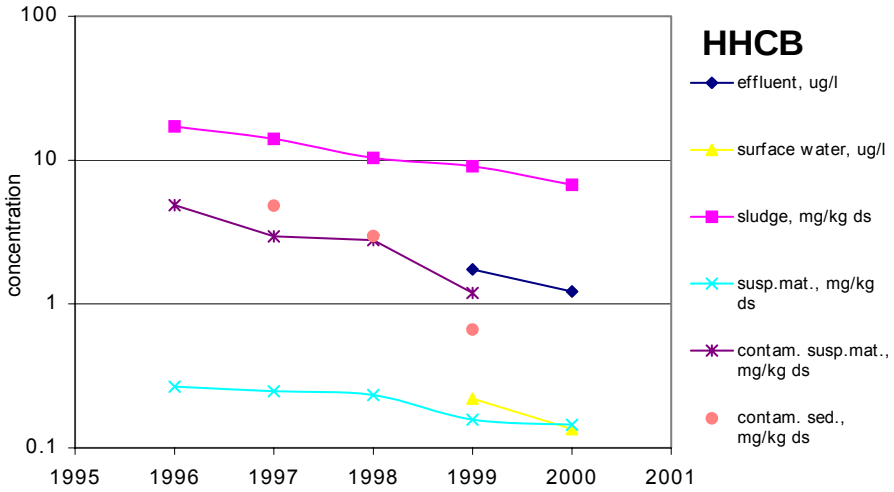


Figure 3.5. Trend in the median concentrations of HHCB in various environmental compartments, Hessen, Germany (based on data from HLUG 2001) (Remark: Contaminated suspended matter: mean for 3 sites; contaminated sediment: only one site. Logarithmic scale).

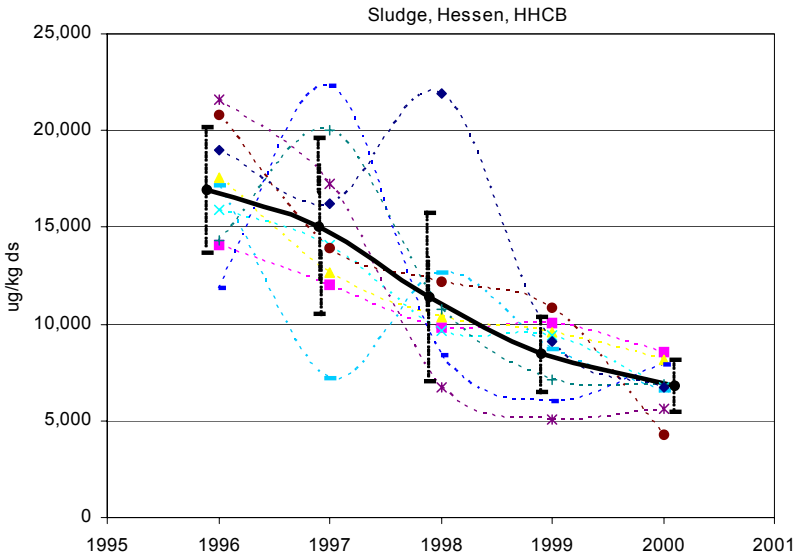


Figure 3.6. Trend in the concentrations of HHCB in wasted sludge from 9 domestic STPs, Hessen, Germany (HLUG 2001). The solid line connects the median per year. *Standard deviations are included.* Please note that each data point is one observation per STP per year. They are connected with a dotted line to show the individual STP, not to suggest a trend. The difference between the years 1996-2000 was statistically significant (t-test, $\alpha = 0.01$, $P < 1E\text{-}06$).

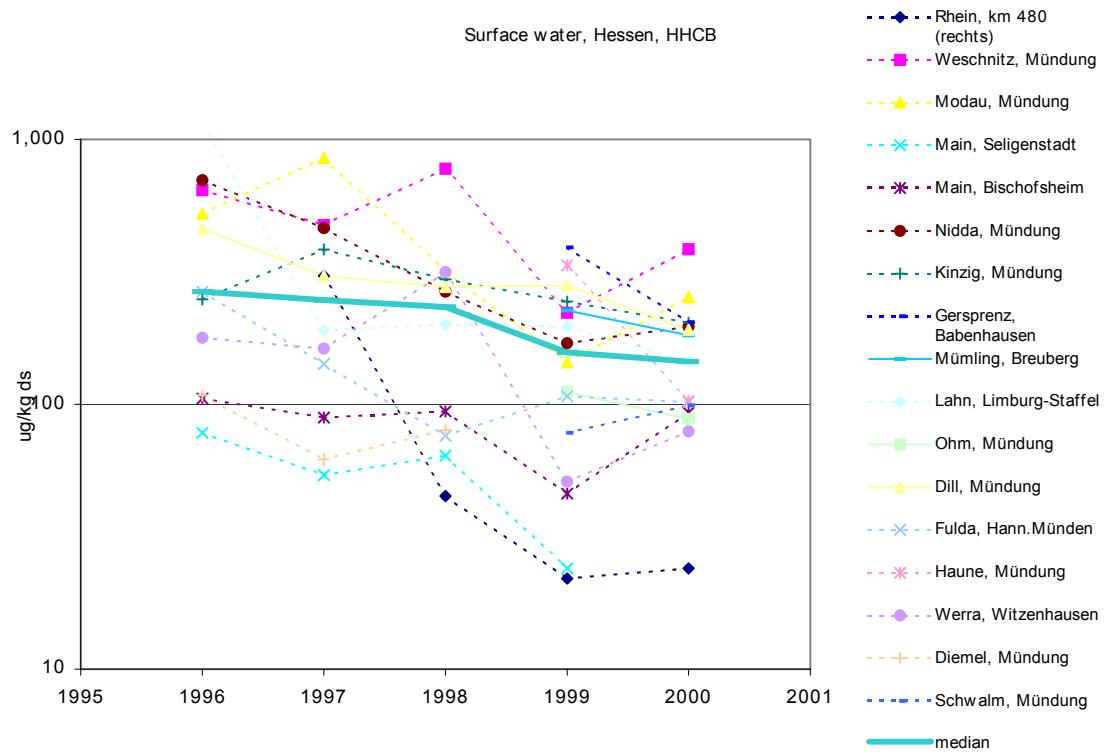


Figure 3.7. Concentrations of HHCB in suspended material in water systems in Hessen from 1996 – 2000 (based on data from HLUG 2001). Please note that the points represent one sample per year. Per river the points are connected by dotted lines; these are not suggesting the trend between the sampling periods. (Logarithmic scale)

A similar trend is observed in the concentrations in the STPs along the Teltow canal and the receiving compartments. Concentrations measured in the 1996-1997 were relatively high and stimulated further research. The results are summarised in **Table 3.19**. Between 1996/97 and year 2004 the concentrations in effluent and in sediment have decreased by a factor of 3 to 4. Data for sludge are from 2000 and the concentrations in 2004 did not deviate from 2000.

This decrease is also observed in other regions, but not documented as completely. Therefore the year of sampling is an important piece of information of a sample.

The downward trend is also reflected in biotic samples, see section 3.1.7.2.

Table 3.19. Decrease of the concentrations in samples from STPs along Teltow Canal and sediment, Berlin (source: Heberer 2002, Fromme et al. 2000, Fromme et al. 2001a, Blok et al. 2005)

		1996/1997	2000	2004	reduction factor
Effluent µg/l	median 90th-perc. max	6.7 10.8 13.3		1.6 2.1	4
Sludge mg/kg dwt	median 90th-perc. max		8.3 11.5	10.2 12	1
Sediment mg/kg dwt	median 90th-perc. max	0.9 1.9 2.2		0.26 1.1	3.5

3.1.4.3 Comparison between predicted and measured levels

When comparing measured concentrations with predicted concentrations, it should be realised that:

- measured concentrations in water are sometimes reported as total, sometimes as dissolved concentrations. However, with a suspended solids concentration of 10 mg/l in surface water, the fraction sorbed is less then 10%, and in general this source of variation is considered negligible;
- measured concentrations vary on a spatial scale, where higher concentrations are clearly related to the discharge of STP effluents into surface water;
- measured concentrations in Hessen, Germany, show a decrease in time, 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 of sample and in comparison with a prediction it determines the scenario to which it should be compared;
- environmental data are highly variable. Simply taking the highest value does not reflect a representative exposure. Therefore the data are summarised in median and 90th-percentile values if possible in boxplots where the source of the data and periods of sampling can be taken into account. For a conservative comparison, the 90th-percentile of the data is compared to the predicted concentrations.

For a proper comparison, the PEC based on use volumes for the year 2000 should be compared to concentrations measured in recent monitoring campaigns. When values from older studies are considered, it should be kept in mind that concentrations may have decreased by a factor of 2 to 4 since 1996. The aim of this section is, in fact, to identify the PECs that should be taken along in the risk characterisation section.

3.1.4.3.1 Influent

The comparison of predicted concentrations and those measured in influents is limited to the more recent data, starting from the year 2000. A large number of observations for HHCB in STP influents is reported from Germany and there are some from other European countries, for example The Netherlands, Switzerland, Austria, Spain and the UK. Influent concentrations as predicted by EUSES are taken from **Table 3.14**. The predicted influent concentrations in the scenarios for northern and southern European countries were based on the calculations where the release factor was reduced to obtain the observed effluent concentrations. **Table 3.20** shows that (1) the predictions by the TGD regional (10%) scenario are too high by almost two orders of magnitude and (2) that the predicted influent concentrations for Southern EU (SEU-15) and Northern EU (NEU-15) are just above the observed concentrations.

Table 3.20. Comparison of measured and predicted concentrations for STP influent (µg/l)

Scenario	Predicted influent STP, µg/l	Observations, location	n	Measured concentration influent	Reference
TGD regional (10%)	391				
southern EU	25.4 (in scaled predictions)	UK 2001	13	mean 11.7, 7.8 – 19.2	Kanda 2003
		Spain 2001-2002	3	2.1 – 3.4	Carballa 2004

Scenario	Predicted influent STP, µg/l	Observations, location	n	Measured concentration influent	Reference
		Spain 2003	1	2.2	Mitjans 2004
northern EU	6.7 (in scaled predictions)	Switzerland 2002	14	mean 4.5 – 6.9	Brändli 2002
		The Netherlands 2001	4	median 3.4, max 4.3	Artola 2002
		Germany 2002	5	1.4 – 2.3	Bester 2004
		Germany 2003	2	2.5 – 4.6	Fahlenkamp 2004
		Germany 2000	42	0.25 – 4.9	Müller 2002
		Austria 2000	7	0.14 – 3.2	Kreutzinger 2004

3.1.4.3.2 Effluent

Concentrations measured in the effluent of STPs were reported for larger campaigns in Germany, The Netherlands, Switzerland, Austria, Spain, Italy and Greece and from the USA, see **Table 3.16**.

The estimations from the TGD regional (10%) scenario (see **Table 3.13**) predicted PECeffluent = 87.5 µg/l. For the Northern EU-15 Scenario the recent data for Germany (Hessen HLUG 2001, Müller et al. 2002, Fahlenkamp et al. 2004, Bester 2004, Dsikowitzky 2002, and Blok et al. 2005) were used as the start of the calculations for the Northern EU-15 Scenario (90^{th}-percentile, 1.4 µg/l) whereas the data for Italy, Spain and Greece were the basis for the calculations of the Southern EU-15 Scenario (90^{th}-percentile, 4.7 µg/l, Blok et al. 2005).

The results of larger recent campaigns are summarised in a boxplot in **Figure 3.8**. For each country the boxplot shows the median, the interquartile range, outliers and extremes (values between 1.5 and 3 boxlengths or more then 3 boxlengths from the upper edge, respectively). The boxplot in the figure shows that the value of 4.7 µg/l used in the SEU-15 Scenario is higher than the levels in the other countries. It suggests that only Switzerland and Germany suit the Northern EU-15 Scenario for HHCB.

When the level of 4.7 µg/l used for the Southern EU-15 Scenario is compared to the other data listed in **Table 3.16**, some studies report observations above this level, e.g., reports from Germany on the Elbe, Sachsen-Anhalt and Berlin. However, all these studies were carried out before the year 2000 and later studies have shown that concentrations in those regions had decreased significantly. Recently reported data from Austria and Sweden are also in line with the Northern EU-15 Scenario, see **Table 3.16**. The maximum of 9.4 µg/l observed in Spain (considered as an outlier in **Figure 3.8**) is above the 90^{th}-percentile of Southern EU-15 by a factor of 2.

For the risk characterisation the Southern EU-15 Scenario is used:

PECeffl = 4.7 µg/l (total).

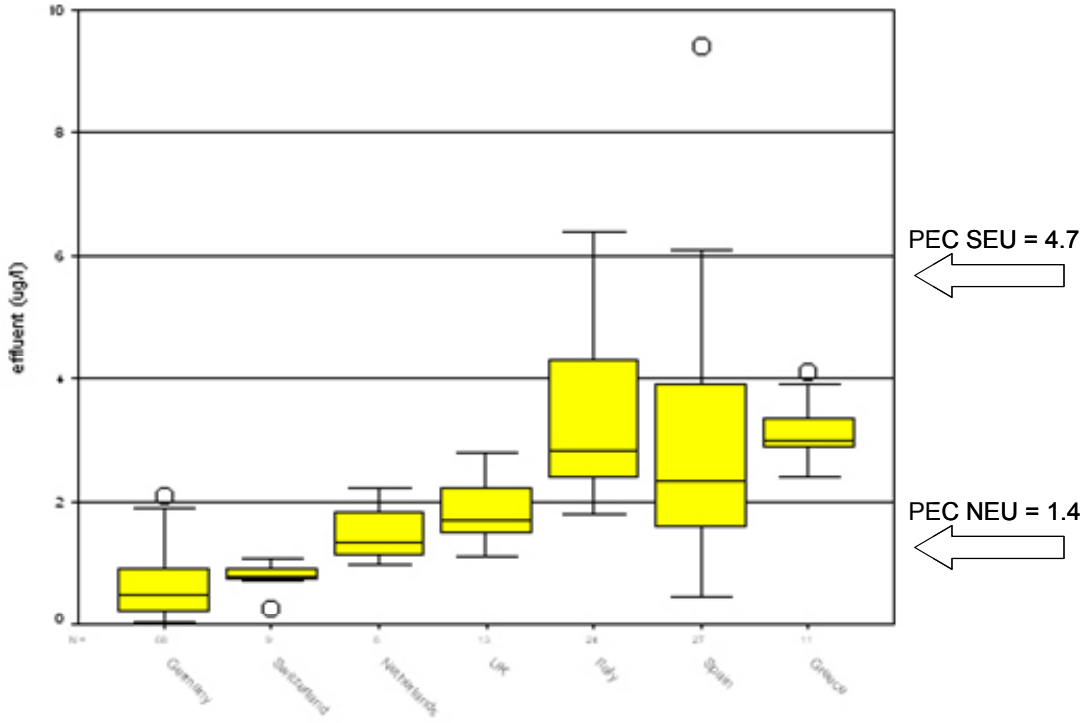


Figure 3.8. Concentrations in effluents in recent campaigns (Source: HLUG 2001, Müller 2002, Blok et al. 2005, Brändli 2002, Mitjans 2004, Simonich 2002, Artola 2002)

3.1.4.3.3 STP sludge

Concentrations measured in sludge were reported from The Netherlands, Germany, Switzerland, UK, the Nordic countries Denmark, Sweden, Norway, Finland, Iceland and a from southern countries Greece, Spain and Italy, see **Table 3.17**.

The concentration in sludge predicted by the TGD regional (10%) scenario = 665 mg/kg dwt. For the Northern EU-15 Scenario the calculations were made using the 90th-percentile of recent data for Germany: 11.5 mg/kg dwt (HLUG 2001, Heberer 2002, Müller et al. 2002, Friedrich et al. 2004, Bester 2004, Blok et al. 2005) and for the Southern EU-15 Scenario, the overall 90th-percentile of the results in Spain, Italy and Greece was used, 46.9 mg/kg dwt (Blok et al. 2005).

The boxplot in **Figure 3.9** summarises the results of recent campaigns. The boxplot shows that the value of 47 mg/kg dwt is above the levels in all other countries. It also shows that only Germany, Finland and Iceland belong to the Northern EU-15 Scenario, whereas in all other countries (Norway, Sweden, Denmark, Switzerland, and the southern countries) the concentrations are higher so they belong to the Southern EU-15 scenario. When the other data in **Table 3.17** are considered, it is clear that the results from older studies in Germany were above 11.5 mg/kg dwt and in other countries they were only occasionally above 47 mg/kg.

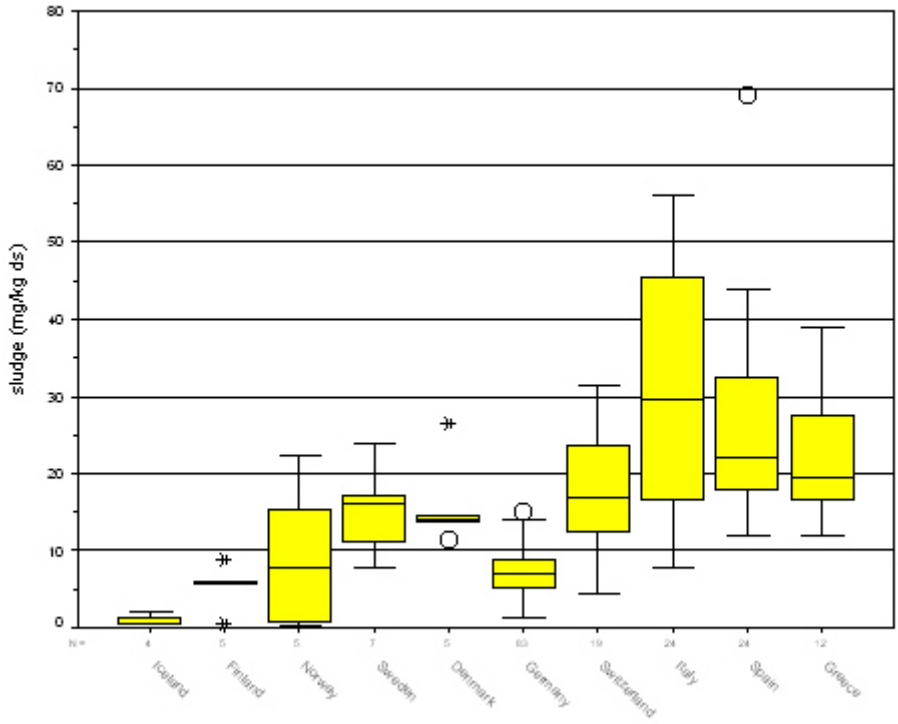


Figure 3.9. Concentrations in sludge in recent campaigns (Source: HLUG 2001, Müller et al. 2002, Blok et al. 2005, Heberer 2002, Kupper 2003a, Friedrich 2004, Mogensen et al. 2004)

It should be remarked that when sludge is applied to soil, fluctuations in sludge concentrations from the same source are smoothed and therefore the median concentration in the wasted sludge is relevant for the calculation of PECsoil. However, in the present calculations the 90th-percentile was used in accordance with the EU-TGD (EC 2003).

3.1.4.3.4 Surface water

Concentrations were measured in a large number of rivers and brooks, lakes and canals in Germany (Ruhr, Elbe including its tributaries, Main and tributaries, Lippe), in The Netherlands (Rhine, Meuse and other main waterways), Switzerland (Aare and tributaries, Basel area, major lakes in midland region and mountains) and outside Europe in Southwest USA, Lake Michigan, Iowa and the San Francisco Bay area and in Japan. The samples are not randomly distributed over the EU-15+2. The results of the monitoring campaigns are summarised in a graph in **Figure 3.10**. The graph shows the median (top of column), 90^{th}-percentile (dash) or maximum (triangle) of the data reported in **Table 3.16**. The higher values observed in surface water are clearly related to the discharge points of treated sewage and they are to be compared to $PEClocal_{water}$. In remote areas the HHCB concentration will have been decreased by losses process and dilution, so concentrations in larger rivers are lower, in between the PEClocalwater and PECregional.

The figure also includes the levels of the predicted local concentrations:
$PEC_{TGD\ regional\ (10\%)}$ = 8.7 μg/l, PEC_{SEU} = 0.49 μg/l and PEC_{NEU} = 0.14 μg/l.

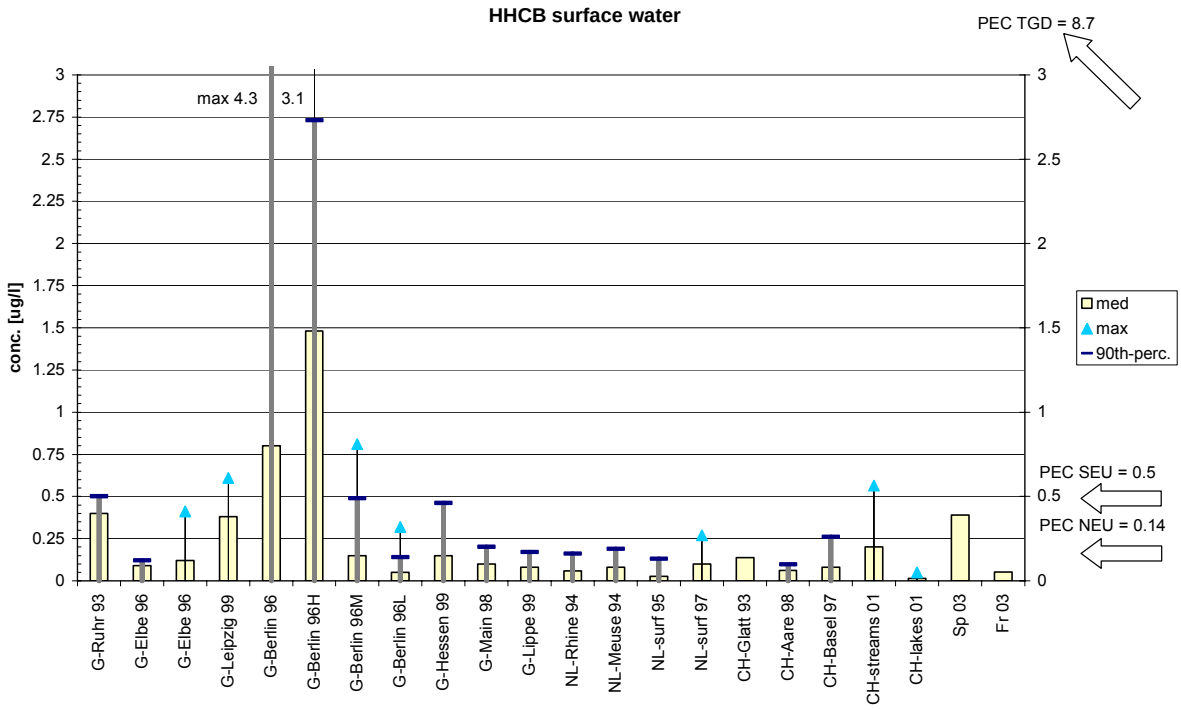


Figure 3.10. Summary of the measured concentrations; median, 90th-percentile and/or maximum

The major part of the samples was taken in Germany and they cover a period of more than a decade. During the past ten years the use of polycyclic musks has decreased and this is reflected in lower levels in the surface water, although it should be remarked that the number of recent monitoring campaigns in surface water is relatively low. The observation is, however, supported by the lower recent *effluent* levels reported in the previous section (3.1.4.3.2). The picture confirms that most recent levels in Germany are at or below the NEU-15 Scenario. Exceptions are the older data from Germany, in particular from the area in Berlin with high effluent input. Since presently the effluent concentrations in the STPs in Berlin have dropped (section 3.1.4.2.3), it is expected that overall the surface water concentrations will have decreased proportionally.

The NEU-15 Scenario predictions are based on effluent concentrations recently measured in Germany by applying a dilution factor of 10. It is concluded that in general these predictions are at the same level as the most recent values in Germany. As the SEU-15 Scenario is also based on recent effluent concentrations, it is concluded that the SEU-15 Scenario is acceptable except maybe for places with a lower dilution potential than the default factor of 10.

For the risk assessment the Southern EU-15 Scenario will be used:

$PEClocal_{water}$ = 0.49 µg/l.

The 90th-percentile of the surface water samples in the high effluent input area in Berlin (1996/1997) was 2.73 µg/l.

3.1.4.3.5 Sediment

Concentrations measured in sediment are reported from Germany, viz. the Elbe, the Berlin area, for contaminated brooks in Hessen, rivers in Niedersachsen, the Lippe, Baden-Würtemmberg. In addition there is one report for sediment taken from the Donau. The predictions of the concentration in sediment are based on the concentrations observed in current effluents and sludge in Germany for the northern EU-15 Scenario: $PEClocal_{sediment}$ = 0.075 mg/kg wwt equalling ~ 0.35 mg/kg dwt. The levels in the contaminated brooks in Hessen in 1996-1998 were clearly above this level as well as the levels from the areas in Berlin highly influenced by effluents in 1996/1997. Later observations in the programme in Hessen showed a clear decrease. A specific study set up to verify the high concentrations in the sediment of the Berlin Teltow Canal (December 2003) also showed that the concentrations had decreased and are around the level of the SEU-15-$PEC_{sediment}$. The other studies report sediment concentrations that are below 0.2 mg/kg dwt, see **Table 3.21**. Thus the predictions based on the current effluent and sediment concentrations predict the current sediment concentrations relatively well. Therefore the sediment concentrations predicted based on effluents and sludge concentrations measured in the Southern EU-15 can be used for the risk assessment:

$PEClocal_{sediment}$ = 0.262 mg/kg wwt ~ 1.21 mg/kg dwt.

The maximum of the concentrations measured in the Teltow Canal in Berlin (2003) is 1.1 mg/kg dwt.

Table 3.21. Comparison of measured and predicted concentrations for sediment

Scenario	$PEClocal_{sediment}$ mg/kg		Measured concentrations in sediment mg/kg dwt	Reference
	wwt	dwt		
TGD regional (10%)	4.73	12.3		
southern EU-15	0.261	1.21		
northern EU-15	0.075	0.35		
			Germany Hessen, contam. 1996 – 1998 n=5 per year range 0.6 – 4.9 Germany Hessen, 1999 – 2000 n=5 per year contam. 0.6	HLUG 2001
			Germany, Berlin area 1977 n=59 low input: median < d.l. 90% < d.l. moder. input: median 0.23, 90% 0.38 high input: median 0.91, 90% 1.90	Fromme 2001a
			Germany, Berlin Teltow Canal 2003 n=9 range < 0.1 – 1.1	Blok et al. 2005
			Germany, Niedersachsen, 1996 n=8 range < 0.0005 – 0.054	Lach and Steffen 1997 cited in Rimkus 1999
			Germany Lippe 1999-2001 n=35 median <0.002, 90-perc. 0.10, max 0.19	Dsikowitzky 2002, Kronimus 2004
			Germany, large rivers Baden-Württemberg 1998-1999 n=39 median 0.006, max 0.110	LfU-BW 2001
			Austria Donau range < 0.001 – 0.005, and 0.052 n=8	Scharf 2004

3.1.5 Terrestrial compartment

The exposure of the terrestrial compartment follows from atmospheric deposition and application of sludge on agricultural land. The following scenarios are used:

- production of HHCB on one site;
- fragrance compounding for the sites described in section 3.1.2.2.1 and for a generic medium/large and a small site;
- end product formulation by a generic large and small formulator (see section 3.1.2.2.2);
- private use in northern and southern EU-15 countries, see section 3.1.2.4.

During production and compounding, no waste is discharged directly to the soil compartment. The wasted sludge produced in the industrial wastewater treatment plant of the production site and on site 4 is treated in an incinerator. On site 6 no waste water is produced.

3.1.5.1 Calculation of PEC_{local}

For the industrial sites discharging to a municipal STP, the concentration of HHCB in sludge is given in **Table 3.11** and **Table 3.12**. The concentration in sludge due to private use is given in **Table 3.14**. **Table 3.22** presents the concentrations in sludge for the scenarios as well as the estimated concentrations in soil.

Table 3.22. Local PECs for the terrestrial compartment.

Scenario	Csludge, mg/kg	$PECsoil_{local}$ mg/kg wwt, 180 d	$PECsoil_{regional}$ mg/kg wwt	$PECgroundwater_{local}$, µg/l
Site				
Production	15.1	0.0184	0.00026	0.0421
Compounding Site 1	68.5	0.0832	0.00026	0.191
Compounding Site 2, 2000	23.7	0.0288	0.00011	0.066
Compounding site 2, 2006	5.78	0.007	0.00011	0.0161
Compounding Site 3	19.6	0.0238	0.00011	0.0545
Compounding Site 4	<143.7>	--	--	--
Compounding Site 5	0.25	0.335 E-03	0.00026	0.0077
Compounding Site 6				
Compounding Site 7 (Large-medium generic)	31.8	0.039	0.00026	0.089
Compounding Site 8 (Small generic)	12.9	0.0157	0.00026	0.036
Formulation Large company	18.5	0.0225	0.00026	0.052

Scenario	Csludge, mg/kg	$PECsoil_{local}$ mg/kg wwt, 180 d	$PECsoil_{regional}$ mg/kg wwt	$PECgroundwater_{local}$, µg/l
Formulation Generic scenario	37.0	0.045	0.00026	0.103
Consumer use				
TGD regional (10%)	665	0.808	0.0053	1.85
southern EU-15	46.9	0.057	0.00026	0.131
northern EU-15	11.5	0.014	0.00011	0.032

3.1.5.2 Measured levels

Few data are available on concentrations measured in sludge amended soils.

An average sludge application rate on a field in Georgetown, DE was 7000 wet gallons per acre (or 0.6 to 1.1 kg sludge per m^2). The HHCB concentration in sludge was in the order of 86 mg/kg dwt. With a ploughing depth of 15 cm the expected initial concentration would be 0.22 mg/kg dwt. The concentration measured immediately after application was 0.07 mg/kg dwt and it had dropped below the quantification limits after 30 days (<0.05 mg/kg dwt). This confirms the fast dissipation of HHCB as observed in the experimental set-up reported in section 3.1.3.1.3 (DiFrancesco et al 2004).

In the state of Baden-Württemberg, Germany, 13 study locations were selected comprising a sewage sludge field with known history of sludge applications and a reference field. Total quantities of sludge applied to the fields ranged from 3.2 ton/ha to 31.5 ton/ha over a known number of years. The load on two experimental plots was 85 and 510 ton/ha. (N.B. the default load according to the EU-TGD is 5 ton/ha during 10 years). Concentrations were expressed as the sum of polycyclic musks (PCM). The concentrations in 6 sludge amended fields measured in 2002 were below 1 µg PCM/kg dwt except on one field (2.1 µg PCM/kg) where the concentration in the reference field was also elevated. Also the concentrations in the reference plots of the experimental areas were elevated: 0.54 µg PCM/kg dwt. The concentrations in the experimental fields were 1.1 and 5.4 µg PCM/kg dwt (LfU-BW 2003).

In the state Saxony-Anhalt, Germany, transects were made through the floodplain of the River Elbe, from the dike to the riverbank and on 6 positions showing elevated concentrations of heavy metals, the depth distribution (to 1.5 m) of other contaminants was investigated. The concentrations of musk fragrances were only in the range of the blank, 0.01 to 0.02 mg/kg (Witter et al. 2003).

3.1.5.3 Comparison between predicted and measured levels

The predicted concentrations in agricultural soil after 10 years of sludge application are 0.014 and 0.06 mg/kg dwt for the Northern and the Southern EU-15 Scenarios. Measured concentrations in soil are scarce and hardly suitable for comparison. The observations from the field in the US where sludge is regularly applied twice per year show concentrations < 0.05 mg HHCB/kg after one month. The study in Baden-Württemberg, Germany suggests that after applications similar to the scenario described in the TGD, concentrations were below 0.001 mg AHTN+HHCB/kg. The concentrations found in the floodplains of the river Elbe

were below 0.01-0.02 mg/kg. It is concluded that all reported concentrations are below PEClocal. The detection levels limit the comparison with PECregional.

For the risk assessment the SEU-15 scenario will be used:

$PEClocal_{soil}$ = 0.06 mg/kg wwt.

3.1.6 Atmosphere

3.1.6.1 Calculation of PEC_{local}

3.1.6.1.1 Emission during production and fragrance compounding

As the production is batchwise in a closed system, emission to air takes place only by exchange of air in the reactors, storage tanks and trucks. The exchanged gas volume is 14,000 m^3 per year. In view of the low vapour pressure, the concentration of HHCB in this gas is expected to be low.

During compounding of HHCB, emission to the atmosphere may take place from the ventilation systems of the plant. The air passes filtration systems before emission, but no information is available on the concentration in filtered air. Therefore estimates are based assuming no removal in the filter systems. The highest emission as% of use estimated in site 1-6 is 0.06%. This emission is taken as a default for the generic scenarios.

Table 3.23. Emission to air

	Air flow/hour * hours/day * days/year	measured conc. in air (indoors)	emission per year	% of use
site 1	74,000 · 10 · 240 = 177.6 · 10^6 m^3	< 0.012 mg/m^3 (extrapolated)	2.1 kg	<0.001%
site 2	50,000 · 16 · 250 = 200 · 10^6 m^3	< 0.03 mg/m^3	6 kg	< 0.0002%
site 3	300,000 · 16 · 250 = 1.2 · 10^9 m^3	max. 10 mg/m^3 total org. material (licence)	max 12,000 kg/yr, 1.2% of use volume is 144 kg	max 0.06%
site 4	total 50,750 m^3/h in various rooms	av. 0.009 – 0.012 mg/m^3	max. 2.725 kg	max. 0.002%
site 5	6000 · 14 · 250 = 21,000 m^3	0.012 mg/m^3 (other site)	0.25 kg	0.0007%
site 6	natural		93.5 kg	0.05% estimated for total loss

3.1.6.1.2 Calculation of PEC_{local} for end product formulation

1427 tons of HHCB are produced per year and the emission factor given in the ESD of the TGD is 0.00002. A specific large end product formulator used 3.5% of the use volume in 345

days per year. Therefore the emission per year is 1427 ton · 0.035 · 0.00002 = 1 kg HHCB/year or 2.9 g HHCB/day (based on formulation 345 days per year).

For a specific small-scale formulator the use volume is 6064 kg per year. Therefore the emission is 0.00002 · 6065 kg = 0.121 kg/year.

3.1.6.1.3 Calculation of PEC_{local} for private use

In the initial calculations the tonnage used in compounding was completely discharged to the sewer, implying that there was only an indirect release to air from the STP and from evaporation after partitioning over the environmental compartments. With the refinements described in sections 3.1.4.1.3 and 3.1.4.1.4, the release factors were adapted in a way that equal fractions were released to waste water and air. The daily release to air and the regional and local concentrations in air due to consumer use of HHCB are summarised in **Table 3.24**.

Table 3.24. Release to air and concentrations in air after consumer use

Scenario	Release factor to air *	Regional release to air, kg · d^{-1}	Regional PECair mg · m^{-3}	Local PECair mg · m^{-3}	Total deposition flux, mg · m^{-2} · d^{-1}
TGD regional (10%)	0	0	1.53 E-06	2.42 E-05	3.37 E-05
southern EU-15	0.065	25.4	3.78 E-07	1.45 E-05	2.32 E-05
northern EU-15	0.017	6.65	8.91 E-08	3.79 E-06	6.07 E-06

* based on Figure 3.4

The predicted concentrations in air **PEClocal $_{air}$** related to private use in northern and southern European countries range from 3.8 to 14.5 ng/m^3.

3.1.6.2 Measured levels

Concentrations were measured in ten ambient air samples taken in the south of Norway in 1998. The concentrations were in the range of 0.1 ng/m^3 (Kallenborn et al. 1999, Kallenborn and Gatermann, 2004). For comparison, median and maximum concentrations in indoor air were 101 and 299 ng/m^3 (Fromme et al. 2004).

Concentrations were measured in ambient air sampled above the water of Lake Michigan during June 1999 and May 2000. Samples were also taken in urban Wilwaukee in June 2001.. The concentration in the gas phase of the urban area was 4.1 ± 1.4 ng/m^3 and 1.1 ± 0.6 over the lake. The airborne particulate-phase concentration was 0.55 ± 0.27 ng/m^3 or 1.3 ± 0.50 ng/mg. The fraction of HHCB in the airborne particulate phase was 13% (Peck and Hornbuckle 2004).

In a study for Greenpeace by TNO, rainwater samples (actual deposition) were taken on 47 locations in The Netherlands, on two locations in Germany and one in Belgium in February 2003. Rainwater was collected in funnels with a diameter of approximately 30 cm during a few weeks. HHCB was found in all samples with concentrations ranging from 7 to 25 ng/l, median 13 ng/l (Peters 2003). In a confirmatory study the concentrations were measured on six locations in the centre of The Netherlands in February 2004. This time the concentrations ranged from 22 to 44 ng/l, median 37 ng/l. The higher levels in 2004 are explained by the weather conditions: sunny weather in 2003, whereas the wet and cloudy weather in 2004 would have caused a higher wash-out of the atmosphere resulting in higher concentrations in the precipitation (Peters 2004).

The concentration in rainwater was also measured in the Nordic countries in 2002. The concentration was above the detection limit but below the limit of quantitation (0.020 ng/l) in 3 out of 23 samples (Denmark, Iceland, Sweden) whereas only once the concentration was above the limit of quantitation of 0.029 µg/l, in one sample out of the four taken on the same site in Denmark, which was a coniferous forest of pristine nature (Mogensen et al. 2004). The validity of this elevated value for a sample taken in pristine area is disputable.

3.1.6.3 Comparison between predicted and measured levels

The concentrations observed in ambient air in Norway are below PECregional air. The concentrations over Lake Michigan were below PEClocal in the SEU scenario and just above PECregional for NEU (but conditions are not related).

From the concentrations measured in rainwater a wet deposition flux may be derived, assuming 700 mm rain/year. 700 mm per year equals 1.92 l of rain per m^2 per day. With the medians of 13 and 37 ng/l of rain, the deposition is 0.025 - 0.071 µg/m^2/d. These results are above the total deposition flux estimated for The Netherlands by a factor of 4 to 10. In view of the variability in weather conditions, rainfall, sunshine, the results seems to match relatively well.

3.1.7 Secondary poisoning

3.1.7.1 Calculation of PECs

The measured BCF for fish is 1584 l/kg. The BCF for earthworms is estimated from K_{ow} (see section 3.1.3.4.3), resulting in a value of 2395 l/kg wwt.

$PECfish = PECwater \cdot BCF_{fish} \cdot BMF$

where:

BCF_{fish} = 1584 l/kg ,

PECwater = 0.5 ($PECwater_{local}$ + $PEC\ water_{regional}$).

BMF, the biomagnification factor, depends on BCF_{fish}. With BCF < 2000, BMF = 1.

Likewise, PECworm is calculated on the basis of the concentration in porewater:

$PECworm = Cporewater \cdot BCF_{worm} \cdot BMF + F$ (F is an additional correction for gut contents)

BCF_{worm} = 2395 l/kg wwt,

$Cporewater = 0.5\ (PECporewater_{local} + PECporewater_{regional})$ (agricultural soil)

BMF = 1.

The results are presented in **Table 3.25**.

Table 3.25. PECs in fish and worm

Scenario	PECwater $_{local}$ (µg/l)	PECwater $_{regional}$ (µg/l)	$PEC_{oral,fish}$ mg/kg wwt	PECground-$water_{local}$, µg/l	PECground-$water_{regional}$ µg/l	$PEC_{oral,worm}$ mg/kg wwt
Production	0.63	0.032	0.528	0.0421	0.0006	0.0469
Compounding Site 1	0.078	0.032	0.0885	0.191	0.0006	0.229
Compounding Site 2 Year 2000	3.7	0.004	2.83	0.066	0.000246	0.0727
Compounding Site 2 Year 2006	0.74	0.004	0.57	0.016	0.000246	0.018
Compounding Site 3	0.007	0.004	0.00926	0.0545	0.000246	0.0602
Compounding Site 4	0.026	0.004	0.024	--		
Compounding Site 5	0.041	0.032	0.0591	0.00077	0.0006	0.0015
Compounding Site 6	0.034					
Compounding Site 7 (Large-medium generic)	0.526	0.032	0.442	0.089	0.0006	0.098
Compounding Site 8 (Small generic)	0.232	0.032	0.203	0.036	0.0006	0.040
Formulation Large company	0.31	0.032	0.275	0.0515	0.0006	0.0572
Formulation Generic scenario	0.58	0.032	0.488	0.103	0.0006	0.114
TGD regional (10%)	8.44	0.348	7.23	1.85	0.0121	2.04
southern EU-15	0.485	0.0318	0.409	0.131	0.00060	0.144
northern EU-15	0.139	0.004	0.114	0.032	0.000107	0.0354

3.1.7.2 Measured levels

For earthworms or other terrestrial invertebrates, no measured concentrations are available.

Monitoring data are available for fish and shellfish, sampled in Germany, The Netherlands, Italy, Czech Republic and Norway. A distinction is made between eel and other fish species because the lipid content of eel is considerably higher than for the other species. Therefore, even though the concentrations of HHCB in fat sometimes seems to be lower for the eel than for the other species, the total body burden of the eel (fresh weight) is always higher.

Fish samples were taken in the River Ruhr, Germany. Species were chub, crucian carp, perch, bream, roach, pike and eel. Concentrations were determined in the edible parts (muscle), in adipose tissue and the liver and the results were expressed as the concentration in fat. median concentrations were 112 µg/kg fresh weight (wwt) for eel and 18 µg/kg wwt for the other fish (Eschke *et al*. 1995a).

Rimkus (1997) presented data for fish sampled from fish ponds in Denmark (rainbow trout), eel, pike and perch from the River Elbe, brown trout from the River Stör, 3 km downstream of a STP and herring from the East Sea and seas around Denmark and Ireland. Using the fat content from the publications, these concentrations were recalculated to fresh weight

concentrations. The concentrations in fish from the Elbe are clearly below those found in the river Ruhr by Eschke et al. (1995a) by a factor of 10. Rimkus as well as Eschke reported elevated concentrations from fish found in effluent ponds that are related to the elevated concentrations found in effluents. In addition Rimkus (1999) reported on concentrations in shellfish: in the blue mussel (*Mytilus edulis*) found on the North Sea coast, the concentration was < 30 – 110 µg/kg lipids, whereas in shrimps (*Crangon crangon*) the concentration ranged from < 40 – 370 µg/kg lipids (HHCB).

Eel caught in the main waters of The Netherlands were analysed by Rijs and Schäfer (1998). Concentrations were below those in the Ruhr by a factor of 4.

Eel and other fish were sampled in Berlin (Fromme et al. 1999, 2001b). A classification was made according to the fraction of effluent input into the water system. Of 165 eel samples, the median was 0.09 mg/kg wwt, 90th-percentile 1.99 mg/kg wwt. In 10% the HHCB concentrations were below the detection limit of 0.03 mg/kg wwt. For the other species sampled (176 samples), the median was 0.04 mg/kg wwt, 90th-percentile 0.26 mg/kg and 23% was below the detection limit. This implies a very skewed distribution of the samples. As compared to the data of Eschke in the Ruhr, median concentrations in eel in the high effluent input areas (up to 97%) were above those in the Ruhr by a factor of 13, and below the eel in the effluent pond by a factor of 6. For the fish species with the highest concentrations, the common bream, the median concentrations were higher than the median in the Ruhr by almost two orders of magnitude. In his review Heberer (2002) gives the overall median and 95th-percentile of 324 fish samples as 0.14 and 2.2 mg/kg wwt respectively, or 3.36 and 54.9 mg/kg lipids.

In Italy, fish were sampled in eight Italian rivers and lakes. The median concentration in 28 fish samples was 0.005 mg/kg wwt. (Draisci et al. 1998).

In the Czech Republic a monitoring programme on fish was set up. In a first publication on the River Elbe, the highest concentrations were found downstream of a detergent production plant: 1.29 mg/kg lipids (Hajslova et al. 1998). Within 4 years, from 1997 to 2000, over 800 fish were collected using electrofishing on four sites on the Elbe River, four sites on the Moldau River and three sites on the Tichá Orlice River. Fish species sampled were Chub, Bream, Barbel, Perch and Trout. The monitoring continued for three or four consecutive years, from 1997 to 2000. Concentrations were reported in mg/kg lipids but other characteristics including the lipid content was reported as well. Median concentrations ranged from 0.3 to 10.8 mg/kg lipids. The highest concentrations were found in Barbel, a bottom-dwelling species. A significant correlation existed between the concentrations in sediment on a site and the fish collected there. It was also observed that different age/size classes (younger and older fish) showed no differences in tissue levels. This demonstrated that the uptake is due to bioconcentration only and not to biomagnification through the food chain (Hajslova and Setkova 2004). A decrease in the concentrations in time was not observed, suggesting that the downward trend observed in northern Europe has not occurred in the Czech Republic.

Eel were sampled in the River Elbe near the Czech border and in the Stör, a tributary downstream of Hamburg, Germany, in 2001. The median concentration in eel muscles caught near the Czech border was 0.022 mg/kg wwt, whereas the maximum was 0.032 mg/kg wwt. More downstream, the median concentration in eel from the Stör was lower, 0.006 mg/kg wwt, maximum 0.067 mg/kg wwt. In 5 tributaries along the Elbe bream were caught in 2000 and 2001. The median concentrations varied from 0.005 to 0.021 mg/kg wwt, whereas the maxima ranged from 0.013 to 0.236 mg/kg wwt, with the highest level found in the River Stör. Flounders were caught on two sites in the Elbe estuary. HHCB was detected in 13 out of

14 flounders caught near the mouth of the Stör and the median concentration was 0.003 mg/kg wwt, the maximum was 0.007 mg/kg wwt. Further out into the sea, HHCB was detected in only 4 out of 16 fish. The median was below the detection level, the maximum 0.007 mg/kg wwt (Wiegel and Stachel 2002).

Samples of marine organisms taken on three sites along the shore of the Wadden Sea, Germany, showed HHCB at detectable levels in bladder wrack (*Fucus vesiculosus*, brown algae) only in one site in out of three years at 0.0003 mg/kg wwt. Between 1994 and 2000, the concentration in eelpout samples ranged from 0.001 to 0.003 mg/kg wwt. In blue mussels (*Mytilus edulis*) the concentration varied between 0.0005 and 0.0017 mg/kg between 1986 and 2000, in general showing a downward trend. At another location the levels turned below detection levels in 1998 – 2000. The concentrations in herring gull (*Larus argentatus*) eggs in the same area ranged from 0.0007 – 0.0026 mg/kg wwt, apparently at the same level of their food organisms (Müller et al. 2004, Rüdel and Schröter-Kermani 2004).

In a recent publication of the same work Rüdel et al. (2006) present extensive results on biotic samples in the German Environmental Specimen Bank from the period of 1986 to 2000, The levels systematically decreased from a maximum around 1994-96 to clearly lower levels in this century. The most conspicuous was the decrease of HHCB in bream in the River Saale which decreased by 64% from 18.4 to 6.7 mg/kg lipids between 1995 and 2003.

A recent report on concentrations in trout from Danish farms showed a downward trend with concentrations of 5.0 µg/kg wwt in 1999 to 1.2 µg/kg wwt in 2003/2004. In 1999, HHCB was detected in at least 98% of all samples, whereas in 2003/2004 it was detected only in 60% of all samples (Duedahl-Olesen et al. 2005).

Fish were caught in remote alpine lakes in Switzerland that are exclusively fed by direct or indirect atmospheric input. In seven alpine lakes between 2000 and 2650 meter above sea level, fish were caught in August and September 2003: brown trout (*Salmo truuta fario*), alpine char (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*). The average fat content of the fish ranged from 1.3 to 3.8% in the different lakes. The sum of the concentration of AHTN and HHCB ranged between 0.064 to 0.12 mg/kg lipids for 6 lakes (Schmid et al. 2004). In the fish in one lake a relatively high level (0.28 mg/kg lipids) was observed which was not at all in proportion to the concentrations of the other substances in the study. This seems to suggest an additional route of entry next to the atmospheric deposition. The study included no analytical control samples so contamination can not be ruled out.

Schmid et al (2007) produced a new publication on the same study results. In this paper the individual polycyclic musks were quantified in fish caught in the different lakes. In comparing both papers we remark that the lake originally showing the relatively high level was now recognised as an area with slight tourism, but at the same time apparently the samples had been exchanged and thus the concentration was reduced to the level found in the other lakes. The elevated concentration is now attributed to a lake nearby a pass road. It is remarkable that in particular HHCB is elevated (0.23 as compared to the median 0.050 mg/kg lipids), whereas for AHTN the increase is less clear (from median 0.027 to 0.05 mg/kg). Usually the concentrations of the different musks are correlated but in this case neither the other polycyclic musks nor the nitromusks show an increase in this sample suggesting that some external factor is the cause of the deviation.

In Nevada in 2001, each month seven to eight carp (*Cyprinus carpio*), 2 kg each, were caught in Lake Maed. The concentration of HHCB as well as the total lipid content was determined.

The concentration of HHCB ranged from 0.0014 mg/kg wwt in October-November (lipid content 11%) to 0.0045 mg/kg wwt in February-March (lipid content 17 – 20%) (Osemwengie and Gerstenberger 2004).

Concentrations found in fish are summarised in **Table 3.26**. Concentrations are presented in mg/kg fresh weight and/or mg/kg lipids.

Table 3.26. Monitoring data for HHCB in fish (mg/kg)

Sample	n	HHCB		Reference
		mg/kg lipids	mg/kg fresh weight	
Germany, River Ruhr Non-eel	7	median 2.8 (1.4 – 3.8)	median 0.018 (0.010 – 0.045)	Eschke et al., 1995a
River Ruhr Eel	2	mean 0.5 (0.4 – 0.6)	mean 0.11 (0.095 – 0.125)	
Non-eel fish effluent pond	8	median 8.0 (1.0 – 19.8)	median 0.15 (0.023 – 0.344)	
Eel from effluent pond	5	mean 35 (6.1 – 63.6)	mean 10 (1.26 – 19.20)	
Denmark Rainbow trout Fish pond	4	mean 0.36	mean 0.011	Rimkus, 1997
Germany Eel River Elbe	5	mean 0.048	mean 0.012	
Non-eel river Elbe	4	mean 2.31	mean 0.012	
Fish river Stör near STP outfall	3	mean 14.3	mean 0.34	
East sea Herring	1	0.75	0.065	
Denmark Herring	1	0.12	0.008	
Ireland Herring	1	<0.01	<0.0008	
The Netherlands Eel	6		median 0.03	Rijs and Schäfer 1998
Germany, Berlin area, Eel	54	low effluent input median 0.198 90-perc. 0.81	low effluent input median 0.050 90-perc. 0.079	Fromme et al. 2001b
	53	moderate effluent input median 0.426 90-perc. 1.405	moderate effluent input median 0.077 90-perc. 0.210	
	58	high effluent input median 5.830 90-perc. 11.483	high effluent input median 1.473 90-perc. 2.812	
Germany, Berlin area, Other fish				
Perch	19	low effluent input median < d.l. max. 0.122	low effluent input median < d.l. max. 0.122	
	9	high effluent input median 33.3 max. 159.9	high effluent input median 0.200 max. 1.215	
Common	37	low effluent input median 3.3	low effluent input median 0.040	

Sample	n	HHCB		Reference
		mg/kg lipids	mg/kg fresh weight	
bream		max. 15.3	max. 0.260	
	10	high effluent input median 90.1 max. 143.3	high effluent input median 1.571 max. 3.426	
Roach	48	low effluent input median <d.l. max. 11.8	low effluent input median < d.l. max. 0.260	
	6	high effluent input median 13.0 max. 55.3	high effluent input median 0.168 max. 1.018	
Pike	12	low effluent input median <d.l. max. 14.2	low effluent input median = d.l. max. 0.098	
	2	high effluent input 61.5 – 66.5	high effluent input 0.366 – 0.370	
Pike perch	25	low effluent input median <d.l. max. 113.0	low effluent input median < d.l. max. 0.113	
	8	high effluent input median 47.3 max. 383.9	high effluent input median 0.190 max. 1.574	
All fish in Berllin area	351		90th percentile 1.50	
Germany, Elbe various tributaries 2000 – 2001	30		eel median 0.006 – 0.022 max 0.032 – 0.067 bream med 0.005 – 0.021 max 0.013 – 0.236 flounder median ≤ 0.001 – 0.003 max 0.007	Wiegel and Stachel 2002
Italy, Various waters,	28	median 0.258 90-perc. 1.163 nd – 5.0	median 0.005 90-perc. 0.034 nd – 0.047	Draisci et al. 1998
Switzerland 2003 7 remote alpine lakes Brown trout, brook trout, alpine char, lake trout		median 0.08 range 0.064 – 0.12 * for sum of AHTN and HHCB		Schmid et al. 2004 * sample with max 0.29 out of range with other substances
		median 0.050 range 0.044 – 0.078 extreme 0.230		Schmid et al. 2007 (revision of Schmid et al. 2004)
Czech Republic				Hajslova and Setkova 2004
Chub	302	median ranges 0.4 – 2.7		
Bream	164	median ranges 1.2 – 8.4		
Barbel	50	median ranges 0.3 – 10.8		
Perch	156	median ranges 0.4 – 5.8		

Sample	n	HHCB		Reference
		mg/kg lipids	mg/kg fresh weight	
Trout	117	median ranges 0.3 – 2.2		
Norway				Kallenborn et al. 2001
Thornback ray filet	1	0.073	0.0006	
Haddock filet	2	mean 0.343	mean 0.0016	
Atlantic cod filet	3	mean 0.035, max. 0.043	mean 0.007, max 0.008	
Saithe filet	1	0.225	0.005	
Thornback ray liver	1	0.021	0.008	
Haddock liver	3	mean 0.243, max. 0.37	mean 0.162, max. 0.25	
Atlantic cod liver	13	mean 0.273, max. 1.51	mean 0.098, max. 0.53	
Saithe liver	1	0.007	0.003	
USA Nevada *Cyprinus carpio*	12· 7		0.0014 – 0.0045	Osemwengie 2004

In Norway a total of 10 pooled Atlantic cod liver samples (*Gadus morhua)* was collected from harbours, fjords and the open coast, winter 1997/1998. The samples were collected in recipients for industrial and municipal wastewater. Seven fish samples were taken from an inner harbour (two thornback ray (*Raja clavata)* and 5 haddock (*Melanogrammus aeglefinus*)), and 8 fish samples (6 Atlantic cod and 2 Saithe (*Pollachius virens*) were from another harbour in 1999. Concentrations were estimated in liver and filet and expressed on the basis of lipid content. With the lipid content given in the paper the figures were converted to freshweight concentrations. HHCB-lactone was detected at variable concentrations, sometimes up to the same level as the parent HHCB (Kallenborn et al. 2001). With the lipid content given in the paper the figures were converted to freshweight concentrations. Due to a lack of details the fresh weight data are probably overestimating the real values.

In a large monitoring programme in the Nordic countries blue mussels were sampled in 20 sites in Denmark, Sweden, Norway, Finland and Iceland in the vicinity of urban as well as pristine areas. The concentrations were below the detection levels (< 0.085 mg/kg lipids) (Mogensen et al. 2004). In the same programme red fox or polar fox livers were sampled and analysed. HHCB was not detected in any of the 15 samples (< 0.39 mg/kg lipids, Mogensen et al. 2004).

The occurrence of HHCB was reported in the liver of a Danish otter. The concentration was 140 mg/kg lipids (Leonards and De Boer 2004).

The concentration of HHCB and HHCB-lactone was determined in Canadian and Arctic samples of Ringed Seal blubber, Arctic char, blue mussels and lake trout. The concentration of HHCB and HHCB-lactone in the trout seemed to be elevated as compared to the background, whereas the concentration in char, mussels and in Ringed Seal blubber were at/below the background level (Hühnerfuss et al. 2002) giving no indication for biomagnification. In the three quantified samples (Ringed Seal blubber and lake trout) the HHCB-lactone level was higher than the HHCB level by a factor of 2.

BAFs and BMFs in Beluga whale (*Delphinuapterus leucas*) tissues were substantially lower than expected based on the physical-chemical properties indicating metabolic transformation (Kelly et al. 2004).

Concentrations were determined in samples of mammals and sharks collected from Japanese coastal waters (Nakata 2005). HHCB was detected in the blubber of 8 adult Finless Porpoises and their foetuses that were stranded along the coastal area or accidentally caught by fishing nets during 1999 and 2002. The lipid content ranged between 76 and 88%. The concentrations varied from 13 to 149 μg/kg wwt. The level in one fetus was comparable to that in the mother (26 vs. 39 μg/kg wwt). The tissue distribution was established in the sample with the highest level in blubber (83% lipid content) of 149 μg/kg wwt. The kidney, with a lipid content of 4.8%, also contained a detectable level of 9.3 μg/kg wwt. There seems to be a relation with the lipid content in both tissues. HHCB was not detected in any other tissue. Hammerhead sharks were collected from a Japanese coastal water in 2004. The HHCB level in their livers (27-58% lipid content) ranged from 52 to 99 μg/kg lipids or 16 – 48 μg/kg wwt (Nakata 2005). These levels were below those in livers of Atlantic Cod in Norway reported by Kallenborn et al. (2001), but levels in fish in the Japanese region are not known.

HHCB concentrations were determined in tissues from marine mammals, water birds and fish collected from US waters. Concentrations were between 1.5 and 5.3 μg/kg wwt in the liver of Seals, Sea Lions, Atlantic Sharpnose Shark, River Otter, Mink, Common Merganser, Lesser Scaup, Greater Scaup and Mallard. The same levels were found in Atlantic Salmon and Smallmouth Bass. The blubber of Bottlenose Dolphin and Striped Dolphins stranded in Florida coastal waters contained an average of 12 – 14 μg/kg wwt, whereas the level in the liver of a Pygmy Sperm Whale was 7 μg/kg. HHCB was not detected (< 1 μg/kg wwt) in the livers of Polar Bear (*Ursus maritmus*) from the Alaskan Arctic (Kannan et al. 2005).

Female Chinese Sturgeons (*Acipenser sinensis*) were caught between 2003 and 2005, ranging in weight from 140 to 263 kg (total length 2.85 – 3.39 m), age 17 to 25 years old (Wan et al. 2007). They migrate between the sea and their spawning grounds in the Yangtze River at intervals of more than 2 years. No details on the sampling place and times were given. The highest levels of HHCB were detected in all 4 adipose tissue samples and in all 11 roe samples. Means were 46 and 28 μg/kg wwt, respectively. Lower levels were found in 3 out of 7 liver samples, 2 of 6 gonad samples and in 2 of 5 gill samples. These concentrations are below those found in fish in Europe but they were higher than those in blubber of dolphins and whales on the coast of Florida. The concentration of HHCB in liver was comparable to those in liver tissues of hammerhead sharks along the Japanese coast. When expressed on a lipid weight basis, the concentrations of HHCB were similar among the different tissues. For fish, increasing trends with age are found for truly persistent substances like DDT, HCB and some PCBs in both female and male brown trout. However, in this study no statistical relationship was found. The concentration ratios for roe to adipose tissue in two fish were 0.38 to 0.55 (Wan et al. 2007). The age-related accumulation is not evident, the variation is very high and r^2 is only 0.17. It is known that fish are able to metabolise HHCB with a short half-life period, so the variable concentrations will reflect the recent exposure more than their life history.

Bioaccumulation of HHCB in a marine food chain was investigated by analyzing marine organisms at various trophic levels including lugworm, clam, crustacean, fish, marine mammal and bird samples collected from tidal flat and shallow water areas of the Ariake Sea, Japan between 2000 and 2005 (Nakata et al. 2007). Tissues of whole body, soft tissue, hepatopancreas, liver or blubber were analysed. The highest concentrations were detected in clams (circa 900 μg/kg lipids) whereas the levels in mallard and black-headed gull were low

(20 µg/kg lipids) and comparable with concentrations in fish and crab. It is concluded that there is no trend of increasing concentrations at higher trophic levels, probably due to the metabolism and elimination of HHCB in higher organisms. Analysis of samples in marine mammals (30 Finless Porpoises and 9 Striped Dolphins) between 1977 and 2005 show an increase since the early 1990. To examine the geographical and global distribution, marine mammals, bird eggs and fish samples from various locations were sampled: Bottlenose Dolphins and California Sealions from the US, Striped Dolphins and Cormorants from the Mediterranean Sea, Ringed Seals Gray Seals and Salmon from the Baltic Sea, a Sperm Whale from the coast of Japan and eggs of a South Polar Skua from Antarctica, collected between 1991 and 2005. The HHCB concentration in a striped Dolphin and a Cormorant from the Italian coast were 1.5 and 1 µg/kg wwt, respectively. HHCB concentrations in dolphins from the US and the Mediterranean Sea were lower than those in the porpoises from Japanese coastal waters. No polycyclic musks were found in Ringed Seal, Gray Seal and Salmon from Finnish coastal waters of the Baltic Sea, nor in Sperm Whale from Japanese coastal waters or in the eggs of South Polar Skua from Antarctica. It is concluded that bioaccumulation in the food chain does not occur and that there is a lack of long-term transportation potential in the environment (Nakata et al. 2007).

3.1.7.3 Comparison between predicted and measured levels

Concentrations measured in fish are reported from both very heavily polluted areas and from more remote regions, in Germany, The Netherlands, Italy, Switzerland, Czech Republic, Norway, the North Sea and USA. HHCB was detected in most samples except in fish caught in remote areas, lakes and on sea. The highest concentrations by far were observed in the areas classified as 'high effluent input' areas in Berlin, Germany, in 1996-1997. These levels are of the order of magnitude of those found in effluent ponds by Rimkus (1999) and Eschke (1995a). The levels found in the Czech Republic (1997-2000) are reported based on the fraction of lipids. The data for the species that are shared with the Berlin study indicate that the maximum levels in the Czech fish are below the fish from the high effluent input area in Berlin by a factor of 10. This confirms that all fish reports other than from the high effluent input area in Berlin are below $PECoral_{fish}$ for the Southern European Scenario (0.41 mg/kg wwt). It had been shown that the levels in effluents discharged into the high input areas in Berlin have decreased considerably, as is also reflected in the current sediment concentrations in the Teltow Canal. Thus it may be expected that also the levels in fish have been reduced considerably.

No recent data are available for comparison with the Northern European Scenario (0.11 mg/kg wwt).

For the risk assessment the Southern EU-15 Scenario will be used, since it covers all monitoring data except for some historic extremes in the Berlin area:

$PECoral_{fish}$ = 0.41 mg/kg wwt.

The 90^{th}-percentile for all fish in the Berlin area (1996/1997) was 1.50 mg/kg wwt.

3.1.8 Marine compartment

For an assessment of the exposure of the marine environment a local exposure assessment was performed for the generic compounding sites (site 7 and 8), for the generic formulators and for the private use scenarios for northern and southern European countries.

3.1.8.1 Compounding and formulation

For a default assessment industrial trade effluents of sites along the coast are not treated in a municipal biological STP. The dilution factor of the effluent in the marine environment is 100 (instead of 10 in the freshwater environment). In the standard freshwater environment the water flow is 18,000 m^3. After discharge of the STP (2000 m^3), the water flow becomes 20,000 m^3 per day. The TGD (EC 2003) assumes that the dilution factor in the marine environment is 100 instead of the factor of 10, so the water flow for dilution in the marine environment is 200,000 m^3 per day. By default the dilution factor for mixing of river water into the coastal sea is 10, so $PECregional_{seawater} \simeq 0.1 \cdot PECregional_{water}$. $PECregional_{seawater}$ is estimated by EUSES.

Since the losses in the trade effluents are not treated in a municipal STP, the amounts released from the generic sites are directly diluted in 200,000 m^3 of seawater. Most of the characteristics of the coastal environment (regional) are similar to the freshwater compartment apart from the suspended matter concentration. The concentration of suspended matter in the local coastal environment is 15 mg/l and in the regional marine zone it is set to 5 mg/l. The results of the default calculations are given in **Table 3.27**.

Table 3.27. PEClocal for the marine environment, compounding and formulation (default scenario without treatment in STP)

Site	Emission kg/d	Sea water volume, m^3	$Clocal_{seawater}$ µg/l (total) → Clocal (diss)	$PECregional_{sea\ water}$ (diss), µg/l	$PEClocal_{sea\ water}$ µg/l	Marine $PEClocal_{sediment}$ (equil. part.) mg/kg wwt
Compounding Site 7 (Large-medium generic)	0.0421	200,000	0.211 → 0.203	0.00286	0.206	0.111
Compounding Site 8 (Small generic)	0.0171	200,000	0.085 → 0.082	0.00286	0.085	0.046
Formulation Large company	0.0246	200,000	0.123 → 0.119	0.00286	0.122	0.066
Formulation generic	0.0485	200,000	0.243 → 0.234	0.00286	0.237	0.128

A survey was carried out to verify whether compounders or formulators using AHTN and HHCB discharge their wastewater into the marine environment only after treatment in a sewage treatment plant (Letter of IFF and PFW, July 2006). The conclusions of the survey are that in the EU-15+2:

- No compounder discharges its wastewater directly into the marine environment;
- Of the more then 1000 formulators, only 16 are located in a radius of 500 metres from the sea/ocean shore. These are among the very small formulators: the **total** amount used by

these formulators is below the volume used in the generic scenario by more than an order of magnitude.

- The treatment of wastewater before discharge is common practice in this industry chain.

When the presence of an STP is taken into account in the calculations, PECmarine roughly equals 0.1 · PECfreshwater (see **Table 3.11**). As the fraction discharged with the effluent is 0.224 (according to EUSES, see **Table 3.7**), the values after treatment are roughly 0.224 of the values predicted in **Table 3.27** for the default scenario, see **Table 3.28**.

Table 3.28. PEClocal for the marine environment, compounding and formulation (scenario with wastewater treatment)

Site	Emission kg/d	Sea water volume, m^3	$Clocal_{seawater}$ µg/l (total) → Clocal (diss)	$PECregional_{sea\ water}$ (diss), µg/l	$PEClocal_{sea\ water}$ µg/l	Marine $PEClocal_{sediment}$ (equil. part.) mg/kg wwt
Compounding Site 7 (Large-medium generic)	0.0421 *0.224 = 0.0094	200,000	0.047 → 0.045	0.00286	0.048	0.026
Compounding Site 8 (Small generic)	0.0171 *0.224 = 0.0038	200,000	0.019 → 0.018	0.00286	0.021	0.011
Formulation Large company	0.0246 *0.224 = 0.0055	200,000	0.028 → 0.027	0.00286	0.029	0.016
Formulation generic	0.0485 *0.224 = 0.0109	200,000	0.054 → 0.052	0.00286	0.055	0.030

3.1.8.2 Local emissions from private use

According to the TGD (EC 2003), it can be assumed that for releases to municipal waste water of substances used for private or public use (IC5 and IC6), the degree of treatment in a biological STP corresponds to the inland scenario. Therefore the effluent concentration from the STP is used as a starting point for the assessment. $PEClocal_{seawater}$ (dissolved) is simply derived from Ceffluent with a dilution factor of 100 and a correction for the sorbed fraction, see **Table 3.29**.

Table 3.29. PEClocal for the marine environment, private use

Scenario	Ceffluent STP, µg/l	$Clocal_{sea\ water}$ µg/l (total) → Clocal (diss)	Marine PECregional (diss) µg/l	$PEClocal_{sea\ water}$ µg/l	Marine $PEClocal_{sediment}$ mg/kg wwt
TGD regional (10%)	87.5	0.875 → 0.844	0.0308	0.877	0.472
southern EU-15	4.7	0.047 → 0.045	0.00286	0.048	0.0261
northern EU-15	1.4	0.014 → 0.0135	0.000367	0.0137	0.0074

3.1.8.3 Secondary poisoning

According to the TGD (EC 2003) the concentration in the food of predators and top-predators is calculated from:

$$PECoral_{predator} = 0.5 \cdot (PEClocal_{seawater} + PECregional_{seawater}) \cdot BCF_{fish} \cdot BMF_1$$

$$PECoral_{top\text{-}predator} = (0.1 \cdot PEClocal_{seawater} + 0.9 \cdot PECregional_{seawater}) \cdot BCF_{fish} \cdot BMF_1 \cdot BMF_2$$

BMF is the biomagnification factor in fish (BMF_1) and in the predator (BMF_2). The value of the BMF is determined by the BCF_{fish} as in this way the potential for metabolism in biota (i.e. fish) is taken into account. The BCF for HHCB is below 2000, leaving both biomagnification factors at 1.

Accordingly, $PECoral_{predator}$ and $PECoral_{top\text{-}predator}$ are calculated for all scenarios taken in consideration for the marine risk assessment, see **Table 3.30**.

Table 3.30. Predicted concentrations in fish, exposure of marine predators

Scenario, mg /d per capita	PECregional seawater, µg/l	PEClocal seawater, µg/l	PECoral predator mg/kg	PECoral top-predator mg/kg
Production, compounding and formulation				
Compounding Site 7 (Large-medium generic)	0.00286	0.206	0.165	0.0367
Compounding Site 8 (Small generic)	0.00286	0.085	0.070	0.0175
Formulation Large company	0.00286	0.122	0.099	0.0234
Formulation generic	0.00286	0.237	0.190	0.0416
Private use				
TGD regional (10%)	0.0331	0.877	0.721	0.1861
southern EU-15	0.00286	0.048	0.040	0.0117
northern EU-15	0.000367	0.0137	0.011	0.0027

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) – RESPONSE (EFFECT ASSESSMENT)

3.2.1 Aquatic compartment (incl. sediment)

It should be noted that HHCB is reported to be photodegradable (See section 3.1.3.1.1). Under atmospheric conditions the half-life of HHCB was determined to be 4.9 hours (Syracuse estimation programme AOP) and in a laboratory test with lakewater a half-life of 109 hours was reported (Buerge et al. 2003).

Generally, the wave length of UV ranges from 180 to 400 nm. The wave length of visible light is > 400 nm. The UV/Vis spectrum of HHCB (IFF (2001), shows that there is absorption at wavelengths below 300 nm with peaks at 202-205, ca. 225, 271 and 280 nm. The molar absorptivity of HHCB was reported as 51,318 cm^{-1} $.M^{-1}$ at 202 nm. HHCB does not absorb at wavelengths above ca. 325 nm.

In the laboratory test on photodegradation in water the light source was a mercury fluorescent lamp emitting UV-radiation between 300 and 460 nm, max 365 nm on the water surface or through a quartz test tube (Buerge et al. 2003). Hence, the photodegradation-induced half-life of 109 hours in the study by Buerge et al. is a consequence of absorption of UV light.

In alga growth inhibition tests generally fluorescent lighting tubes are used emitting wavelengths ranging between 400 and 700 nm at an intensity of approximately 8000 lux. Hence, the wave length of the illumination in the alga test is clearly above 325 nm, thus it will not cause photodegradation of the HHCB molecule. Moreover, there is a glass wall between the alga suspension and the light source, which would disrupt the penetration of UV radiation. The illumination in the other aquatic toxicity tests is at most daylight filtered by glass windows (aquaria) and glass vessel walls (so no UV light) or artificial light in the visible light spectrum. Hence, although photodegradation of HHCB may be considered a relevant degradation process in the atmosphere and in the upper layer of surfacewaters through penetration of UV light, it is concluded that photodegradation is of no significant influence on the outcome of the aquatic toxicity studies performed under normal laboratory conditions since the amount of UV light penetrating the test solutions will be negligible.

In view of the high log K_{ow}, the toxicity was not tested with short-term tests according to the base set, but in tests ensuring longer term exposure. Standard tests were carried out with algae, Daphnia and fish, and in addition a marine crustacean was tested. Because of the low water solubility of HHCB, it was necessary in the aquatic toxicity tests described below to prepare stock solutions using DMF as a solvent and Tween 80 as a dispersant or using triethylene glycol or ethanol as a solvent. These stock solutions were then diluted to reach the desired concentrations in the tests. The residual level of the solvent in the test vessel was always below the maximum level allowed by the test guidelines. Solvent controls containing equivalent levels of the solvents were used in all cases along with undosed water controls. HHCB has a strong tendency to sorb, thereby reducing the concentrations in solutions over time. Final concentrations in water were determined by HPLC or GC or, for radiolabelled HHCB, by LSC and the test concentrations are expressed as measured concentrations. Except

for the highest concentration in the fish growth test, the tested concentrations did not exceed the water solubility limit. The results are summarised in **Table 3.31** [12].

Table 3.31. Aquatic toxicity of HHCB (GLP and completely documented) Results expressed as mean measured concentrations

Test and reference	Results [1] [mg/l]	Remarks [2]
Algae *Pseudokirchneriella subcapitata* [3] 72-h static Van Dijk 1997	NOEC = 0.201 LOEC[5] = 0.466 ErC_{50} > 0.854 EbC_{50} = 0.723 <0.678-0.778>	carrier: 0.005% DMF and 0.005% Tween 80 n=6 HPLC identification start conc. 71-102% of nominal end conc. 54-85% of nominal DQ 1
Daphnia magna 21-d semi-static Wüthrich 1996a	NOECrep = 0.111, LOEC[5] = 0.205 EC_{50}rep = 0.282 <0.260-0.312> IC_{50} = 0.293 <0.204-0.419>[4]	carrier: 0.008% DMF and 0.002% Tween 80 n=5 HPLC identification conc.fresh 82-104% of nominal conc.used 63-91% of nominal DQ 1
Bluegill sunfish *Lepomis macrochirus* 21-d flow-through Wüthrich 1996b	NOECclinical signs = 0.093, LOEC[5] = 0.182 NOECgrowth = 0.182 LC_{50} = 0.452 <0.316-0.911>	carrier: 0.005% DMF and 0.005% Tween 80 n=5 HPLC identification conc. 66-86% of nominal DQ 1
Fathead minnow *Pimephales promelas* 32 days post hatch, 36 days overall Croudace 1997	LOEChatch > 0.140 NOECsurv. = 0.068, LOECsurv. = 0.140 LC_{50} > 0.140 NOECgrowth = 0.068, LOECgrowth[6] = 0.140 NOECdevelop.= 0.068, LOECdevelop.= 0.140	solvent triethylene glycol GC identification conc. 50-104% of nominal DQ 1

[12] For the qualification of the quality of the tests the following classification scheme was used: DQ 1: Valid without restriction. The test is carried out to internationally recognised protocols (or equivalent protocols) and all or most of the important experimental details are available; DQ 2: Use with care. The test is carried out to internationally recognised protocols (or equivalent protocols) but some important experimental details are missing, or the method used, or endpoint studied, in the test means that interpretation of the results is not straight forward; DQ 3: Not valid. There is a clear deficiency in the test that means that the results cannot be considered as valid; DQ 4: Not assignable. Insufficient detail is available on the method used to allow a decision to be made on the validity of the study.

Test and reference	Results [1] [mg/l]	Remarks [2]
Marine copepod *Acartia tonsa* 6d-static, daily feeding Bjørnstad 2007	NOECdevelop. = 0.038 LOECdevelop[5].= 0.075 EC10develop.= 0.044 <0.030-0.055> EC50develop.= 0.131 <0.115-0.153>	Radiolabelled ^{14}C- HHCB solved in ethanol (< 0.01%) n=5 LSC identification conc. > 80% of nominal DQ 1

1 measured concentrations, <95% confidence limits>

2 The number of concentrations tested (n) excludes control and solvent control

3 Former name *Selenastrum capricornutum*

4 Estimated 95% confidence limits after data reported by Wüthrich (1996c)

5 Dunnet's test (p=0.05)

6 Wilcoxon rank sum test (p=0.05)

In addition to the above tests that were carried out under GLP and with concentrations controlled by analyses, in the meantime more test results are reported. The experiments are summarised in **Table 3.32**. These studies have in common that they are presented in a highly aggregated form and sufficient details are not always available. For example, information on control survival or performance, dose-response and variability of replicates or on actually measured concentrations is lacking.

Table 3.32. Other aquatic toxicity tests (details not complete)

Test and reference	Results [1] [mg/l]	Remarks [2]
Worm *Lumbriculus variegatus* 5-d, static Artola 2002, 2003	EC_{50}immob. = 0.394 <0.221 – 0.706 >	solvent isopropanol, static n = 6 GC/MS identification DQ 4: high variability, publication does not specify more details. Insufficient detail to assess validity of test
Midge larvae, 4th instar 96h, semi-static *Chironomus riparius* Artola 2002	LC_{50} = 0.288 < 0.105 – 0.794>	solvent isopropanol, semi-static n = 10 GC/MS identifcation DQ 4: high variability, publication does not specify more details. Insufficient detail to assess validity of test
Marine copepod *Nitocra spinipes* 96-h, static Breitholtz et al. 2003	LC_{50} = 1.9 <1.40 – 2.70>	solvent acetone (<0.01%) No analysis, results expressed as nominal concentrations 96h-LC_{50}: DQ 2
Marine copepod *Nitocra spinipes* 7–8.5 d, partly renewal Breitholtz et al. 2003	NOEC larval devel. = 0.007 NOEC pop. growth > 0.2	solvent acetone (<0.01%) n=5 GC/MS identification, result expressed as nominal concentrations DQ 3: Concentration levels could not be maintained (circa 20% of nominal), presence

Test and reference	Results [1] [mg/l]	Remarks [2]
		of organic matter caused also oral exposure; not a water-only test
Marine copepod *Acartia tonsa* 48-h Wollenberger et al. 2003	LC_{50} = 0.47 <0.40 – 0.60>	solvent acetone (<0.01%) n = 6 no analysis LC50: DQ 2
Marine copepod *Acartia tonsa* 5-d, partly renewal Wollenberger et al. 2003	EC_{50} = 0.059 <0.044 – 0.079> EC10 larval development ratio = 0.037	DQ 3: No analysis of concentration levels algae added as food source were left as residue. potentially causing oral exposure; not a water-only test. Test was repeated, see **Table 3.31**
Freshwater mussel *Lampsilis cardium* 48 h Gooding et al. 2004	glochidia: 24h-LC_{50} = 0.56 and 48h-LC_{50} =0.98 juveniles: small dose dependant impact on growth	no data on preparation, no details on effects GC/MS analysis and identification actual conc. 5 to 19% of nominal DQ 4: no details, LC_{50} 24h < LC_{50} 48h
Zebrafish *Brachydanio rerio* 48-h, static Carlson and Norrgren 2004	NOEC heart rate $\underline{>}$ 1 [3] NOEC larval devel. > 1.0	solvent DMSO (0.1%) n = 7(devel.) no analysis, nominal concentrations DQ 2: Development: actual concentrations are missing
Zebrafish *Brachydanio rerio* 96 h Dietrich and Chou 2001	EC_{50}hatching 0.39	DQ 4: no details
Zebrafish *Brachydanio rerio* 3 d Schreurs et al. 2001	NOECdevelopment 0.4	DQ 4: no details
Clawed frog *Xenopus laevis* 96 h Dietrich and Chou 2001	LC_{50}embryo-adult> 2.0 LC_{50}embryo growth> 2.0 LC_{50}embryo-development > 4.0	DQ 4: no details

1 <95% confidence limits>

2 The number of concentrations tested (n) excludes control and solvent control

3 heart rate: biological significance not clear

3.2.1.1 Toxicity test results for aquatic organisms

3.2.1.1.1 Fish

A 21-d prolonged toxicity test was carried out with bluegill sunfish (*Lepomis macrochirus*) according to OECD Test Guideline 204 under flow-through conditions (Wüthrich, 1996b). Test parameters included fish growth (weight and length). The fish weights at the start of the experiment varied between 1.4 and 1.6 g. Nominal concentrations ranged from 0.125 to 2.0 mg/l (step size 2). Concentrations were measured at the start, halfway through and at the end of the test period. Survival of the fish was not significantly affected up to and including 0.182 mg/l. However, at 0.182 mg/l (and above) clinical signs of irregular respiration, bottom and tail dominated swimming, loss of equilibrium and righting reflex were observed. Mortality was 10% at the next higher concentration of 0.393 mg/l and coincided with significantly reduced growth. In 0.830 mg/l, mortality reached 100% after 14 days and in 1.566 mg/l at day 2. The 21-d LC_{50} was 0.452 mg/l. The overall NOEC of the test was 0.093 mg/l as determined by the onset of clinical signs at the next higher concentration.

An early life stage test was carried out with fathead minnow (*Pimephales promelas*) according to OECD Test Guideline 210 under flow-through conditions (Croudace et al. 1997). Eggs less than 24 h old were exposed to nominal concentrations ranging from 0.0125 to 0.2 mg/l (step size 2). Concentrations were measured 13 times at regular intervals during the 36-day test period. Egg hatchability was not significantly affected in any of the test concentrations. Larval survival after 32 days was not affected in concentrations of 0.068 mg/l and below. In the highest concentration of 0.140 mg/l mean larval survival was 78%. Larval growth was not affected in concentrations of 0.068 mg/l (-11% weight, not statistically significant). At 0.140 mg/l, mean length and weight were reduced by 20 and 54%, respectively, as compared to the solvent control. Larvae surviving in the highest concentration (0.140 mg/l) were recorded to be generally smaller, less well developed and appeared less active, exhibiting some erratic swimming behaviour and loss of balance. Therefore, the NOEC for HHCB from this e.l.s. fish study is 0.068 mg/l.

Apart from the completely reported GLP studies, some other test results are available on zebrafish. A short test on an early life stage was carried out with zebrafish by Carlson and Norrgren (2004). Nominal concentrations ranged from 1 to 1000 µg/l. Concentrations were not measured. Selected newly laid zebrafish eggs were individually exposed in 96-well styrene plates in 250 µl of test medium during 2 days. No effects were found on development of the embryos after 2 days. A slight but not significant effect was found on heart rate that was decreased by 5% in 1000 µg/l. The biological significance of this parameter however is unclear and it is unsuitable for risk assessment.

In an early life stage test with zebrafish ventro-dorsal curvature of the tail was observed after 96h of exposure to concentrations ≥ 0.45 mg/l. The 96h-EC_{50} for embryo hatching was 0.39 mg/l (Dietrich and Chou 2001).

In another early life stage test with zebrafish, four hours to three days after fertilisation of eggs, abnormal tail curving was observed at a concentration of 0.8 mg HHCB/l and not at 0.4 mg/l (Schreurs et al. 2001).

3.2.1.1.2 Aquatic invertebrates

For *Daphnia magna*, a semi-static 21-d toxicity test was carried out according to OECD Test Guideline 202, part II, proposed updated version of June 1993 (Wüthrich, 1996a). The test medium was refreshed three times per week. Nominal concentrations ranged from 0.062 to 1.0 mg/l (step size 2). Concentrations were measured at the start and end of the first and last exposure period. Mobility of the parent generation was not affected at concentrations up to and including 0.205 mg/l, whereas 100% were immobile in the next higher concentration – 0.419 mg/l. At 0.111 mg/l the mean reproduction was inhibited by 21% as compared to the solvent control. According to the Dunnett test ($P<0.05$), this was statistically not significantly different from the control. At the next higher concentration, 0.205 mg/l (LOEC) the mean reproduction was inhibited by 26%. At 0.419 and 0.842 mg/l, reproduction of the surviving adults was inhibited completely.

The chronic toxicity to marine copepod *Acartia tonsa* was tested according to OECD Draft Guideline for Testing of Chemicals (2004) describing a life cycle test, with specific adaptations to prevent volatilisation and accumulation of organic debris (Bjørnestad 2007). For this test HHCB was mixed with 0.1% of radio-labelled [3-^{14}C]-HHCB with a radiochemical purity of 94.8%. Ethanol was used as a solvent. Test concentrations were 37.5, 75, 150, 300 and 600 µg/l, with four replicates per concentration and 6 replicates in the control as well as in the solvent control. For the test 110-ml glass flask were used, closed with screw caps with Teflon seals and filled almost completely with natural seawater, salinity 30.2 ‰, and test substance leaving a headspace of 5 ml. The test was carried out at 20 ± 1.0°C in a climate room with a daily light/dark period of 16:8 hours. One day before addition of the eggs, the flasks were saturated with the appropriate test concentration. The test was initiated with approximately 80 eggs in each flask and approx. 7,000 cells/ml of the algae *Rhodomonas salina.* The algal concentration was readjusted daily to 7,000 cells/ml and the flasks were slowly rotated to keep the algae from settling. This was confirmed to be the optimal design to prevent the formation of organic debris and the loss of test substance by sorption and volatilisation. Samples of the test solution for LSC were taken daily. After 5.5 days the number of nauplii, copepodites and non-hatched eggs was counted and the lengths of the larvae was measured.

The actual test concentrations were >80% of nominal during the whole study, implying that the nominal concentrations can be used for the calculations. The larval mortality in the control was below below the quality criterion of 30%: 27.1 % and 29.1% in the control and solvent control, respectively. The larval development ratio (LDR) was 73% in the control and 72% in the solvent control. The NOEC(LDR) was 37.5 µg/l with an inhibition of 7.5% as compared to the solvent control. The inhibition was 27% in 75 µg/l (LOEC). At 300 µg/l, the length of the nauplii was significantly smaller than in the controls. Moreover no copepodites were observed. Up to 150 µg/l, there was no impact on growth. The EC10(LDR) was 43.8 µg/l (95% confidence interval 30.1 – 55.3 µg/l), and the EC50 was 115 µg/l (131-153 µg/l).

Apart from these completely reported GLP studies, results are available for a number of other invertebrate species, see **Table 3.32** .

The GLP-study on *A. tonsa* described above was initiated as a result of the publication of Wollenberger et al. (2003) on this species. The effects of HHCB on the larval development rate of the marine copepod *Acartia tonsa* was investigated according to draft ISO/DIS 14669 (1997). The 48h-LC_{50} was 0.47 mg/l, whereas 5d-EC_{50} for larval development was 0.059 mg/l and the 5d-EC_{10} was 0.037 mg/l (Wollenberger et al. 2003). The test showed a number of methodological deficiencies, e.g., (1) the test concentrations were not measured and it is known from a similar set-up that concentrations were decreased to only 10-30% after 2 days;

(2) the renewal of the medium by only 50% combined with daily feeding probably left residual algae and faeces in the medium to which the test substance could readily sorb. This probably lead to oral exposure in addition to the intended exposure throught the water phase. In view of the newer test results of the GLP study with this species, the results of the Wollenberger study are not used for the risk assessment.

A population growth experiment was carried out with the marine harpactoid copepod *Nitocra spinipes*. After short-term exposure (96 h) the LC_{50} was 1.9 mg/l (1.40-2.70). To investigate the chronic toxicity, eight replicates of 8-10 nauplii per concentration were tested in 10 ml test medium. The test was carried out at 20 °C in artificial seawater (low salinity: 6‰). Every other day, 70% of the medium was renewed and new food (salmon feed) was added. The test included the period between hatching of the F_0 to hatching of F_1-generation. Concentrations in the chronic test ranged from 0.002 to 0.2 mg HHCB/l. The larval development ratio was established after 7 to 8.5 days and the exposure for the life-cycle experiment was terminated at day 22, although for ovigerous females the exposure was continued until day 26. Survival was not affected but the larval development rate was reduced by circa 50% in 0.2 mg/l. At lower concentrations a statistically significant reduction was observed as well but without a clear concentration-effect relation. No effect was observed on the true population parameter intrinsic rate of increase (r_m) up to and including 0.2 mg/l (Breitholtz et al. 2003). Measurements showed that the initial concentrations were variable (57-117% of nominal) and that concentrations were not maintained at all after the 2 days interval before renewal (2 – 19% of nominal). The loss of HHCB is partly explained by volatilisation, as in the above described *Acartia* test, the concentrations were well maintained in a closed system. An explanation of the variability of the concentrations may be the sorption to organic residues remaining in the system after incomplete (70%) refreshment of the medium. *Nitocra spinipes* is a benthic organism so it would also be exposed to the test substance sorbed to organic residues. The test conditions did not ensure a 'water-only' exposure and therefore the results of this test can not be expressed on the basis of the concentration in water. This is also remarked by the authors in Breitholtz et al. (2003).

A more detailed evaluation of the long-term aquatic toxicity study with *Nitocra spinipes* is included in Appendix 1 of this RAR. In this evaluation it is reasoned that *Nitocra spinipes* could be more sensitive for HHCB than the other sediment-dwelling organisms tested. However, this calculation is prone to some uncertainties. From the acute tests with water-only exposure it can not be concluded that *Nitocra spinipes* is more sensitive than other benthic species. For this reason, there is no need for a new toxicity study with *Nitocra spinipes* tested in sediment (regardless of the fact that no test protocol is available for such study).

Although also in the *short term* tests with *Acartia tonsa* (Wollenberger et al. 2003) and *Nitocra spinipes* the test concentrations have not been measured, in view of the relatively short exposure period, the LC50 values based on nominal concentrations are considered valid (only) for the classification and labelling of the substance.

The toxicity of HHCB to midge larvae *Chironomus riparius* was determined in a 96h test with mortality as endpoint. Per concentration 10 midge larvae were exposed individually. The test medium was renewed every 24 hours. HHCB was solved in isopropanol and 6 concentrations were tested, ranging from 0 to 6.6 µMol (1.7 mg/l). The concentrations were chemically analysed in freshly prepared medium and before renewal. The LC_{50} was 0.288 mg/l. The measured body residues in the surviving animals were all below 0.02 mmol/kg wwt. They were independent of aqueous concentrations and far below reported lethal body burdens for narcosis type compounds indicating that HHCB is indeed biotransformed but does not act directly by narcosis (Artola 2002, Artola et al. 2003).

The same author also reported on the toxicity to the worm *Lumbriculus variegatus* in a 120h static test with immobilisation as endpoint. HHCB was solved in isopropanol and 6 concentrations were tested, ranging from 0 to 6.6 µMol (1.7 mg/l). The concentrations were chemically analysed in freshly prepared medium and at termination. Ten worms were exposed in triplicate. The LC_{50} was 0.394 mg/l. The concentrations measured in surviving organisms in the highest test concentration (27 mmol/kg wwt) are somewhat higher than the critical body residues for narcosis (2-10 mmol/kg wwt). This was explained by the relative insensitivity of *L. variegatus* (Artola 2002).

The effect of HHCB on early life stages of the freshwater mussel *Lampsilis cardium* was studied. Glochidia (larvae) were tested in 24 and 48-h static tests with concentrations up to 1.6 mg/l. The concentrations were measured by GC/MS and were shown to drop to only 5 to 19% of the nominal concentrations. The LC_{50} values ranged from 0.56 to 0.98 mg/l. In tests with juveniles there was no significant mortality, but a slight dose dependant reduction of growth rate was observed (Gooding et al. 2004). As the experimental details are not available the interpretation cannot be evaluated.

3.2.1.1.3 Algae

The toxicity to algae was studied in a static test according to OECD Test Guideline 201 with *Pseudokirchneriella subcapitata* (Van Dijk 1997). Concentrations were measured at the start and end of the test. Maintenance of the concentrations proved to be difficult and in some cases pH-values rose to above 10 indicating an insufficient buffering capacity of the medium. The loss of test material in the algal test was studied for a related polycyclic musk and it was shown that sorption to algal cells was the most relevant 'sink', whereas sorption to glass might play a role as well as volatilisation (see Appendix I, Table A1-2). The results were expressed as the mean concentration over the exposure time. Both biomass production and growth rate were not significantly inhibited up to and including 0.201 mg/l. Growth rate was inhibited by 9 and 20% (both statistically significant) at 0.466 and 0.854 mg/l. The NOEC was 0.201 mg/l (-9%). The inhibition based on Area Under the Curve was 35% and 56% at the highest two concentrations. .

3.2.1.1.4 Microorganisms

For microorganisms no specific toxicity tests have been carried out. In the standard tests on biodegradation that have been conducted (see section 3.1.3.1.2), the toxicity control proved that HHCB was not toxic to the inoculum at concentrations far above the water solubility. Thus NOEC > 20 mg/l.

3.2.1.1.5 Amphibians

The acute toxicity of HHCB was tested on the South African clawed frog larvae (*Xenopus laevis*) in a procedure analogous to ASTM guideline E 1439-91. The 96h-LC_{50} for embryo-adult was > 2.0 mg/l, the 96h-EC_{50} was > 2.0 mg/l for embryo growth and > 4.0 mg/l for embryo malformation (Dietrich and Chou 2001).

3.2.1.2 Calculation of Predicted No Effect Concentration ($PNEC_{water}$)

For the determination of the PNEC various results of prolonged toxicity tests are available for algae, the invertebrates Daphnia and Acartia, and fish that were fully reported and carried out according to GLP requirements. Tests are also available for other species of the class of crustaceans, insects, molluscs, annelids and amphibians, however, the validity of these data cannot be established as critical pieces of information are lacking (information on actual test concentration, concentration-response, variability of replicates, control survival, etc.). Based on the results of the tests summarised in **Table 3.31** and **Table 3.32** (See **Table 3.33**) the lowest value is the EC_{10} of is 0.044 mg/l for the larval development of the marine crustacean *Acartia tonsa*. Therefore with an assessment factor of 10, **$PNEC_{water}$ is 4.4 µg/l**.

Table 3.33. Summary of aquatic toxicity data from prolonged tests

	Test organisms	Results [mg/l]
Algae	*Pseudokirchneriella subcapitata*	72h-NOEC = 0.201
Crustaceans Cladocera Copepods	*Daphnia magna* *Acartia tonsa*	21d-NOEC(rep) = 0.111 5d-EC_{10} = 0.044
Fish	Bluegill sunfish *Lepomis macrochirus*	21d-NOEC = 0.093
Fish	Fathead minnow *Pimephales promelas*	36d-NOECgrowth, develop.= 0.068,

For microorganisms no specific toxicity tests have been carried out. In the biodegradation tests, no inhibition was observed, implying that the NOEC is above 20 mg/l. With an assessment factor of 10, the **$PNEC_{STP}$** would be > 2 mg/l. This PNEC is above the water solubility of HHCB of 1.75 mg/l.

3.2.1.3 Toxicity test results for sediment organisms

Toxicity tests were carried out with three species of sediment organisms, according to or in line with the OECD TG 218 (Draft December 2002): Sediment-water chironomid toxicity test using spiked sediment. The sediment was formulated from 5% Sphagnum moss peat, 75% quartz sand (>50% in range 50-200 µm), 20% kaolinite clay and 0.05% calcium carbonate to adjust the pH between 6.5 and 7.1. The organic carbon content was 2%. At the same time 0.2-0.25% Urtica powder was added as feed. The formulated sediment was conditioned for 7 days prior to application of the test material. The test material was solved in acetone to prepare the stock solutions for each concentration. The proper volumes were mixed first with dry quartz sand allowing the solvent to evaporate. Next the sand was mixed with the formulated sediment to achieve the intended nominal concentration levels. Each glass vessel contained a layer of 1.5 to 3 cm of sediment and the water (Elendt medium M4) volume was 3.5 to 4.5 times the sediment volume. Both a control and a solvent control were included. The test animals were introduced after an equilibration period of 1 week. The tests were carried out at 20 ºC under a 16/8 hours L/D cycle with a light intensity of 400 to 600 lux. The overlying water was slightly aerated during the test. No additional food was given during the test. Test concentrations were measured. Samples of porewater and overlying water were extracted by

SPE using Speedisks. Sediment samples were freeze-dried and analysed by GC/MS after solvent extraction. The results are reported by Belfroid and Balk (2005). These tests were carried out under GLP and they are completely documented. The results are summarised in **Table 3.34**.

Table 3.34. Sediment toxicity of HHCB (GLP and completely documented)

Test and reference	Results [1] [mg/kg dwt] <95% c.l.>	Remarks [2]
Insectae	NOEC ≥ 1000 (development rate)	solvent: acetone, 2.6% OC
Chironomus riparius	NOEC = 250 (emergence ratio)	n=5
28 d	LOEC = 500 (emergence ratio)	identification by GC/MS
Egeler & Gilberg 2004a	EC_{50} = 402 <260 – 829> (emergence ratio)	start conc. 80% of nominal
		end conc. 82% of start
Crustaceae, Amphipoda	NOEC = 34.7 (survival)	solvent: acetone, 1.8% OC
Hyalella azteca	LC_{50} = 62.5 <49.8 – 78.4>	n=5
28 d	NOEC = 14.5 (growth, nominal)	identification by GC/MS
Egeler 2004	NOEC = 7.1 (growth, measured)	start conc. 54% of nominal
	LOEC = 34.7 (growth)	end conc. 96% of start
	EC_{50} = 53.5 <53.0 – 54.0> (growth)	
Oligochaeta	NOEC ≥ 140 (survival)	solvent: acetone, 2.1% OC
Lumbriculus variegatus	NOEC = 26.5 (reproduction)	n=5
28 d	LOEC = 60.9 (reproduction)	identification GC/MS
Egeler & Gilberg 2004b	EC_{50} = 74.1 <42.5 – 165.7> (reproduction)	start conc. 60% of nominal
	NOEC = 60.9 (biomass)	end conc. 85% of start
	LOEC = 140 (biomass)	

1 nominal concentrations; <95% confidence limits>

2 The number of concentrations tested (n) excludes control and solvent control

For the midge larvae *Chironomus riparius* the test was carried out with five concentrations ranging from 62.5 to 1000 mg/kg dwt with step size 2 (Egeler and Gilberg 2004a). Twenty animals, first instar larvae, were used in each of the four replicates per test concentration, in the control and in the solvent control. The test concentrations were measured on day 0, 14, 20 and 28 in the control, 125 and 1000 mg/kg. After the equilibrium period the test concentration was on average 80% of the intended nominal test concentration. At termination of the test, the average concentration was 82% of the concentration at the start. The development rate was not affected up to 1000 mg/kg for both males and females. The emergence ratio was the more sensitive endpoint. A clear dose response relation was found for the emergence of the midges. The data for males and females were pooled for the statistical analysis. The NOECemerg. was 250 mg/kg (-4%), the EC_{15} was 259 mg/kg with 95% confidence limits <164 – 435> and the EC_{50} was 402 <260 – 829> mg/kg dwt. The actual concentrations remained largely within 80% of the initial concentrations at day 0. The NOEC in this study was 80% of 250 mg/kg or **200 mg/kg dwt (measured concentration)**.

The Amphipoda *Hyalella azteca* was tested in five concentrations ranging from 6 to 200 mg/kg sediment with step size 2.4 (Egeler 2004). The test animals were 7 to 14 days old, and between 355 and 500 μm. Four replicates each with 10 animals were used per test concentration and in the solvent control, whereas six replicates were used in the control. The

test concentrations were measured on day 0, 9, 19 and 28 in the control, 35 and 200 mg/kg. At the start of the test the test concentration was on average 54% of the intended nominal test concentration. At termination of the test, the average concentration was 96% of the concentration at the start. Survival was not affected up to 35 mg/kg, whereas the mortality in 83 mg/kg was 88% and 100% in 200 mg/kg. Thus the LC_{50} was 62.5 mg/kg. Growth was inhibited at 35 mg/kg (LOEC). The length of the amphipods was 9% below the pooled control at 35 mg/kg, whereas the inhibition for the (total) biomass per replicate was 15%. At the NOEC, 14.5 mg/kg, the inhibition was 3% for length and none for biomass. The EC_{50} for biomass was 53.5 mg/kg. The actual concentrations at day 0 were on average 49% of the initial concentrations and remained at this level during the test. The actual concentration on day 0 at the LOEC level was measured: 16.3 mg/kg. The actual concentration at the level of the **NOEC** (nominal 14.5 mg/kg) **was 7.1 mg/kg** in this study.

The aquatic oligochaete worm *Lumbriculus variegatus* was tested in concentrations ranging from 5 to 140 mg/kg with step size 2.3 (Egeler and Gilberg 2004b). The test animals were 'synchronised' before the start of the test to avoid high variation in the test results. Four replicates each with 10 regenerated animals were used per test concentration and in the solvent control, whereas six replicates were used in the control. The test concentrations were measured on day 0, 9, 20 and 28 in the control, 26.5 and 140 mg/kg. At the start of the test the test concentration was on average 60% of the intended nominal test concentration. At termination of the test, the average concentration was 85% of the concentration at the start. Survival was not affected up to the highest test concentration. The total number of worms (including adult and regenerated worms) at the end of the test was evaluated as a parameter of reproduction. As compared to the solvent control, reproduction was significantly inhibited by 50% in 61 mg/kg, whereas the inhibition was 8% at 26.5 mg/kg (NOECrepr.). The $EC_{50repr.}$ was 74.1 <42.5 – 165.7> mg/kg, the $EC_{15repr.}$ was 24.1 <1.8 – 42.1> mg/kg. Growth as measured by biomass was significantly inhibited by 80% at 140 mg/kg, whereas at 61 mg/kg the inhibition was 13% (NOECbiomass). The EC_{50}(biomass) was 97.7 <77.7 – 119.7> mg/kg, the EC_{15}(biomass) was 62.4 <37.7 – 78.3> mg/kg. The NOEC in this study was 26.5 mg/kg. The actual concentration on day 0 at the level of the NOEC was measured: 16.2 mg/kg sediment. The NOEC in this study is taken to be **16.2 mg/kg (measured concentration)**.

Data are also available for sediment organisms (*Chironomus riparius* and *Lumbriculus variegatus*) tested in water during 4 to 5 days. They were summarised under 3.2.1.1.

3.2.1.4 Calculation of Predicted No Effect Concentration ($PNEC_{sediment}$)

PNECsediment is determined from the results of the three tests described in section 3.2.1.3, with the midge larvae, amphipoda and worms. These tests were carried out, according to the protocol, in a substrate containing 2% organic carbon. In the TGD, PECsediment is derived for a sediment containing 5% organic carbon and thus NOEC needs to be standardised to 5% organic carbon.

This is shown in **Table 3.35**. The lowest NOEC is 19.7 mg/kg for the growth of *Hyalella azteca*. Since there are tests with benthic species of three different taxonomic groups, an assessment factor of 10 is applied to the lowest of the NOECs, giving **$PNEC_{sediment}$** of **2.0 mg/kg dwt.**

Table 3.35. Summary of sediment toxicity data

	Test organisms	Results [mg/kg dwt], measured, 2% OC	Result standardised, [mg/kg dwt], 5% OC
Insecta	*Chironomus riparius*	28d-NOECemergence = 200 (OC 2.6%)	28d-NOECdevelopment = 385
Crustaceans Amphipoda	*Hyalella azteca*	28d-NOECgrowth = 7.1 (OC 1.8%)	28d-NOECgrowth = 19.7
Worms Oligochaeta	*Lumbriculus variegatus*	28d-NOECgrowth = 16.2 (OC 2.1%)	28d-NOECgrowth = 38.6

For comparison, $PNEC_{sediment}$ was also derived according to the equilibrium partitioning theory:

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

where:

$PNEC_{sediment}$: PNEC for sediment-dwelling organisms (kg/kg_{wwt})

$PNEC_{water}$: PNEC for aquatic organisms (kg/m^3)

$K_{susp.-water}$: suspended matter-water partition coefficient (619 m^3/m^3)

RHO_{susp}: bulk density of suspended matter (1150 kg_{wwt}/m^3)

With $PNEC_{water}$ = 4.4 μg/l, $PNEC_{sediment}$ = 2.36 mg/kg wet weight. This is converted to dry weight by multiplication with a factor of 4.6 (susp.solids) and thus **$PNEC_{sediment,\ EqP}$ = 10.9 mg/kg dry weight**.

The $PNEC_{sediment}$ based on sediment tests and the one derived by equilibrium partitioning from $PNEC_{water}$ differ by a factor of 5.5.

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity test results for terrestrial organisms

3.2.2.1.1 Plants

No data available. In section 3.1.3.4.4 it was noted that in a mixture of soil and sludge with a total concentration of 30 mg/kg dwt the growth of plants was inhibited (Müller et al. 2002). However, the cause and the extent of the inhibition were not clarified.

3.2.2.1.2 Earthworm

Toxicity tests were carried out with earthworms and springtails (see **Table 3.36**). The test materials at appropriate concentrations were dissolved in equal amounts of acetone, mixed with the quartz sand and allowed to slowly evaporate and mixed with the standard soil containing 10% Sphagnum peat, 20% kaolinite clay, approximately 70% fine quartz-sand (grain size 0.1-0.5 mm) and 0.5% calcium carbonate to adjust to pH 6.0±0.5. After preparation of the test concentrations and an equilibrium period of one week, the test organisms were added to the soil. The test medium was not refreshed during the test period. The earthworm test was carried out according to ISO 11268 (Gossman 1997). Adult worms (*Eisenia fetida*) were exposed to nominal concentrations in soil of 8, 19, 45, 105 and 250 mg/kg. Weights of the adult worms ranged between 340 and 540 mg, but did not differ more than 100 mg within this range in each test container. The worms were fed weekly with finely ground cattle manure. Adult worms were removed after 4 weeks of exposure, counted and weighed. The remaining offspring remained in the test containers for another four weeks. Mortality of the adults was not affected after 4 weeks in concentrations up to 250 mg/kg. In the range finding test mortality was 100% after 14 days exposure to 1000 mg/kg. Growth was significantly inhibited (15%) in the highest concentration of 250 mg/kg. Reproduction was not significantly affected up to concentrations of 45 mg/kg (7% inhibition, NOEC). At the level of the LOEC (105 mg/kg), the reproduction was 57% of the control, whereas in the highest concentration reproduction was inhibited completely.

3.2.2.1.3 Microorganisms

No data are available on the toxicity to specific microorganisms in soil.

3.2.2.1.4 Other terrestrial organisms

The springtail test was carried out according to the draft ISO/CD 11267 (Klepka, 1997). The conditions were as described for the earthworm. The test was started with juvenile springtails of the species *Folsomia candida* 10 to 12 days of age and survival and reproduction after 28 days were determined. Nominal test concentrations were 1, 3, 8, 19, 45 and 105 mg/kg soil. The animals were fed with granulated dry yeast. No significant mortality was observed in soils containing up to 45 mg/kg, but mortality was 72% in 105 mg/kg. Reproduction was inhibited by 23% in 45 mg/kg. However, this inhibition was not statistically significantly different from the control. The reproduction in 105 mg/kg was 16% of the control. Thus, the NOEC for HHCB is 45 mg/kg based on survival.

Table 3.36. Toxicity data for soil organisms

Test and reference	Results for HHCB (nominal concentrations)	Remarks
Earthworm	8wk-NOEC = 45 mg/kg,	inital weight adults 0.34-0.54 g
Eisenia foetida	LOEC[2] = 105 mg/kg,	test range 8-250 mg/kg
ISO 11268	reproduction and food consumption	solvent: acetone
(OECD 207)	4wk-$NOEC_{growth}$ = 105 mg/kg,	artificial soil pH 6.1,
	LOEC = 250 mg/kg	10% sphagnum DIN[1]
Gossmann 1997	4wk-$NOEC_{survival} \geq$ 250 mg/kg	temp. 17-23°C

Test and reference	Results for HHCB (nominal concentrations)	Remarks
Springtail *Folsomia candida* ISO /CD 11267 Klepka 1997	4wk-NOEC = 45 mg/kg, LOEC[2] = 105 mg/kg, mortality and reproduction	10-12 d old juveniles test range 1-105 mg/kg solvent: acetone temperature 17-25°C artificial soil, 10% sphagnum DIN[1]

[1] Sphagnum DIN standard: organic material minimum 90%, organic carbon 52%

[2] Dunnet's test (p=0.05)

[3] Student's t-test (p=0.05)

3.2.2.2 Calculation of Predicted No Effect Concentration ($PNEC_{soil}$)

For HHCB two long term tests are available, allowing an assessment factor of 50 to be applied to the lowest NOEC. However, first this lowest NOEC is normalised to the standard soil of the TGD containing 3.4% of organic material: 45 / 0.1 ·0.034 = 15.3 mg/kg. Therefore **$PNEC_{soil}$ = 0.31 mg/kg dwt or 0.28 mg/kg wwt**.

If $PNEC_{soil}$ were derived from $PNEC_{aqua}$ by equilibrium partitioning, $PNEC_{soil, equil}$ = 1.28 mg/kg wwt or 1.54 mg/kg dwt.

3.2.3 Atmosphere

No data are available on exposure of organisms via the air. Therefore, no $PNEC_{air}$ can be derived.

3.2.4 Secondary poisoning

No specific toxicological data are available on e.g. (fish-eating) birds. The PNEC for secondary poisoning will therefore be based on mammalian toxicity data for HHCB.

3.2.4.1 Effect data

See relevant paragraphs in Human Health Chapter (4.1.2). Two relevant NOAEL values were identified: a NOAEL of 150 mg/kg bw/d was derived from the 90-day oral study with rats and a NOAEL of 50 mg/kg in a 21-day reproduction and development toxicity study.

3.2.4.2 Calculation of Predicted No Effect Concentration ($PNEC_{oral}$)

A NOAEL of 150 mg/kg bw/d was derived from a 90-day oral study with rats (see Chapter 4.1.2.). As toxicity is based on the P-generation (rats > 6 weeks) a conversion factor of 20 has to be used resulting in a NOEC of 3000 mg/kg food (e.g., in fish). For the derivation of $PNEC_{oral}$, the test duration of 90 days implies an assessment factor of 90, giving a $PNEC_{oral}$ = 33.3 mg/kg food.

In a 21-day reproduction and development toxicity study, the NOAEL was 50 mg/kg/d. With the same conversion as above, the NOEC in food is 1000 mg/kg. The assessment factor for a 28 day test is 300 and then the result is 3.33 mg/kg food:

Thus $PNEC_{oral}$ ranges from 33.3 to 3.33 mg/kg food.

In the current risk assessment $PNEC_{oral}$ = 3.33 mg/kg food was used.

3.2.5 Marine effects assessment

For the marine effects assessment, the available toxicity data of freshwater and marine test organisms may be pooled. According to the TGD (EC 2003) there are no reasons to believe that a systematic bias to freshwater or marine species would exist. In view of the larger diversity of taxa in the marine environment it is expected that there is a wider distribution of the species sensitivity. This is reflected by a larger assessment factor in the derivation of the PNEC for the marine environment.

Results are available from long-term tests with species from at least three trophic levels: algae as the primary producers, *Daphnia* and Acartia as the primary consumers and fish as secondairy consumers (see **Table 3.33**). Therefore the assessment factor is 100 (instead of 10 used in the freshwater compartment), applied to the lowest EC_{10} of 44 µg/l for the marine copepod *Acartia tonsa*. Therefore the **$PNEC_{marine\ water}$ = 0.44 µg/l**.

The PNEC for the marine sediment is derived from three long-term sediment tests with species representing different living and feeding conditions, implying that an assessment factor of 50 is applied to the lowest NOEC of 4.3 mg/kg wwt (OC-normalised), see section 3.2.1.3. Thus **$PNEC_{marine\ sediment}$ = 0.086 mg/kg wwt or 0.394 mg/kg dwt.**

3.2.6 Other effects

This section summarises other effects reported in literature, such as endocrine disruption and other subcellular interactions. Studies on endocrine interactions are also described in section 4.1.2.9.3 of the Human Health Risk Assessment. Here the studies with possible environmental relevance are described.

3.2.6.1 Endocrine interactions

In vitro studies

The activity of HHCB on the estrogen induced vitellogenin (Vtg) production in carp (*Cyprinus carpio*) was investigated by using cultured primary hepatocytes from genetically uniform strains of carp. Vtg production was measured by indirect competitive ELISA, using a polyclonal antiserum against goldfish Vtg that cross-reacts with carp Vtg (described in Smeets et al., 1999). The Vtg production by carp hepatocytes, a sensitive marker of estrogenic activity, was not affected by HHCB (cited in Seinen et al., 1999).

The interaction of HHCB (purity unknown) with the hepatic estrogen receptor(s) of rainbow trout, carp and the amphibian *Xenopus leavis* was investigated in a competitive binding assay. In the *X. leavis* assay a concentration of 258 mg HHCB/l did not give competitive inhibition of the binding of 17β-estradiol to the receptor up to 1 mmol (258 mg/l). Very weak binding of

HHCB was found in the rainbow trout receptor-binding assay. No competitively binding took place in the carp receptor-binding assay, corroborating the results cited by Seinen et al., 1999 (Dietrich and Chou, 2001).

The ecsysteroid agonist and antagonist activity of HHCB was assessed in an assay with the *Drososphila melanogaster* BII-cell line. The concentration of 20-hydroxyecdysone used in the antagonist assay was $5 * 10^{-8}$ M (i.e. 0.024 mg/l). HHCB did not show specific agonistic or antagonistic activity in this bioassay up to the highest concentration (10^{-4} M i.e. 26 mg/l). However, HHCB was cytotoxic at concentrations $\geq 2.5 * 10^{-5}$ M (6.5 mg/l) (Breitholtz et al., 2003).

Human embryonal kidney 293 (HEK293) cells were transient transfected with plasmids containing the human and zebrafish estrogen receptor isoforms (hERα and hERβ, and zfERα, β and γ) and an estrogen responsive reporter gene construct (ERE-luciferase). HHCB was tested up to 10 µM. HHCB alone showed a marginal transcriptional activation of hERα at the highest test concentration. The other receptors were not affected at all. The anti-estrogenic activity was tested using a submaximal dose of 0.01 nM estradiol (E2) for hERα, 0.1 nM E2 for hERβ and zfERγ and 1 nM E2 for zfERα and zfERβ, together with 0.1, 1 or 10 µM HHCB. A dose-dependent suppression by HHCB of E2 induction was shown toward hERβ and zfERγ. A weak antagonistic effect could be observed on hERα and zfERβ only at the highest test concentration of 10 µM (i.e. 2.6 mg/l), whereas no effect was seen at zfERα (Schreurs et al., 2004).

In vivo studies

This same group also investigated the possible anti-estrogenic effects in zebrafish *in vivo* (Schreurs et al., 2004). Transgenic zebrafish, containing a similar reporter gene construct as used in the above mentioned *in vitro* experiments, were exposed to HHCB with and without E2. HHCB at a concentration of 10 µM was toxic to the fish. HHCB did not show any estrogenic effect with the tested concentrations of 0.01, 0.1 and 1 µM. The concentrations of 0.1 and 1 µM (258 µg/l) resulted in a dose-dependent antagonistic effect on E2 (at 0.01 µM E2) in the juvenile zebrafish. The repression was down to 20% of the E2 induction at the highest test dose. The authors state that the actual concentrations at which they observe anti-estrogenic effects are around or below the no-observed effect levels in the e.l.s tests and growth tests with fish and they conclude that no developmental disorders were or will be observed at the concentrations used in their transgenic zebrafish assay (Schreurs et al., 2004).

3.2.6.2 Other effects

The interaction of HHCB with multixenobiotic resistance (mxr) transporters was studied in gill tissue of the marine mussel *Mytilus californianus* (Luckenbach et al. 2004a). Mxr transporters are ATP dependant efflux pumps that remove a broad spectrum of chemically unrelated xenobiotics from the cell. A competitive substrate transport test using rhodamine B (RB), a fluorescent substrate of mxr transporters, was used to assay modulation of transport activity by HHCB. If the efflux of RB is inhibited, it accumulates in the cell to a higher degree which is indicated by increased fluorescence. Discs were cut from gill tissue and mucus was removed. The tissue was incubated in seawater with 1 µmol RB and HHCB for 90 min at 15 °C and next rhodamine B was extracted and measured. HHCB caused increased accumulation of RB in a dose-dependent way compared to control levels. The IC_{50} was listed as 2.43 µMol ~ 0.6 mg/l. The IC10 was 0.095 mg/l. The reversibility of the inhibitory effect seemed to be delayed by 24 hours (Luckenbach and Epel 2004b). This may be related to the

high lipophilicity of the polycyclic musks. Effective inhibitory concentrations were similar to quinidine and approximately 100 times higher than for verapamil. The relevance of these *in vitro* observations for the risk assessment is not clear. The mucuous membrane plays a protective role. Removal of this membrane in this test system raises questions as to the relevance of these observations.

The transporter activity in mussel gill is as sensitive as the effects observed in the standard toxicity tests with aquatic organisms. Thus at the exposure level where the protective transporter efflux is decreased rendering the cell more accessible to other potential toxicants, other effects of the synthetic musks show up also in algae or fish tests studying development and growth.

3.3 RISK CHARACTERISATION [13]

The PECs were discussed in section 3.1.4.3 for the aquatic environment, in section 3.1.5.3 for the terrestrial compartment and section 3.1.7.3 for secondary poisoning. In most cases the results of the Southern European scenario were selected for the risk assessment for private use. For completeness the results of measurements in Berlin in 1996/1997 are also included but it should be realised that the situation has improved since then. For comparison, also other scenarios are shown, like the TGD regional (10%) and the scenario for the northern European countries. The data used for the risk assessment are underlined in the tables.

3.3.1 Aquatic compartment (incl. sediment)

Surface water and STP

The PEC/PNEC ratios for the aquatic compartment are presented in **Table 3.37**. The PNECs used are > 2000 mg/l for the STP and 4.4 µg/l for aquatic organisms. The PECs were discussed in section 3.1.4.3. For private use, the results of the SEU-15 scenario are used. All ratios are below 1, hence a **conclusion (ii)** is drawn for all scenarios.

Table 3.37. PEC/PNEC ratios for STP and surface water (µg/l)

	PEC_{STP}	**$PEC/PNEC_{STP}$** **PNEC > 2000**	**$PEC_{surface\ water}$**	**RCR Surface water** **PNEC = 4.4**
Production, formulation and compounding				
Production	2.3	< 1.2E-03	0.66	0.15
Compounding Site 1	10.6	< 5.3E-03	0.078	0.02
Compounding Site 2 (year 2006)	0.80	< 0.4E-03	0.738	0.17
Compounding Site 3	3.0	< 1.5E-03	0.007	0.002
Compounding Site 4	22.3 (WWTP)	< 0.011	0.026	0.006
Compounding Site 5	0.039	< 2.0E-05	0.040	0.009

[13] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

	PEC_{STP}	**$PEC/PNEC_{STP}$** **PNEC > 2000**	**$PEC_{surface\ water}$**	**RCR Surface water** **PNEC = 4.4**
Compounding Site 6				
Compounding, Large-medium generic	4.9	< 2.5E-03	0.526	0.12
Compounding, Small generic	2.0	< 7.0E-04	0.232	0.05
Formulation Large company	2.9	< 1.5E-03	0.32	0.07
Formulation Generic scenario	5.7	< 2.9E-03	0.58	0.13
Private use				
southern EU-15	**4.7**	**< 2.4E-03**	**0.485**	**0.11**
Private use (other scenarios)				
northern EU-15	1.4	< 7.0E-04	0.139	0.03
TGD regional (10%)	(87.5)	(< 0.044)	(8.70)	(1.98)
PECregional SEU-15			0.0336	0.008
PECregional NEU-15			0.0044	0.001
measured 90th percentile Berlin high effluent input area 1996/1997			2.73	0.62

Sediment

The results of the comparison of PEC with PNEC are shown in **Table 3.38**. $PNEC_{sediment}$ = 0.43 mg/kg wet weight or 2.0 mg/kg dry weight, derived directly from toxicological data, where the intake of HHCB by ingestion of food is taken into account. Thus the risk characterisation expressed as PEC/PNEC without an additional factor. The PECs were discussed in section 3.1.4.3.

Table 3.38 shows that for all compounding and formulation scenarios as well as for the production scenario, PEC/PNEC is below 1. Also for the private use scenario which is based on the Southern EU-15 Scenario, the ratio is below 1 (underlined in table).

The assessment based on recent measured concentrations is carried out for the sediment in the Teltow Canal which was a cause for concern in earlier risk assessments. The current data show that PEC/PNEC is below 1. For completeness the measurements in Berlin in 1996/1997 are included. Also here the risk quotient is below 1. In the tiered approach taken in this risk assessment report the availability of sediment toxicity data has generated a higher PNEC value, now resulting in a ratio below 1 also for these historic samples.

Table 3.38. PEC/PNEC ratios for sediment (mg/kg wwt)

	$PEC_{sediment}$ **mg/kg wwt**	**RCR Sediment** **PNEC = 0.43 mg/kg wwt** **PNEC = 2.0 mg/kg dwt**
Production, formulation and compounding		
Production	0.358	0.83
Compounding Site 1	0.042	0.10

	$PEC_{sediment}$ mg/kg wwt	**RCR Sediment PNEC = 0.43 mg/kg wwt PNEC = 2.0 mg/kg dwt**
Compounding Site 2 (year 2006)	0.397	0.92
Compounding Site 3	0.004	0.009
Compounding Site 4	0.014	0.03
Compounding Site 5	0.021	0.05
Compounding Site 6	0.017	0.04
Compounding, Large-medium generic	0.283	0.66
Compounding, Small generic	0.125	0.29
Formulation Large company	0.17	0.40
Formulation Generic scenario	0.33	0.77
Private use		
southern EU-15	**0.261**	**0.61**
Private use (other scenarios)		
TGD regional (10%)	(4.73)	(11.0)
northern EU-15	0.0749	0.17
measured 90th-perc. Berlin high effluent input area 1996/1997	(1.90 mg/kg dwt)	(0.95)
measured max. Berlin, Teltow Canal 2003	**1.1 mg/kg dwt**	**0.55**

Conclusions to the risk assessment for the aquatic compartment:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to Production, compounding, formulation and private use.

3.3.2 Terrestrial compartment

The PEC/PNEC ratios for the soil compartment are presented in **Table 3.39**. PNECsoil is 0.31 mg/kg dwt or 0.28 mg/kg wwt. . The risk ratios for production, compounding and formulation are all below 1.

For the risk assessment of the private use the Southern European Scenario is used. The PEC/PNEC ratio for this scenario is below 1. Therefore **conclusion ii** is justified.

Table 3.39. PEC/PNEC ratios for the terrestrial environment

	PECsoil, mg/kg wwt	**RCR Soil** **PNEC = 0.28 mg/kg wwt** **PNEC = 0.31 mg/kg dwt**
Production, formulation and compounding		
Production	0.0184	0.07
Compounding Site 1	0.0832	0.30
Compounding Site 2 (year 2006)	0.007	0.03
Compounding Site 3	0.0238	0.09
Compounding Site 4	--	
Compounding Site 5	0.335 E-03	0.001
Compounding Site 6		
Compounding, Large-medium generic	0.0387	0.14
Compounding, Small generic	0.0157	0.06
Formulation Large company	0.0225	0.08
Formulation Generic scenario	0.045	0.16
Private use		
southern EU-15	**0.057**	**0.20**
Private use (other scenarios)		
TGD regional (10%)	(0.808)	(2.89)
northern EU-15	0.014	0.05

Conclusions to the risk assessment for the terrestrial compartment:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to Production, compounding, formulation and private use.

3.3.3 Atmosphere

As no $PNEC_{air}$ could be derived, a risk characterisation for the atmosphere is not possible.

3.3.4 Secondary poisoning

The PNECoral for the assessment of secondary poisoning is 3.3 mg/kg. This PNEC is compared with PEC_{oral} for fish as well as for worms. The concentrations selected for the risk assessment were discussed and selected in section 3.1.7.3. The PECs for private use are based on the SEU-15 scenario (underlined). In addition, PNEC is compared to levels measured in fish in the area of Berlin (see **Table 3.40**).

All PEC/PNEC ratios are below 1 (**conclusion ii**).

Table 3.40. PEC/PNEC ratios for fish-eating and worm-eating predators.

	PECoral,fish mg/kg wwt	**PEC/PNEC fish** **PNEC = 3.33**	**PECoral,worm mg/kg wwt**	**PEC/PNEC worm** **PNEC = 3.33**
Production, formulation and compounding				
Production	0.528	0.16	0.0469	0.01
Compounding Site 1	0.0885	0.03	0.229	0.07
Compounding Site 2 (year 2006)	0.57	0.17	0.018	0.005
Compounding Site 3	0.00926	0.003	0.0602	0.02
Compounding Site 4	0.024	0.01		
Compounding Site 5	0.0591	0.02	0.0015	0.0005
Compounding Site 6				
Compounding, Large-medium generic	0.442	0.13	0.098	0.03
Compounding, Small generic	0.203	0.06	0.0401	0.02
Formulation Large company	0.275	0.08	0.0572	0.02
Formulation Generic scenario	0.488	0.15	0.114	0.03
Private use				
southern EU-15	**0.409**	**0.12**	**0.144**	**0.04**
Private use (other scenarios)				
TGD regional (10%)	7.23	(2.17)	2.04	(0.61)
northern EU-15	0.114	0.03	0.0354	0.01
measured 90th-percentile all fish Berlin 1996/1997	1.50	0.45		

Conclusions to the risk assessment for secondary poisoning:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to production, compounding, formulation and private use.

3.3.5 Marine compartment

3.3.5.1 Aquatic marine compartment

With the approach using additional assessment factors of 10 to derive a marine PNEC and a simple approach of a conservative additional dilution factor of 10 in the marine environment, the risk for the marine environment is screened, see **Table 3.41**. For the private use scenario

the marine PEC/PNEC ratios are similar to the freshwater and marine sediment ratios, well below 1.

As indicated in the TGD, a generic scenario for an industrial site must use a default assessment, unless site specific information is available, for PEClocal. This default assumes that industrial effluents are not treated in a municipal biological STP but are discharged directly to the marine aquatic environment. As described in section 3.1.8.1 the results of a survey confirmed that compounders and formulators using AHTN and HHCB discharge their wastewater into the marine environment only after treatment in a sewage treatment plant (Letter of IFF and PFW, July 2006). Therefore the default marine scenario used in the calculations is not realistic. When the presence of an STP is taken into account in the calculations, the PECs for marine water and sediment are considerably lower and thus all PEC/PNEC ratios are well below 1.

Table 3.41. PEC/PNEC ratios for the aquatic marine environment

	Default PEClocal seawater, µg/l	**Default PEC/PNEC seawater**	**With STP PEC/PNEC seawater**	**Default Marine PEC local sediment, mg/kg wwt**	**Default PEC/PNEC sediment**	**With STP PEC/PNEC sediment**
		PNEC = 0.44			**PNEC = 0.086**	
Compounding and formulation						
Compounding, Large-medium generic	0.206	0.56	0.11	0.111	1.3	0.29
Compounding, Small generic	0.085	0.23	0.05	0.046	0.53	0.05
Formulation, Large company	0.122	0.33	0.07	0.066	0.77	0.07
Formulation, Generic scenario	0.237	0.64	0.13	0.128	1.5	0.08
Private use						
southern EU-15	**0.048**		**0.11**	**0.0261**		**0.30**
Private use (other scenarios)						
TGD regional (10%)	0.877		2.0	0.472		(5.5)
northern EU-15	0.0137		0.03	0.0074		0.09

Conclusions to the risk assessment for the aquatic marine compartment:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to compounding, formulation and private use.

Note that for compounding and formulation this conclusion is now based upon evidence that currently no medium-large compounders or formulators located within a radius of 500 m from a sea or ocean shore discharge their waste water directly (i.e. without treatment) into the marine environment. If such would be the case a risk would be identified and the need for limiting the risks would be indicated (conclusion iii). See **Table 3.41**.

3.3.5.2 Secondary poisoning

The risk for food chain effects is expressed as the PEC/PNEC ratio for a predator in the marine food chain and for a top-predator, see **Table 3.42**. The risk ratios are below 1 for the private use scenario. Also for the default compounding and formulation scenarios the PEC/PNEC ratios are below 1. Therefore no calculations were performed with inclusion of the STP. The concentrations measured in marine fish in Norway are also below the PNEC.

Table 3.42. PEC/PNEC ratios for predators in the marine environment

	PECoral predator mg/kg wwt	**PEC/PNEC predator** **PNEC = 3.3**	**PECoral top-predator mg/kg wwt**	**PEC/PNEC top-predator** **PNEC = 3.3**
Compounding and formulation				
Compounding, Large-medium generic	0.165	0.05	0.0367	0.01
Compounding, Small generic	0.070	0.02	0.0175	0.005
Formulation, Large company	0.099	0.03	0.0234	0.007
Formulation, Generic	0.190	0.06	0.0416	0.01
Private use				
southern EU-15	0.040	0.01	0.0117	0.004
Private use (other scenarios)				
TGD regional (10%)	0.721	0.22	0.1861	0.06
northern EU-15	0.011	0.003	0.0027	0.001

Conclusion to the risk assessment for secondary poisoning in the marine food chain:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to compounding, formulation and private use.

3.4 PBT ASSESSMENT

In order to protect the marine environment against unpredictable or irreversible long-term effects, substances must be submitted to a so-called PBT-assessment. Available data must be tested to the PBT-criteria in the TGD (EC 2003). For substances that do not fulfil all three PBT criteria, but are known to be persistent and bioaccumulating, vPvB (very persistent and very bioaccumulating) criteria are set.

3.4.1 Persistence

The TGD (EC 2003) criteria for persistence for PBT substances as well as vPvB substances are summarized in **Table 3.43**.

Table 3.43. PBT criteria for persistence

PBT		vPvB	
criterion (days)	**environment**	**criterion (days)**	**environment**
t ½ > 60	marine waters	t ½ > 60	water (marine and freshwater)
t ½ > 40	fresh waters	t ½ > 180	sediment (marine and freshwater)
t ½ > 180	marine sediments		
t ½ > 120	fresh water sediments		

For HHCB no data are available from tests that simulate the marine environment in water or sediment. Evidence for rapid degradation is based on various die-away studies in river water (with 0.5 to 5 µg HHCB per liter), resulting in 60% disappearance of the parent material in 28 days (Langworthy et al. 2000, Federle et al. 2002, Schaefer 2005). Overall t½ in river water die- away tests ranged from 33 to 100 hours. The rapid primary degradation was characterised by the formation of more polar metabolites which were slowly mineralised. Moreover, it was also shown that the substance is rapidly metabolised in fish (t½ 1–3 days) and in midge larvae.

A study was carried out in freshwater sediment (Envirogen 1998). In this test only 4% of the parent compound was present after one year. Assuming a first-order reaction, a half-life value of 79 days was estimated. This is below the limit of 120 days. The results of the soil tests confirm these results.

It was also shown that the substance is rapidly metabolised to polar metabolites in fish (t½ 1 – 3 days) and in midge larvae.

Photodegradation in water is observed by Buerge et al. (2003) and Sanchez-Prado et al. (2004). It may be expected that this photodegradation also takes place in the upper water layer of the marine environment. In atmospheric conditions a half-life of 3.7 hours is determined (Aschmann et al. 2001).

It is concluded that HHCB does not meet the persistence criterion.

3.4.2 Bioaccumulation

The bioaccumulation criterion for PBT substances is BCF > 2000. For vPvB substances, the criterion is BCF > 5000.

The bioconcentration in Bluegill sunfish (*Lepomis macrochirus*) was studied according to OECD Guideline 305E. The BCF is 1584 (Van Dijk 1996). This study was carried out under GLP. In another bioconcentration study with zebrafish (*Brachydanio rerio*) according to OECD Guideline 305E the measured BCF is 624 (Butte and Ewald 1999). BAF values determined from actual measurements in fish and surface water are available. The individual BAF in 165 eel samples in the Berlin area ranged from 201 to 1561 (Fromme et al. 2001b). BAF values for rudd (20), tench (510), crucian carp (580), eel (290) and zebra mussel (620) are also reported (Gatermann et al., 2002a).

Therefore, the BCF for fish is below 2000 although there is an indication that it may accumulate in a lower invertebrate species that is not capable of metabolising the substance.

Evidence for the absence of food chain accumulation or biomagnification is presented in section 3.1.7.2. In general concentrations in predatory organisms in Arctic and marine species were reported to be substantially lower than expected based on the physical-chemical properties of HHCB. In the beluga whale concentrations were relatively low indicating metabolic transformation (Kelly et al. 2004). The concentrations were at or below the background level in the Ringed Seal (Hühnerfuss et al. 2002), Alaskan Polar Bear (Kannan et al. 2005), Sperm Whale and South Polar Skua eggs (Nakata et al. 2007), Red Fox and/or Polar Fox livers (Mogensen et al 204). The extensive studies of Kannan et al. (2005) and Nakata et al. (2007) show that bioaccumulation in the food chain does not occur.

It is concluded that HHCB does not to meet the criterion for bioaccumulation

3.4.3 Toxicity

The criterion for environmental toxicity for PBT substances is NOEC (long term) < 0.01 mg/l.

Several tests on aquatic species were performed under GLP:

Algae: the toxicity to algae was studied in a static test according to OECD Test Guideline 201 with *Pseudokirchneriella subcapitata* (Van Dijk 1997). The measured 72 hours NOEC was 0.201 mg/l.

Invertebrates: for *Daphnia magna*, a semi-static 21-d toxicity test was carried out according to OECD Test Guideline 202, part II, proposed updated version of June 1993 (Wüthrich, 1996a). The measured 21 days NOEC was 0.111 mg/l.

For the marine copepod *Acartia tonsa*, a 5-d toxicity test was carried out with larval development ratio as endpoint according to a specific adaptation of the draft OECD Test Guideline (2004). The 5 days EC_{10} was 0.044 mg/l.

Fish: A 21-d prolonged toxicity test was carried out with bluegill sunfish (*Lepomis macrochirus*) according to OECD Test Guideline 204 under flow-through conditions (Wüthrich 1996b). The measured 21 days NOEC was 0.093 mg/l.

An early life stage test was carried out with fathead minnow (*Pimephales promelas*) according to OECD Test Guideline 210 under flow-through conditions (Croudace, et al. 1997). The 36 days NOEC from this early life stage study is 0.068 mg/l.

Conclusion: the lowest (long-term) experimentally derived NOEC is 0.044 mg/l. Thus, based on the results of 5 GLP studies, HHCB does not does not meet the criterion for environmental toxicity within the scope of the PBT assessment.

The criterion for human toxicity for PBT substances is classification with one or more of the following R-phrases: 25, 28, 40 (carc. cat. 3), 45, 46, 48, 60, 61, 62, 63, 64, 68 (mut. cat. 3). All toxicological tests performed on mammals only justify no classification.

The criterion for endocrine disrupting effects for PBT substances is evidence of ED potential, e.g. listed in the Community Strategy for Endocrine Disrupters. There is no evidence of ED potential; HHCB is not listed in the Community Strategy for Endocrine Disrupters (COM(2001)262final) as a substance with suspected or proven ED potential.

It is concluded that HHCB does not meet the criteria for PBT substances.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General introduction

See also section 1 for general substance information and physico-chemical substance properties and section 2 for general information on exposure and the use pattern of HHCB.

HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran and related isomers) is a member of a group of substances used in fragrances and known collectively as the polycyclic musks.

The manufactured purity of undiluted HHCB, as stated in Chapter 1, is >95% as a mixture of related isomers.

Undiluted HHCB is a highly viscous liquid that is impractical to use and handle as a fragrance ingredient. In order to make it manageable for application, it is fluidised with an odour neutral diluent. The product name Galaxolide or Galaxolide 50 refers to HHCB diluted with diethyl phthalate (DEP), the most common form of commercial HHCB. This product is at times referred to as Galaxolide 50 DEP. In some cases, the HHCB is diluted with benzyl benzoate (BB) or isopropyl myristate (IPM). When so diluted, it is designated as Galaxolide 50 BB and Galaxolide 50 IPM, respectively. These dilutions in various solvents are manufactured by blending approximately 65 weight parts of undiluted HHCB with 35 weight parts of diluent. Because Galaxolide is the primary item of commerce, much of the safety testing was conducted on the solution rather than the pure material. Where this is so, the final concentrations of HHCB have been adjusted to reflect the 65% dilution.

HHCB is used to make a fragrance long lasting and have a positive technical effect on its balance. The balance is important because HHCB possesses fragrance properties that bring the initial and residual smell into harmony. Because of these properties, it is used in many fragrances and is found in a wide variety of consumer products like soaps, shampoos, detergents, cosmetics and perfumes.

HHCB is produced in one plant in the UK and is transported to Ireland for dilution to the commercial product.

4.1.1.2 Occupational exposure

From the use pattern described in section 2.2 the following stages of the life cycle will be described in this chapter by appropriate scenarios:

Scenario 1. Production and dilution of HHCB (section 4.1.1.2.1)

Scenario 2. Compounding of fragrance oils (section 4.1.1.2.2)

Scenario 3. Formulation of consumer products that contain fragrance oils (section 4.1.1.2.3)

Scenario 4. The use of cleaning agents by professional cleaners (4.1.1.2.4)

For each of the scenarios measured exposure data (where available) and exposure models have been used to estimate the exposure levels. Where the EASE model is used, estimates were calculated using EASE 2.0 for Windows.

4.1.1.2.1 Scenario 1. Production and dilution

The primary production of HHCB in Europe occurs in one plant in the UK. Dilution is a final step in the production process and occurs mainly in another plant in Ireland. The following specific description of worker exposure is based on site visits and audits by an independent consultant to both plants (Balk 2001a-b).

The production is partly continuous and partly batch-wise depending on the process step. The nominal batch size is circa 7 tonnes. Each batch takes 10 – 15 hours. Production is continuous during 24 hours a day, 7 days a week for 48 weeks in a year (approximately 330 days).

The following activities are described:

- Process operation (3 operators in each shift)
- Dilution (1 person in each shift)
- Analytical measurements (1 person in each shift)
- Odour Quality control (2 persons, daily basis)
- Wastewater treatment (1 person, daily basis)

1. Process operation

Activities performed

Process operation is by remote control, where valves are opened and closed to fill and drain reactors and distillation columns. The plant is a typical outdoor chemical plant.

Sampling of small volumes (100 ml, or buckets from distillation tanks) in various stages of the process is performed manually. Due to the use of formaldehyde in the process, adequate Local Exhaust Ventilation (LEV) is provided at a specific sampling place.

The tanker trucks are filled with product at a temperature of 75° C by remote control. Opening and closing of the manhole is manual. About 4 tanker trucks are filled per week.

Reaction vessels are not cleaned as these are dedicated to HHCB production. An inspection of the glass-lined vessels is conducted 2-3 times per year after washing with hot water to remove the oil. Distillation columns are washed and the washing is drained. This involves no manual handlings.

Exposure at process operation

Operators wear heavy-duty gloves and overalls in accordance to their heavy mechanical duties. Operators do not handle any products except during sampling operations. Proper use of gloves to prevent exposure is assumed, because of the high temperature of the product when handled.

Exposure by inhalation of vapour may also occur 3 times per 12 hours during sampling. The exposure time is less than a minute and the concentration is reduced by the outdoor ventilation or by LEV. Concentrations in inhaled air are not quantified.

2. Dilution

Activities performed at production plant

A small fraction (<500 tonnes) of the undiluted product is diluted in the bulking area at the production site. Addition of solvents and filling of drums and tanker trucks is completely automated and takes place in closed tanks. This occurs on average once per month. The operator controls the equipment, labels the batches and takes samples by glass tubes.

Exposure at production plant

Exposure of skin to liquids is negligible, because there is no contact with the substance because of the automated procedures.

Exposure by inhalation of vapour may be incidental and lasts a few minutes. The concentration is not quantified.

Activities performed at the dilution plant

Road tankers with 18 to 22 tonnes each of undiluted HHCB at a temperature of 55 °C are delivered twice a week.

Connections are made with dedicated hoses to a manifold. The pumping area is external to the main plant. Undiluted HHCB is pumped to an outside tank that already contains diethyl phthalate as a diluent. The pumping takes about two hours. Near the end of the transfer, the dilute HHCB is sampled, any corrections adjusted by additional pumping and finally the tanks are closed.

Exposure at dilution plant

There is no direct contact with hoses or tubes while pumping at the manifold outside. Any possibly spilled material below the connection points is collected in buckets. Only indirect contact via the bucket handle or valve handle is imaginable. Workers use heavy-duty rubber gloves with internal lining. Exposure of skin to liquid is improbable as adequate precaution is taken when working with HHCB at high temperature.

Exposure to vapour is incidental (twice a week) during the few minutes involved in the outdoor sampling and (de)connecting of hoses. Concentrations are not quantified.

3. Analytical measurements

Activities performed

The five analysts handle together about 900 samples a week, of which 28 contain HHCB. So on average each analyst handles about one sample a day that contains HHCB.

Samples are heated to become pourable and diluted to a 10 % solution for GLC determination and determination of acidity.

The sample bottles are delivered and kept clean (which is strictly maintained because otherwise the bottles may become sticky, and the odour quality control will be disturbed). The analysts wear gloves.

Exposure

Both dermal and inhalation exposure are negligible because of the very small volumes used, the low vapour pressure and the controls (including gloves) to prevent contact with the heated material.

4. Odour quality control

Activities performed

Of a total of 400 samples that are controlled daily, 6 or occasionally 10 contain HHCB.

Thus each controller sniffs on average 3 – 5 times a day a sample with HHCB.

The sample bottles are kept dry and clean, which is very important in view of the disturbing effect of the odour quality. A drop of liquid is absorbed on the tip of a piece of paper and sniffing takes a few seconds. Since HHCB and diluted HHCB are tested during a short time (less than 10 seconds), inhalation exposure during this activity is estimated to be negligible.

Exposure

The exposure by inhalation of vapour or to liquid on the skin is negligible.

5. Wastewater treatment

Activities performed

The operator checks the equipment a few times daily. There is no direct handling involved.

Exposure

There is no direct contact and therefore there is no exposure of the skin to liquids or inhalation of vapour.

Quantitative evaluation of exposure at Scenario 1 - Production and dilution

Inhalation exposure

Inhalation of vapour may occur incidentally several minutes a day for process operators and for bulk operators during sampling and dilution activities. It is assumed that these activities represent the worst case situation for exposure during production.

Measured data

In a TNO report (Beijer et al.,1998) measured values are mentioned for similar substances during drumming or filling of tank trucks during the manufacturing process. During production (including drumming) of dibutyl-phtalate (DBP), a substance with a vapour pressure of 9.7 10^{-6} kPa, the 8-hour TWA value was 0.003 ppm (N=114). During drumming of toluene diisocyanate (TDI) with a vapour pressure of 4.10^{-3} kPa, the mean 8-hour TWA was 0.007 ppm (N=95). During production and filling of tank-trucks of trichlorotoluol with a vapour pressure of 0.02 kPa, the 8-hour TWA mean was 0.005 ppm (N=33). None of these estimates is directly comparable with taking samples from a manhole.

Modelled data

At 25 °C the vapour pressure is 0.07 Pa and with a molecular weight of 258.4, the vapour-saturated air has a concentration of 7.4 mg/m^3. At 75 ^oC a vapour pressure of 5 Pa (0.04 mm

Hg) is calculated by the SRC MpBp Win V 1.30. This would correspond to a satured air concentration of 447 mg/m^3. At 55 ^{o}C (during delivery) the vapour pressure will be in between the values at 25 ^{o}C and 75 ^{o}C.

For the estimation of exposure during sampling or dilution activities, the following input parameters for the EASE 2.0 model are used. Assuming non-dispersive use, no aerosol formation, direct handling and local exhaust ventilation (because this is assumed to be relevant for outdoor activities such as delivery) and a vapour pressure of 5 Pa (worst case), the estimated range is 0.5-1 ppm. Because the vapour pressure is at the lower end of the range used in EASE, the value of 0.5 ppm (4.5 mg/m^3) is the best estimate with the model.

The exposure time will be less than a few minutes and about twice a week for the same worker.

Dermal exposure

Operators wear heavy-duty gloves and overalls during the mechanical duties. Products are not handled except during sampling. In the dilution of HHCB, there is no direct contact with hoses or tubes while pumping at the manifold. Any possibly spilled material below the connection points is collected in buckets. Only indirect contact via the bucket handle or valve handle is imaginable. Workers use heavy-duty rubber gloves with internal lining. Exposure of skin to liquid is improbable as adequate precaution is taken when working with HHCB at high temperature.

Measured data

No data available.

Modelled data

Assuming non-dispersive use, direct handling and incidental contact, dermal exposure is estimated with the EASE 2.0 model to be 0 – 0.1 mg/cm^2/day. Considering the elevated temperature of the substance and the use of heavy-duty gloves (default protection factors are not available for this type of gloves) it may be assumed that dermal protection will be adequate and dermal exposure is negligible.

Conclusions

From all activities performed during production, sampling and diluting form the worst case situation. A short term inhalation exposure is estimated with the EASE model to be 4.5 mg/m^3 during a few minutes per day. If an exposure of 3 minutes is assumed at 4.5 mg/m^3, an 8 hour exposure would be estimated at 0.03 mg/m^3. Measured values of similar substances are available, that are not representative for sampling from a manhole. The EASE estimate, extrapolated to 8 hours, and the measured values for other activities with very low vapour pressure liquids appear to agree reasonably well. Exposure during other activities, like chemical analysis, odour quality control and waste water treatment are considered to be negligible, compared to the above value.

Dermal exposure is considered to be negligible because adequate personal protection will be used because of the elevated temperature of the substance.

4.1.1.2.2 Scenario 2. Compounding of fragrance oils

To describe the workers scenario for compounding, six out of about 39 large and medium size compounding sites were visited and audited (Balk 2001b-f, 2002a). The six plants together consumed in 2000 an amount equivalent to 1024 ton/y undiluted HHCB. If taken into account that 1427 tonnes are produced for destinations inside EU and 14 % of the EU use volume is directly used by the detergent formulators, and 6 % is used by small size compounders, the use volumes of the six large compounders represents 1024/1142, i.e. 90% of the large and medium size compounding activity.

Based on the six different specific descriptions supplied separately to the rapporteur, a generic scenario is described for the large and medium size compounding facilities.

The small size companies have not been visited. The number of sites in EU is about 140. The description of activities and possible exposure of workers is considered to be completely similar for small size and medium/large size compounders. This view is based on the fact that in both situations the exposure is mainly the result of manual handling procedures for small size batches. The production of large size batches that occurs mainly at the large and medium size facilities is highly automatic and does not contribute significantly to the workers exposure. For the exposure via inhalation, the ventilation rate may be lower or LEV may not be used. As a worst-case approach it is assumed that the inhalation exposure is a factor of five higher than in the observed large size plants.

In the six large compounding sites, fragrance oils are produced in volumes that vary between 1 and 20,000 kg. The operations in these plants are expected to be representative for the operations in all large and medium size compounding plants.

This scenario for compounding of very complex mixtures does not include the use by detergent and cosmetic industry for bulk products. This type of use represents about 14 % of the HHCB use volume and occurs mainly at three plants [source: IFF]. For these use types, the scenario for formulation will be more adequate.

Activities performed

Worker scenarios differ for the following four types of activity:

- Delivery
- Filling of stock tanks
- Compounding of fragrance oils
- Analytical determinations
- Odour control

1. Delivery

The amounts delivered to each of the six plants vary between 67 and 460 tonnes of 65 % solutions and together 1585 tonnes/y of the 65% solution was delivered to these plants in 2000.

In one plant, HHCB is delivered mainly as undiluted. This amount is not used for compounding but in a diluted form, it is supplied to fragrance industry.

Larger quantities are delivered in 15 – 20 ton tanker trucks, smaller amounts in 1 ton containers or in 200 l drums. Altogether, the frequency of deliveries is between once in two weeks and once in six weeks. The activity of the worker includes connecting hoses, pumping and sampling. They use heavy-duty gloves. The delivery of diluted or undiluted HHCB in tanker trucks is at a temperature of 75 °C or lower. The working area is outdoors. Drums are sampled indoors, also using sturdy gloves. Handling generally takes less than a minute.

Workers handle many ingredients and any cross contamination is carefully prevented.

2. Filling stock tanks

Activities performed

Membrane pumps fill smaller stock tanks. Pumps and tubes are washed by pumping alcohol or hot water with detergents. Handling normally takes less than one minute. Workers always wear heavy-duty rubber gloves. The filling of stock tanks is done with dilutions of 65%, at a temperature between 25 and 75 °C. The filling of stock tanks follows delivery in the same frequency: between one in two and one in six weeks.

3. Fragrance compounding

In a typical compounding facility, about 15 to 60 compounders work often in shifts, with a rotation of tasks. The activities include, retrieving of tanks and containers, pouring and weighing, pushing lorries, mixing, sampling and washing.

In general, several thousands (typically 3000) of ingredients are mixed in fragrance mixtures that contain on an average some 40 ingredients each. Between 1/3 and 2/3 of the final products contain HHCB. The average final weight in HHCB containing products is about 3 – 4 %. Stock solutions of HHCB are between 10 and 65 %.

Batches vary between 0.5 L to 20 m^3; each worker prepares one to several HHCB containing batches per day. The level of automation varies for the size of the batches and between the different plants ranging from 50 to 100 % of the weighings. Manual weighing is limited to the small batches, which will be limited to the preparation of one batch per day per worker.

Workers have no fixed position in the room but walk around with mobile tanks to different storage tanks, mixing area or washing area or push containers below a series of valves.

The room for compounding is centrally vented and on several spots equipped with additional local ventilation (LEV). Workers wear impermeable gloves in most cases but not 100% of the time. Gloves are changed and hands are washed 3 to 5 times per day (at each break). As the risk of cross contamination should be eliminated as much as possible the working discipline and accuracy is high. Paper tissue may be used occasionally to clean the outside of vessels or bottles.

- Bulk volumes are compounded either automatically or by using membrane pumps.
- Vessels for 500 L are on lorries and moved below a series of automatic valves.
- Volumes of 0.2 L are poured manually by cups or volumes of 10 – 20 L poured from buckets into larger size containers.

- Small volumes are taken automatically or by single use pipettes and added to 0.5 L bottles. In a typical example, this occurs by rotation of tasks approximately 8 times per year during one week. In that week, about 10 – 12 samples of HHCB are taken per day.

Mixing is performed by mounting mechanical stirrers on top of the open tanks. Tanks are mixed for about ½ hour, often without covering. The mixing area has additional LEV. The HHCB containing fragrance oils that are mixed contain on average 4 % HHCB.

This procedure with mechanical mixers is limited to medium size batches of 25 – 500 litres. With a normal rotation of tasks among compounders, each worker will handle in this way not more than one HHCB containing batch per day.

Washing of tanks and smaller vessels is made by washing machines. The machines are loaded and unloaded by hand. Larger tanks may be cleaned by mobile systems using hot water with detergents. Workers do not clean or wash tanks by hand. The outside of the tanks is clean. In one plant the vessels are not cleaned by washing, but reused for dosing dilution ingredients to the same batch. The dilution ingredient cleans the vessels before they are used for a next batch. In some cases, the Teflon lined empty tanks are rinsed with alcohol and the few remaining droplets are wiped dry with a cotton rag. During this procedure, gloves are always worn. Tubes and pumps are flushed with alcohol before they are used in a new batch. The alcohol is collected for waste incineration.

Floors are cleaned regularly or continuously by liquid vacuum cleaners.

Exposure to vapour will occur during the workday, i.e. 8 hours each working day. The concentrations are not regularly monitored. Concentrations can be estimated on the basis of a few studies (see below).

Exposure of the skin to HHCB containing liquid may occur incidentally. As the workers are well trained to work neatly and accurately, small spills of liquid are usually prevented. However where automation is not yet 100 % and gloves are not always used, it cannot be guaranteed that there will never be any drops on the hands. As compounders prepare one to several batches per day and half of these batches contain HHCB, a reasonable worst-case scenario for the Risk Assessment of the frequency of possible skin contact with HHCB is once per day. Such contacts will be limited to a maximum of 100 cm^2 (about 50 % of the skin surface of the palm of one hand) and for the duration of less than 2 hours, because of the frequency of hand washing. The residual amount that may stay for a longer time in the pores of the skin will not contribute significantly to the exposure and is not considered.

Therefore, the exposure on skin will be highly variable between workers and over time. Some estimation on the exposure can be based on a specific study for a similar compounding plant in the US (see below) .

4. Analytical measurements

The scenario of analytical work is more or less identical to the description given for the production and dilution. The total number of samples per plant may vary by a factor of 6 in relation to the production volume, but the number of HHCB containing samples per analyst will be roughly identical.

5. Odour quality control

The exposure scenario during odour control is more or less identical to the description given for the production and dilution. The total number of samples per plant may vary by a factor of 6 in relation to the production volume, but the number of HHCB containing samples per analyst will be roughly identical. In practice, these activities are not conducted for extended periods, because the capacity of odour perception decreases after some time. The worker performs other tasks in between in order to recover the sensitivity of smelling. Since only final fragrance mixtures with on average 4% HHCB are tested during a short time (less than 10 seconds), inhalation exposure during this activity is estimated to be negligible.

Quantitative evaluation of exposure at Scenario 2 - Compounding of fragrance oil

Inhalation exposure

Measured data

In several plants air monitoring in the working area has been conducted.

Plant 1 - Measurements in 1991/1992 for limonene 1- 6 mg/m^3.

The room was vented with 74,000 m^3/h, which implies an exchange rate of six times per hour. As limonene and HHCB are used in the same room and often occur in the same batches the dilution by ventilation will be similar for both substances and concentrations can be estimated on the basis of partial vapour pressures or vapour pressures multiplied by the average concentrations.

The vapour pressures for limonene and for HHCB differ by a factor 3,000 (2.1 mbar = 210 Pa versus 0.07 Pa). Assuming that HHCB occurs on average at 4 % and limonene occurs at 10 % in the fragrance oils, and assuming proportionality to both vapour pressure and these concentrations according to Henry's law, would give HHCB concentrations in the air of 3000 * 10/4 = 7500 times below that of limonene (= 0.13-0.8 µg/m^3)

It should be remarked, however, that at higher concentrations in a mixture Henry's law cannot be applied.

Based on experimental observations in plant 4, the ratio of the concentration of HHCB and limonene is 1/500. Applying this ratio for plant 1 as well, the concentration of HHCB would be in the range of 2-12 µg/m^3.

Plant 2 - Extensive air monitoring study in 1988.

The duration was 4 – 5 months, once per week over an 8-hours workday. The ventilation rate is 50,000 m^3/h, which means a refreshment of five times per hour. Both stationary and personal monitoring was carried out. At the detection level of 30 µg/m^3, no HHCB was found.

Plant 3. - Personal monitoring programme in 1995.

Five persons were monitored 8h/d during 1 week. The total flow of the air through the building was 300,000 m^3/h and air renewal rate is 4 times per hour.

The concentration of Limonene was 0.34 – 0.41 ppm (1.9-2.3 mg/m^3).

Taking into account the much lower vapour pressure and assuming proportionality to the vapour pressure and the concentrations in the final products, this would correspond to about 0.1 µg HHCB/m^3.

If the factor of 500 is applied on the basis of data from plant 4, the HHCB concentration would be in the range of 3.8-4.6 µg/m^3.

Plant 4 - Static measurements in 1999 during 11 working days for 8 hours a day.

The various rooms have a common ventilation of altogether 5,100 m^3/h. Air renewal rates of the rooms are between 5 and 11 times per hour.

A total of 34 samples from 16 spots in the working room have been analysed.

In the majority of the samples, the HHCB concentration was below the detection limit of 8 µg/m^3. In 6 samples the HHCB level varied between 11 and 13 µg/m^3 and in one sample the value was 58 µg/m^3. The median value was < 8 µg/m^3.

The outlier of 58 µg/m^3 concerns a moment just after filling of the stock flasks in the small robot filling station. In another sample at the same station, the concentration was below detection limit. Thus, this value should be considered as incidentally occurring.

Limonene was measured in the same samples. In 39 samples limonene varied between 0.3 and 23.2 mg/m^3, with a median value of 2.8 mg/m^3. Limonene constituted 15 – 60 % of the Volatile Organic Carbon in the air. If HHCB was measured above the detection limit the ratio between Limonene and HHCB was about 500 : 1. This ratio shows that concentrations in air are not proportional to vapour pressure and concentrations in liquids.

Figure 4.1 shows the relation between the concentrations of HHCB and limonene.

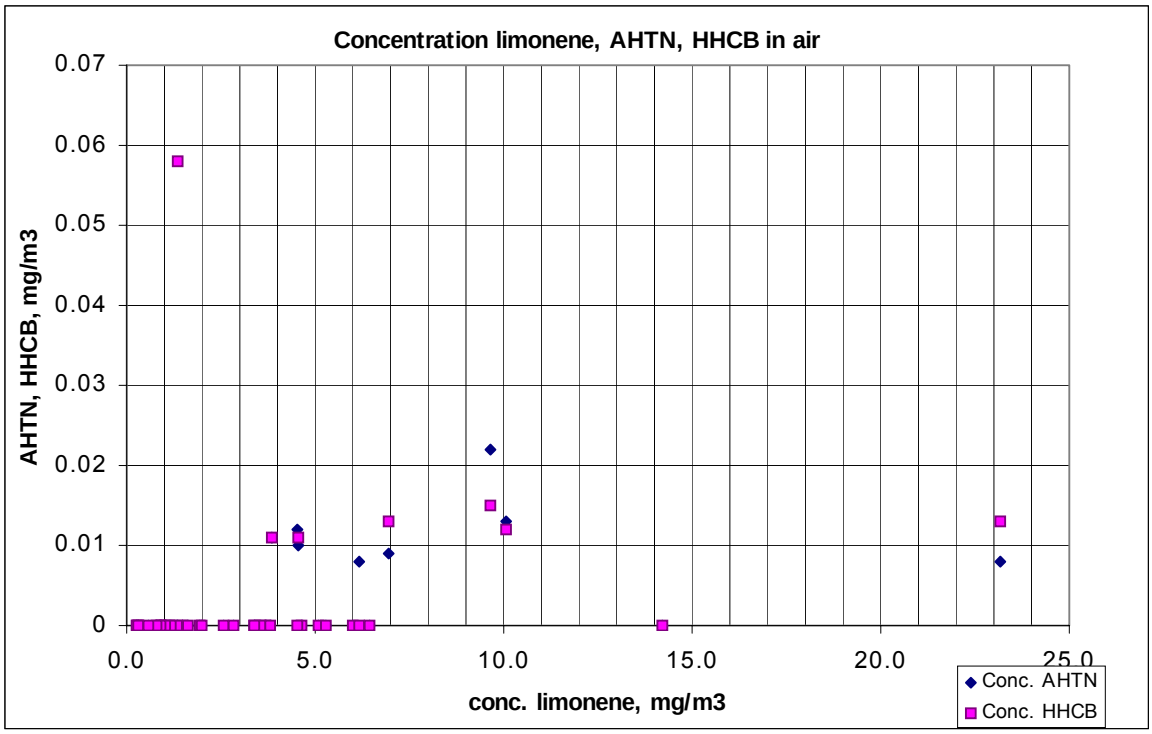


Figure 4.1. Concentrations measured in air, plant 4.

Monitoring data for limonene are available for three plants. These are indicative and show that the conditions in the various plants are quite similar. In one plant limonene and HHCB were measured simultaneously. Using a ratio of 500 on the basis of these measurements and applying this ratio to the other two plants would give a concentration of HHCB < 4.6 μg/m^3 and < 12 μg/m^3 respectively. In one plant, limonene was not quantified but HHCB was below the detection limit of 30 μg/m^3.

The monitoring program at plant 4 indicated levels of HHCB from below the detection limit (8 μg/m^3) and 58 μg/m^3. In about 20 % of the samples the level varied between 11 and 13 μg/m^3. In the majority of samples the value was below 8 μg/m^3 and in one sample 58 μg/m^3.

For the small size compounding activities it cannot be assumed that the ventilation rates in the compounding rooms are similar as to large size plants, or the LEV may not be used. Therefore as a worst-case approach for this risk assessment, it is assumed that the 8 hours average exposure via inhalation is a factor of five higher (65 μg/m^3). This is a rather conservative estimate. The ventilation rate in the various plants varied between 4 – 11 per hour for the plants 1, 2, 3 and 4. A normal ventilation rate in a workers room should be 1/hour and if odorous substances are handled this will normally be designed at 3/hour.

Modelled data (filling of stock tanks)

During delivery and filling of stock tanks, exposure is estimated with the EASE 2.0 model to be 10-20 ppm, assuming direct handling with dilution ventilation (because there is no certainty of the availability of LEV) of 65% HHCB at a temperature of 75 °C. The vapour pressure used is 0.65 * 5 Pa = 3.3 Pa. This situation represents a worst case because the temperature in reality is below 75°C. The vapour pressure may therefore be below 1 Pa, in which case the exposure is estimated as 0-0.1 ppm (0-0.9 mg/m^3). The temperature of the process and the concentration of HHCB lead to two different EASE estimates that are rather far apart. Therefore, a pragmatic approach is chosen by comparing these values with an estimate based on the saturated vapour concentration. At a vapour pressure of 3.3 Pa, the saturated vapour concentration is approximately 295 mg/m^3. Assuming that the.saturated vapour in the headspace of the stock tank is diluted by a factor of 10 to calculate exposure in the area around the stock tank appears to be a reasonable worst case. This leads to an estimate of approximately 30 mg/m^3 that will be used as the value in risk characterisation.

Dermal exposure

Measured data

In a study by Cohen and Wolff (1998) conducted in a compounding facility in the USA a model substance (isobornyl acetate with vapour pressure 13.3 Pa) was used to measure the exposure of the hands. Isobornyl acetate was dispensed and weighed in volumes varying from small (milligram – gram) quantities up to bulk volumes (ten to hundreds of kilograms) using pipettes or pumps. Dispensing procedures are identical to the description given for two of the visited sites

The measurement was based on extraction of the gloves, hand rinsing and extraction of tissues for wiping of the face. The sampling was conducted as a one-day study. A total of 11 workers were monitored and sampled. Nine of the workers were engaged in compounding of fragrances, which included weighing, dispensing and mixing of fragrance ingredients. The remaining 2 workers were material handlers with job duties such as transporting fragrance ingredients in metal drums and periodically collecting aliquots of samples from large drums

for QC purposes. The total number of samples was approximately 214 including baseline samples. Four to six hand rinse samples were taken from each worker at each work-break. Two to six glove samples were collected from each worker in the course of the day and 3 face wipes were obtained from each worker at the end of the work shift.

Cumulative extracted amounts over several pairs of gloves used over the day reached 0.32 – 39 mg (on average 9 mg). The highest observed value of 39 mg is very close to the estimate for one drop of 65% dilution (32.5 mg) and the average of 9 mg is very close to 4 drops of 4 % dilution. This result supports the concept that incidentally one drop of 65 % HHCB dilution may be spilled on the hands and usually the frequency is less than once every two hours. A higher frequency up till once per 2 hours may occur with the average dilution of 4 %.

The study of Cohen and Wolff (1998) showed that there is no correlation between the exposure and the volume of fragrance oils produced or handled and exposure is not related to a special task of the compounders during the day.

The three different estimates given above, result in a rather similar picture. The worst case scenario with 65 % HHCB present in concentrated stock solutions results in a skin exposure of 39 mg/day on a surface area of 105 cm^3 and at a frequency of once/day. This exposure will be used for the risk assessment.

Quantification of the exposure by handling of cotton rags:

The wiping procedure concerns mixing vessels for compounding. The average concentration of HHCB in the final mixtures is 4 % HHCB. The vessels are Teflon lined to minimize the amount of residue after pouring into larger recipients. After the Teflon lined containers are emptied it can be assumed that less than 0.5 ml of fragrance oil is left. After rinsing with 50 ml alcohol the concentration in the residual 0.5 ml alcohol is less than 1/100 of 4 % and this will be absorbed on the cotton. So the absolute amount is 0.2 mg. In a realistic worst case the cotton rag may be taken in the hand at the wet side, so the 0.2 mg HHCB is spread over a surface of half of one hand (105 cm^2) to give a dose of 0.002 mg/cm^2.

With the production of one HHCB containing batch per person per day, this may occur not more than once a day and the exposure may hold for a maximum of two hours before the hands are washed.

Modelled data

During delivery and filling of stock tanks, dermal exposure is estimated with the EASE 2.0 model to be 0 – 0.1 mg/cm^2/day, assuming non-dispersive use, direct handling and incidental contact. Considering the elevated temperature of the substance and the use of heavy-duty gloves (default protection factors are not available for this type of gloves) it may be assumed that dermal protection will be adequate and dermal exposure is negligible.

Incidental handling of liquid drums (non dispersive use) is assumed to result in an exposure of 0 – 0.1 mg/cm^2/day, or based on the surface of hands and forearms (1300 cm^2): 130 mg/day.

Based on the scenario description the spills on the hand will be limited to a few drops of 50 µL each. One drop of a stock dilution of 65% will contain 32.5 mg HHCB, whereas one drop of a final fragrance oil with 4 % HHCB will contain 2 mg HHCB. If the amount is spread over the surface of half of one hand (105 cm^2) the concentration on the skin is 0.02 – 0.3 mg/cm^2.

Conclusions

During delivery and filling of stock tanks, the results of the pragmatic calculation will be used: 30 mg/m^3. This is a pragmatic value calculated from concentrations in based on saturated vapour pressure and a dilution factor. The value is in between values calculated by two different EASE estimates that are made for situations with a vapour pressure just above or just below 1 Pa. The frequency of this activity is up to once in two weeks. The duration of the exposure during delivery is estimated to be up to 3 minutes per day of delivery. Recalculation of this value to an 8 hours exposure leads to a value of 0.19 mg/m^3.

The results of measured values will be used for fragrance compounding. For large and medium sized companies, a reasonable worst-case value for the 8 hours average the level of 13 µg/m^3 will be used in the risk assessment. The short-term exposure will be set at 15 minutes inhalation of 100 µg/m^3. For small sized plants, due to the lower ventilation rate, a worst-case approach for the risk assessment is assumed of 65 µg/m^3.

A short-term exposure, derived from measured values can be set at 100 µg/m^3 .

For other activities in this scenario, such as wiping of rinsed vessels, analysis of samples and odour control, exposure will be estimated to be negligible, compared to the above values.

Dermal exposure during delivery and filling stock tanks is estimated to be negligible because it is assumed that adequate personal protection will be used due to the elevated temperature of the substance.

For dermal exposure during compounding the results of measured values is used: 39 mg/day, assuming an exposed area of half of one hand (105 cm^2).

During wiping of rinsed vessels the result of measured values will be used: 0,2 mg/day over an exposed surface of half of one hand (105 cm^2).

4.1.1.2.3 Scenario 3. Formulation of consumer products that contain fragrance oils

The drummed liquid fragrance oil is used in the cosmetic industry for production of toiletries, shampoos, soap, and the household cleaning products industry for production of detergents and cleaning agents etc. It is assumed that the production is highly automated with little or no exposure to polycyclic musks. Exposure may be possible during handling of the drums and during cleaning and maintenance of the equipment. Because of the risk of contamination with an odour, it is expected that the procedures limiting exposure in a similar way as described for the compounding of fragrance oils are followed.

Quantification of exposure in scenario 3

Inhalation exposure

Measured data

No data available.

Modelled data

As the systems are closed and the bulk volume of the products handled will contain not more than 1% of a 4 % solution (0.004 % HHCB has a partial vapour pressure of 28 * 10^{-7} Pa), the EASE model gives an estimate of 0-0.1 ppm. During cleaning the liquids are even further diluted so exposure by inhalation can be considered negligible.

Dermal exposure

Measured data

No data available.

Modelled data

The EASE model estimates for the direct handling of liquids assuming non-dispersive use and for incidental contact a dermal exposure of 0 – 0.1 mg/cm^2/day. With the palms of both hands (together 420 cm^2) and a maximum concentration of 4 % for the undiluted fragrance oil this results in 1.7 mg HHCB/day.

For cleaning and maintenance, it is assumed in the EASE model that there is previous rinsing which lowers the concentration by a factor of 10. With a standard default exposure for direct handling and non-dispersive use and extensive contact (up to 5 mg/cm^2/day) on both hands and part of the forearms (1300 cm^2) and a concentration of 4 % HHCB in undiluted fragrance oil, the dermal exposure is 26 mg HHCB per day.

Based on one site visit (Balk 2002b), the exposure at cleaning is quantified as follows:

Empty vessels of fragrance oil are rinsed with water that is added to the product.

The rinsed vessels are filled with hot water and put aside for some time. Before cleaning the water is drained to the sewer.

The extensive contact during cleaning with a generic (default) exposure of 5 mg/cm^2 and 1300 cm^2 skin on hands and fore arms concerns 6500 mg water with some HHCB in diluted form. The two rinsing steps can be considered as a dilution of 5/500 ml in each step so in total a factor of 10^4. This brings the amount of HHCB at 4 % * 10^{-4} * 6500 mg = 0.026 mg.

The difference between the former estimate and the EASE approach is the dilution before cleaning. It can be argued that the procedure with two rinsing steps before cleaning is a normal practice with this type of extremely expensive substances.

For that reason the 0.026 mg, as predicted by EASE is used for a single exposure.

The extensive contact during cleaning will not be continuous. Not more than several HHCB containing vessels will be cleaned per day and after the first flush the vessels may be flushed with clean water. As a worst-case approach it can be assumed that not more than ten HHCB containing vessels are cleaned on a day. Thus if the separate doses are accumulated over the day, the daily dose will be less then ten times a single dose of 0.026 mg or 0.26 mg and this figure will be used in the risk assessment.

Conclusions

Because of the very low vapour pressure of the substance after dilution, inhalation exposure is estimated as negligible.

Dermal exposure during handling of liquids is estimated to be 1.7 mg/day, based on modelled data. The palms of both hands are exposed (420 cm^2).

Based on the EASE model and observations in practice dermal exposure during cleaning and maintenance is estimated to be 0.26 mg/day. The exposed body surface is both hands (1300 cm^2).

4.1.1.2.4 Scenario 4. The use of cleaning agents by professional cleaners

Professional cleaners may be exposed to HHCB in the cleaning products.

Inhalation exposure

Measured data

No measured data are available. Modelled data

For inhalation, exposure without aerosol formation, the EASE model estimates an exposure of 0-0.1 ppm. Due to the very low vapour pressure of the diluted substance however, the exposure is assumed to be negligible.

Dermal exposure

Measured data

No data available.

Modelled data

For dermal exposure assuming extensive contact and wide dispersive use the exposure according to EASE ranges from 5 – 15 mg/cm^2/day on both hands (840 cm^2) so 4200 – 12,600 mg water with cleaning agent diluted 1 to 50.

The final professional cleaning products contain 0.1 % fragrance oil, which in turn contains on average 4 % HHCB. Thus the (50 times) diluted cleaning agent will contain a maximum of 0.8 mg HHCB per litre (0.1 % * 4 % * 0.02 = 0.8 mg/l) and the highest daily exposure is 0.01 mg/d (12,600 mg water with 0.8 mg/l =0.01 mg). This exposure is not a single dose, but the outer liquid may be replaced repeatedly if the hands are immersed. Although these workers will wear rubber gloves for hygienic reasons and to prevent damage to the skin due to a constant exposure to detergents, it can be assumed that the liquid on the skin is refreshed every 15 minutes and that each dose is accumulated over the day. This gives a daily dose of 0.32 mg/d.

Conclusions

For inhalation, exposure is considered negligible .

For dermal exposure, the exposure level from the use of cleaning agents by professional cleaners is 0.32 mg/day for an 8-hour workday as a worst-case approach. The exposed area is 840 cm^2 (both hands).

4.1.1.2.5 Summary of inhalation and dermal occupational exposures

Table 4.1 Conclusions of the occupational exposure assessment

Workers scenario	Inhalation	Dermal
Scenario1 Production and dilution		
- process operator	Short term (3 min/day) 4.5 mg/m^3	Negligible
- blending & dilution	Short term (3 min/day) 4.5 mg/m^3	Negligible
Scenario 2 Compounding of fragrance oils		
- delivery & stocking	Short term (3 min/day; once in 14days) 30 mg/m^3	Negligible
- compounding		
- *large and medium size plants*	8 h/d 0.013 mg/m^3	2 hours/day 39 mg/day
	Short term (0.25 h/d) 0.1 mg/m^3	
- *small size plants*	8 h/d 0.065 mg/m^3	2 hours/day 39 mg/day
	Short term (0.25 h/d) 0.1 mg/m^3	
- wiping of rinsed vessels	Negligible	2 hours/day 0,2 mg/day
Scenario 3 formulation		
- handling	Negligible	4 hours/day 1.7 mg/d/420 cm^2
- cleaning & maintenance	Negligible	4 hours/day 0.26 mg/d/1300 cm^2
Scenario 4 professional cleaning		
- handling	Negligible	8 hours/day 0.32 mg/d/ 840 cm^2

4.1.1.3 Consumer exposure

4.1.1.3.1 Introduction

Consumer exposure occurs from consumer products to which HHCB is added intentionally as a component of the fragrance that enhances the product. It is used as an ingredient in commercial preparations (fragrance oils) intended to be used to fragrance a wide variety of consumer products such as perfumes, creams, toiletries, soaps and shampoos (SCCNFP, 24 October 2000).

Two scenarios for direct consumer exposure are discussed. Scenario 1 considers exposure as a result of the use of HHCB in fragrances in cosmetics and Scenario 2 considers exposure via other perfumed household products. In both cases it is necessary to know the levels of perfume oil used in the various products and the reasonably worst-case (97.5^{th} percentile HHCB in perfume oil) level of use of that perfume that may be HHCB. It is also necessary to understand the various patterns of use of these products.

For Scenario 1, cosmetic use, the levels of perfume in the various classes of cosmetics and the 97.5^{th} percentile use level (30%) of HHCB in the perfume were the results of industry surveys (IFRA, survey of use of HHCB in the fragrance industry; private communication via COLIPA, 1996 to the SCCNFP) and are shown in **Table 4.2**. These are the same figures that were used in the report of the SCCNFP (SCCNFP, 24 October 2000).

Table 4.2 Use levels (97.5 percentile use) of HHCB in cosmetic products.

Type of cosmetic product	Fragrance oil in product (%)	HHCB in fragrance oil (%)	HHCB in product (%)
Body lotion	0.4	30	0.12
Face cream	0.3	30	0.090
Eau de toilette	8.0	30	2.40
Fragrance cream	4.0	30	1.2
Antiperspirant /deodorant	1.0	30	0.30
Shampoo	0.5	30	0.15
Bath products	2.0	30	0.60
Shower gel	1.2	30	0.36
Toilet soap	1.5	30	0.45
Hair spray	0.5	30	0.15

Scenario 2: HHCB is also used in household and laundry cleaning products and air fresheners (Balk and Ford, 1999). Both the fragrance manufacturing industry and the consumer product industry were surveyed by the International Fragrance Association (IFRA, 2002) to determine the use levels of fragrance oils in these product types and the levels of HHCB that are used to formulate these oils (**Table 4.3**). Exposure calculations based on use of household products are reported in Scenario 2.

Table 4.3 Use levels of HHCB in household cleaning products. Results of a survey including data from manufacturers of fragrances as well as household cleaning products

Product category	Median percent of fragrance oil in product	97.5 percentile use level of HHCB [a]	Level of HHCB in product
Laundry regular powder	0.33	15%	0.05%
Laundry liquid	0.80	15%	0.12%
Laundry compact (tabs)	0.33	15%	0.05%
Laundry compact (powder and other)	0.28	15%	0.04%
Laundry liquid concentrate	0.85	15%	0.13%
Fabric softener (conditioner)	0.43	15%	0.06%
Fabric softener concentrate	0.80	15%	0.12%
Laundry additive, powder bleach	0.20	15%	0.03%
Laundry additive, liquid bleach	0.20	15%	0.03%
Laundry additive, tablet	0.30	15%	0.05%
Hand dishwashing liquid	0.23	15%	0.04%
Hand dishwashing liquid concentrate	0.45	15%	0.07%
Machine dishwashing powder	0.15	15%	0.02%
Machine dishwashing liquid	0.15	15%	0.02%
Machine dishwashing tablet	0.15	15%	0.02%
Surface cleaner liquid	0.60	15%	0.09%
Surface cleaner powder	0.25	15%	0.04%
Surface cleaner gel	0.75	15%	0.11%
Surface cleaner spray	0.13	15%	0.02%
Toilet cleaner powder	0.30	15%	0.05%
Toilet cleaner liquid	0.35	15%	0.05%
Toilet cleaner gel (concentrate)	0.38	15%	0.06%
Toilet cleaner tablet	0.30	15%	0.05%
Toilet rim block or gel	6.0	15%	0.9%

[a] .97.5 percentile use level of HHCB in fragrance oils used in household and detergent products

Using any of the above products results in some exposure to HHCB, either dermally through direct contact, orally as a result of residues migrating from dishes and pots into food and drinks, or by inhalation of aerosols from cleaning sprays. All of these potential exposures are addressed below with explanations as to how the estimates were made. However, exposure calculations have shown that because of the low contents of HHCB and the use patterns of these products, the resulting exposures to HHCB are negligible in comparisons to the exposures from cosmetic uses (HERA draft risk assessment, 2003). Only exposures from laundry detergents and dish washing liquid are shown here.

For the estimates of consumer exposure to laundry detergents and dish washing liquids, the consumer exposure models developed by the Association Internationale de la Savonnerie, de la Détergence et des Produits d'Éntretien (International Association for Soaps, Detergents &

Maintenance Products) (A.I.S.E) and the European Chemical Industry Council (CEFIC) as part of their program on Human & Environmental Risk Assessment (HERA) of ingredients of household cleaning products and presented in the HERA guidance document are used along with the data presented in the Table of Habits and Practices for Consumer Products in Western Europe, an appendix to that document (HERA, 2002). This table presents use data for cleaning products in grams/task, use frequency, duration of task and other intended uses. While minimum, maximum and typical use frequencies and amounts are given, only the maximum figures are used for the exposure estimations with the understanding that further refinement will be possible if necessary.

In addition, the use of HHCB in fragrance oils in air fresheners and inhalation exposure from such use is discussed.

4.1.1.3.2 Exposure from uses

Inhalation exposure

Measured data

The occurrence of musk fragrances was tested in air samples from 74 kindergartens and in household dust from 30 apartments in Berlin in 2000 and 2001. In indoor air, HHCB levels were above the detection limit (10 ng/m^3) in all 74 samples. The median value was 101 ng/m^3 with a maximum concentration found of 299 ng/m^3 and a 95th percentile of 245 ng/m^3. In household dust, HHCB was detected in 63% of the samples (detection limit 0.5 mg/kg) with a median value of 0.7 mg/kg, a maximum of 11.4 mg/kg and a 95th percentile of 5.0 mg HHCB/kg (Fromme et al., 2004).

Synthetic musks were determined in ambient and indoor air by Kallenborn and Gatermann (2004). Five outdoor samples were taken at Kjeller in Norway at different time points in 1998. HHCB concentrations ranged from 110 to 223 pg/m^3. For comparison a few indoor samples were taken. This resulted in HHCB levels of 44.3 ng/m^3 at a hairdresser, 19.0 ng/m^3 at a rest facility, 18.9 ng/m^3 at a toilet, 35.3 ng/m^3 at a cafeteria and 2.5 and 5.6 ng/m^3 at two laboratories.

Synthetic musks were determined in 35 house dust samples from vacuum cleaner bags in Germany. Sample processing consisted of sieving the contents of the vacuum cleaner bags to <63 μm, discarding coarse matter and fiber. AHTN levels ranged from 0.34 to 94 mg/kg with a median of 0.69 mg/kg (Butte, 2004).

Modelled data

Scenario 1 - Cosmetic products

As is shown below, the largest exposure to HHCB as used in cosmetics is by dermal contact. Also cosmetic exposure via aerosols (perfume sprays) will end up mostly on the skin. The exposure by inhalation from this type of exposure would be expected to be negligible compared to that from dermal exposure to cosmetics. The evaporation from the skin is also considered negligible due in part to the low vapour pressure (0.000727 hPa at 25 ^{0}C) of HHCB and also because of the relatively small amounts used (see **Table 4.4)** below in the dermal exposure section). However, if such evaporation were considered, it would be necessary to lower the dermal exposure by the amount evaporated.

For hairspray, the following calculation can be made, assuming that most of the hairspray will end up on the skin (90%) based on Bremmer et al. (2002) and the exposure time will be short e.g. 5 minutes.

2 x 5000 mg x 0.0015 x 0.1 x 0.083 x 1 x 1 / 60 = 0.002 mg/kg bw /day

With:

Events per day:	2
Amount of hairspray per event:	5000 mg
Amount of HHCB in hairspray:	0.15%
Aerosol in air:	10% of hairspray ends up in the air (0.1)
Time of exposure:	0.083 hr (= 5 min)
Inhalation rate (light activity, TGD default):	1 m^3/hr
Emission volume (around the head):	1 m^3
Body weight:	60 kg

Scenario 2 - Household products

Exposure as used in fragrances in air fresheners

Air freshener aerosol may contain up to 1% of fragrance. The 97th percentile use level of HHCB in the fragrance oils is 15% (IFRA, 2002). The estimated worst case exposure to HHCB in an aerosol air freshener is 0.01 mg/kg bw/day assuming 5 g air freshener/event (comparable to hair spray), one event/day, in a living room of 58 m^3 with an exposure period of 16 hr and one event/day in a bedroom of 16 m^3 and an exposure period of 8 hr (Bremmer and van Veen, 2000), combined with an inhalation rate of 20 m^3/day, not taking into account deposition and assuming 100% absorption on inhalation. The initial concentrations must be adjusted for a standard ventilation rate of 0.5/hr for the living room and 1/hr for the bedroom (Bremmer and Veen, 2000). The ventilation rate of 0.5/h means that every hour half of the amount in the living room will be removed, following the equation

$$C = (A / V \times T \times Q) \times (1 - e^{-Q\,t})$$

With: C = concentration
A = amount
V = volume room
T = exposure time
Q = ventilation rate

The first hour the exposure will be:

5000 mg * 1% * 15% /58 m^3 * 20 m^3/24 hr * 1 hr = 0.108 mg

Every next hour roughly half of the amount will be removed. After 16h resulting in a total exposure of 0.216 mg per person, which will be 0.0036 mg/kg bw/day assuming 60 kg bw.
For the bedroom a similar calculation can be made:

5000 mg * 1% * 15% /16 m^3 * 20 m^3/24 hr * 1 = 0.39 mg

Roughly the next hour the total amount will be removed. Therefore the total exposure is 0.39 mg. This results in a 0.0065 mg/kg bw/day assuming 60 kg bw.

These exposures must be considered gross exaggerations because they are based on the assumptions of no deposition, 100% absorption on inhalation and the consumer being only in the bedroom or living room 24 hr/day.

Estimates of long-term exposure from a constant diffusion (electric plug-in type) have also been made (Cadby et al., 2002, some details not given) using maximal observed weight loss data (12 mg/hr) and a number of highly conservative assumptions. A 20 m^3 room with 110 m^3/hr internal airflow and 54.4 m^3/hr external air flow using the SCIES model (Versar Inc., 1991) gives inhalation exposure to the fragrance well below 100 µg fragrance/kg bw/day which would be equivalent to 0.015 mg HHCB/kg bw/day if used at 15% in the fragrance oil.

Summary/statement of the inhalation exposure level

Using the highest measured level of 299 ng/m3, the exposure to indoor air would result in a level of 76.3 x 10^{-6} mg/kg bw/day (229 ng/m3 x 24 hr x 20 m3/24 hr (ventilation rate)/ 60 kg). Compared to the calculated data, this is considered to be negligible.

For risk characterisation we used the calculated data, where under realistic worst-case conditions, these exposures by inhalation of products containing HHCB result in 0.0085 mg/kg bw/day (= 0.002 + 0.0065).

Dermal exposure

Modelled data

Scenario 1 - Cosmetic products

Because the products containing the highest levels of HHCB, eaux de toilette, fragrance creams, antiperspirants and deodorants, are intended for use on the skin and because of the low volatility of HHCB, the principal exposure to consumers can be considered to be via the skin. Upper use levels in a variety of consumer products have been reported to range from 0.09 to 2.4% (SCCNFP, 24 October 2000). These exposures are based on usage data supplied by COLIPA (1996) to the SCCNFP along with data on the use of fragrance oils in consumer products and a 97.5th percentile use level of HHCB in such oils of 30% (IFRA, private communication via COLIPA, 1996 to the SCCNFP). The resulting exposure to HHCB on the skin from the use of a combination of all classes of consumer products on a daily basis was calculated to result in a "worst case situation" of 0.85 mg/kg bw/day (**Table 4.4**) and this value will be used for the risk characterisation.

The 97.5th percentile was chosen to represent an upper level that could be encountered in cosmetic products. However, it is highly unlikely that a series of consumer products exist all of which would contain the same fragrance ingredient at the 97.5th percentile use level. Furthermore, it is unreasonable to consider that a consumer could consistently use all of the classes of cosmetic products over their entire lifetime, all of which are perfumed with the upper 97.5th percentile level of the fragrance ingredient.

Table 4.4 Overview of products and uses that can contain HHCB adapted from the SCCNFP

Type of cosmetic product	Application quantity in grams per application	Application frequency per day	Retention factor (%)[5]	HHCB in product (%)	Exposure to HHCB (mg/day)	Exposure for 60 kg person mg/kg bw/day)
Body lotion [1]	8	0.71	100	0.12	6.83	0.114
Face cream [2]	0.8	2	100	0.090	1.44	0.024
Eau de toilette [3]	0.75	1	100	2.40	18.0	0.30
Fragrance cream [1]	5	0.29	100	1.2	17.4	0.29
Antiperspirant /deodorant	0.5	1	100	0.30	1.5	0.025
Shampoo	8	1	1	0.15	0.120	0.002
Bath products [4]	17	0.29	1	0.60	0.30	0.005
Shower gel [4]	5	1.07	10	0.36	1.93	0.032
Toilet soap	0.8	6	10	0.45	2.16	0.036
Hair spray	5	2	10	0.15	1.5	0.025
				Total	**51.2**	**0.85**

1. Assumes use of conventional body lotion 5 times a week and a fragranced cream twice a week.

2. Including make up and foundation.

3. Including perfume and after shave, but these three products are not used concurrently. The quantity used is inversely proportional to the fragrance concentration so these values include all hydroalcoholic products.

4. Assumes use of bath products twice a week and an average use of shower gel 1.5 times a day, 5 times a week.

5. Proportion of product remaining on the skin.

Scenario 2 - Household products

Direct skin contact from hand-washed laundry

Hand-washed laundry is a common consumer habit. During this procedure, the HHCB-containing laundry solution with an estimated product concentration of 10 mg/ml comes in direct contact with the skin of hands and forearms. A hand-washing task typically takes 10 minutes (Table of Habits and Practices - HERA, 2002). This table also reports a maximum frequency of 18 times per week (3 times/day) when using laundry powder, which seems highly exaggerated but nevertheless is used here as a worst case scenario. The table gives a lower frequency of hand washing with laundry liquid of 10 times per week (1.43 times/day), which still is considered exaggerated. Because the use level of HHCB is different in powder (0.05%) from that in liquid (0.12%) both scenarios are calculated here.

The exposure to HHCB is estimated according to the following algorithm from the HERA guidance document.

$$\mathbf{Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW}$$

For this exposure estimate, the terms are defined with following values for the calculation considering a worst-case scenario:

F_1	percentage weight fraction of substance in product	0.0005 or 0.0012 (**Table 4.3**)
C	product concentration in mg/ml:	10 mg/ml
Kp	dermal penetration coefficient	4.29 x 10^{-5} cm/h*
t	duration of exposure or contact	10 min (0.167h) [HERA, 2002]
S_{der}	surface area of exposed skin	1980 cm^2 [TGD, 1996]
n	product use frequency (tasks per day)	3 or 1.43 [HERA, 2002]
BW	body weight	60 kg

* The dermal penetration coefficient was calculated from the dermal flux (10.3 µg/cm^2) which was determined in an in vitro dermal penetration (Green and Brain, 2001) according to the following algorithm: Kp = dermal flux/(exposure time x concentration of test solution); Kp = (0.0103 mg/cm^2)/(24h x 10 mg/cm^3) = 4.29 x 10^{-5} cm/h

For powder use:

$$\mathbf{Exp_{sys}} = [0.0005 \times (10\ mg/ml) \times (4.29 \times 10^{-5}\ cm/h) \times (0.167h) \times 3 \times (1980\ cm^2)] / 60\ kg =$$
$$\mathbf{0.0035\ \mu g/kg\ bw/day}$$

For liquid use:

$$\mathbf{Exp_{sys}} = [0.0012 \times (10\ mg/ml) \times (4.29 \times 10^{-5}\ cm/h) \times (0.167h) \times 1.43 \times (1980\ cm^2)] / 60\ kg =$$
$$\mathbf{0.0040\ \mu g/kg\ bw/day}$$

Direct skin contact from pre-treatment of clothes

Consumers typically spot-treat clothing stains by hand using either a detergent paste (i.e. water/laundry powder = 1:1) or a laundry liquid, which is applied undiluted (i.e. concentration = 1000 mg/ml) directly on the garment. In this exposure scenario, only the skin surface of the hand (~ 840 cm^2) is exposed.

The exposure to HHCB is estimated according to the same algorithm from the HERA guidance document as is used above using the liquid detergent since this is the highest concentration of HHCB.

F_1	percentage weight fraction of substance in product	0.0012 (laundry liquid -(**Table 4.3**)
C	product concentration in mg/ml:	1000 mg/ml
Kp	dermal penetration coefficient	4.29 x 10^{-5} cm/h [Green and Brain, 2001]
t	duration of exposure or contact	10 min (0.167h) [HERA, 2002]
S_{der}	surface area of exposed skin	840cm^2 [TGD, 1996]
n	product use frequency (tasks per day)	0.5 [HERA, 2002]
BW	body weight	60 kg

$$\mathbf{Exp_{sys}} = [0.0012 \times (1000\ mg/ml) \times (4.29 \times 10^{-5}\ cm/h) \times (0.167h) \times (840\ cm^2) \times 0.5] / 60\ kg =$$
$$\mathbf{0.060\ \mu g/kg\ bw/day}$$

This exposure estimate is very conservative in that it does not recognise use of water to dilute the detergent, a common practice and the fact that only a fraction of the two hands' surface skin will actually be exposed.

Direct skin contact from hand dishwashing

The determination of HHCB exposure from hand dishwashing also uses the same algorithm to calculate the dermal exposure to HHCB from hand dishwashing. The following assumptions have been made as a reasonable worst-case scenario:

F_1	percentage weight fraction of substance in product	0.0007 (**Table 4.3**)
C	product concentration in mg/ml:	2 mg/ml
Kp	dermal penetration coefficient	4.29 x 10^{-5} cm/h [Green and Brain, 2001]
t	duration of exposure or contact	45 min (0.75h) [HERA, 2002]
S_{der}	surface area of exposed skin	1980 cm^2 [TGD, 1996]
n	product use frequency (tasks per day)	3 [HERA, 2002]
BW	body weight	60 kg

$$\mathbf{Exp_{sys}} = [0.0004 \text{ x } (2 \text{ mg/ml}) \text{ x } (4.29 \text{ x } 10^{-5} \text{ cm/h}) \text{ x } (0.75\text{h}) \text{ x } (1980 \text{ cm}^2) \text{ x } 3] / 60 \text{ kg} = \mathbf{0.0025\ \mu g/kg\ bw/day}$$

Measured data

HHCB has been found in certain consumer products at the following concentrations: laundry detergents 0.4 – 120 ppm; shower gels 0.5 – 500 ppm; fabric softeners 3 – 17 ppm; alcohol based cosmetics <0.1 – 24 ppm (Eschke et al., 1995b). Additionally, the levels found in a variety of domestic and occupational products including soap, detergents, shampoos, etc. have been reported to be 6-346 ppm (Rastogi et al., 2001). These reported levels are substantially lower than those used in **Table 4.2** and **Table 4.3** indicating the conservative nature of the modelled exposures.

In a recently performed study, among other chemicals, levels of HHCB were measured in some consumer products. Levels found are presented in **Table 4.5**. In other tested body care products, perfumes or air fresheners, the levels were lower than the detection limit of 0.5 mg/kg. (Peters, 2003).

Table 4.5 Levels of HHCB in some consumer products.

Product description	HHCB (mg/kg)
A shampoo	351
An eau de toilette spray	6248
An eau de totilette	7992
An eau de parfum vaporisateur spray	73
A baby shampoo	131
A kids shampoo	537
A car air-freshener	6.3

Summary/statement of the dermal exposure level

Dermal exposure of consumers to products containing HHCB can mainly be attributed to cosmetics (worst-case estimate 0.85 mg/kg bw/day). Compared to this exposure the dermal exposure of consumers to household products is negligible.

Oral exposure

As fragrance ingredients are not intended for oral ingestion, extensive oral exposure is not to be expected. Unintentionally, exposure could result from food and drinks as a result of residues migrating from dishes and pots which are previously washed with HHCB containing concentrates. However, this exposure is considered negligible.

4.1.1.3.3 Summary of consumer exposure

The resulting exposure to HHCB on the skin from the use of a combination of all classes of consumer products on a daily basis was calculated to result in a "worst case situation" of 0.85 mg/kg bw/day (**Table 4.4**). The inhalatory exposure of consumers to HHCB in household cleaning products and air fresheners is lower, in total 0.0085 mg/kg bw/day. These figures are taken forward to the risk characterisation.

4.1.1.4 Humans exposed via the environment

The daily human intake resulting from indirect exposure via the environment takes into account exposure to HHCB in food, drinking water and inhaled air. Thus the indirect human exposure is estimated using concentrations in fish, root and leaf crops, meat, milk, drinking water and in air.

In the EUSES model, a log Kow value of 5.3 has been used to assess the distribution in the environment. A measured fish bioconcentration factor of 1584 L/kg (see Section 3.1.1.3) has been used in the EUSES model to estimate the concentration in wet fish. For other parts of the food chain, particularly root crops, leaf crops, meat and milk, EUSES estimates the concentrations in these food products using methods that, similar to the fish BCF, rely on log Kow as no equivalent measured accumulation factors exist for these routes.

For the assessment of the indirect exposure to HHCB via the environment the release from the scenarios with the highest environmental concentrations will be taken into account. These are production, compounding at site 2 (2006), compounding large-medium generic, formulation generic and private use in Southern EU. The daily human intake from food, water and air is calculated for these local scenarios as well as for the regional scale. In **Table 4.6** the estimated concentrations in food and other intake media are shown. The estimated daily human intake using these figures is shown in **Table 4.7**.

Table 4.6 Estimated concentrations of HHCB in human intake media

	Estimated concentration in human intake media						
Lifecycle step	**Wet fish (mg/kg)**	**Root crops (mg/kg)**	**Leaf crops (mg/kg)**	**Drinking water (mg/l)**	**Meat (mg/kg)**	**Milk (mg/kg)**	**Air (mg/m^3)**
Production	0.91	0.07	3.4E-02	1.4E-04	1.2E-02	3.7E-03	3.4E-04
Compounding Site 2 (2006)	1.01	2.5E-02	1.1E-02	1.6E-04	3.7E-03	1.2E-03	1.1E-04
Compounding large-medium generic	0.63	0.14	7.1E-03	1E-04	2.5E-03	8.0E-04	7.3E-05

	Estimated concentration in human intake media						
Lifecycle step	**Wet fish (mg/kg)**	**Root crops (mg/kg)**	**Leaf crops (mg/kg)**	**Drinking water (mg/l)**	**Meat (mg/kg)**	**Milk (mg/kg)**	**Air (mg/m³)**
Formulation generic scenario	0.73	0.16	5.4E-03	1.2E-04	1.9E-03	6.1E-04	5.5E-05
Private use, SEU scenario	0.77	0.20	1.4E-3	1.5E-5	5.8E-4	1.8E-4	1.5E-5
Regional, SEU scenario	0.05	0.001	4.5E-5	8.7E-6	1.9E-5	5.9E-6	4.6E-7

Table 4.7 Estimated human daily intake of HHCB via environmental routes

	Estimated human daily intake (mg/kg body weight/day)[1]							
Lifecycle step	**Wet fish**	**Root crops**	**Leaf crops**	**Drinking water**	**Meat**	**Milk**	**Air**	**Total**
Production	1.49E-03	3.64E-04	5.74E-04	4.1E-06	5E-05	2.94E-05	9.74E-05	0.0026
Fraction of total daily dose	0.56	0.15	0.22	0.0016	0.019	0.011	0.037	
Compounding site 2 (2006)	1.65E-03	1.36E-04	1.84E-04	4.54E-06	1.61E-05	9.49E-06	3.11E-05	0.002
Fraction of total daily dose	0.81	0.067	0.09	0.0022	0.0079	0.0047	0.015	
Compounding large-medium generic	1.04E-03	7.53E-04	1.22E-04	2.9E-06	1.09E-05	6.42E-06	2.08E-05	0.002
Fraction of total daily dose	0.53	0.39	0.06	0.0015	0.006	0.003	0.011	
Formulation generic scenario	1.2E-03	8.76E-04	9.2E-05	3.3E-06	8.3E-06	4.9E-06	1.6E-05	0.0022
Fraction of total daily dose	0.55	0.40	0.04	0.002	0.004	0.002	0.007	
Private use, SEU scenario	0.0013	0.0011	2.45E-5	3.73E-6	2.5E-6	1.48E-6	4.16E-6	0.0024
Fraction of total daily dose	0.53	0.46	0.01	0.0016	0.001	0.0006	0.0017	
Regional, SEU scenario	9.0E-5	5.6E-6	7.8E-7	2.47E-7	8.1E-8	4.7E-8	1.32E-7	9.7E-5
Fraction of daily dose	0.93	0.06	0.008	0.003	0.0008	0.0005	0.001	

Note 1: Daily intake of: drinking water 2 L/day. fish 0.115 kg/day, leaf crops 1.2 kg/day, root crops 0.384 kg/day, meat 0.301 kg/day, dairy products 0.561 kg/day. Inhalation rate: 20 m³/day. Bioavailability for oral uptake: 0.5. Bioavailability for inhalation: 1. Body weight of human: 70 kg. SEU = Southern Europe

From the estimated human daily intake doses it can be concluded that the total human daily intake for the scenarios with the highest environmental concentrations are all in the same order. Man will be mainly exposed via the intake of fish and crops. The highest local human

daily intake is estimated for the production scenario and is 2.6 µg/kg bw/day. This value will be taken forward to the risk characterisation. It should be noted that the other scenarios have comparable intake doses. For the regional scenario a value of 0.097 µg/kg bw/day will be taken forward to the risk characterisation.

4.1.1.4.1 Exposure via air

EUSES estimates the highest local air concentration of 3.4E-4 mg/m^3 or 340 ng/m^3 for the production scenario. This is far above the concentrations measured in ambient air samples taken in the south of Norway (0.1 ng/m^3) and the water above Lake Michigan (0.55 ± 0.27 ng/m^3), see section 3.1.6.3. The regional concentration estimated by EUSES (0.46 ng/m^3) is in the same order as the measured concentrations.

4.1.1.4.2 Exposure via food and water

The EUSES model predicts concentrations in drinking water ranging from 0.015 µg/l from private use to 0.16 µg/l from compounding site 2. HHCB has not been detected in drinking water (< 0.01 µg/l) based on measurements in several countries, see section 3.1.4.2.2.

EUSES predicts concentrations in fish ranging from 0.63 (compounding large-medium generic) to 1.01 mg/kg for the compounding site 2 scenario. Monitoring data for HHCB in fish are summarised in 3.1.7.2. As a very conservative approach, the 90th-percentile level of fish from Berlin in the 1990s could be used: 1.5 mg/kg. The assumption is that a citizen would consume only fish caught in high effluent input areas and then only the fish with the highest load. With a daily consumption of 0.115 g fish the intake is 2.5 µg/kg bw/day. This is comparable to the estimated figures (**Table 4.7**) which give human daily intake doses for fish ranging from 1.04 to 1.65 µg/kg bw/day.

No data are available on concentrations measured in vegetables cultivated under normal conditions.

Exposure via mother's milk

In 1999 a study on synthetic musk fragrance ingredients in human milk was carried out by Sönnichsen et al. From 107 women, milk was taken and analysed for several polycyclic musks and nitromusks. A mean and a median fat content of 3.67 and 3.40%, respectively, were found in the mother's milk. The concentration of HHCB in the milk showed a mean value of 80 µg/kg milk fat with a standard deviation of 149. The minimum and maximum values found were close to zero and 1316 µg/kg milk fat, respectively. Arrangements were made to prevent contamination. For more details, see section 4.1.2.1.2.

In 2001, Zehringer and Herrmann published data on 53 milk samples obtained in 1998/1999 from 29 mothers living around the city of Basle. HHCB was detected in 52 milk samples, in a range from not-detectable to 281 µg HHCB/kg fat. The average fat content was 3.3%. This resulted in a mean level of 73 µg HHCB/kg fat.

Other literature reports show levels of 310 and 360 µg/kg fat (only 2 samples measured) (Eschke et al., 1995b) and 16-108 µg/kg fat (5 samples) (Rimkus and Wolf, 1996).

In a case control study for risk factors of early miscarriages in the Uppsala County in Sweden, during the period 1996-2003, women donated milk samples to be analysed for the presence of

various polycyclic- and nitromusks. Women were also asked to fill out extensive questionnaires about lifestyle, medical history and dietary habits and a sub-population was also asked to fill out an additional questionnaire about use of perfumes and perfumed deodorants, skin lotions, laundry- and washing detergents. Milk was collected at the beginning and at the end of the breast-feeding sessions. The goal was to collect 500 ml from each mother during the 3rd week after delivery. It was not mentioned whether measurements were taken to prevent contamination of the milk samples from musks present on the mother's skin. In total 101 milk samples were analysed. For 42 of these, useful data on use of perfumed products were available. HHCB was found in concentrations ranging from 2.8 to 268 µg/kg milk (mean ± SD: 78 ± 55; median 64 µg/kg milk fat). No correlation could be demonstrated between concentration of HHCB in milk samples and life style factors, medical history or dietary habits and no time-trend was observed. The results may indicate that concentrations in milk samples from women using perfumed products were higher that in those collected from non-users. However, the differences were only significant for use of perfume during pregnancy (≥ 1/week) as compared to no use, but the significance was driven by only three samples, in the otherwise rather small sample population (Lignell et al., 2004).

In a pilot study in the Czech Republic, 59 milk samples were collected from nursing mothers (living but not necessarily born in Prague). The manual sampling (milk expressed from the breast into a clean container) was conducted in accordance with WHO guidelines. Using a detailed questionnaire, relevant information on parameters, such as age, dietary habits (specifically consumption of freshwater/marine fish), use of perfumed cosmetics, frequency of contacts with detergents, etcetera were collated. The lipid content in the human milk samples ranged from 1.5 to 4.2 wt %. The detection limit for the musks was 10 µg/kg fat. HHCB was found in all samples. Concentrations ranged from 13 to 720 µg/kg fat, with a median value of 149, a mean value of 214, and a 90th percentile of 509 µg HHCB/kg fat. No correlations were found between the musk levels and personal data of the mothers obtained by the questionnaire (Hajslova and Setkova, 2004). Milk samples were collected in 1999 at Hvidovre Hospital in Denmark from 10 primiparous mothers (25-29 year of age) 14-26 weeks after birth. HHCB was found at levels from 38.0 to 422 µg/kg fat. Fat content of the milk samples had an average of 3.5% ±2.5% (2.1%-4.8%). The median level of HHCB was 147 µg/kg fat and the average level was 179 µg/kg fat (Duedahl-Olesen et al., 2005). The study of Sönnichsen et al. (1999) showing the highest maximum level of 1316 µg/kg of all studies and a mean level of 80 µg HHCB/kg milk fat will be used for the risk characterisation. This study is well performed by excluding contamination from cosmetic products as much as possible and it involved a significantly larger population than other studies. Other studies did not give information on excluding contamination. It could be argued that actual exposure of an infant might even be to higher concentrations than the maximum found in the Sönnichsen study, because of the sampling procedure, but this is not directly supported by the other data available. Other maximum values were 281 (Zehringer and Herrmann, 2001), 360 (Eschke et al, 1995b), 108 (Rimkus and Wolf, 1996), 268 (Lignell et al., 2004), 720 (Hajslova and Setkova, 2004) and 422 (Duedahl Olesen et al., 2005) in µg HHCB/kg fat. Furthermore, no correlation is found between use of cosmetic products and the HHCB concentration in milk fat.

4.1.1.5 Combined exposure

See under 4.1.3.5 Combined exposure – Risk characterization.

4.1.2 Effects assessment: Hazard identification and dose (concentration)-response (effect) assessment

In many of the tests described in this section, the test material was obtained from the manufacturer or a supplier under the trade names Galaxolide (most common) or Galaxolide 50 and tested as such. Thus, as explained in section 4.1.1.1, the actual doses of HHCB were only 65% of that stated in the report or publication and all such doses are adjusted to account for the diluent when the test material is described as Galaxolide.

4.1.2.1 Toxicokinetics, metabolism and distribution

All studies in this section, with the exception of the analyses of human fat and milk samples (Eschke et al., 1995b and Rimkus and Wolf, 1996) and the *in vitro* dermal absorption studies with excised rat skin (Ashcroft and Hotchkiss, 1996), followed the basic guidance outlined in OECD Method 417.

4.1.2.1.1 Studies in animals

In vivo studies

All available studies in this section were evaluated for information on the absorption, distribution, excretion and metabolism of HHCB in *in vivo* animal studies. Within the subheadings based on the route of exposure, these sections were further subdivided into these fate processes to assist in the evaluation of HHCB in these studies.

Also, additional sections were added to address the available intravenous animal studies, the animal and human milk studies, and the human fat studies.

Inhalation

No data available.

Dermal

Absorption: In a GLP compliant study, the absorption, distribution and excretion of radioactivity have been determined by topical application (occluded with aluminum foil after evaporation of the solvent (not stated for how long) with 4.5 mg/kg bw of ^{14}C-HHCB (uniformly labelled in the aromatic ring – radiochemical purity 97.2%) in 200 µl of 70 % aqueous ethanol solution to the shaven backs of 18 male pigmented rats (Lister Hooded, bodyweight *ca* 200g, age 5-7 weeks). The application rate was 0.1 mg/cm^2 over an area of 9 cm^2. (This experiment was conducted for the purpose of obtaining ethical approval for the human simulated exposure experiment (see below) and, thus, skin exposure was limited to 6 hours.) After the 6 hr application the dressing was removed and the remaining dose at the treated area washed off with cotton wool swabs moistened with 70% alcohol. Another occlusive dressing (aluminum foil) was placed on the skin of the animals until sacrifice.

Urine, faeces and expired air were collected for rats killed at 6 hr after start of dosing or later and were analysed for radioactivity and metabolite identification. Pairs of rats were killed at 0.5, 1, 3, 6, 12, 24, 48, 72, and 120 hr after start of dosing. Prior to sacrifice blood was withdrawn for analysis. At sacrifice, tissues (including untreated and treated skin) as well as

the remaining carcass were removed for analysis of radioactivity. Urine was collected at 0-6, 6-12 and 12-24 hr and every 24 hr thereafter until 120 hrs. Faeces was collected every 24 hr until 120 hr, and air was collected during 0 – 48 hr post dosing. Average recovery of radiolabel (6 – 120 hr) was 97%.

A majority (mean of 77%) of the applied material remained on the surface of the skin at the time of washing - 6 hr. At that time, about only 3.9% of the applied dose could be detected in excreta and tissues (excluding application site). Although exposure was only for 6 hr, the results indicate that a significant skin reservoir of material (up to approximately 10%) was formed from which material continued to be absorbed up to 120 hr resulting in trace organ levels still being present at this time. Analysis of the dressings applied after surface dose removal, indicated that approximately half of the material in the reservoir was lost to the dressing applied after dose removal. Residual radioactivity in the treated skin declined from 10.54% of the dose at 6 hr to 2.02 % of the dose at 120 hr with a half-life of about 2 days. Based on the amount of radiolabel excreted (see below in excretion section) combined with that remaining in the tissues and carcass, but not including the amount remaining in the skin at the treatment site, at 120 hr (2% of the dose), ~13.7% of the applied dose had been absorbed.(Ford et al., 1999; Hawkins et al., 1995).

Distribution: In the rat *in vivo* dermal absorption study described above (Ford et al., 1999, Hawkins et al., 1995), plasma levels peaked at about 6 hr (time of removal of dose from surface). Analyses of tissue levels indicated that ~0.7% of the absorbed radiolabel was present at 120 hr; however, ~2% still remained in a skin reservoir at the treatment site at 120 hr. The large majority of the absorbed radiolabel was found in the large and small intestines and their contents (**Table 4.8**) consistent with biliary excretion. Organ levels essentially reflected plasma levels only. Fat levels peaked later at 24 hr declining after that. Average recovery of radiolabel (6 – 120 hrs) was 97%. Peak tissue levels of less than 0.2 µg equiv./g tissue were seen generally at 6 hr in adrenals, bone marrow, brain, eye, heart, kidney, lung, lymph node (peak 12 hr), muscle, pancreas (12 hr), skin (12 hr), spleen, testis, thymus, and thyroid.

Table 4.8 Distribution of radioactivity in selected tissues during 0.5 to 120 hours after dermal application of 14C-HHCB to male rats at a dose of 4.5 mg/kg bw over an area of 9 cm^2 (as µg equivalents/g of tissue)

Tissues	Time (hr after initial application)								
	0.5	1	3	6	12	24	48	72	120
LI + contents	0.011	0.061	0.48	1.82	8.01	7.74	3.95	2.13	0.67
SI + contents	0.076	0.37	3.31	5.64	5.71	5.07	3.09	1.64	0.56
Stomach + contents	0.033	0.073	0.401	1.11	1.22	0.598	0.810	0.440	0.123
Liver	0.018	0.083	0.213	0.310	0.306	0.228	0.135	0.107	0.053
Fat	0.004	0.023	0.101	0.238	0.367	0.415	0.291	0.242	0.145
Plasma	0.005	0.022	0.054	0.073	0.059	0.046	0.024	0.019	0.009
Adrenal glands	0.028	0.091	0.158	0.161	0.086	0.090	0.043	0.031	0.013
Kidneys	0.016	0.063	0.131	0.193	0.130	0.096	0.039	0.028	0.012
Thyroid	nd	0.068	0.108	0.094	0.074	0.043	nd	nd	nd
Untreated Skin	0.001	0.006	0.073	0.019	0.031	0.019	0.010	0.009	0.002

LI = Large intestine, SI = Small intestine

Excretion: In the rat *in vivo* dermal absorption study (GLP) in rats (6 hr application under occlusion) described above, after 120 hr, 13% of the applied dose had been excreted (primarily in the faeces - 11.6%, with the remainder in the urine - 1.27%, cage wash - 0.08%, and expired air – 0.06%) with the majority excreted within 48 hr (8.3%) (all mean values). No attempt was made to characterize possible metabolites. Average recovery of radiolabel (6 – 120 hrs) was 97% (Ford et al., 1999; Hawkins et al., 1995).

Metabolism: No data available.

Oral

Absorption: No data available. A default value of 50% will be used.

Intravenous

Distribution: In a GLP compliant study, groups of four female Sprague Dawley CD rats (bodyweight range 213-230 g – age 10-11 weeks) received a single intravenous administration of 2 mg/kg bw ^{14}C-HHCB (uniformly labelled in the aromatic ring – radiochemical purity 99%) in a 0.4 mg/ml ethanol/Emulphor EL 620/isotonic saline (1:1:7) solution in the tail vein and were sacrificed at 5, 15, 30 min and 1, 2, 4, 6, 12, 24 and 48 hr and 7, 14 and 28 days. Tissues (fat, kidney, liver) were weighed and blood was collected by cardiac puncture. Urine, faeces and air were collected from the 4 animals that were sacrificed at day 7 after every 24 hrs until 168 hours (air up to 48 hrs). The recovery of radioactivity in these 4 animals represented 91.8 % of the dose administered: 89.3% in excreta plus cage washings, 2.14% in the carcass and 0.25% in the liver.

Maximum concentrations of radioactivity were observed in all tissues at 5 min (earliest time of measurement) except for the fat where the maximum was at 2 hr (**Table 4.9**). Between 48 hr and 14 days, radioactivity in the plasma and fat decreased with apparent half-lives of elimination of 2.1 and 2.6 days respectively. In the fat, the majority of radioactivity (57-77%) was associated with parent HHCB. In whole blood, concentrations declined between 7 and 28 days with a half-life of 8.5 days with the majority of the radioactivity being associated with the cells while at earlier times it was primarily associated with the plasma. In the kidneys, the decline between 7 and 28 days was with a half-life of 8.6 days (Hawkins et al., 1997a).

Table 4.9 Concentrations of radioactivity in tissues after an intravenous doses of ^{14}C- HHCB of 2 mg/kg bw to rats (in µg equivalents/g tissue).

Time	Tissues				
	Plasma	Whole Blood	Liver	Kidney	Fat
5 min	2.57	1.58	8.83	4.65	1.21
15 min	1.94	1.17	5.90	3.11	1.84
30 min	1.54	0.914	4.73	2.38	3.29
1 hr	1.46	0.845	4.03	1.94	5.27
2 hr	1.31	0.716	3.00	1.26	6.64
4 hr	1.06	0.584	2.32	0.914	5.55
6 hr	1.04	0.565	2.39	0.817	4.20
12 hr	0.564	0.332	1.99	0.503	4.75
24 hr	0.249	0.148	1.09	0.227	3.66

Time	Tissues				
	Plasma	Whole Blood	Liver	Kidney	Fat
2 days	0.102	0.0644	0.548	0.0920	2.17
7 days	0.011	0.0108	0.121	0.0237	0.575
14 days	0.00199	0.00438	0.0407	0.00985	0.0989
28 days	0.00050	0.00185	0.0221	0.00415	0.0260

In a GLP compliant study, one male domestic pig (Sus scrofa of Large White Hybrid strain – age 8-12 weeks, bodyweight 33 kg) received a nominal dose of 0.1 mg/kg bw (actual dose 0.101 mg/kg bw) ^{14}C- HHCB (uniformly labelled in the aromatic ring – radiochemical purity >99%) in ethanol/Emulphor EL 620/isotonic saline (1:1:7) solution by intravenous injection into the ear vein. Urine was collected at 0-6 hr and 6-24 hr and every 24 hr up to 14 days and faeces were collected at 24-hr intervals up to 14 days. Blood was collected at 10, 20 and 40 min, 1, 2, 4, 8, 12, 24 hr, 2, 3, 5, 7, 14, 21, and 28 days. Biopsies of skin and underlying fat tissue were taken at 9, 16 and 28 days (day of sacrifice). The recovery of radioactivity via the excreta was 88.1 % of the administered dose. The maximum concentrations of radioactivity in whole blood and plasma were observed at 10 min (earliest collection) (see **Table 4.10**).

Table 4.10 Concentrations of radioactivity in blood and plasma after an intravenous doses of ^{14}C- HHCB to a pig of 0.1 mg/kg bw (in ng equivalents/g).

Time (hr)	Whole blood	Plasma
0.17	69.9	108
0.33	60.6	98.2
0.67	50.5	77.7
1	37.5	58.8
2	21.3	34.1
4	11.9	18.2
8	6.8	10.6
12	5.8	8.6
24	3.3	4.9
48	1.8	2.6
72	1.2	1.9
120	0.9	1.4
168	0.7	1.0
336	<0.5	0.5
504	0.4	<0.3
672	<0.3	<0.3

Radioactivity decreased rapidly in blood and plasma during the initial distribution phase with half-lives of ca. 1.1 hr. Thereafter concentrations declined at a slower rate. After 48 hr up to

168 hr the apparent half-life of elimination was about 90-94 hr. At later times (336-672 hrs), concentrations were close to or below the limits of accurate determination. There was no obvious accumulation of radioactivity in blood cells. In fat, the maximal concentration (earliest collection) was at 9 days. After that, the fat concentration decreased slowly and it was < 3.1 ng equiv./g 16 days after injection and < 0.5 ng equiv./g after 28 days. In skin, the maximal concentration (earliest collection) was at 9 days (3.8 ng equiv./g) declining to 0.8 ng eq/g at 16 days and to below the limit of accurate measurement (<0.5 ng eq/g) at 28 days (Hawkins, 1997b).

Excretion: In the rat intravenous study (GLP) described above (Hawkins et al., 1997a), the majority of the radioactivity (53% of the dose via faeces and 23% of the dose via urine) was excreted during the first 72 hr or 48 hr post-dosing for faeces and urine, respectively. Over the entire collection period (168 hr), the excretion via these routes amounted to 61% and 28.1% for faeces and urine. Exhalation of radioactivity could not be detected.

In the pig intravenous study (GLP) described above (Hawkins, 1997b), the majority of the radioactivity (63% of the dose via urine and 10% of the dose via faeces) was excreted during the first 48 hr. Over the entire collection period (336 hr) the excretion via these routes amounted to 74% and 14.6 % for urine and faeces. Exhalation of radioactivity was not monitored.

Metabolism: The urine collected from the two (rat and pig) intravenous studies (GLP) described above were analysed for metabolites by Thin Layer Chromatography using several solvent combinations with solvent E (chloroform/methanol/water/formic acid - 75/25/3/3 by volume) giving good separation. Chromatography of urine samples with Solvent E revealed no unchanged HHCB but at least 10 metabolites in the pig and 12 metabolites in the rat (**Table 4.11**). Chromatography of urine samples with Solvent H (chloroform/methanol/ ammonia 80/20/1 by volume) revealed at least 14 metabolites in pig urine and 10 metabolites in rat urine (**Table 4.12**). None of these metabolites was characterized other than by retention times (R_f).

No change in abundance of any of the metabolites was observed when urine was treated with aryl sulphatase. In contrast, chromatography with Solvent E revealed decreases in the abundance of the 4 of the 5 main pig metabolites (R_f 0.24, 0.33, 0.42 and 0.47) after treatment with β-glucuronidase indicates that these were glucuronide conjugates. Two of these (R_f 0.33 and 0.42) were also seen after similar extraction of the rat urine but only one (R_f 0.42) decreased after treatment, indicating that pig R_f 0.33 is different from rat R_f 0.33. With the exception of one other metabolite in the rat (R_f 0.59), β-glucuronidase treatment caused no significant decreases. Treatment of either pig or rat urine with β-glucuronidase resulted in the increase of the abundance of metabolites that were also detected without enzymatic treatment, except for a pig metabolite with R_f 0.90 and a rat metabolite with R_f 0.97, which were only excreted as conjugates. The principal urinary metabolite in the pig (R_f 0.47) was not seen in the rat nor was the principal metabolite in the rat (R_f 0.70) seen in the pig.

Table 4.11 Proportion of urinary metabolites as a percentage of dose after intravenous injection in rats or a pig – solvent E

R_f value	Pig urine (0-48 hr)		Rat urine (0-24 hr)	
	Untreated	Enzyme treated	Untreated	Enzyme treated
0.12	*	*	0.31	0.25
0.18	*	*	0.96	0.86

R_f value	Pig urine (0-48 hr)		Rat urine (0-24 hr)	
	Untreated	Enzyme treated	Untreated	Enzyme treated
0.20	*	*	2.91	3.22
0.24	6.30	1.22	*	*
0.33	10.69	1.28	2.98	3.19
0.42	4.77	0.29	0.43	*
0.47	19.56	0.47	*	*
0.59	0.23	0.40	2.99	0.85
0.63	2.34	6.89	0.52	0.86
0.70	*	*	3.79	3.45
0.74	3.04	14.36	0.5	0.68
0.80	3.45	8.46	*	*
0.83	*	*	0.56	0.93
0.85	6.66	24.9	*	*
0.90	*	1.44	0.89	1.06
0.97	*	*	*	0.46
Others	5.39	2.75	0.72	1.76
Total in urine	62.45		17.56	

*Not detected

Chromatography with solvent H, revealed 6 metabolites greater than 1% in pig urine, the principal of which (Rf 0.06) decreased significantly on enzyme treatment but the remaining 5 all increased (**Table 4.12**). In the rat urine, again the metabolite with Rf 0.06 decreased on enzyme treatment but there were no other significant decreases. Two new metabolites (Rf 0.75 and 0.99) appeared in the enzyme treated sample (Hawkins, 1998). Faecal metabolites were not studied (although in the rat faecal excretion is quite important).

Table 4.12 Proportion of urinary metabolites as a percentage of dose after intravenous injection in rats or a pig – solvent H

R_f value	Pig urine (0-48 hr)		Rat urine (0-24 hr)	
	Untreated	Enzyme treated	Untreated	Enzyme treated
0.06	41.74	4.58	3.89	2.08
0.12	1.40	2.13	2.79	2.93
0.21	0.64	0.89	2.15	2.19
0.25	0.88	0.28	2.37	2.57
0.34	0.49	0.95	3.62	3.33
0.49	0.66	1.00	*	*
0.52	0.44	0.70	*	*
0.62	1.19	1.74	0.38	0.39
0.69	0.69	2.67	*	*
0.75	3.72	15.09	*	0.69

R_f value	Pig urine (0-48 hr)		Rat urine (0-24 hr)	
	Untreated	Enzyme treated	Untreated	Enzyme treated
0.82	2.23	6.61	0.48	0.54
0.88	8.05	24.57	0.24	0.57
0.92	0.10	0.97	1.16	1.28
0.99	*	*	*	0.28
Others	0.22	0.29	0.47	0.71
Total in urine	62.45		17.56	

*Not detected

Animal milk studies

In a GLP compliant study, designed to measure plasma and milk levels that would be reached as a result of oral dosing, ^{14}C- HHCB (uniformly labelled in the aromatic ring – radiolabel purity 98.0%) was administered by gavage to pregnant Charles River CD rats (n=18/group – bodyweights *ca* 250-500 g – age 10-15 wks) at 2.0 or 20 mg/kg bw as a solution in corn oil (nominal dose levels; actual dose levels were about 10% higher), daily from day 14 of gestation up to 7 days post-parturition. The dosing regimen was designed to achieve steady state prior to parturition but not to have exposure during organogenesis even though it is recognised that some organogenesis occurs after day 14. Milk samples of *ca*. 0.5 ml (after administration of oxytocin) and blood samples of about 4 ml were obtained from 3 dams per dose level at 4, 8 and 24 hr after dosing with HHCB, on days 3 and 7 post-parturition. Milk and blood samples were analysed for radiolabel. In plasma, the highest mean levels of radiolabel were found in the 4 hr samples, declining to about 35% of that level by 24 hr after dosing (see **Table 4.13**). Lower levels were consistently seen after 7 days as opposed to 3 days indicating no significant accumulation in plasma. Levels were roughly proportional to dose with levels at 20 mg/kg bw/day approximately 7 fold higher than those at 2 mg/kg bw/day.

Table 4.13 Analysis of total radioactivity in plasma after daily oral administration of 2 or 20 mg/kg ^{14}C-HHCB in µg equivalents HHCB/ml plasma

Time after parturition	Time after oral administration (hours)	Mean level after oral doses of 2 mg/kg/day	Mean level after oral doses of 20 mg/kg/day
Day 3	4	1.90 ± 0.87	11.08 ± 1.86
	8	1.05 ± 0.43	7.24 ± 0.53
	24	0.33 ± 0.12	2.66 ± 0.62
Day 7	4	1.21 ± 0.09	8.76 ± 1.57
	8	0.66 ± 0.25	5.06 ± 0.70
	24	0.23 ± 0.05	1.62 ± 0.55

In milk, levels of total residue (**Table 4.14**) were also highest at 4 hr after dosing declining significantly by 24 hr. Similar levels were seen after 7 days dosing as compared to after 3 days dosing. Additionally, the major residue in the milk was associated with a peak, which appeared to co-chromatograph with HHCB (the radiolabelled HHCB peak elutes at a retention time of 18-19.5 minutes whereas in the extract, the peak elutes at 18-21 minutes). Although not fully characterised, the HHCB peak in the milk extracts is considered authentic as the metabolites are expected to be more polar and would therefore precede HHCB on the C18 polar column. About 52 - 70% and 47 - 59% of the radioactivity was associated with other materials (metabolites) at the low and high dose, respectively (Hawkins et al., 1996a).

Table 4.14 Analysis of total radioactivity and unchanged HHCB in milk after daily oral administration of 2 or 20 mg/kg ^{14}C-HHCB in µg equivalents HHCB/ml milk (ppm)

Milk collection time after parturition	Time after oral dosing (hours)	After oral doses of 2 mg/kg bw/day			After oral doses of 20 mg/kg bw/day		
		Total radiolabel Mean	HHCB Mean	Ratio HHCB/total residue	Total radiolabel Mean	HHCB Mean	Ratio HHCB/total residue
Day 3	4	1.71 ± 0.20	0.82 ± 0.11	0.48 ± 0.03	32.8 ± 10.9	17.57 ± 6.4	0.53 ± 0.08
	8	0.88 ± 0.20	0.27 ± 0.09	0.32 ± 0.13	12.4 ± 4.4	4.95 ± 1.48	0.41 ± 0.02
	24	0.27 ± 0.11	nd	-	1.69 ± 0.37	nd	-
Day 7	4	2.28 ± 0.66	0.99 ± 0.49	0.41 ± 0.11	25.0 ± 7.0	11.56 ± 4.6	0.45 ± 0.06
	8	1.09 ± 0.20	0.33 ± 0.13	0.30 ± 0.07	16.1 ± 3.55	8.30 ± 2.92	0.52 ± 0.13
	24	0.15 ± 0.03	nd	-	1.34 ± 0.48	nd	-

nd : not detected due to low radioactivity levels

In vitro studies

Absorption: The *in vitro* absorption of ^{14}C-HHCB (position of label not indicated) was measured (non-GLP) using full thickness dorsal skin (male F344 rat) in flow-through diffusion cells. A receptor fluid containing 50% v/v aqueous ethanol to enhance absorption flowed across the underside at a rate of 1.5 ml/hour. Dose solutions of 0.1% and 0.5% in an ethanol/DEP (75:25) vehicle (15 µg/cm^2 and 78 µg/cm^2, respectively) were applied to occluded (Teflon caps) and non-occluded systems. Receptor fluid was collected at 2 hr intervals for up to 72 hr. After 24 or 48 hr radioactivity on the skin, in the skin, in the receptor fluid and in the skin support system was determined by liquid scintillation spectrometry. HHCB was poorly absorbed through non-occluded skin after 24 hr (0.07% of applied dose). Occlusion enhanced absorption at 24 hours to 5.55%. After 24 hr, about 47 or 66% of the applied radioactivity was present in the skin after unoccluded and occluded exposure, respectively. Over 48 hr HHCB continued to be absorbed into the receptor fluid and total absorption was greatly enhanced by occlusion. No data were presented with respect to the 48-72 hr period. Total recovery of radioactivity was not presented but was stated to be generally > 80% (Ashcroft and Hotchkiss, 1996). These results cannot be used as determinates of dermal absorption because of the use of a non-physiologically relevant receptor fluid, no report of testing the integrity of the skin (the data were taken from a poster presentation) and poor recovery of radiolabel.

4.1.2.1.2 Human data

In vivo studies

Human adipose tissue studies

In a study (non-GLP) to measure residues of HHCB, two human fat samples (origin not specified) were extracted with hexane and the extracts analysed by selective ion trap gas chromatography (GC)/mass spectrometry (MS) for residues of HHCB. Residues were found in both samples at levels of 145 and 149 µg/kg fat (Eschke et al., 1995b).

In a similar study (non-GLP), human adipose samples were obtained from 8 females and 6 males in Germany between 1993 and 1995. These samples were extracted with a mixture of water/acetone/petroleum ether and analysed for HHCB residues by GC/mass spectroscopy. HHCB was found in all 14 samples at concentrations ranging from 28 to 189 µg/kg fat (ppb) (mean 82). Although the small number of samples and wide range of data preclude meaningful statistical evaluation, a visual inspection of the data reveals no clear correlation with sex or age (Rimkus and Wolf, 1996).

In a non-GLP study, human fat samples obtained over the years 1983/1984 and –1994 in Switzerland from corpses of 10 females and 5 males (age group 3-100 years) were analysed for residues of HHCB by homogenisation followed by extraction with cyclohexane/ethyl acetate (1:1) and analysed by GC/MS. HHCB was detected in all samples with a range of 12 – 171 µg/kg fat (mean 66 µg/kg) (Müller et al., 1996).

Human blood studies

Blood samples from 413 German subjects (85 men and 328 women) were extracted (details not reported) and analysed by GC-MS for levels of HHCB as well as for musk ketone and musk xylene for comparison. The average level of HHCB (Galaxolide) was 722 ng/l with a range of <100 (3 samples) to >1600 ng/l (23 samples). The 95-percentile level was 1651 ng/l (Bauer and Frössl, 1999).

Blood samples from 100 Austrian volunteers (55% woman, median age 23, average age 25.5, range from 19 to 43 years) were analysed by GC-MS for the determination of levels of musk fragrance substances. A questionnaire on the use of cosmetics, household products and food was completed by these volunteers. Blood samples were analysed using GC-MES ananlysis after extensive sample extraction and clean-up. HHCB was not detected in 9% of the samples, with a detection limit of ±30 ng/l. The average level of HHCB found was 594 ng/l with a maximum level of 4100 ng/l. A weak correlation was found between the levels of musk fragrances in blood and the use of cosmetics (Sattelberger et al., 2003; Hutter et al., 2005).

Blood samples from 91 Dutch volunteers (48 males and 43 females, age ranging from 19 till 78 years) were analyzed by GC-MS for the determination of levels of musk fragrance substances after solvent extraction. HHCB was found in all 91 samples. The median level of HHCB was 1.3 ng/g serum with a range of 0.2 to 9.2 ng/g serum. The 95-percentile level was 3.6 ng/g serum (Peters, 2004).

Human milk studies

In a study (non-GLP) to determine residues of HHCB, two human milk samples (origin not specified) were extracted with hexane and the extracts analysed by GC/MS for residues of HHCB. Residues were found in both samples at levels of 310 and 360 µg/kg fat or 3.3 and 1.5

µg/kg (ppb) whole milk based on measured fat contents of 1.06 and 0.41%, respectively (Eschke et al., 1995b).

In a similar study (non-GLP), five milk samples were obtained from 4 nursing mothers and were extracted according to an AOAC method (Helrich, 1990) and analysed for HHCB residues by selective ion trap GC. All samples contained some HHCB at concentrations ranging from 16 – 108 µg/kg milk fat (Rimkus and Wolf, 1996). The fat contents of these samples were not reported.

In another, larger study (non-GLP) of HHCB residues, milk samples (mean 34 g) were obtained from 107 nursing mothers in Germany (mean age 31.5 years, mean body mass index 24.5 kg/m^2 at time of child birth and 23.2 kg/m^2 at time of milk sampling) under conditions designed to minimize contamination (all equipment was carefully cleaned and the breast area was cleaned 3 times with cotton wool swabs immersed in propylene glycol). All were asked to report on their use of various household products including soaps, detergents and cosmetics as well as their consumption of fish products. As established in a separate study, the back-ground level of HHCB in cyclohexane extracts of cotton swabs was 0.8 ng/ml before these swabs were used for skin cleaning purposes. After the first, second, third and fourth cleaning steps of breast surface with cotton swabs soaked in propylene glycol, HHCB levels were 127, 182, 136 and 97 ng/ml respectively. Because of the decline after 3 cleanings and in consideration with the mothers, the study authors decided to take milk samples after 3 cleaning steps. HHCB was detected in 82% of all samples. HHCB was detected in the milk samples at levels from zero to maximum 1316 µg/kg of fat with a mean of 80 µg/kg of fat. Based on the reported mean fat level of 3.67 %, this corresponds to a maximum level in the whole milk of 48 µg/kg milk (ppb) with a mean of 2.9 µg/kg milk (ppb).

Higher concentrations of HHCB were seen in subjects with a higher body mass index (BMI), either at parturition or the time of milk sampling. This could suggest that an increased fat storage in the body causes an accumulation of synthetic musk fragrances, which in turn leads to a higher concentration of these substances in the breast milk. If this were true, one would find some positive correlations between the BMI change and/or body weight loss versus musk concentrations in breast milk, however, this turns out to be not the case. Another important biological variable is maternal age, which was found to have no bearing on the musk concentrations in milk fat. There was also no correlation shown with number of siblings, complete time of breast feeding, diet or use of household products and cosmetics (Sönnichsen et al., 1999).

In 2001, Zehringer and Herrmann published data on 53 milk samples obtained in 1998/1999 from 29 mothers living around the city of Basle. HHCB was found in concentrations ranging from not-detectable (1 sample) to 281 µg HHCB/kg fat. The average fat content was 3.3%. This resulted in a mean level of 73 µg HHCB/kg fat. No special arrangements were made to prevent contamination of milk samples by HHCB present on the skin.

In a case control study for risk factors of early miscarriages in the Uppsala County in Sweden, during the period 1996-2003, women donated milk samples to be analysed for the presence of various polycyclic- and nitromusks. Women were also asked to fill out extensive questionnaires about lifestyle, medical history and dietary habits and a sub-population was also asked to fill out an additional questionnaire about use of perfumes and perfumed deodorants, skin lotions, laundry- and washing detergents. Milk was collected at the beginning and at the end of the breast-feeding sessions. The goal was to collect 500 ml from each mother during the 3rd week after delivery. It was not mentioned whether measurements were taken to prevent contamination of the milk samples from musks present on the mother's

skin. In total 101 milk samples were analysed. For 42 of these, useful data on use of perfumed products were available. HHCB was found in concentrations ranging from 2.8 to 268 μg/kg milk (mean ± SD: 78 ± 55; median 64 μg/kg milk fat). No correlation could be demonstrated between concentration of HHCB in milk samples and life style factors, medical history or dietary habits and no time-trend was observed. The results may indicate that concentrations in milk samples from women using perfumed products were higher that in those collected from non-users. However, the differences were only significant for use of perfume during pregnancy (≥ 1/week) as compared to no use, but the significance was driven by only three samples, in the otherwise rather small sample population (Lignell et al., 2004).

In a pilot study in the Czech Republic, 59 milk samples were collected from nursing mothers (living but not necessarily born in Prague). The manual sampling (milk expressed from the breast into a clean container) was conducted in accordance with WHO guidelines. Using a detailed questionnaire, relevant information on parameters, such as age, dietary habits (specifically consumption of freshwater/marine fish), use of perfumed cosmetics, frequency of contacts with detergents, etcetera were collated. The lipid content in the human milk samples ranged from 1.5 to 4.2 wt %. The detection limit for the musks was 10 μg/kg fat. HHCB was found in all the samples. Concentrations ranged from 13 to 720 μg/kg fat, with a median value of 149, a mean value of 214, and a 90th percentile of 509 μg HHCB/kg fat. No correlations were found between the musk levels and personal data of the mothers obtained by the questionnaire (Hajslova and Setkova, 2004).

Milk samples were collected in 1999 at Hvidovre Hospital in Denmark from 10 primiparous mothers (25-29 year of age) 14-26 weeks after birth. HHCB was found at levels from 38.0 to 422 μg/kg fat. Fat content of the milk samples had an average of 3.5% ±2.5% (2.1%-4.8%). The median level of HHCB was 147 μg/kg fat and the average level was 179 μg/kg fat (Duedahl-Olesen et al., 2005).

Inhalation

No data available.

Dermal

In a GLP compliant study, the absorption and excretion of total radioactivity was determined in 3 human male volunteers. ^{14}C- HHCB (uniformly labelled in the aromatic ring – radiochemical purity 98.3%) was applied to the skin of human volunteers under conditions intended to simulate a typically high exposure from the use of alcohol based products such as perfumes or eaux de toilette, i.e. 0.4% in 70% ethanol. A mean of 1.76 mg ^{14}C- HHCB dissolved in 70 % ethanol (0.48 ml) was applied to 100 cm^2 (0.018 mg/cm^2) area of skin on the upper back. After 30 min to allow the ethanol to evaporate, the area was covered with light gauze dressing. Six hr after application, the dressing was removed and the treated area washed with cotton wool swabs, moistened with 70% ethanol. An area of 6.25 cm^2 was stripped by 5 successive applications of adhesive tape to determine the amount of total radioactivity in the upper level of the horny layer. The treated site was again covered with fresh dressings up to 120 hr after compound application at which time the dressings were taken off and another skin area of 6.25 cm^2 was stripped to determine the remaining total radioactivity in the upper stratum corneum. Samples of blood (at 0.5, 1, 2, 4. 6, 8, 10, 12, 24, 36, 48, 72, 96 and 120 hr) and excreta (urine at 0-2, 2-4, 4-6, 6-12, 12-24, 24-48, 48-72, 72-96 and 96-120 hr intervals, and faeces at 24 hr intervals) were collected during the five-day period.

The majority of the applied material (~56%) was still on the surface of the skin at the time of washing - 6 hr. The first tape stripping at time of removal of the dose indicated that approximately 11% of the applied radioactivity (AR) remained in the upper layers of the stratum corneum. Recovery in the faeces was below the limits of accurate detection (< 0,1 % applied radioactivity, AR) and only one of the three subjects excreted measurable radiolabel in the urine (0.1 % AR). Concentrations in whole blood and plasma were also below the limits of accurate measurement (i.e. < 2.76 ng/ml) at all sampling times. A further 19.5% AR was detected in dressings at the 120 hr time suggesting that considerable radioactivity remained in the skin after washing but was not significantly absorbed. Tape stripping at 120 hr indicated that only trace amounts (0.27% of the dose) remained in the upper layers of the stratum corneum at that time. Assuming that the radioactivity found on the strippings is representative for the entire application site, a total recovery from excreta, dressings, swabs and skin strips of ~ 86.44% AR can be calculated. A separate study indicated that approximately 22% of the HHCB may evaporate under experimental conditions similar to those for this human unoccluded dermal uptake study (Hawkins et al., 1996b; Ford et al., 1999).

Oral

No data available.

In vitro studies

The dermal absorption (non-GLP, but with QA statement) of HHCB was determined over a 24-hr period according to the methodology of the SCCNFP. Radiolabelled HHCB (uniformly labelled in the aromatic ring – radiochemical purity 99.3%) was applied in 1% solution in ethanol (96% v/v) to human epidermal membranes (prepared from female breast or abdominal skin and assayed for integrity with tritiated water) supported on a piece of filter paper (for strength) in glass diffusion cells (n=12). The area of the membrane available for absorption was approximately 1 cm^2 and the average applied dose was 20±0.2 $\mu L/cm^2$. The amount of material absorbed into the receptor phase, 6% Volpo N20 (to enhance solubility) in pH 7.4 phosphate buffered saline, after 24 hr was 0.40±0.06% of the applied dose. The majority of applied HHCB (81±2% of the applied dose) was found in the 24-hr surface wipe and donor chamber wash plus wipe. The stratum corneum tape strips contained 5.8±0.8% of the applied dose and the remaining stratum corneum plus epidermis 4.5±0.6% of the applied dose. Levels of HHCB in the remaining stratum corneum plus epidermis, filter paper (on which the epidermis samples rested) and permeated HHCB were combined to produce a total absorbed dose value of 5.2±0.6% of the applied dose. Overall recovery of radioactivity was 92±0.8% (Green and Brain, 2001).

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

There are no data available on the toxicokinetics of HHCB after oral and inhalation exposure. Taking into account physico-chemical properties neither no nor complete oral absorption is likely. Hence, an intermediate default percentage of 50% for oral absorption is taken forward to the risk characterisation. For inhalation exposure, an assumption of 100% absorption is used in the risk characterization.

In the *in vivo* human study, a 6 hr unoccluded exposure with 0.4% HHCB in 70% ethanol, intended to simulate a typically high exposure from the use of alcohol-based products such as perfumes or eaux de toilette, resulted in considerable absorption *into* the skin (approximately

20%). However, most of the material in this skin reservoir was not absorbed but was recovered from dressings over the site of exposure over a 120 hr period presumably from reverse diffusion and/or desquamation. Based on amounts excreted, primarily in the urine, approximately 0.1% was actually absorbed under the conditions of this experiment. In addition, because the application was unoccluded about 22% of the applied dose may evaporate under the conditions of the test, which may explain that about 14% of the radioactivity was not recovered in the *in vivo* human dermal absorption study. An *in vivo* study in rats supports the assumption that a good indication of the amount absorbed is the amount excreted.

A similar picture (although, as expected, with considerably higher absorption) was seen *in vivo* with rats where the material was applied for 6 hr under occlusion in 70% alcohol. Here again, a reservoir in the skin of about 10% of the applied dose was formed after the six-hr application with about 5% of this reservoir being lost presumably from reverse diffusion and/or desquamation to the dressing 120 hr after dose removal. Based on the amount remaining in the tissues, including that at the site of dosing, at sacrifice (2.7%) and the amount excreted (13%) almost all (11.6%) of which was in the faeces, a total absorption under the conditions of this experiment of ~ 16% can be concluded. The principal differences from the human study were the much larger absorption as a result of the application under occlusion and the well-known fact that rat skin is more permeable than human. For risk characterisation, a value of 16% will be used to estimate the dermal absorption via rat skin. This value also includes the continued absorption from the dermal reservoir at 120 hr (2% of the dose).

Recently, an *in vitro* absorption study using 1% HHCB in 96 % alcohol with human epidermal membranes was performed according to the recommendations of the SCCNFP. In this study, 0.4% of the applied dose was found in the receptor fluid after 24-hr, however, 4.5% of the applied dose remained in the epidermis. Adding these amounts to the small amount remaining on the filter paper used to support the membranes leads to the calculation of total absorption of 5.2% of the applied dose. From a separate study, it appeared that under similar conditions about 2.4% of an applied dose might evaporate. Because this study used a 24-hr application and because of the limitations of the human simulated exposure study (primarily the small number of subjects) this figure is used as a conservative estimate for absorption of HHCB via human skin in the risk characterisation.

The intravenous studies in rats and the pig showed that HHCB is rapidly distributed and is excreted primarily in the faeces by the rat as was seen in the dermal study (~ 68% of total excretion as opposed to ~90% after dermal exposure) but in the pig the principle route of exposure is in the urine. In neither of these studies was any evidence of accumulation seen. However, clearance from the fat was considerably slower than from other tissues. It is noteworthy that in neither of these studies was any of the urinary radioactivity present shown to be present as unmetabolised HHCB, however the faeces, which is the major excretion route of the rat, was not analysed for metabolites or parent.

An oral study with pregnant and later lactating rats shows that orally dosed HHCB and HHCB metabolites can end up in the milk. The levels seen in the milk of the lactating dams can aid in the interpretation of the study (see **Table 4.14** above) where neonate rats were exposed to HHCB and its metabolites through nursing.

HHCB is also found in human milk at levels up to 1316 μg/kg fat (equivalent to 48 μg/kg whole milk based on a measured fat content of 3.67%) and in adipose tissue at levels ranging from 12 – 189 μg/kg fat.

In summary, for the purpose of risk characterisation, 50% absorption will be used for oral exposure and 100% for inhalation. For dermal absorption of HHCB in rats and humans, values of 16 and 5.2% are taken forward to the risk characterisation.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

In vivo studies

Table 4.15 Acute toxicity studies for HHCB

Study	Solvent	LD50 (mg/kg bw)	OECD	GLP	Reference
Rat dermal	None	>6500	No	No	Minner and Foster, 1977
Rabbit dermal	None	>3250	No	No	Moreno, 1975
Rat oral gavage	None	>3000	No	No	Minner and Foster, 1977
Rat oral gavage	None	>3250	No	No	Moreno, 1975
Rat intraperitoneal	None	3160	No	No	Minner and Foster, 1977
Mice intraperitoneal	Corn oil	2135	NA	Yes	Gudi and Ritter, 1997

NA -Not aplicable

Inhalation

No data available.

Dermal

Galaxolide 50 (65% HHCB in DEP) was administered undiluted by inunction to the shaved skin (area not reported) of groups of five female Charles River Sprague Dawley rats (initial bodyweight 108–187 g) in doses of 0.464, 1.0, 2.15, 4.64 or 10.0 g/kg bw (equivalent to 0.30, 0.65, 1.4, 3.0, 6.5 g/kg HHCB) that were then observed for 7 days. There were no deaths at any dose but all animals in the high dose group exhibited urine staining on their fur. A dermal LD_{50} of >10.0 g/kg bw (equivalent to >6.5 g/kg bw HHCB) was reported (Minner and Foster, 1977). This study was conducted prior to GLP and OECD guidelines but was conducted according to acceptable procedures at the time.

In a limitedly reported dermal acute toxicity study, Galaxolide 50 (65% HHCB in DEP) was applied to the skin of groups of 7 albino rabbits at a dose of 5000 mg/kg bw (equivalent to 3250 mg/kg bw HHCB). There were no deaths at that dose therefore the dermal LD50 can be listed as >3250 mg/kg bw. In all animals, moderate redness of the skin was seen, in 6/7 animals moderate oedema of the skin and in 1/7 animals slight oedema of the skin was seen. No control animals with only solvent were included. (Moreno, 1975). This study was conducted prior to GLP and OECD guidelines and specific details regarding the study are not available. However, the study was reported by the Research Institute for Fragrance Materials (RIFM) and was conducted in accordance with OECD Guideline 401. No solvent would have been used since the material as tested was a liquid (Personal communication, RIFM).

Oral

Galaxolide 50 (Non-GLP; 65% HHCB in diethyl phthalate (DEP)) was administered undiluted (hence, there was variation in volume of dosing) by oral intubation at doses of 0.215, 0.464, 1.0, 2.15 or 4.64 g/kg bw (equivalent to doses of HHCB of 0.14, 0.30, 0.65, 1.4, 3.0 g/kg when corrected for the 65% dilution) to groups of 5 female Charles River Sprague Dawley rats (initial bodyweight 104–141 grams) that were then observed for mortality and signs of effects for 7 days. There was one death as a result of gavage error at 1.0 g/kg but no deaths at any other dose. One animal at 2.15 g/kg appeared distressed shortly after dosing but appeared normal after 2 hr. There were no effects at the high dose. An LD_{50} of >4.64 g/kg bw (equivalent to >3 g/kg bw HHCB) was calculated (Minner and Foster, 1977). This study was conducted prior to GLP and OECD guidelines but was conducted according to acceptable procedures at the time.

In a limitedly reported oral gavage non-GLP study, Galaxolide 50 (65% HHCB in DEP) was administered to 10 rats at a dose of 5000 mg/kg bw (actual dose of HHCB – 3250 mg/kg bw) followed by a 14-day observation. At the end of a 14-day observation period, only one rat had died (on day 2). The oral LD50 can be listed as >3250 mg/kg bw (Moreno, 1975). This study was conducted prior to GLP and OECD guidelines and specific details regarding the study are not available. However, the study was reported by the Research Institute for Fragrance Materials (RIFM) and was conducted by a standard protocol that was state of the art at the time (Personal communication, RIFM).

Intraperitoneal

Groups of five female rats (Charles River Sprague Dawley – weighing 106-155 g) were dosed with Galaxolide 50 (65% HHCB in DEP) by intraperitoneal injection at doses of 0.1, 0.215, 1.0 or 4.64 g/kg bw, (equivalent to 0.065, 0.14, 0.65 or 3.0 g/kg bw HHCB) and observed for 7 days. At 1.0 g/kg, 3 animals were observed to be in a depressed condition within two hr of dosing but returned to normal at 24 hr. There were no deaths at this dose. Lethargy and depression were observed in 4/5 animals at the high dose within 2 hr and all were found dead at 24 hr. The remaining animal was prostate at 24 hr and found dead the next day. Based on these observations an IP LD_{50} of 3.16 g/kg bw (equivalent to 2.1 g/kg bw HHCB) was calculated (Minner and Foster, 1977). This study was conducted prior to GLP and OECD guidelines but was conducted according to acceptable procedure at the time.

In a range finding study in preparation for a micronucleus test (see section 4.1.2.7 below), groups of 5 male and 5 female ICR mice were dosed with 500, 1000, 3000 or 5000 mg/kg bw of HHCB in corn oil by intraperitoneal injection at a constant volume of 20 ml/kg bw. Based on no mortality at 500 or 1000 mg/kg bw and deaths of 4/5 males and 5/5 females dosed at 3000 mg/kg and the death of all 5 male and female mice dosed at 5000 mg/kg bw, a LD_{50} of 2135 mg/kg bw was calculated by probit analysis (Api and San, 1999a; Gudi and Ritter, 1997).

<u>In vitro studies</u>

No data available.

4.1.2.2.2 Human data

No data available.

4.1.2.2.3 Summary of acute toxicity

The data provided are considered sufficient to meet base set requirements for acute toxicity. Based on the oral and dermal LD_{50} values of >3000 mg/kg bw, there is no need to classify HHCB for acute toxicity.

Data for acute inhalation toxicity are not available.

4.1.2.3 Irritation including photoirritation

It is common practice for *in vivo* tests for irritation and photoirritation to test more than one substance on the same animal or human subject. That is the case in most of the studies reported in this section. Rarely are the identities of these additional substances revealed. They are not mentioned here unless they affect in some way the results with the reported substance.

4.1.2.3.1 Skin

Irritation

Studies in animals

HHCB has been tested in a battery of skin tests in rabbits. **Table 4.16** is a summary of these tests.

Table 4.16 *In vivo* rabbit studies of the dermal irritation of HHCB

Species	Number Tested	Concentration of HHCB	Results – Primary irritation	Reference
Rabbit	3	65% HHCB in DEP	Avg. erythema = 1.3, Avg. oedema = 0.4	Haynes, 1984
Rabbit	4	65% HHCB in DEP	Avg. erythema = 2.1, Avg. oedema = 1.5	Haynes, 1985
Rabbit	4	65% HHCB in DEP, 32.5% HHCB in DEP, 65% HHCB in BB, and 32.5% HHCB in BB	Avg. erythema/oedema scores: 1.8/1.3, 1.3/0.3, 1.8/0.8 and 1.3/0.7 respectively.	Haynes, 1986
Rabbit	3	65%. 32.5 and 16.25% HHCB in DEP	Moderate primary irritant*	Levenstein, 1973a
Rabbit	3	65%, 32.5% and 16.25% HHCB in DEP was tested	Mild primary irritation at 65%, very mild irritation at lower concentrations*	Levenstein, 1975a

* Because of the length of the application, this result cannot be used in the classification of HHCB.

A series of GLP compliant studies, have been performed according to directive 79/831EEC using New Zealand White female rabbits.

In the first of these studies, 0.5 ml of Galaxolide (65% HHCB in DEP) was applied over an area of approximately six cm^2 for 4 hr under semi-occlusive lint patches (held in place with Elastoplast plastic adhesive bandage 10 cm wide) on the dorsal skin (clipped free of fur) of three rabbits for a period of 4 hrs. Undiluted DEP and benzyl benzoate (BB) were similarly applied to groups of three rabbits. Scores for erythema and oedema per animal were given after 1, 24, 48, 72 and 168 hrs and the average scores per animal for erythema and oedema over 24, 48 and 72 hrs were calculated. The results for Galaxolide were an erythema score of 1.3 (on all three) and an oedema score of 0.4 (highest score 1). After 168 hrs, erythema was still observed in two animals, while slight desquamation of the skin surface at the treated site was seen in all three rabbits. For DEP and for BB, the scores were zero for erythema and oedema on all three rabbits (Haynes, 1984).

In another study 0.5 ml of Galaxolide 50 (65% HHCB in DEP), DEP and BB were placed evenly over a 2.5 cm^2 of surgical lint, which was then placed on the skin of each of 4 rabbits and held by an Elastoplast adhesive bandage 10 cm wide for a period of 4 hrs. Scores of skin results were given after 1, 24, 48, 72, and 168 hrs. An average score (average of all 4 animals over 24, 48, and 72 hrs) for erythema of 2.1 and for oedema of 1.5 was calculated for Galaxolide. After 168 hrs erythema and oedema were still observed in 4/4 and 3/4 animals, respectively. The average scores for DEP were 0.2 for erythema and zero for oedema while the corresponding scores for BB were 1.2 and 0.4 (Haynes, 1985).

In the final study, 0.5 ml of either undiluted or 50% solutions of Galaxolide 50 DEP (65% HHCB in DEP) or Galaxolide 50 BB (65% HHCB in benzyl benzoate) were placed evenly over a 2.5 cm^2 patches of surgical lint. These patches were then placed on the skin of 4 rabbits and held by an adhesive bandage 10 cm wide for a period of 4 hrs. The average erythema/oedema scores calculated over 1, 24, 48 and 72 hours were 1.8/1.3 for 100% Galaxolide 50 DEP (reversible after 168 hrs), 1.3/0.3 for 50% Galaxolide 50 DEP in DEP (reversible in 3/4 animals after 168 hrs), 1.8/0.8 for 100% Galaxolide 50 BB (reversible in 3/4 animals after 168 hrs), and 1.3/0.7 for 50% Galaxolide 50 BB in BB (reversible after 168 hrs), respectively. In this study, there were no solvent controls. However, the results can be compared to the results with the two solvents seen in the previous two studies (Haynes, 1986).

A non-GLP test for irritancy was conducted with undiluted Galaxolide-50 (65% HHCB in DEP) on 3 albino rabbits (strain not specified). A single application of 0.5 ml of the test material was applied to the skin (area specified as 2x2 with no units) which had been clipped free of hair and, on a site that was abraded so as to penetrate the stratum corneum and an unabraded site. The site of application was covered with a Webril patch and sealed with Blenderm Surgical tape for 24 or 72 hr during which the rabbits were immobilized in racks for the first 24 hr. At the end of the 24-hr patch period and again 48 hr later, the sites were scored according to Draize. Average scores of 1 for erythema were observed at both 24 and 72 hr and at both abraded and unabraded sites but no erythema or oedema was seen with Galaxolide 50. It was concluded that Galaxolide 50 was a moderate irritant (Levenstein, 1973a).

In another non-GLP test under identical conditions to the preceding, three rabbits were treated with solutions of 25% or 50% Galaxolide 50 (equivalent to 16% or 33% HHCB) in Alcohol SDA 39C as well as undiluted Galaxolide 50 (65% HHCB in DEP) for 24 or 72 hr. No solvent control was reported. The 25% solution produced no erythema or oedema at either abraded or unabraded sites. The 50% solution produced a score of 1 for erythema at 24 hr on

abraded skin but no erythema or oedema at any other site or time period. It was concluded that this solution could be considered a very mild irritant. The undiluted Galaxolide 50 produced scores of 1 for erythema at 24 hr on both abraded and unabraded sited but no erythema at 72 hr and no oedema at any time on either site (Levenstein, 1975a).

Studies in humans

During the induction phase of Human Repeated Insult Patch Test (HRIPT) for sensitisation, a semi-occlusive patch of 100% neat HHCB was applied on the upper arms of the 42 subjects for 24 hr three times per week for three weeks. 0.5 ml of the test substance was applied to a 1x1 inch Webril patch, which was affixed to the centre of a 1x2 inch elastic bandage and applied to the upper arms of the panelists. Reactions were scored at 24 and 72 hr after patch removal. No irritation was observed in any of the 42 subjects (group 117) even after repeated occlusive applications of undiluted material (Guillaume et al., 1973b).

Forty subjects were tested with HHCB and evaluated for irritation as part of sensitisation study. Nine semi-occlusive induction applications of 3.75% Galaxolide were made on the upper arms of the subjects, 3 times a week for 3 weeks. Little or no primary irritation was observed under the conditions of this study (Rubenkoenig and Ede, 1964).

Photoirritation

Because HHCB absorbs in the UV region, several studies to detect a possible photoirritation hazard have been conducted (see **Table 4.17**). Several of these were designed for method development. Up to now, there are no validated *in vivo* tests for photoirritation. However, draft testing guidelines for photoirritation have been circulated by OECD both for *in vivo* as well as *in vitro* tests. These draft guidelines have been used to facilitate the interpretation of the studies cited below. The test described in the draft guideline for *in vitro* testing has also been discussed and adopted by the EU-SCCNFP. Also the (USA) Cosmetic, Toiletry, and Fragrance Association (CTFA) has developed a guideline for photoirritation studies. One test has been performed according to this CTFA guideline. The available data for this test indicate that the CTFA guideline is less comprehensive than the draft OECD guideline for *in vivo* testing.

Studies in animals

Table 4.17 *In vivo* animal studies of the photoirritation of HHCB

Species	GLP	Method	Results	Reference
Rabbits and guinea pigs	No	None	Slightly positive reactions at Concentrations of 13 or 32%	Sato et al., 1978
Guinea pigs	No	None	Positive in 5/20 animals at 6.5%	Guillot et al., 1985
Mice	No	None	Negative at 65%	Forbes et al., 1978
Rabbits and guinea pigs	No	None	Positive reactions observed at >3.25%	Ogoshi et al., 1980, 1981

In tests for photoirritation, 0.02 ml HHCB (purity unknown) in ethanol or diethyl phthalate (DEP) were applied evenly to 1.5 x 1.5 cm areas on both sides of the shaved, depilated backs of rabbits or guinea pigs. One side of the animal's back was used as a control side and covered with aluminum foil. Three rabbits and 3 guinea pigs (strains not reported) were treated with 5 (in ethanol), 10, 20 and 50 % (in DEP) of a commercially available sample of

HHCB (equivalent to 3.25% in ethanol and 6.5, 13 or 32.5% HHCB in DEP). The rabbits were treated only with the lower 2 concentrations, Treatment was followed (time after dosing not specified) by glass-filtered UV-A irradiation for 110 min from six Toshiba 40 WFL BLB lamps (300-400 nm; peak at 360 nm) at a distance of 10 cm. Readings were taken at 24, 48 and 72 hr after irradiation. The degree of difference between the average scores of irradiated and non-irradiated sites was evaluated to determine photoirritation. No significant effects were observed at either of the 2 lower doses of HHCB with rabbits or guinea pigs. The 2 higher doses produced reactions only in guinea pigs. At 32.5% in DEP the average score over 24, 48 and 72 hrs for erythema/oedema was 2.2/1.7 compared to 0.5/0.0 at control sites (presumably solvent only). At 13% in DEP, the average score for erythema/oedema was 0.8/0.6 compared to no reaction at control site. No positive control was tested. According to classification into categories given by the author of the study, based on the scores, the 32.5% solution of HHCB is concluded to be moderately photoirritant and the 13.5% solution to be very weakly photoirritant (Sato et al., 1978).

The photoirritant potential of HHCB (purity unknown) was evaluated in 10 male and 10 female young adult albino Dunkin-Hartley guinea pigs. The animal's fur on back and flank was clipped and depilated, 24 hr prior to dosing. A single application of a 10% solution of a commercial sample of HHCB (65% in DEP) (so the actual concentration was 6.5%) in ethanol (0.5 ml) on a gauze pad of 2 cm^2 was applied to the skin on the back for 1.3 hr. The gauze pad was kept in contact with the skin by an adhesive hypoallergenic patch under an occlusive aluminum foil sheet of 5x5 cm. Another (not-treated) part of the dorsal skin was also covered with aluminum foil to protect it from unwanted irradiation. Three male and two female guinea pigs were maintained as a control group and were treated with the same solution of HHCB but were not irradiated. The treated patches were irradiated for 5 min using a system of 2 fluorescent lamps with continuous UV-A spectral emission of 310-400 nm (peak at 360 nm) and 285-350 nm (UV-B; peak at 310 nm) delivering energy of 12.5 J/cm^2 (99% UV-A; 1% UV-B), as dosimetrically determined. This amount of irradiation was the minimal erythematous dose. Readings of erythema and oedema were carried out 6 and 24 hr after irradiation. The readings were performed in a blinded way. Erythema and oedema were scored on a 5-unit scale (no effect – very pronounced effect). Erythema and oedema scores were considered positive if it was 2 units greater than the one attributed to the control sites. Negative and doubtful scores were equal to that or slightly higher than that in the control group, respectively. Positive histopathological readings were those representing "sun burned" type of lesions. The final score for each treated and irradiated animal was made based on both macroscopic and histopathological examinations. Macroscopically, in 2/20 and in 12/20 animals, respectively, a positive or a doubtful response was observed. Histopathology confirmed that 5 of these responses were positive and 15 were negative. In conclusion, evidence of photoirritation was observed in 25% of the animals receiving a dose of 6.5% HHCB (Guillot et al., 1985).

An aliquot of 20 µl of Galaxolide (65 % HHCB in DEP) was applied to 5 cm^2 of normal skin in SKF Hairless-1 mouse (number of animals not given). At 30 min after the application, the centre 1 cm diameter circle of the application site was irradiated for 30 min with simulated sun light using a filtered (Schott WG 320) Osram XBF 6000 w Xenon lamp, or was irradiated for 1 hr with a bank of F40T12BL fluorescent black light, (glass-filtered to eliminate "sunburning UV light" (<320 nm)) to produce a very slight erythema. A sample of citrus lime oil was used as a positive control. Skin reactions were assessed at 2, 4, 24, 48 and 72 hr after irradiation. No skin photoirritant reactions were observed with HHCB but the positive control gave symptoms of photoirritation at 24-72 hr post irradiation exposure (Forbes et al., 1978).

(The following was taken from a symposium paper presented in 1980 and from the published paper, in Japanese, presumably reflecting the same data.) Galaxolide 50 BB (65% HHCB in benzyl benzoate) at a concentration of 1, 5, 10 or 20% in either Vaseline or 99.5 % ethanol (actual HHCB concentrations 0.65 %, 3.25 %, 6.5 % and 13 %) was tested in rabbit and guinea pig. The number of animals used in the study is not included in the study summary. 50 µg sample of the test mixtures were applied to 4 cm^2 shaved skin area for 2 hr and then the animals were irradiated for 30, 60, or 120 min by five Toshiba FL20sBLB fluorescent lamps, 300-430 nm. Non-irradiated sample patches were used as control sites. The total irradiation dose was 1.6 -7.6 J / cm^2. Skin reactions were assessed 3 days after irradiation. There were no photoirritant effects observed at 0.65 % and 3.25%. Photoirritation was observed at 6.5% and 13% only in guinea pigs after 120 min of irradiation. In rabbits, 6.5% produced photoirritant reactions after 60 min of irradiation and 13 % produced photoirritant reactions after 30 min of irradiation. No data were given on strength of the reactions. Irradiated test materials had a stronger irritating effect than the corresponding unirradiated test material. Therefore, the strength of the reactions tended to depend on the quantity of the irradiated light and the concentration of the substance tested. Based on these data, Galaxolide 50 BB was classified by the authors as equivocal (±) as to photoirritation at the highest concentration of 20% (equivalent to 13% HHCB) (Ogoshi et al., 1980, 1981).

Studies in humans

There is no standardized protocol for conducting photoirritation screening in humans. Because of this, several tests were conducted in different laboratories using their standard protocol and standard operating procedures.

Table 4.18 *In vivo* human studies of the photoirritation of HHCB

Number of subjects	GLP	Method	Results	Reference
26, 25 & 9	No	None	Negative at 65%	Lindstrum et al., 1978a, 1978b, 1978c
10	No	None	Negative at 6.5%	Harrison and Stolman, 1986
10	No	None	Negative at 6.5%	Gabriel and Mark, 1987
26	No	None	Negative at 6.5%	Folk and Dammers, 1987
10	No	None	Negative at 6.5%	Shanahan and Alworth, 1987
27	No	None	Negative at 25%	Mills, 1997

Galaxolide 50 (65% HHCB in DEP) undiluted or as a 50 or 25% solution in ethanol (was applied under occlusive Webril patches of approximately 2 cm^2 area on the backs of 26 female volunteers. The volume of the material applied for each patch ranged from 0.2 to 0.4 ml. Patches were allowed to stand for 30 min for the evaporation of the solvent-ethanol before application. Control exposures to natural sunlight were conducted at the approximate minimal erythematous dose (MED) for each subject. No solvent controls were included. The MED for an individual subject is defined as the exposure time in min that induced a faintly perceptible erythema at the irradiated site, 16 to 20 hr following sun exposure. After *ca*. 21 to 23 hr of HHCB treatment, the treated skin sites were scored, and treated sites as well as untreated sites were exposed to natural sunlight at the MED. 16 and 40 hr after exposure to sunlight the HHCB treated and control sites were evaluated. A positive photoirritant reaction is characterised by sharply demarcated erythema at a test site, which has been treated with test material and 1 MED of sunlight. Subjects who did not show perceptible erythema at the

untreated site were excluded from the study. No evidence of photoirritation was observed after skin exposure to HHCB containing samples followed by irradiation with natural sunlight. Positive control sites exposed to a solution of 8- methoxypsoralen in ethanol showed signs of photoirritation (Lindstrum et al., 1978a).

Another study following the same method was conducted in 25 female panelists using 0.3 ml per site of Galaxolide 50 BB (65% HHCB in benzyl benzoate) undiluted showed no evidence of photoirritation (Lindstrum et al., 1978b).

In a third study following the same method with 9 female panelists, 0.4 ml per site of undiluted HHCB, Galaxolide 50 IPM (65% HHCB in isopropyl myristate), or Galaxolide BB (65% HHCB in benzyl benzoate) were tested using the same natural sunlight method, with no evidence of any photoirritation (Lindstrum et al., 1978c)

The photoirritant potential of Galaxolide 50 (65% HHCB in DEP) was evaluated in 10 volunteers (2 males and 8 females) who were treated with duplicate occlusive patches (Redi-Bandage) containing 0.2 ml of a 10% solution of Galaxolide 50 in ethanol/DEP (3:1) (resulting concentration of HHCB - 6.5 %) applied to each volar forearm (area not reported) for 24 hr. After patch removal, one of the forearms was irradiated with UVA from 4 F40BL fluorescent tubes (output at 360 nm of 1.23 W/10 nm of wavelength). The dose delivered was 0.22 J/cm²/min. The sites were scored immediately after irradiation and at 24 and 48 hr later. No visible reactions were seen at any time on any subject (Harrison and Stolman, 1986).

Galaxolide 50 BB (65% HHCB in benzyl benzoate) and Galaxolide 50 DEP (65 % HHCB in DEP) were tested as 10 % solutions in ethanol/DEP (3:1) (actual concentrations of HHCB - 6.5%). The vehicle control was 75% ethanol: 25% DEP. Before application of the preparation for each of the subjects the Minimal Erythemal Dose (MED) was determined using UV light irradiation from a xenon arc solar light simulator. Subsequently, areas on the back of 10 female subjects (ages 18-39) were stripped 3 times to remove the superficial stratum corneum. This was followed by application of 20 µl of the test solution to 2 designated test sites, each approximately 1.5 cm in diameter. In addition, three of the 10 subjects were randomly selected and treated with 20 µl of a 0.2 mg/ml solution of 8-methoxypsoralen in ethanol as a positive control. After 30 min of exposure to the test material, one site was irradiated with UVA followed by UVA + UVB calibrated xenon lamp and the other site remained unirradiated. The test material was maintained on the skin sites. A third site was used as untreated, irradiated control site (UVA + UVB only). Vehicle treated irradiated and unirradiated control sites were included. Based on the previously determined MED, each individual subject was exposed to UVA light for a time period of 10 MED equivalents. Followed by exposure, to 0.5 MED of UVA ± UVB light. The sites were scored 5 min after irradiation and thereafter were lightly covered. The sites were re-examined at 3, 24, 48 and 72 hr after irradiation. Sites were re-covered after the 3 hr reading through the 24 hr reading and were uncovered thereafter. There were no significant reactions to either preparation of HHCB but all three subjects exposed to 8-MOP showed clear positive reactions (Gabriel and Mark, 1987).

In another photoirritation study, 26 volunteers (male and female Caucasians) received single applications of duplicate sets of (Parke-Davis with Webril) patches with 0.3 ml of a 10% ethanol/DEP (3:1) solution of Galaxolide BB (65% HHCB in benzyl benzoate), (final concentration of HHCB of 6.5 %). The test material was applied to areas of approximately 1.5 cm in diameter (~1.75 cm^2) on the back under occlusion for 24 hr. The test site was irradiated with 16-20 J/cm² of UVA from a filtered xenon arc solar simulator within 10 min after patch removal. Prior to irradiation, any excess test material remaining on the skin was wiped off

with a wet towel. All sites were evaluated 1, 24, 48, and 72 hr after irradiation. Treated unirradiated control sites, and vehicle treated irradiated and unirradiated control sites were included. No significant reactions due to HHCB were observed (Folk and Dammers, 1987).

According to another photoirritation study, a 10% solution Galaxolide 50 BB (65% HHCB in benzyl benzoate) in ethanol/DEP (3:1), (resulting concentration of HHCB - 6.5%), was tested in ten volunteers (8 females and 2 males). Inner aspects of their forearm were tape stripped 3-4 times to remove the outer stratum corneum. 0.2 ml of the test material was placed directly on the skin (surface of treated skin area was not reported), which was then subjected to ultraviolet radiation, receiving a UVA light dosage over a period (~60 min) sufficient to deliver 15-20 joules of energy. The light exposure was from fluorescent bulbs. The contact site was then covered with an occlusive patch (Parke-Davis Readibandage^{R}), with ~0.2 ml additional test material for 24 hr. 0.2 ml of the test material on a Parke-Davis Readibandage^{R} occlusive patch was applied to non-irradiated control sites and left in place for 24 hr. No significant reactions to HHCB were observed (Shanahan and Alworth, 1987).

Photoirritation in 27 subjects was evaluated as part of a photosensitisation study using 25% HHCB in ethanol/DEP (75:25). First the MED for each subject was determined. 0.2 ml of the sample was applied occlusively and irradiated after 24 hours with 16 J/cm^{2}UVA and 0.75 x MED of UVB radiation. Dermal irritation was observed in a few panelists but the reactions were seen in sample-treated sites, control only-treated sites as well as blank sites. Therefore these reactions are attributed to UV exposure beyond the subject's MED and are therefore, indicative of a sunburn effect (Mills, 1997).

Studies *in vitro*

Table 4.19 *In vitro* studies of photoirritation of HHCB

Study Type	GLP	Method	Results	Reference
Mouse Fibroblasts 3T3 Assay	Yes	EC/COLIPA	Negative	Harbell et al., 2001
Photohemolysis of human RBCs	No	None	Positive	Sugiyama et al., 1994
Yeast	No	None	Positive	Sugiyama et al., 1994
Yeast	No	None	Negative	Tenenbaum et al., 1984
Yeast	No	None	Positive	Forbes et al., 1978
Yeast	No	None	Negative	Weinberg and Springer, 1981
Yeast	No	None	Positive	Bagley et al., 1988

In a GLP compliant study according to the draft OECD guideline / adopted EU-SSCNFP guideline mentioned above, Balb/c 3T3 mouse fibroblasts were exposed to 50 µl aliquots of HHCB (purity not reported in report, confirmed to be >95% pure undiluted material, IFF personal communication with RIFM) in Hank's Balanced Salt Solution (HBSS) containing 0.5% ethanol (concentrations of 1.77 – 100 µg/ml) for 1 hr followed by irradiation with UVA light for 50 minutes for a total irradiation dose of 5 J/cm^2. Duplicate slides were kept in the dark for the 50-minute period. After the irradiation period, the test solutions were decanted from the plates and the cells were washed with HBSS. Assay medium was then added to the cells and the cells were incubated for 24 hr at which time the assay medium was decanted from the cells and 100 µl of filtered Neutral Red solution added. After a 3 hr incubation, the

cells were washed, scored for Neutral Red uptake and the IC_{50}, the Mean Photo Effect (MPE) and Photo-Irritation Factor (PIF) were calculated. The average (over 2 runs) IC_{50} for HHCB was 8.84 µg/ml with and 11.2 without irradiation. The MPE was 0.016 (<0.1 is considered non-photoirritant) for each run and the PIF was 1.32 and 1.12 (<2.0 is considered non-photoirritant). Chlorpromazine was tested as a positive control. The average IC_{50} was 30.1 µg/ml with irradiation and 1.84 without. The MPE was 0.64 for each run and the PIF was 18.36 and 13.62 (Harbell et al., 2001).

In vitro photohemolysis studies with Galaxolide (65% HHCB in DEP) and red blood cells were conducted. Cells obtained from healthy volunteers were washed, centrifuged, and suspended in a buffer. HHCB in serial five fold dilutions was added to a microplate along with the red blood cell suspension and irradiated with long wavelength ultraviolet light (UVA, 25 J/cm² - time not given). Hemolysis of the red blood cells was measured after irradiation. HHCB produced positive effects as indicated by 15.9 % photohemolysis of the red blood cells. Details on the concentration of HHCB used are not provided. None of the three (*in vivo*) positive controls, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 6-methylcoumarin (6-MC) gave any measurable photohemolysis and were classified as negative in this assay (Sugiyama et al., 1994).

The same workers conducted an *in vitro* yeast (*Saccharomyces cervisiae*) growth inhibition assay for photoirritation with a five-fold dilution of Galaxolide (65% HHCB in DEP). The plates were incubated for 72 hr after irradiation with 50 J/cm^2 of UVA (time of irradiation not reported). A 2.1 mm growth inhibition was observed on the plates indicating positive effects (a positive reaction was defined as a 2 mm inhibition) were seen with HHCB. Details on the concentration of HHCB used and applied in serial five-fold dilutions are not provided. Three positive controls, 8-MOP, 5-MOP and 6-MC gave inhibitions of 14.1, 10.0 and 6.4 mm, respectively (Sugiyama et al., 1994).

In a similar photoirritation assay, *Saccharomyces cervisiae* cultures were covered with filter paper impregnated with a 5% solution of Galaxolide (65% HHCB in DEP - 3.25% actual concentration of HHCB) in methanol. The plates were exposed to UVA radiation for 18 hr (320-400 nm; peak 350 nm) and incubated for 48 hr. 8-MOP (0.0005-0.001%) was used as a positive control. No effects were seen with HHCB but 8-MOP gave positive results (Tenenbaum et al., 1984).

Galaxolide 50 (65% HHCB in DEP) was evaluated in an *in vitro* test using plates seeded with yeast (*Saccharomyces cervisiae*). The Galaxolide 50 was applied to filter paper plates and placed on culture media, which had been seeded with yeast. The plates were then grown for 24 hr under 10 W/m² UVA and observed at 24-hr intervals for four days. A 6.5% v/v/ Galaxolide solution after irradiation caused growth inhibition in the area adjacent to the plate and was concluded to be positive in the yeast assay. This was a marginal positive value. Inhibition greater than 2 mm was considered a positive response. HHCB resulted in a growth inhibition of 2.1 mm. Details on the concentration of HHCB and the solvent used to dissolve HHCB are not provided (Forbes et al., 1978).

An *in vitro* study on Fleischman's active dry yeast (*Saccharomyces*) was conducted with 0.1, 1.0 and 10% solutions of Galaxolide (65% HHCB in DEP) in methanol. The solution (40 µl) was added uniformly to a paper disk and allowed to dry for 15 min. The disk was then applied to the yeast suspension, and the plate was then exposed to UVA (320 - 400 nm, peak 370 nm) for 18 hr with a flux of 1.5 W/cm². Forty-eight hr after inoculation or when contrast is adequate, zones of inhibition were measured. The positive control was 8-MOP but several other known photoirritants gave positive reactions as well. No effects were observed up to the

maximum tested concentration of 10% Galaxolide (equivalent to 6.5% HHCB) in methanol (Weinberg and Springer, 1981).

A modified *in vitro* yeast cell assay was conducted using *Saccharomyces cervisiae*. Paper discs were treated with 25 µl of a solution of Galaxolide (65% in DEP) (lowest concentration tested - 0.3%; higher concentrations not given) in methanol and then placed on plates that had been seeded with yeast. The plates were then grown for 18 hr under UVA (320 - 400 nm, peak at 340 - 360 nm) and then incubated at 32° C. After incubation, zones of inhibition were measured. 8-MOP (concentrations of 0.001% to 0.01%) was the positive control. Positive effects due to Galaxolide were observed at the lowest dose tested (no details of the effect at this or higher doses were given) (Bagley et al., 1988).

4.1.2.3.2 Eye

Studies in animals

Galaxolide (65% HHCB in DEP) was tested in the eyes of 6 rabbits by a procedure essentially equivalent to OECD 405 (no observation at 1 hr and no wash at 24 hr). 0.1 ml of Galaxolide was instilled into the right eye (the left serving as control) of healthy young adult albino New Zealand rabbits. Both eyes were scored according to the method of Draize at 24, 48, 72, 96 and 168 hr. Four rabbits had no ocular changes at any time. One had a small central opacity (score 2) at 24 hr, which was fainter at 48 hr and cleared at 72 hr. The same animal showed also score 1 for effect on the iris at 24 hr only and slight conjunctival redness and discharge (both score 1) at 24 hr only. Another animal had slight redness of the conjunctivae (score 1) at 24 and 48 hr but no effects at later times. Primary eye irritation score at 24, 48, and 72 hr was 3.5, 1.17 and 0 (average of three time points was 1.6). Based on the irritation scores the test material (Galaxolide) is considered as practically nonirritating to the eye (Sauer, 1980).

Table 4.20 Eye irritation studies with HHCB

Species	Number tested	Concentration of HHCB	Results	Reference
Rabbit	6	65% HHCB in DEP	Practically non irritating	Sauer, 1980
Rabbit	3	65% HHCB in DEP	Non irritating	Levenstein, 1975b
Rabbit	3	65% HHCB in DEP in ethanol	Effects seen in controls	Levenstein, 1973b
Rabbit	3	65% HHCB in DEP in ethanol	Effects seen in controls	Wolven and Levenstein, 1963

In an incompletely reported study, 0.1 ml of Galaxolide 50 (65% in DEP) was tested in the eyes of three rabbits with an observation time of 168 hours by a method that seemed similar to that above. No irritation was seen in all three rabbits at any observation time (Levenstein, 1975b).

In an incompletely reported study, 0.1 ml of a 50% solution of Galaxolide 50 (65% HHCB in DEP) in ethanol (final concentration of HHCB was 32.5%) was tested in the eyes of three rabbits with an observation time up to 168 hr by a method, which seemed to be similar to those above. In all three rabbits conjunctival irritation (redness, chemosis and discharge) (scores 2 to 1 at 24 hr) was seen which was cleared in two rabbits by 48 hr and in the third by 168 hr. Primary eye irritation index for one rabbit was 4.7 (average 24, 48, and 72 hr) and for the other two 2.7 and 2.7. The rabbit with the highest irritation index in this study started to

show corneal opacity (score 2) with an area score of 2 (>25%<50%) after 96 hr persisting up to termination of the study after 168 hr. In the control study with ethanol, the primary eye irritation index was 7.3, 5.3 and 2.3 over 24, 48, and 72 hrs (Levenstein, 1973b).

In a similar study, a dilution of Galaxolide (65% HHCB in DEP) in ethanol (concentration unknown) (Wolven and Levenstein, 1963a) was tested with an observation time up to 168 hr. Conjunctival irritation (redness, chemosis, discharge) with scores 1 to 2 was seen in all three rabbits at 24 hr. Discharge disappeared after 48 hr, whereas redness and chemosis persisted up to 96 hr.

As ethanol has eye irritant potential, the relevance of these last two studies is questionable.

Human data

No data available.

4.1.2.3.3 Respiratory tract

No data available.

4.1.2.3.4 Summary of irritation

GLP compliant studies of skin irritation have been performed according to directive 79/831/EEC to groups of either 3 or 4 New Zealand White female rabbits. All tests showed very slight to well-defined erythema and very slight oedema. In only one of the tests did the mean erythema score for Galaxolide 50 DEP exceed 2.0 (the calculated score was 2.1). The solvent in this study, DEP, scored 0.2 for erythema and zero for oedema. The irritating effect was not reversible in 7 out of in total 15 animals in these three studies during an observation period of 7 days, as at that time point still some erythema and/or oedema was seen. Unfortunately, the observation period was not sufficiently long (14 days, according to the test method guideline in Annex V) to evaluate fully the reversibility of the effects. However, the test method guideline also states that the irritation scores should be evaluated in conjunction with the nature and severity of lesions and their reversibility or lack of reversibility. Taking that also into account, the results of the animal studies do not indicate that HHCB is a skin irritant. There is a difficulty though, because according to Annex VI of Directive 67/548, inflammation of the skin is also significant if it persists in at least 2 animals at the end of the observation period [without specifying the length of the observation period and the severity of the effects]. If this guidance is followed strictly, the animal studies would warrant classification as a skin irritant. This issue was discussed at the TC-C&L meeting of November 2005, where it was concluded that it is not warranted to classify HHCB as a skin irritant.

In a Human Repeated Insult Patch Test (HRIPT) for sensitisation, a semi-occlusive patch of 100% neat HHCB showed no irritation after repeated application during the induction phase of the study. Other HRIPTs with diluted HHCB also showed no signs of irritation.

There are some indications from animal studies that HHCB could be a photoirritant. The results in human tests do not indicate a photoirritating effect in humans. Also, an *in vitro* phototoxicity test (in compliance with test guideline B.41 (EU/COLIPA Test)) was negative. No criteria on classification of photoirritating substances are available in Annex VI.

HHCB has been tested for ocular irritation in rabbits in several studies. Some studies used ethanol, a known eye-irritant as solvent, and are not used. In other relevant studies, some ocular irritation was found. However, the effects were not severe enough to require classification according to EU guidelines.

No data on respiratory tract irritation are available.

No further studies are deemed necessary.

4.1.2.4 Corrosivity

In three skin irritations studies in rabbits (see under 4.1.2.3) no corrosivity was observed (Haynes, 1984, 1985, 1986).

HHCB does not need to be classified as corrosive.

4.1.2.5 Sensitisation

Sensitisation and photosensitisation studies are discussed here.

4.1.2.5.1 Studies in animals

Skin

In vivo studies

Sensitisation

Table 4.21 *In vivo* studies of sensitisation and photoallergy of HHCB

Species	Number tested	Concentration of HHCB	Results	Reference
Guinea pig	10	65% HHCB in DEP, vehicle 70% acetone/PEG400, induction-100%, 25% for challenge	1 equivocal response, non – sensitising in remaining animals	Basketter, 1996
Guinea pig	12	65% HHCB in DEP, vehicle ethanol, 1% HHCB in ethanol	Not a photosensitiser	Parish, 1988

Galaxolide (65% HHCB in DEP) has been subjected to a non-GLP guinea pig maximization test. The used doses of Galaxolide were 0.5% in 0.01% dodecylbenzene sulphonate in 0.9% saline (DOBS/saline) for the intradermal injection, 100% for the induction patch, and 25% in 70% acetone/30% polyethylene glycol 400 (acetone/PEG400) for the challenge patch. These doses were selected based on preliminary irritation tests using 0.1, 0.25, 0.5, 1.0 and 2.0% Galaxolide concentrations for intradermal injections, however the selection criteria were not clear. The actual concentrations of HHCB are 0.325%, 65%, and 16.25%, respectively. Ten (six male, four female) Albino Dunkin/Hartley guinea pigs (weight 316-350g) were tested on a 2cm x 4cm area of skin in the dorsal shoulder area, clipped free of fur. Induction consisted of a 0.1 ml intradermal injection of 0.325% HHCB in DOBS/saline and 0.1 ml 50% Freund's Complete Adjuvant in 0.9% saline. This was followed one week later by a 48 hr occluded

patch (filter paper attached by adhesive tape to polythene backing) saturated with 65% HHCB The patch was applied at the same 2 cm by 4cm area after freshly shaving the skin. Challenge applications were made 14 days later at a freshly shaved naïve site by saturation of an 8mm diameter filter paper patch with 16.25% HHCB in 70% acetone/30% PEG 400. Eight animals were treated as controls and received induction and challenge treatments similar to the test pigs minus the test material. Two repeat challenges at weekly intervals were conducted. At 24 hours, very faint erythema (score 0.5) was found in 2/10 animals at challenge 1, 3/10 animals at challenge 2, and 1/10 at challenge 3. At 48 hours, 3/10, 1/10 and 0/10 had very faint erythema. At challenge at 24 hours, only one animal showed very faint erythema to faint erythema. Except for one equivocal response in one animal, no evidence that the material was a sensitiser was seen (Basketter, 1996).

Photosensitisation

In a GLP compliant study, Galaxolide (65% HHCB in DEP) was tested for its allergenic and photoallergenic potential in 12 albino Hartley strain guinea pigs (418 to 487 g) with Freund's adjuvant injection at the shaved interscapular region. Four injections of 0.1 ml Freund's Complete Adjuvant were administered to the four corners of a 9 cm^2 shaved site. 0.1 ml of 1% Galaxolide in ethanol (resulting in an actual HHCB concentration of 0.65%) was dermally applied to the site. The used doses were selected based on preliminary photoirritation studies to be the maximum non-photoirritant or slightly photoirritant doses. After 25 min, the sites were exposed to ultraviolet light using fluorescent black lamps for about 1 hr and 34 min (ca. 1.8 mW/cm^2; 10 J/cm^2). The procedure, excluding adjuvant injections, was repeated 24 hr later. A control group of 12 guinea pigs (422 to 547 g) were treated in the same way except that the treatment with test substance was replaced by treatment with solvent. Ten to 14 days after induction, the guinea pigs (both test and control animals) were challenged with 1, 0.3 or 0.1% Galaxolide in ethanol (actual HHCB concentrations 0.65%, 0.2%, and 0.065%) by dermal application to the shaved lumbar region. Thirty min later, the animals were irradiated as above, after which the test material was applied to fresh sites to check for contact sensitivity, and the sites scored at 24 and 48 hr. A second challenge was carried out 6 or 7 days later. In 1/12 a very faint trace of erythema was found at 1.0% and 0.3% Galaxolide with UVA at challenge 1 and 2. Under the conditions of this test, Galaxolide is not a photosensitiser in guinea pigs (Parish, 1988). This study was conducted prior to but essentially similar to the OECD 406 guideline except only 12 animals were used and another scaling of grades was used.

In vitro studies

No data available.

4.1.2.5.2 Human data

In vivo studies

Table 4.22 Human studies of sensitisation and photosensitisation of HHCB

Study Type	Number tested	Concentration of HHCB	Patch details	Result	Reference
HRIPT	40	3.75% Galaxolide in alcohol	Semi-occlusive, 9 applications of 24-Hr each	Negative	Rubenkoenig and Ede, 1964
HRIPT	43	100% 50% Galaxolide in alcohol SDA 39Cl	Semi-occlusive, 8-9 applications of 24-Hr each	Negative	Guillaume et al., 1973a
HRIPT	42	100% neat HHCB, no vehicle	Semi-occlusive, 8-9 applications of 24-Hr each	Negative	Guillaume et al., 1973b
Maximization	10	65% HHCB in DEP, vehicle-petrolatum	Occlusive, 48-hr patch on 5 alternate days.	Negative	Epstein, 1974
Maximization	24	65% HHCB in DEP, vehicle-petrolatum	Occlusive, 48-hr patch on 5 alternate days.	Negative	Epstein, 1979
Patch testing in patients	179	25% Galaxolide in petrolatum	Silver Patch Testers	Negative, 3/179 false-positives	DeGroot et al., 1985
Patch testing in patients	28	3% Galaxolide in petrolatum	Occlusive patches	Negative	Meynadier et al., 1986
Patch testing in patients	100	1 and 5% Galaxolide 50 in petrolatum	Finn Chambers	Negative	Frosch et al., 1995
Photo-sensitisation	27	25% HHCB in vehicle ethanol/DEP 75:25	Occlusive –24 hour patches	Negative	Mills, 1997

Sensitisation

A Human Repeated Insult Patch Tests (HRIPT) was performed with 3.75% HHCB and a cream control. Nine inductions were made by application of semi-occlusive patches (Webril patch affixed to the centre of a 1x3 inch elastic bandage) containing 0.5 ml of the preparation for 24-hr periods to the upper arms of the subjects, 3 times a week for 3 weeks. After a rest period of two weeks, a 24-hr challenge patch identical to the induction patches was made on a site previously not exposed. Reactions were scored 48 or 72 hr after the challenge patch application. Little or no primary irritation and no allergic potential was evidenced among the 40 subjects tested with Galaxolide (Rubenkoenig and Ede, 1964).

In another HRIPT test, 43 subjects (group 118) were tested with a 50 % solution of Galaxolide 50in ethanol by application of 0.5 ml of the solution on test patches (1x1 inch Webril swatch affixed to the centre of a 1x2 inch elastic bandage) to the upper arms for 24 hrs 3 time/week for 3 weeks (9 applications) to the same site if possible. Approximately 2 weeks after removal of the final patch, challenge duplicate patches were applied, one to the original site, one to a fresh skin site. Scores were recorded at 48 or 72 hours after application of the patches. HHCB and the solvent control resulted in little or no primary irritation and no sensitisation (Guillaume et al., 1973a).

The same authors performed a HRIPT test using the same protocol but with 100% neat HHCB without vehicle on 42 subjects (group 117). Neat HHCB resulted in little or no primary irritation and no sensitisation (Guillaume et al., 1973b).

In a limitedly reported study, a human maximization test was performed using Galaxolide 50 (65% HHCB in DEP) probably in petrolatum (which was used as a control). Galaxolide 50 was applied (volume not reported) under occlusion on the volar aspects of the forearm under occlusion (patch not described) of volunteers on 5 alternate days for 48 hours. Patch sites were pretreated with 5% sodium lauryl sulfate (SLS) under occlusion for 24 hr to enhance penetration. Challenge patches were applied under occlusion on the back after a 10-14 day rest period with and without pretreatment for 30 min with 2% SLS. After 48 and 72 hours the sites were scored. Galaxolide demonstrated no potential for irritation or sensitisation in any of the 10 subjects (Epstein, 1974). Further detail of this study are not available however, it was reported by the Research Institute for Fragrance Materials by a methodology widely recognised at the time for screening of fragrance materials.

In a similar study using Galaxolide 50 with 24 Japanese American subjects, no reactions were found that were considered irritant or allergic. (Epstein, 1979). Further detail of this study are not available however, it was reported by the Research Institute for Fragrance Materials by a methodology widely recognised at the time for screening of fragrance materials.

A study was performed in order investigate the potential of Galaxolide to elicit potential allergic reactions in sensitive patients. Patch tests were conducted on 179 patients (144 women, 34 men) suffering from dermatitis in which cosmetic allergy was suspected. Exposure to 25% Galaxolide in petrolatum was performed using Silver Patch Testers. Reactions were evaluated after 48 and 72 hr. Positive reactions were observed in 3/179 patients (1.7%), however, the authors note occasional false-positive reactions due to the Excited Skin Syndrome (DeGroot et al., 1985).

Twenty-eight patients allergic to perfumes and sweet smelling constituents were studied by patch testing in 21 tests recommended by the International Contact Dermatitis Research Group. Galaxolide (purity unknown) was tested at 3% in petrolatum. No sensitisation reactions were observed (Meynadier et al,. 1986).

A multicentre study with patch tests with 48 fragrance materials was reported. Galaxolide 50 (65% HHCB in DEP) was tested in a 1 and 5% solution in petrolatum on 100 patients. The material was applied to the back for 2 days using Finn Chambers on Scanpor tape, and the reactions were evaluated on days 2 and 3 or on days 2 and 4. For Galaxolide 50, no sensitisation was observed. One questionable reaction was noted at 1%. (Frosch et al., 1995).

Photosensitisation

A human allergenicity and photoallergenicity study by a modified repeated insult patch test according to the procedure of Kaidbey and Kligman (1980) was conducted in accordance with applicable Good Clinical Practice guidelines. Prior to induction, the minimal erythema dose (MED) for the radiation to be used was determined for each subject. A 25% HHCB (purity not reported) solution in ethanol/diethyl phthalate (75/25) and a vehicle control were applied under occlusive patches (Webril covered by an occlusive hypoallergenic tape) along with an untreated patch twice per week for 3 weeks, on the back of 27 panelists. The patch sizes were 4 cm^2 and the aliquot of sample used was 0.2 ml. After 24 hr, the patches were removed and the sites were irradiated with UVB with 5% UVA at approximately 2 times the MED, using a 1000 watt Xenon Arc Solar Simulator with UV-A/UV-B filters (time period not given). Following a 2-week rest period, a single application (identical to that used during induction)

of duplicate patches was made to naïve sites. After 24 hr the patches were removed and one of the duplicate patch sites was exposed to 16 J/cm^2 of UV-A and 0.75 × MED of UV-B. The test sites were evaluated at approximately 1, 24 and 48 hours following irradiation and non-irradiated patch removal. A total of 27 panelists (6 males, 21 females) completed the study. Slight to one or two strong signs of dermal irritation were obtained during the induction period. However, these reactions were observed in HHCB-treated and vehicle-treated at about the same rate, and even more in blank sites. There was no increase, rather a decrease in the severity of the dermal irritation during the progress of the induction phase. After the challenge, two subjects showed skin responses to the sample, vehicle control as well as blank (untreated site). These reactions are attributed to UV exposure beyond the subject's MED and are therefore, indicative of a sunburn effect (Mills, 1997).

In vitro studies

No data available.

Respiratory tract

No data available

4.1.2.5.3 Summary of sensitisation

Although some questionable elicitation reactions have been reported as a result of patch tests in dermatological clinics on sensitive patients, the available data with guinea pigs and humans (HRIPT and maximization tests) provide no evidence of potential for induction of sensitisation for HHCB. HHCB need not be classified as a skin contact sensitiser.

There is no evidence from studies in experimental animals or with humans, that HHCB is a photosensitiser.

No further testing is deemed necessary.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

In vivo studies

Inhalation

A group of 20 female CD rats was exposed by inhalation (whole body) to a fragrance mixture at a nominal concentration of 5 mg/m^3 for 4 hr per day, 5 days per week for 6 weeks (mixture B). A group of 24 female SD rats was exposed to 50 mg/m^3 for 4 hr per day, 5 days per week for 13 weeks (mixture G). These fragrance mixtures were aerosolised with a compressed air nebulizer (particle size was not determined for this particular study, but was assumed to be between 0.5 and 7.5 μm, based on similar studies with 5 other fragrance mixtures). HHCB (purity not reported) was part of these fragrance mixtures, and the level of HHCB to which the animals were exposed was 5.7 μg/m^3 for mixture B and 132 μg/m^3 for mixture G. Exposure to either mixture did not result in mortality, skin reactions or effects on body

weight, behaviour or physical appearance, haematology and clinical chemistry, organ weights and gross pathology (including uterus and ovaries), or histopathology (uterus but not ovaries examined) (Fukayama et al., 1999). These studies were not conducted according to GLP. Remark: the study is of limited value because the animals were not exposed to HHCB alone, but to mixtures of fragrances. In these mixtures HHCB was only present at rather low levels.

Dermal

Two subchronic dermal non-GLP studies have been conducted but limitedly reported. In the 13 week study, groups of 15 female rats (Crl:COBS CD (SD) BA strain; weight 156-232 g) were exposed topically unoccluded (gentle inunction to the anterior dorsal shaven skin) to dose levels of 1, 10 and 100 mg Galaxolide (65% HHCB in DEP) /kg bw per day as a 2% (w/v) solution in ethanol. In the 26 week study (with one 13 week interim sacrifice), groups of 20 female rats (Crl:COBS CD (SD) BA strain; weight 156-232 g) were similarly exposed to dose levels of 0, 9, 18 and 36 mg Galaxolide/kg bw/day as a 2% solution in ethanol (area of application not reported). Untreated controls and ethanol controls were included. Observations included mortality, clinical signs, behavioural and motor function and (limited) haematology, serum chemistry, organ weights, macroscopy and histopathology. Special neuropathological examination of brain, spinal cord, and peripheral nerves was included for 2 animals per dose group.

In the 13-week study, there were no reported adverse clinical signs, no variation in biochemistry or haematological parameters, no effects on bodyweight and no histological changes at any dose. However, increased absolute and relative liver weights were seen at 100 mg Galaxolide/kg bw per day, but no actual data were presented so the degree of these changes is unknown. No effects were reported quantitatively from the 26-week study. It was reported that a decrease in bodyweight gain was seen with other test materials and that a similar effect was seen at both interim and terminal sacrifice in the 26 week study for animals treated with 36 mg/kg Galaxolide. No microscopic changes were observed in the nervous tissues of animals treated with Galaxolide at any of the dose levels employed. These studies were primarily designed to screen for neurotoxicity and a positive control, 7-Acetyl-6-ethyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene (AETT) a known rat neurotoxin, was similarly dosed at 0.1, 0.3, 1, 3, 10, 30 or 100 mg/kg bw/day for 13 weeks and 3, 18 or 36 mg/kg bw/day for 26 weeks. Clear evidence of neurotoxicity, both clinically and pathologically, was seen with the positive control but no such evidence for HHCB was seen in either study at any dose level (Gressel et al., 1980).

Because of 1) the uncertainties in the significance of the effects reported, 2) the study was conducted without collar or occlusion to prevent oral intake of compound making it impossible to determine actual exposures and 3) the area of application was not reported, this study should not be used to determine a dermal NOAEL.

In a third non-GLP study also designed to screen for neurotoxic potential, a 10% solution of Galaxolide 50 (65% HHCB in DEP) in 95% ethanol was applied daily unoccluded to groups of 15 male (236-288 g) and 35 or 38 female (178-226 g) Charles River CD rats at doses of 50, 100, 200 mg Galaxolide/kg bw per day for 26 weeks (with 6 and 13 week interim sacrifices). Untreated controls and ethanol controls were included. One group was dosed for 13 weeks and left to recover till autopsy at 26 weeks. Haematology, clinical chemistry and urinalysis were performed, organ weights were determined and histopathology was carried out on 26 different tissues. Treatment with Galaxolide at 100 or 200 mg/kg bw per day led to the appearance of crusty white or brown material and scabbed areas on the dorsal surface of a few rats. Treatment with 50 mg/kg bw /day led to no unusual observations. At 200 mg/kg bw male

rats showed a trend toward decreased body weight gain early in the study accompanied by a decreased food consumption but the differences were not significant. No statistically significant effects were observed on haematology, biochemical parameters or urinalysis. Organ weights were not significantly changed at week 6, 13 and 26 weeks (both treated and recovery group), except for an increase in relative liver weight in females at week 26 (with 11 and 23% in the 100 and 200 mg/kg bw/day groups) and kidney weight in the high dosed males (37%). This was not accompanied by any effect in histopathology. In this study, oral ingestion or removal by grooming was not prevented so the received doses cannot be determined. Following the same study protocol with doses of 25, 50 or 100 mg AETT (a rat neurotoxin)/kg bw/day showed clear signs of neurotoxicity both in behavioural changes and on microscopic examination. No evidence of neurotoxicity was seen with HHCB at any dose level (Estes et al., 1980).

However, because 1) neither collars nor occlusion were used to prevent oral intake making it impossible to determine actual exposures, 2) the area of application was not reported, and 3) there was no adverse effect dose, it is impossible to conclude a true NOAEL in terms of dermal toxicity.

Oral

A two-week range finding study was conducted in groups of 5 male and female Crl:CD (SD)Br rats receiving HHCB (purity not reported in report, confirmed to be >95% pure undiluted material, IFF personal communication with RIFM) by dietary admixture at achieved doses of 0, 341, 598, and 679 mg/kg bw/day for males and 0, 352, 633 and 980 mg/kg bw/day for females. In this preliminary study, a progressive dose-related decrease in body weight was observed at the two highest dose-levels in males as well as females. A significantly and dose-related increase in absolute and relative liver weight was reported in males and females at all dose-levels. Histopathology revealed moderate centrilobular hypertrophy in the liver of 1/5 males and 2/5 females in the high dose group. Based on these findings, a 90-day study at 5, 15, 50, 150 mg/kg bw/day was conducted (Api and Ford, 1999b; Hopkins et al., 1996).

A 13-week oral toxicity study in accordance with OECD guideline 408 and conforming to GLP was conducted in 150 Crl:CD (SD)Br rats (5 groups of 15 males (weight 182-260 g) and 15 females (weight 152 and 201 g)). They received HHCB (purity not reported in report, confirmed to be >95% pure undiluted material, IFF personal communication with RIFM) by dietary admixture at 0, 5, 15, 50, or 150 mg/kg bw/day. HHCB was added to the diet. Analyses of diet indicated that desired homogeneity was reached. The concentrations of HHCB in the test diets were adjusted weekly based on bodyweight and food consumption from the previous week. The mean achieved daily intakes were 5.4, 15.7, 51.8 and 155.8 mg HHCB/kg bw for males and 5.1, 15.6, 51.9 and 154.6 mg HHCB/kg bw for females. After the treatment period, 3 males and 3 females from the control and the high dose groups were maintained for a treatment-free period of 4 weeks.

Observations included mortality and clinical signs (daily), body weight and food consumption (weekly), ophthalmoscopy (before start, at week 13 and at the end of the treatment-free period, only controls and high dose animals), urinalysis (at week 6 and 12 of treatment and at the end of the treatment-free period), haematology and clinical chemistry (at week 7 and 13 of treatment and at the end of the recovery period), macroscopy, organ weights and histopathology (on all tissues from controls and high dose animals, on all gross lesions, and on lungs, liver, kidneys and male and female reproductive and accessory organs from all animals). There were no mortalities or adverse clinical signs. Body weight and food consumption of treated groups were similar to those observed in the control group. No

changes in ophthalmologic evaluation were observed and no significant histopathological findings at any dose in any tissue including reproductive organs and bone marrow (Api and Ford, 1999b; Hopkins et al., 1996).

A variety of statistically significant differences between control and test animals were seen in haematology (see **Table 4.23**) and blood chemistry although these differences were all small, often not proportional to dose, often seen only at one time point and/or in one sex, and, with two exceptions (noted below), well within historical controls. There were no significant differences seen at the end of the treatment-free period. A statistically significant higher mean partial thromboplastin time (PTT) was seen in the females at 150 mg/kg bw/day (16.6 sec.) and 50 mg/kg bw/day (16.5 sec.) compared to controls (14.3 sec) at week 13. These values were slightly higher than the high end of the historical controls. The increase in PTT can indicate acquired hypofibrinogenemia. However, in this study, the abnormal height of control fibrinogen might explain the increase in PTT. Moreover, there is no clear dose-response relationship, a lack of other corresponding findings such as effects on liver cells (absence of histomorphological alterations, absence of liver enzyme activation), a lack of any other indication of abnormal (retarded) coagulation and no signs of uncontrolled spontaneous bleeding such as abnormal occurrence of petechia or hemorrhagia in organs, skin or mucous membranes. There were no significant differences in prothrombin times in any of the groups (males and females) in comparison with their respective controls. All groups of treated females had statistically significant lower fibrinogen than controls, without a clear dose-response, while the mean fibrinogen level for the female control group was significantly higher than the highest historical control value. At the end of the treatment-free period, no significant effect was found on either fibrinogen, partial thromboplastin time or prothrombin time in females nor males. For these reasons, the deviations in PTT are not considered of toxicological relevance.

The mean red blood cell counts in males but not females at week 7 in the 150 and 50 mg/kg bw/day groups were significantly slightly lower than in the controls. At week 13, the males did not differ significantly from the controls in any group however the high dose females were slightly lower than controls.

The total leucocyte count was significantly lower for the females in the 15, 50 and 150 mg/kg bw/day groups compared to controls at week 7, without any dose-response relationship.

Statistically significant lower monocytes were reported for males, but not females, at all but the low dose group at week 13 but not 7. The imprecision of the reported values makes significance questionable.

The packed cell volumes for males at 50 and 150 mg/kg bw/day were statistically significantly lower than controls at week 7 but not week 13. There was no correlation with dose. The mean haemoglobin concentrations for all dosed groups at week 13, all dosed females at week 7 and for the two highest dosed male groups at week 7 were statistically significantly higher than the respective controls but with no correlation with dose and all values, except for that of the males at 50 mg/kg bw/day at week 7, were within the historical control ranges.

Table 4.23 Selected haematological parameters for HHCB (n=15/group)

Dose (mg/kg bw/day)	Partial thromboplastin time (sec)	Partial thromboplastin time (sec)	Fibrinogen (mg/dl)	Fibrinogen (mg/dl)	RBC	RBC	RBC	RBC	WBC	WBC	WBC	WBC
	Male W13	Female W13	Male W13	Female W13	Male W7	Female W7	Male W13	Female W13	Male W7	Female W7	Male W13	Female W13
0	17.0 ± 1.46	14.3 ± 1.72	303 ± 39.5	302 ± 68.2	8.7 ± 0.30	8.2 ± 0.34	9.0 ± 0.35	8.6 ± 0.34	14.6 ± 4.10	12.6 ± 3.14	13.0 ± 2.74	8.5 ± 1.23
5	17.9 ± 2.02	15.8 ± 3.93	317 ± 54.9	250 ± 40.6b	8.5 ± 0.30	8.1 ± 0.29	8.8 ± 0.43	8.4 ± 0.30	15.4 ± 3.11	11.2 ± 3.13	14.3 ± 3.12	8.1 ± 3.62
15	16.2 ± 1.63	15.5 ± 1.75	301 ± 52.1	253 ± 29.0b	8.5 ± 0.29	8.2 ± 0.32	8.9 ± 0.38	8.5 ± 0.44	15.4 ± 3.97	9.5 ± 2.74b	12.6 ± 2.88	6.7 ± 1.42
50	17.8 ± 2.78	16.5 ± 2.65a	307 ± 25.3	250 ± 38.2b	8.3 ± 0.33a	8.1 ± 0.28	8.9 ± 0.32	8.4 ± 0.24	14.3 ± 3.39	8.5 ± 3.05b	12.6 ± 3.07	7.6 ± 2.41
150	17.9 ± 3.52	16.6 ± 2.28a	298 ± 40.9	241 ± 41.5b	8.4 ± 0.35a	8.3 ± 0.31	8.8 ± 0.36	8.3 ± 0.31a	15.2 ± 5.29	10.0 ± 3.76b	12.4 ± 3.52	7.8 ± 3.14
HH	19.7	16	394	285	8.9	8.6	9.8	9.2	18.0	15.0	21.0	14.6
HM	15.1	12.6	292	234	7.7	7.6	9.0	8.4	12.7	10.0	14.2 ±	9.5
HL	10.5	9.2	191	182	6.5	6.6	8.2	7.6	7.4	5.0	7.4	4.4
	Mono	**Mono**	**Mono**	**Mono**	**PCV**	**PCV**	**PCV**	**PCV**	**MCHC**	**MCHC**	**MCHC**	**MCHC**
	Male W7	**Female W7**	**Male W13**	**Female W13**	**Male W7**	**Female W7**	**Male W13**	**Female W13**	**Male W7**	**Female W7**	**Male W13**	**Female W13**
0	2 ± 1.2	2 ± 1.6	4 ± 1.5	2 ± 1.9	45.2 ± 1.31	44.8 ± 1.98	46.1 ± 1.40	46.4 ± 2.01	36.9 ± 0.52	36.5 ± 1.05	36.2 ± 0.57	36.2 ± 0.50
5	3 ± 1.8	2 ± 1.4	4 ± 1.7	1 ± 1.1	44.6 ± 1.10	43.5 ± 1.36	45.5 ± 1.79	45.1 ± 1.88	37.0 ± 0.39	37.6 ±0.50c	36.6 ± 0.55a	36.7 ± 0.58a
15	2 ± 1.1	2 ± 1.4	2 ± 2.0a	2 ± 1.5	44.7 ± 1.15	43.5 ± 1.07	46.2 ± 1.70	45.2 ± 1.64	37.0 ± 0.77	37.3 ±0.77c	36.4 ± 0.61a	36.6 ± 0.73a
50	3 ± 1.5	2 ± 2.1	2 ± 1.8b	2 ± 1.6	43.5 ± 1.36b	43.7 ± 1.24	45.0 ± 1.52	45.6 ± 1.01	37.7 ± 0.46b	37.1 ±0.46c	36.9 ± 0.46c	36.8 ± 0.52b
150	2 ± 1.4	2 ± 1.7	3 ± 1.7a	1 ± 1.1	44.1 ± 1.49b	44.3 ± 1.36	45.2 ± 1.64	45.2 ± 1.59	37.3 ± 0.53b	37.4 ±0.54b	36.9 ± 0.37c	36.9 ± 0.45b
HH	5	5	5	4	49.7	48.1	50.7	49.3	37.5	37.9	37.4	37.5
HM	2	2	2	1	44.5	43.7	46.9	45.2	35.1	35.6	35.2	35.3
HL	0	0	0	0	39.3	39.3	43.1	41.1	32.7	33.3	33.0	33.1

M: male rats; F: female rats; W7: week 7 observations; W13: week 13 observations HH: historical high values; HM: historical mean values; HL: historical low values

(for measurements at W7, values from historical animals 0-3 months of age are used and for measurements at W13, values from animals 4-6 months are used.)

RBC: Red blood cells count (*10^6/µl) WBC: Total leucocyte count (*10^3/µl)

Mono: Monocytes (%) PCV: Packed cell volume (%)

MCHC: Mean cell haemoglobin conc. (g%) a, b, c: significantly different from control, $p<0.05$, $p<0.01$, $p<0.001$

ALT levels were statistically significantly lower in females but not males in the two higher dosed groups at week 7. They were not significantly different in males or females at week 13. AST levels were statistically significantly lower than controls in males at all dose levels but only in the highest dosed females at week 7. However, at week 13, all but the lowest dosed females had statistically significantly lower AST levels with no significant differences in any male group.

There were statistically significant lower triglyceride levels relative to controls seen in the males at all but the lowest dose group (5 mg/kg bw/day) at both week 7 and week 13 and the values were dose related. There were no significant differences with females. There were, however, statistically significant lower glucose levels in all but the lowest dose group in both males and females at week 7 but with no clear dose relationship. At week 13, the females and males at 50 and 150 mg/kg bw/d were still lower but statistically significantly only in the males. While these differences appear to be attributable to HHCB administration, they are considered of minor toxicological significance and likely reflect slight nutritional effects.

There were no statistically significant differences in any haematological parameter or blood chemistry values between test and control animals at the end of the treatment free period (Api and Ford, 1999b; Hopkins et al., 1996).

The haematology and blood chemistry effects were all small, often not proportional to dose, often seen only at one time point and/or in one sex, and, with two exceptions, well within historical controls and are not considered to "reduce the capacity of an organism or a component of an organism to function in a normal manner" (Abadin et al., 1998). This, and the fact that these findings were not accompanied by any adverse histopathology (e.g. in the bone marrow) or other related findings, leads to the conclusion that they are not adverse effects.

It is concluded there were no significant adverse effects at any dose level up to the highest tested level of 150 mg/kg bw/day (NOAEL ≥ 150 mg/kg bw/day).

Intraperitoneal

Galaxolide (65% HHCB in DEP) was administered to 6 female rats (CRL:COBS CD(SD)BR) as part of a fragrance oil dissolved in ethanol via the intraperitoneal route at a dose of "approximately" 36 mg/kg bw/day (equivalent to 23.4 mg/kg bw/day HHCB) for a period of 4 days. The purpose of this study was to determine if HHCB caused blue coloration similar to acetyl ethyl tetramethyl tetralin (AETT) a polycyclic musk identified as a neurotoxic ingredient and which induced blue coloration to the internal organs of dosed animals. HHCB did not induce blue coloration of the internal organs in the treated animals (Minner and Foster, 1976b).

An additional comparative study was conducted in groups of 5 female rats (CRL:COBS CD(SD)BR). Each animal was administered a daily injection of 0.6 ml/kg bw of a 6% solution in absolute ethanol of Galaxolide (23.4 mg/kg bw HHCB) daily (5/wk) for a at least a 10-day period. Galaxolide was not found to induce blue coloring in this test, however similar administration of Versalide (AETT) did. (Minner and Foster, 1976a).

In vitro studies

No data available.

4.1.2.6.2 Human data

No data available.

4.1.2.6.3 Summary of repeated dose toxicity

In an adequate 90-day oral study, there were no mortalities or adverse clinical signs. Body weight and food consumption of treated groups were similar to those observed in the control group. No changes in ophthalmologic evaluation were observed and no significant histopathological findings at any dose.

The haematology and blood chemistry differences from controls were all small, often not proportional to dose, often seen only at one time point and/or in one sex, and, with two exceptions, well within historical controls and are not considered to "reduce the capacity of an organism or a component of an organism to function in a normal manner" (Abadin et al., 1998). This, and the fact that these findings were not accompanied by any adverse histopathology or other related findings, leads to the conclusion that they are not adverse effects.

A NOAEL of ≥150 mg/kg bw/day, the highest dose tested, for HHCB in rats is concluded.

Three dermal subchronic studies are available. In two of these there was some evidence of liver weight increases (at 100 mg/kg bw/day for 13 weeks) and body weight decreases (at 36 mg/kg bw/day for 26 weeks) but the magnitude of these effects were not reported and their significance cannot be determined. In a third dermal 26-week study, no effects were seen up to and including the highest dose administered (200 mg/kg bw/day). However, because 1) neither collars nor occlusion were used to prevent oral intake making it impossible to determine actual exposures, 2) the area of application was not reported, and 3) the lack of significance in the findings reported in the first two studies and the lack of an adverse effect dose in the third, it is impossible to conclude a true NOAEL in terms of dermal toxicity.

When administered as part of a fragrance mixture, inhalation exposure to HHCB up to a maximum tested dose of 132 μg/m^3 for 4 hr per day, 5 days per week for 13 weeks did not result in any toxicity. This study is of limited value because HHCB was only present at rather low levels in the mixtures.

4.1.2.7 Mutagenicity

Table 4.24 Mutagenicity studies available for HHCB

Type	Species	Activation	Doses	Results	GLP	OECD	Reference
in vitro Bacterial Reverse Mutation Assay	*Salmonella typhimurium* (TA98,100, 1535, 1537, 1538) & *E. Coli* (WP2 UVRA)	With and without S-9	10, 33, 100, 333, 1000, 3333, 5000 μg/plate	negative	Yes	471	Api and San 1999a; San et al., 1994
in vitro Bacterial Reverse Mutation assay	*Salmonella typhimurium* (TA97, 98, 100, and 102)	w & w/o S-9	5, 16.6, 50, 166.6 or 500 μg/plate	negative	No		Mersch-Sunderman, 1998a
in vitro SOS	*E.coli* PQ37	w & w/o	0.39, 0.78, 1.56,	negative	No		Mersch-

Type	Species	Activation	Doses	Results	GLP	OECD	Reference
Induction		S-9	3.125, 6.25, 12.5, 25 or 50 µg/assay				Sunderman, 1998b
in vitro Cytogenetic Assay	Chinese hamster ovary cells	w & w/o S-9	9, 17, 34 µg/ml & 23, 28, 30 µg/ml	negative	Yes	473	Api and San, 1999a; Curry and Putman, 1995
in vitro Micronucleus Test	human lymphocytes	w & w/o S-9	0.05, 0.49, 4.85, 48.5, 97 or 194 µM = 0.013. 0.13, 1.25, 12.5, 25 and 50 µg/ml	negative	No		Kevekordes et al., 1997
in vitro Micronucleus test	human hepatoma cells	None added, some inherent	0.1, 0.97, 9.7, 97, 194 and 387 µM = 0.036, 0.25,2.5, 25, 50, and 100 µg/ml	negative	No		Kevekordes et al., 1997
in vitro Sister Chromatid Exchange Assay	human lymphocytes	w & w/o S-9	0.025, 0.25, 2.43, 24.25, 48.5, 97 µM = 0.007, 0.07, 0. 6, 6, 12.5, 25 µg/ml	negative	No		Kevekordes et al., 1998
in vitro Unscheduled DNA synthesis	primary rat hepatocytes	inherent	0.15, 0.5, 1.5, 5, 15, 50 µg/ml	negative	Yes	482	Api and San, 1999a; San and Sly, 1994
in vivo Micronucleus test	mice		380, 750, 1500 mg/kg bw	negative	Yes	474	Api and San, 1999a; Gudi and Ritter, 1997

4.1.2.7.1 Studies in vitro

HHCB (>99% pure) in acetone was tested in the Ames test in absence or presence of Aroclor-induced rat liver S9 at a dose ranging from 10 to 5000 µg/plate according to OECD guideline 471 using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and Escherichia Coli strain WP2 UVRA and appropriate positive controls. Based on preliminary range-finding studies, doses of 10 (not tested in confirmation assay), 33, 100, 333, 1000, 3333 (only tested in confirmation assay) or 5000 µg HHCB/plate were used. Slight precipitation was seen at the three highest doses (≥333 µg/plate). All dose levels of HHCB, acetone (negative control) and positive controls were plated in triplicate. All positive controls gave positive responses to the systems within acceptable ranges. No significant increase in the number of revertant colonies was observed for HHCB at any dose with any of the six strains with or without activation (San et al., 1994; Api and San, 1999a).

A second Ames test was conducted with Galaxolide (65% HHCB in DEP) using *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 with and without rat liver S-9 (Aroclor 1254-induced) metabolic activation and with appropriate positive controls. The method used resembled OECD guideline 471. The vehicle was DMSO. The doses were 5, 16.6, 50, 166.6 or 500 µg/plate (limit of solubility). All positive controls significantly increased the number of revertants. No significant increase in revertants was seen with HHCB at any dose with or without activation (Mersch–Sundermann et al., 1998a).

A cytogenetic assay with Chinese Hamster ovary cells (CHO-K_1) was conducted on HHCB (purity >99%) according to OECD Guideline 473. Concentrations of 5, 10 and 20 µg HHCB/ml were used without metabolic activation, using 4/20, 20/20 and 44/44 hr exposure/harvest periods. In the study with metabolic activation (S9 from rat liver induced by Aroclor 1254), dose levels of 8.7, 17.3 and 34.5 µg HHCB/ml were tested for the 4-hr period with a 20-hr harvest time and dose levels of 22.6, 28.2 and 30.0 µg/ml for the 4-hr period with a 44-hr harvest time. At the 20 and 44-hr harvest times, the cells were assessed for structural chromosome aberrations, and at the 44-hr harvest time, also for numerical chromosome aberrations. The mitotic index was significantly lowered at the highest dose in all cases. N-methyl-N'-nitro-N-nitrosoguanidine was used as a positive control in the non-activated study and benzo(a)pyrene in the activated study. Positive controls caused increases in structural (significantly) aberrations in all cases. No significant increase in structural or numerical chromosome aberrations was observed with or without activation with HHCB at any dose. HHCB was concluded to be negative for chromosome aberrations in this test (Curry and Putman, 1995; Api and San, 1999a).

The ability of Galaxolide (65% HHCB in DEP) to induce sister-chromatid exchange (SCE) was evaluated using cultured human lymphocytes obtained from healthy non-smoking donors ranging in age from 25-35 years. The method used resembled OECD guideline 479. Cultures were treated with concentrations of 0.025, 0.25, 2.43, 24.25, 48.5 or 97 µM (solvent DMSO) for 2 hours with rat liver S9 activation (Aroclor 1254-induced) or for 24 hours without metabolic activation. After harvest, the cells were scored for SCEs. Cyclophosphamide at 0.1 µM was used as a positive control and produced a significant increase in SCEs. Concentrations of HHCB up to 48.5 µM produced no effects (97 µM was too cytotoxic to be evaluated) (Kevekordes et al., 1998).

An *in vitro* unscheduled DNA synthesis (UDS) assay in accordance with OECD guideline 482 was conducted with HHCB (purity >99%) in acetone in primary rat hepatocytes at concentrations of 0.15, 0.50, 1.5, 5.0, 15 µg/ml (50-5000 µg/ml proved too toxic to test). The positive control 7,12-dimethylbenz(a)anthracene induced a significant increase in the average net nuclear grain count over controls. No increase in net nuclear grain count was seen for HHCB up to and including 15 µg/ml although this dose did induce significant cytotoxicity as determined by LDH leakage. 50 µg/ml proved too toxic to be evaluated. (San and Sly, 1994; Api and San, 1999a).

A *in vitro* micronucleus test was conducted with Galaxolide (65% HHCB in DEP) at concentrations of 0.05, 0.49, 4.85, 48.5, 97 or 194 µM using human peripheral lymphocytes cultures obtained from healthy non-smoking donors aged 25-35 years. After induction of mitosis, HHCB (in DMSO) was added to the cultures with and without rat liver S-9 (Aroclor 1254 induced) metabolic activation for 48 hr. After harvest, the cells were scored for micronuclei in binucleated cells. The positive controls (mitomycin –S9, cyclophosphamide +S9) significantly increased the frequency of micronuclei. No significant increase in the frequency of micronuclei was seen with HHCB at concentrations up to 97 µM (194 µM was too cytotoxic to score) (Kevekordes et al., 1997).

Another *in vitro* micronucleus test was conducted with Galaxolide (65% HHCB in DEP) at concentrations of 0.1, 0.97, 9.7, 97, 194 and 387 µM in DMSO using human hepatoma cells (Hep G2 line) which are capable of some metabolism. After a two hr incubation, the cells were harvested and scored for micronuclei in binucleated cells. The positive control cyclophosphamide (1.0 µM) significantly increased the frequency of micronuclei. No

significant increase in the frequency of micronuclei was seen with HHCB up to 194 μM (387 μM was too toxic to score) (Kevekordes et al., 1997).

An SOS chromotest was conducted by incubating *Escherichia coli* PQ37 with Galaxolide (65% HHCB in DEP) in DMSO at concentrations of 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25 or 50 (limit of solubility in this assay) μg/assay (volume per assay is 310 μl) with and without rat liver S-9 (Aroclor 1254 induced) metabolic activation. 4-Nitroquinoline-N-oxide (-S9) and benzo[a]pyrene (+S9) were used as positive controls. After 2 hr incubation, enzyme activities of β-galactosidase and alkaline phosphatase were measured. Inducing factors, IF, were calculated relative to negative controls (solvent only). Both positive controls significantly increased IF but no inducing potency nor toxicity was seen with HHCB at any dose (Mersch-Sundermann et al., 1998b).

4.1.2.7.2 Studies in vivo

In a micronucleus test according to OECD guideline 474, groups of 5 male (28.1-37.2 g) and 5 female ICR mice (24.5-31.0 g) were dosed with 0, 376 750, or 1500 mg/kg bw HHCB (in corn oil - purity >99%) by intraperitoneal injection at a constant volume of 20 ml/kg bw. The high dose was selected to be approximately 70% of the estimated intraperitoneal LD_{50}. The positive control was cyclophosphamide. Bone marrow was harvested at 24, 48 and 72 hr after dosing and examined for micronucleated polychromatic erythrocytes (PCE). No mortality was seen. Lethargy was observed in all animals on 1500 mg/kg bw, in 4/15 males and 4/15 females on 750 mg/kg, and 1/15 males and 0/15 females on 376 mg/kg bw. Moderate reductions (up to 25%) in the ratio of PCE to total erythrocytes were observed in groups on 1500 mg/kg bw after 48 and 72 hrs indicating toxicity and bioavailability to the bone marrow target. The positive control induced a significant increase in micronucleated PCE in both male and female mice at 24 hr (the only harvest time for this group). No significant increase in micronucleated PCE in HHCB-treated groups relative to the respective vehicle control group was observed in male or female mice at 24, 48 or 72 hr after dose administration. (Api and San, 1999a; Gudi and Ritter, 1997).

4.1.2.7.3 Summary of mutagenicity

HHCB has been tested in a wide array of *in vitro* tests and in an *in vivo* mouse micronucleus test. *In vitro*, HHCB was negative in gene mutation tests with bacteria, in an SOS chromotest with bacteria, in SCE and micronucleus tests with human cells, in an UDS test with primary rat hepatocytes and in a chromosome aberration assay in CHO cells. HHCB also did not induce significant chromosome aberrations in the *in vivo* micronucleus test. Hence, it can be judged that HHCB is a non-genotoxic substance.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

No studies are available.

4.1.2.8.2 Human data

No studies are available.

4.1.2.8.3 Summary of carcinogenicity

There are no carcinogenicity data available. HHCB is demonstrated to be not genotoxic. There are no indications from repeated dose toxicity studies that could be used to judge carcinogenic potential.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Studies in animals

No multiple generation study is available. In the 13-week oral study (see repeated dose toxicity, section 4.1.2.6), administration of doses of up to 150 mg/kg bw/day (NOAEL ≥150 mg/kg bw/day) had no effects on the reproductive organs of male or female rats (Api and Ford, 1999b; Hopkins et al., 1996). No effect on reproduction performance was found in a peri/postnatal study, see 4.1.2.9.2 (Ford and Bottomley, 1997; Jones et al., 1996).

Human data

No studies available.

4.1.2.9.2 Developmental toxicity

Studies in animals

A dosage-range finding study was conducted to provide information for the selection of dosages to be used in a developmental study. In this study, groups of 8 pregnant Sprague-Dawley rats were administered HHCB (purity not reported in report, confirmed to be >95% pure undiluted material, IFF personal communication with RIFM) in corn oil by gavage (5 ml/kg bw/day) at doses of 100, 250, 500 or 1000 mg/kg bw/day on days 7 through 17 of pregnancy. A control group of 19 pregnant rats received corn oil only. Three rats in the high dose group died on days 10, 10 and 12, respectively. One rat in the highest dose group, found dead on day 12, showed at autopsy six pinpoint dark areas in the fundic mucosa of the stomach. Decreased motor activity, localized alopecia and urine stained fur were observed in dams on 1000 and 500 mg/kg bw per day. In addition, at the 1000 mg/kg bw per day group, red perioral substance, ungroomed coat and changes in defecation (soft or liquid faeces) were observed. Dams on all dose-levels showed reduced body weight gain during the treatment period (day 7-18 of gestation). Dams on 500 and 1000 mg/kg bw showed reduced body weight gain during treatment plus post treatment period (day 7-20 of gestation). Reduced absolute and relative food consumption was seen in dams of all treated groups during

treatment period (day 7-18) and during treatment plus post treatment period (day 7-20). Mean foetal body weights were 89.3% of controls at 1000 mg/kg bw/day. No other treatment related effects were seen (Christian et al., 1997; 1999)

Based on the range-finding study, HHCB (purity not reported in report, confirmed to be >95% pure undiluted material, IFF personal communication with RIFM) in corn oil was administered by gavage to groups of 25 female Sprague-Dawley rats on days 7 through 17 of presumed gestation at dosages of 0, 50, 150 and 500 mg/kg bw/day in a GLP compliant study. The dams were observed for signs of toxicity and body weights and feed intake were recorded. On day 20 of gestation, the dams were sacrificed and gross necropsy was performed. The number of corpora lutea in the ovaries was recorded and the uteri were examined for pregnancy, number and distribution of implantations, live and dead foetuses and early and late resorptions and the placenta were examined. All foetuses were weighed and examined for sex and gross external abnormalities. One half of the foetuses in each litter were examined for soft tissue alterations. The remaining foetuses were examined for skeletal alterations.

The 500 mg/kg bw/day dosage group had four to nine ($p \leq 0.01$) rats with excess salivation (9 animals), urine-stained abdominal fur (7 animals), red or brown substance on the forepaws (4 animals) and alopecia (6 animals). Dams on 500 and 150 mg/kg bw/day showed statistically significant dosage-dependent reductions in maternal body weight gains for the entire dosage period (days 7 to 18 gestation), to 78% and 91% of body weight gain in controls, respectively. These reductions in weight gain reflected significant weight loss (-10.9 g compared to +10.5 g in the control group) on days 7 to 10 at 500 mg/kg bw per day, while on days 10 to 12 the weight gain was increased (+17.9 g compared to +11.8 g in the control group). At 150 mg/kg bw/day, a significantly reduced weight gain (+5.5 g) was also found on days 7 to 10. For the remainder of the study, body weight gains at 150 and 500 mg/kg bw per day were comparable to the control group values. Body weights were significantly reduced compared to controls on GD20 by 2.5, 3,3 and 4.6% for 50, 150 and 500 mg/kg bw/day, respectively.

Foetuses in the 500 mg/kg bw/day dosage group showed significantly reduced body weights, 3.24 g compared to 3.48 g in the control group (7% reduction). Significant increases in foetal incidences of skeleton (vertebral/rib) malformations were found (2.0 compared to 0 in the control group). In addition, significant increases in foetal incidences of incomplete ossification and/or no ossification of sternal centra and a significantly decreased number of ossification sites in the metatarsals were seen at 500 mg/kg bw/day (5.4 compared to 0.5 in the control group). For litters, these incidences were also increased, 14.3 compared to 0 in the control group for the skeleton malformations, and 23.8 compared to 4.0 in the control group for the ossification problems. No other Caesarean-sectioning and litter parameters were affected by administration of HHCB to the dams at doses as high as 500 mg/kg bw/day. The litter averages for corpora lutea, implantations, litter sizes, live/dead foetuses, early and late resorptions, percent resorbed conceptuses and percent live male/female foetuses were comparable among the four dosage groups and did not differ significantly. No dam had a litter consisting of only resorbed conceptuses and there were no dead foetuses.

Based on a reduction in maternal body weight gains for the dosing period (days 7 to 18 of gestation), the maternal no-observable-adverse effects level (NOAEL) for HHCB was concluded to be 50 mg/kg bw/day. Based on a reduction in foetal body weight, increased incidences of foetal-skeletal (vertebral/rib) malformations, and decreased ossification of sternal centra and metatarsals seen at 500 mg/kg bw/day, the developmental NOAEL was 150 mg/kg bw/day. Because adverse effects on development occurred only at dosages that

produced toxic effects in the dams, HHCB is likely not selectively toxic to development. This study was conducted in accordance with GLP and evaluated ICH Harmonized Tripartite Guideline stages C and D (Christian et al., 1997; 1999).

In a study designed to determine the effects of HHCB (purity not reported in report, confirmed to be >95% pure undiluted material, IFF personal communication with RIFM) on the neonate when exposed through nursing, HHCB was administered at dosages of 0, 2, 6 or 20 mg/kg bw/d once daily by gavage in corn oil to groups of 28 time-mated rats (Crl:CD BR VAF/Plus strain) from Day 14 of pregnancy (end of organogenesis) through to weaning on Day 21 post partum. The females were allowed to litter and rear their young to weaning. From these litters, selected offspring were retained (24 males and females per group) to maturity and assessed for behavioural changes and reproductive capacity. The F1 generation was only exposed to HHCB *in utero* during the perinatal phase and through transfer in the milk of the lactating dams. The exposure of the F1 foetuses through mother's milk can be estimated based on a pharmacokinetic study in pregnant/lactating rats (Hawkins et al., 1996a reported in section 4.1.2.1.1, under heading: Animal milk studies). HHCB levels in mother's milk up to 2.28 and 32.8 mg HHCB equivalents (including also metabolites)/l were found at oral doses of 2 and 20 mg 14C-HHCB/kg bw per day, respectively, to the dams (see **Table 4.14**). Actual intakes cannot be determined because milk consumption during nursing was not measured.

After parturition, the young were counted, sexed, weighed and examined for external abnormalities. On day 4 *post partum* the pups were weighed and all litters containing more than 8 pups were culled to 8 retaining, where possible 4 males and 4 females. During the pre-weaning period, all pups were examined to determine the age of reaching certain developmental stages by examining surface righting reflex, startle reflex, air righting reflex and pupil reflex. This F1 generation was also evaluated for behavioural effects by examining changes in motor coordination and balance, activity and avoidance. When the F1 generation reached approximately 84 days of age (having been continuously observed for signs of adverse health) they were mated one male to one female avoiding brother-sister pairings. The females were examined before and after mating to determine time of pregnancy, marked anomalies of the oestrous cycle, median pre-coital time, whether pregnancy had occurred and terminated and duration of pregnancy.

The offspring (F2 generation) were examined for abnormalities at parturition and periodically until day 21 *post partum* at which time the study was terminated.

There were no effects of treatment in any of the treated parent females during pregnancy or lactation. No effects were apparent on development of the F1 generation during the late pre-natal phase, or on postnatal growth, no changes in post weaning behavioural tests or mating performance were seen and post mortem examination of F1 males and females, reproductive capacity, litter data and macroscopic post mortem examination of F2 pups did not reveal abnormalities. The highest dose administered, 20 mg/kg bw/day produced no adverse effects. This study was conducted in accordance with GLP and based on the guidelines endorsed by the ICH Steering Committee on the Detection of Toxicity to reproduction for Medicinal Products (Ford and Bottomley, 1997; Jones et al., 1996).

It should be noted that this dose cannot be considered a NOAEL for the purpose of risk characterisation for the evaluation of neonatal exposure via milk, since it is the dose received by the dams and the study was designed to detect adverse effects on the pups. However, the top dose from this study can be used to assess the risk for pup weight, pup survival and postnatal death in neonates resulting from maternal exposure. Since no adverse effects were

detected, no LOAEL can be established, and the NOAEL is higher than or equal to the highest dose tested, thus ≥20 mg/kg bw/day.

Human data

No data available.

4.1.2.9.3 Endocrine interactions

In vitro

In a non-GLP study, HHCB (purity not reported) in ethanol was added to transiently human estrogen receptor (ER)-α- or ERβ- transfected human embryonal kidney 293 cells for 24 hr. HHCB weakly stimulated the transcriptional activities (about 6 orders of magnitude less than estradiol) with ERα- but not with ERβ- transfected cells (Seinen et al., 1999).

In a non-GLP study, HHCB (purity 98%) in ethanol (10 mmole/L) was added to estrogen receptor-positive human mammary carcinoma cells (MCF-7) and incubated for 6 days according to the method for the E-screen assay of Soto et al. (1995). It was tested at 5 different concentrations, the highest being 10 µM with a solvent concentration of 0.1% at the highest. The rate of proliferation of the cells was compared to that of a hormone-free control sample as determined by photometric analysis of the total protein content of the fixed cells. The relative rate of proliferation (test substance relative to control) was then compared to that of 17β-estradiol. HHCB showed a slightly higher but statistically insignificant rate of proliferation relative to the hormone-free control (Bitsch et al., 2002).

In a non GLP-study, HHCB was again tested in human embryonal kidney 293 (HEK293) and human osteoblastic (U2-OS) cells which were stably transfected with plasmids containing the human ER isoforms α and β and transiently transfected with an estrogen responsive reporter gene construct (ERE-luciferase). In both cell types only the highest HHCB concentration (10 µM) was able to only marginally activate the transcripton of hERα and not hERβ. HHCB in 0.01, 1 and 10 µM concentrations did repress the 17β-estradiol induced transcription in both cell types and with both receptor isoforms dose-dependently (Schreurs et al., 2002).

Human embryonal kidney 293 (HEK293) cells were transient transfected with plasmids containing the human and zebrafish estrogen receptor isoforms (hERα and hERβ, and zfERα, β and γ) and an estrogen responsive reporter gene construct (ERE-luciferase). HHCB was tested up to 10 µM. HHCB alone showed a marginal transcriptional activation of hERα at the highest test concentration. The other receptors were not affected at all. The anti-estrogenic activity was tested using a submaximal dose of 0.01 nM estradiol (E2) for hERα, 0.1 nM E2 for hERβ and zfERγ and 1 nM E2 for zfERα and zfERβ, together with 0.1, 1 or 10 µM HHCB. A dose-dependent suppression by HHCB of E2 induction was shown towards hERβ and zfERγ. A weak antagonistic effect could be observed on hERα and zfERβ only at the highest test concentration of 10 µM, whereas no effect was seen at zfERα (Schreurs et al., 2004).

In a non GLP-study, HHCB was tested in human osteoblastic (U2-OS) cells, which were stably transfected with either the androgen receptor (AR) or the progesterone receptor (PR), and a sensitive reporter gene construct (3xARE-TATA-luciferase). Towards the AR, HHCB at concentrations of 1 and 10 µM could dose-dependently repress the transcriptional activity induced by 0.1 nM dihydrotestosterone to a maximum of 30% at the highest dose. Towards

the PR, HHCB at concentrations of 0.01, 0.1, 1 and 10 µM could dose-dependently repress the transcriptional activity of 30 pM ORG2058, a stable PR agonist. HHCB did not show any agonistic effects towards the AR or PR in this cell line (Schreurs et al., 2005a).

In a non GLP-study, HHCB was tested in human embryonal kidney 293 (HEK293) cells stably transfected with either hERα or hERβ, and a sensitive estrogen responsive reporter gene construct (3xERE-TATA-luciferase). Furthermore, HHCB was tested in human osteoblastic (U2-OS) cells stably transfected with hAR and an androgen responsive reporter gene construct (3xARE-TATA-luciferase). As seen in previous studies of the same research group, HHCB showed dose-dependent antagonism towards hERβ only, with an IC50 value of 2.4 µM. Towards the hAR, HHCB showed dose-dependent antagonism with an IC50 value of 2.9 µM. No agonistic or antagonistic effects were observed towards the aryl hydrocarbon receptor (dioxin receptor) (Schreurs et al., 2005b).
Gomez and colleagues have studied substances found in cosmetic formulations on their estrogenic effects. A human cervical epithelioid carcinoma HeLa cell line was chosen as the host cell line and these cells were stably transfected with human ERα and β (then called HELN). HHCB was tested from 10^{-7} to 10^{-5} M. HHCB weakly induced luciferase expression in HELN ERα cells and was not active in HELN ERβ cells (Gomez et al., 2005).

In vivo

In a non-GLP study, weanling (21 days old) female Balb/c mice (6 per dose group) were maintained on a diet containing 0, 50 or 300 mg HHCB (purity not reported)/kg diet for 2 weeks. This resulted in mean daily intakes of about 0, 6 or 40 mg/kg bw/day. At the end of 2 weeks, the mice were sacrificed and uterus, thymus, liver and bodyweights were recorded. Positive control mice were injected with 17β-estradiol on days 1, 5, 9 and 12 of the study. Possible uterotrophic activity of HHCB was assessed according to the protocol of Thigpen et al. (1987) (as referenced in Seinen et al., 1999), a standardised method to demonstrate estrogenic activity. The estradiol treated mice had significantly increased uterine weights and decreased thymus weights. HHCB had no significant effects on body weight or weights of uterus or thymus. Relative liver weights were increased significantly at 50 (with 8%) and 300 mg HHCB/kg of diet (with 22%) (Seinen et al., 1999). No histopathology was performed.

This same group also investigated the possible anti-estrogenic effects in zebrafish *in vivo* (Schreurs et al., 2004). Transgenic zebrafish, containing a similar reporter gene construct as used in the above mentioned in vitro experiments, were exposed to HHCB with and without E2. HHCB at a concentration of 10 µM was toxic to the fish. HHCB did not show any estrogenic effect with the tested concentrations of 0.01, 0.1 and 1 µM. The concentrations of 0.1 and 1 µM resulted in dose-dependent antagonistic effects on E2 in the juvenile zebrafish.

4.1.2.9.4 Summary of toxicity for reproduction

No multigeneration study is available.

In an oral peri/postnatal toxicity study (exposure of only the F_1-generation to HHCB in *utero* during the perinatal phase or through any transfer in the milk of the lactating dams), no toxicity in dams or their F1 and F2 offspring (including behavioural and reproductive capacity of the F1 animals) was seen at dose levels of 2, 6, or 20 mg HHCB/kg bw per day. The exposure of F1 foetuses through mother's milk can be estimated based on a pharmacokinetic study with pregnant/lactating rats given oral doses of 2 and 20 mg 14C-HHCB/kg bw per day.

Levels up to 2.28 and 32.8 mg HHCB equivalents (i.e. HHCB + metabolites)/l of whole milk were reported, for maternal oral doses of 2 and 20 mg/kg bw/d, respectively (**see Table 4.14**). In an oral developmental study there were signs of maternal toxicity at 150 mg/kg bw/day and higher. There was an increased incidence of skeletal malformations and decreased ossification in foetuses at the highest dose of 500 mg/kg bw/day. The NOAEL for maternal toxicity is 50 mg/kg bw/day and for developmental toxicity the NOAEL is 150 mg/kg bw/day. From the peri/postnatal study described above, a NOAEL of ≥20 mg/kg bw/day can be established for pup weight, pup survival and postnatal death, the highest dose tested. These effects are not included in the oral teratogenicity study. Since this NOAEL is also lower than the NOAEL for teratogenic effects generated during earlier periods of foetal development (150 mg/kg bw/day; see above), this NOAEL (≥20 mg/kg bw/day) will cover also these early teratogenic events. A NOAEL for developmental toxicity of ≥20 mg/kg bw/day will be taken forward to the risk characterization.

No effects on reproductive organs of male or female rats were seen in a 13-week oral study at doses up to 150 mg/kg bw/day (NOAEL ≥150 mg/kg bw/day).

HHCB has a very weak estrogenic and anti-estrogenic potency *in vitro*, dependent on the estrogen receptor type. Marginal repressing effects were also found *in vitro* on the androgen and progesterone receptor. However, *in vivo* no estrogenic effects were seen in the uterotrophic assay.

4.1.3 Risk characterisation

4.1.3.1 General aspects

There are no data available on the toxicokinetics of HHCB after oral or inhalation exposure. Taking into account physico-chemical properties neither no nor complete oral absorption is likely. Hence, an intermediate default percentage of 50% for oral absorption is taken forward to the risk characterisation. For inhalation exposure, 100% absorption is taken forward.

Route-to-route extrapolation introduces an additional uncertainty, not taking into account eventual first pass metabolism.

An *in vitro* dermal absorption study with ^{14}C-ring-labelled HHCB using human epidermal membranes indicated that 5.2% of the applied dose is absorbed over 24-hr. This figure is taken forward to the risk characterisation. This is considered to be a worst-case assumption, even for damaged skin, because the *in vivo* data indicate a much lower dermal absorption in humans.

The initial plasma elimination half-life in rats and pigs after intravenous administration of ^{14}C-ring-labelled HHCB is approximately 2.1 hr and 1.1 h, respectively but longer half-lives were noted in both species after initial measurements with the pig showing a half-life of approximately 90 hr (48-672 hr after administration). No data on plasma half-life in humans are available for HHCB.

HHCB is found in human milk in several studies. Values for risk characterization were chosen from the Sönnichsen study (1999), because this study is well performed by excluding contamination of skin products as much as possible (although possibly not ruled out) and it involved a fairly large study population (107 volunteers). HHCB was detected in milk

samples at levels from zero to 1316 µg/kg fat (equivalent to 48 µg/kg whole milk based on a median measured fat content of 3.67%) with a mean of 80 µg/kg fat (2.9 µg/kg milk).In adipose tissue HHCB was found at levels ranging from 12 – 189 µg/kg fat.

The oral LD_{50} for rats and the dermal LD_{50} for rabbits are both greater than 3000 mg/kg bw. Data for acute inhalation toxicity are not available. The data provided are considered sufficient to meet base set requirements for acute toxicity. There is no need to classify HHCB for acute toxicity.

GLP compliant studies of skin irritation have been performed according to directive 79/831/EEC to groups of either 3 or 4 New Zealand White female rabbits. All tests showed very slight to well-defined erythema and very slight oedema. In only one of the tests did the mean erythema score for Galaxolide 50 DEP exceed 2.0 (the calculated score was 2.1). The solvent in this study, DEP, scored 0.2 for erythema and zero for oedema. The irritating effect was not reversible in 7 out of in total 15 animals in these three studies during an observation period of 7 days, as at that time point still some erythema and/or oedema was seen. Unfortunately, the observation period was not sufficiently long (14 days, according to the test method guideline in Annex V) to evaluate fully the reversibility of the effects. However, the test method guideline also states that the irritation scores should be evaluated in conjunction with the nature and severity of lesions and their reversibility or lack of reversibility. Taking that also into account, the results of the animal studies do not indicate that HHCB is a skin irritant. There is a difficulty though, because according to Annex VI of Directive 67/548, inflammation of the skin is also significant if it persists in at least 2 animals at the end of the observation period [without specifying the length of the observation period and the severity of the effects]. If this guidance is followed strictly, the animal studies would warrant classification as a skin irritant. This issue was discussed at the TC-C&L meeting of November 2005, where it was concluded that it is not warranted to classify HHCB as a skin irritant.

In a Human Repeated Insult Patch Test (HRIPT) for sensitisation, a semi-occlusive patch of 100% neat HHCB showed no irritation after repeated application during the induction phase of the study. Other HRIPTs with diluted HHCB also showed no signs of irritation.

There are some indications from animal studies that HHCB could be a photoirritant. The results in human tests do not indicate a photoirritating effect in humans. Also, an *in vitro* phototoxicity test (in compliance with test guideline B.41 (EU/COLIPA Test)) was negative. No criteria on classification of photoirritating substances are available in Annex VI.

HHCB has been tested for ocular irritation in rabbits in several studies. Some studies used ethanol, a known eye-irritant as solvent, and are not used. In other relevant studies, some ocular irritation was found. However, the effects were not severe enough to require classification according to EU guidelines.

No data on respiratory tract irritation are available.

Although some questionable elicitation reactions have been reported as a result of patch tests in dermatological clinics on sensitive patients, the available data with guinea pigs and humans (Human Repeated Insult Patch Test and maximisation tests) provide no evidence of potential for induction of sensitisation for HHCB. HHCB need not be classified as a skin contact sensitiser.

There is no evidence from studies in experimental animals or with humans, that HHCB is a photosensitiser.

In an adequate 90-day oral study, there were no mortalities or adverse clinical signs. Body weight and food consumption of treated groups were similar to those observed in the control group. No changes in ophthalmologic evaluation were observed and no significant histopathological findings at any dose.

The haematology and blood chemistry differences from controls were all small, often not proportional to dose, often seen only at one time point and/or in one sex, and, with two exceptions, well within historical controls and are not considered to "reduce the capacity of an organism or a component of an organism to function in a normal manner" (Abadin et al., 1998). This, and the fact that these findings were not accompanied by any adverse histopathology or other related findings, leads to the conclusion that they are not adverse effects.

A NOAEL of ≥150 mg/kg bw/day, the highest dose tested, for HHCB in rats is concluded.

Three dermal subchronic studies are available. In two of these there was some evidence of liver weight increases (at 100 mg/kg bw/day for 13 weeks) and body weight decreases (at 36 mg/kg bw/day for 26 weeks) but the magnitude of these effects were not reported and their significance cannot be determined. In a third dermal 26-week study, no effects were seen up to and including the highest dose administered (200 mg/kg bw/day). However, because 1) neither collars nor occlusion were used to prevent oral intake making it impossible to determine actual exposures, 2) the area of application was not reported, and 3) the lack of significance in the findings reported in the first two studies and the lack of an adverse effect dose in the third, it is impossible to conclude a true NOAEL in terms of dermal toxicity.

When administered as part of a fragrance mixture, inhalation exposure to HHCB up to a maximum tested dose of 132 $\mu g/m^3$ for 4 hr per day, 5 days per week for 13 weeks did not result in any toxicity. This study is of limited value because the animals were not exposed to HHCB alone, and HHCB was only present at rather low levels in the mixtures.

HHCB has been tested in a wide array of *in vitro* tests and in an *in vivo* mouse micronucleus test. *In vitro*, HHCB was negative in gene mutation tests with bacteria, in an SOS chromotest with bacteria, in SCE and micronucleus tests with human cells, in an UDS test with primary rat hepatocytes and in a chromosome aberration assay in CHO cells. HHCB also did not induce significant chromosome aberrations in the *in vivo* micronucleus test. Hence, it can be concluded that HHCB is a non-genotoxic substance.

There are no carcinogenicity data available. HHCB is demonstrated to be not genotoxic. There are no indications from repeated dose toxicity studies that could be used to judge carcinogenic potential.

No multigeneration study is available.

In an oral peri/postnatal toxicity study (exposure of only the F_1-generation to HHCB in *utero* during the perinatal phase or through any transfer in the milk of the lactating dams), no toxicity in dams or their F1 and F2 offspring (including behavioural and reproductive capacity of the F1 animals) was seen at dose levels of 2, 6, or 20 mg HHCB/kg bw per day. The exposure of F1 foetuses through mother's milk can be estimated based on a pharmacokinetic study with pregnant/lactating rats given oral doses of 2 and 20 mg 14C-HHCB/kg bw per day. HHCB levels up to 2.28 and 32.8 mg HHCB equivalents (including also metabolites)/l of mother's milk, respectively, were reported (see **Table 4.14**).

In an oral developmental study there were signs of maternal toxicity at 150 mg/kg bw/day and higher. There was an increased incidence of skeletal malformations and decreased ossification in foetuses at the highest dose of 500 mg/kg bw/day. The NOAEL for maternal toxicity is 50 mg/kg bw/day and for developmental toxicity the NOAEL is 150 mg/kg bw/day. From the peri/postnatal study described above, a NOAEL of ≥20 mg/kg bw/day can be established for pup weight, pup survival and postnatal death, the highest dose tested. These effects are not included in the oral developmental study. Since this NOAEL is also lower than the NOAEL for teratogenic effects generated during earlier periods of foetal development (150 mg/kg bw/day; see above), this NOAEL (≥20 mg/kg bw/day) will cover also these early teratogenic events. A NOAEL for developmental toxicity of ≥20 mg/kg bw/day will be taken forward to the risk characterization.

No effects on reproductive organs of male or female rats were seen in a 13-week oral studies at doses up to 150 mg/kg bw/day (NOAEL ≥150 mg/kg bw/day).

HHCB has a very weak estrogenic potency *in vitro* but such effects were not seen *in vivo.*

4.1.3.2 Workers

4.1.3.2.1 Introduction

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers is limited to the dermal and inhalation routes of exposure.

4.1.3.2.2 Comparison of exposure and effects

Acute toxicity

Dermal exposure

Given the dermal LD_{50} in rabbits (>3000 mg/kg bw) and the highest anticipated short-term exposure level of 39 mg/d/100 cm^2 (or 39 mg / 70 kg bw = 0.55 mg/kg bw) for scenario 2 (compounding) it is concluded that that HHCB is of no concern for workers with regard to acute dermal toxicity (**conclusion ii**).

Inhalation exposure

There are no data on acute inhalation toxicity. Given the highest anticipated short-term exposure of 0.03 mg/kg bw (30 mg/m^3 x 0.05 h x 1.25 m^3/h x 1/70 kg^{-1}) and the oral and dermal LD50 values (both >3000 mg/kg bw), it is concluded that there are no indications for concern with respect to acute toxicity by inhalation exposure (**conclusion ii**).

Irritation including photoirritation and corrosivity

Skin irritation

Acute dermal irritation

Based on the available data, HHCB is judged not to be a skin irritant. Hence, there is no concern for workers (**conclusion ii**).

Photoirritation

In available tests for photoirritation, HHCB was not identified as a photoirritating substance. Therefore, **conclusion ii** may be drawn for this endpoint.

Corrosivity

HHCB is not corrosive to the skin (**conclusion ii**).

Dermal irritation after repeated exposure

Based on the available data, HHCB is judged not to be a skin irritant. Hence, there is no concern for workers (**conclusion ii**).

Eye irritation

HHCB is not an eye irritant (**conclusion ii**).

Respiratory tract irritation

No data are available on local effects in the respiratory tract after acute exposure. Given the lack of skin and eye irritation potential, no significant respiratory irritation potential is expected (**conclusion ii**).

Sensitisation including photosensitisation

Sensitisation

HHCB is not a skin sensitiser (**conclusion ii**).

Photosensitisation

HHCB is not a photosensitiser (**conclusion ii**).

Repeated dose toxicity

With regard to conclusions regarding the risk characterisation for local effects after repeated exposure to HHCB it is referred to the sections ‘irritation including photoirritation and corrosivity’ and ‘sensitisation including photosensitisation’.

Unlike local effects, systemic effects depend on the internal body burden. Doses or exposures at different routes of application may be converted to an internal body burden by taking into account the different absorption factors. NOAELs are not available for dermal repeated dose toxicity or for toxicity upon repeated doses via inhalation. The starting point for the risk assessment is the oral NOAEL of ≥150 mg/kg bw/day from the oral 90-day repeated dose study with rats. Assuming an oral absorption value of 50% for rats, this NOAEL corresponds to an internal no-effect dose of ≥75 mg/kg bw/day. For exposure after inhalation the absorption is assumed to be 100 %. Although it is recognized that quite different dermal exposure conditions exist between the different scenarios, e.g. in terms of exposure times and area doses, a value of 5.2% is taken for dermal absorption in all worker scenarios.

Inhalation exposure

The values of the MOS between the inhalation exposure levels and the internal no-effect dose for repeated dose toxicity are mentioned in **Table 4.25**. The table shows that for each scenario the MOS is above the minimal MOS of 100 so **conclusion ii** applies to all scenarios.

Table 4.25 Occupational risk assessment of inhalation exposure to HHCB for repeated dose toxicity

Workers scenario	exposure	IBB mg/kg bw/d [A]	MOS [B]	Conclusion [C]
Scenario1 Production and dilution				
- process operator	4.5 mg/m^3 (rwc: 3 min/d)	4 x 10^{-3}	18750	ii
- blending & dilution	4.5 mg/m^3 (rwc: 3 min/d)	4 x 10^{-3}	18750	ii
- analysis	Negligible	-	-	ii
- odour control	Negligible	-	-	ii
- wastewater treatment	Negligible	-	-	ii
Scenario 2 Compounding of fragrance oils				
- delivery & stocking	30 mg/m^3 (rwc: 3 min/d; once in 14 days)	0.03	2500	ii
- compounding				
large and medium size plants	0.013 mg/m^3 (8 h/d)	1.8 x 10^{-3}	≥41667	ii
	0.1 mg/m^3 (rwc 15 min/d)	4.5 x 10^{-4}	≥166667	ii
small size plants	0.065 mg/m^3 (8 h/d)	9.2 x 10^{-3}	≥8152	ii
	0.1 mg/m^3 (rwc 15 min/d)	4.5 x 10^{-4}	≥166667	ii
- wiping of rinsed vessels	Negligible	-	-	ii
- analysis	Negligible	-	-	ii
- odour control	Negligible	-	-	ii
Scenario 3 formulation				
- handling	Negligible	-	-	ii
- cleaning & maintenance	Negligible	-	-	ii
Scenario 4 professional cleaning				
- handling	Negligible	-	-	ii

rwc: reasonable worst case

[A] Estimated internal body burden (IBB) via inhalation exposure in mg/kg bw/d assuming a worker body weight of 70 kg, a respiratory volume of 1.25 m^3/hr and an inhalation absorption value of 100%

[B] Based on comparison of internal body burden to an internal no-effect dose of 75 mg/kg bw/d.

[C] Conclusion is reached considering the magnitude of the MOS in comparison with a minimal MOS of 100 (which is based upon the default values for assessment factors as specified in the draft version of the TGD (2005): an interspecies factor of 10 (4 for metabolic size differences * 2.5 for remaining differences), an intraspecies factor of 5 and a factor of 2 for semichronic to chronic exposure extrapolation).

Dermal exposure

The values of the MOS between the dermal exposure levels and the internal no-effect dose for repeated dose toxicity are mentioned in **Table 4.26.**

Table 4.26 Occupational risk assessment of dermal exposure to HHCB for repeated dose toxicity

Workers scenario	**exposure**	**IBB* mg/kg bw/d [A]**	**MOS [B]**	**Conclusion [C]**
Scenario1 Production				
- pr. Operator	Negligible		-	ii
- blending & dilution	Negligible		-	ii
- analysis	Negligible		-	ii
- odour control	Negligible		-	ii
- wastewater treatment	Negligible		-	ii
Scenario 2 Compounding				
- delivery & stocking	Negligible		-	ii
- compounding large and medium size	39 mg/d	0.029	≥2600	ii
- compounding small size	39 mg/d	0.029	≥2600	ii
- analysis	Negligible		-	ii
- odour control	Negligible		-	ii
- wiping of rinsed vessels	0.2 mg/d	1.5×10^{-4}	≥500,000	ii
Scenario 3 formulation				
- handling	1.7 mg/d	1.3×10^{-3}	≥58,000	ii
- cleaning & maintenance	0.26 mg/d	1.9×10^{-4}	≥395,000	ii
Scenario 4 professional cleaning				
- handling	0.32 mg/d	2.4×10^{-4}	≥313,000	ii

[A] Taking a value of 5.2 % dermal absorption and a worker body weight of 70 kg into account (IBB = internal body burden).

[B] Based on comparision of internal body burden to an internal no-effect dose of 75 mg/kg bw/d

[C] Conclusion is reached considering the magnitude of the MOS in comparison with a minimal MOS of 100 (which is based upon the default values for assessment factors as specified in the draft version of the TGD (2005): an interspecies factor of 10 (4 for metabolic size differences * 2.5 for remaining differences), an intraspecies factor of 5 and a factor of 2 for semichronic to chronic exposure extrapolation).

It can be concluded that for each scenario the MOS is above the minimal MOS of 360 so **conclusion ii** applies to all scenarios.

Combined exposure

The total internal body burden is determined by uptake after dermal exposure as well as exposure by inhalation of HHCB. This combined exposure should not be applied if a simultaneous exposure can be excluded. Combination of various exposure routes is only

relevant for the compounding scenario. The combined total body burden from skin contact and inhalation is 0.0309 mg/kg bw/day. For small size compounders the total may be 0.0383 mg/kg bw/day. The resulting MOS values are ≥2400 and ≥2000, respectively. Based on a comparison with a minimal MOS of 100 (see above), no additional risks are expected for repeated dose toxicity upon combined exposure for all worker scenarios (**conclusion ii**).

Mutagenicity

HHCB is a non-genotoxic substance (**conclusion ii**).

Carcinogenicity

There are no data available on the carcinogenic potential of HHCB. The mutagenicity data on HHCB do not indicate a concern with regard to carcinogenicity nor does HHCB possess any structural features that would raise a concern (**conclusion ii**).

Toxicity for reproduction

There are no indications for effects on fertility in the oral 90-day study with rats although in this study investigation was limited to histological examination of the reproductive organs and no adverse effects were reported up to the highest dose tested (the NOAEL was ≥150 mg/kg bw/day).

Dermal developmental studies are lacking.

In an oral developmental toxicity study with rats, developmental toxicity only occurred at maternal toxic dose levels ($NOAEL_{developmental\ toxicity}$ 150 mg/kg bw/day, $NOAEL_{maternal\ toxicity}$ 50 mg/kg bw/day). The peri/postnatal study, including endpoints as pup weight, pup survival and postnatal death, resulted in a NOAEL (highest dose level) of ≥20 mg/kg bw/day. Based on an oral absorption rate of 50 % this corresponds to an internal no-effect dose of ≥10 mg/kg bw per day for maternal toxicity.

Given this internal no-effect dose and the highest internal body burden (scenario 2) of 0.0093 mg/kg bw/d for inhalation exposure and 0.029 mg/kg bw/d for dermal exposure, the resulting MOS values are ≥1075 and ≥345 respectively. For combined exposure, a combined internal body burden of 0.0383 mg/kg bw/d results in a MOS of ≥261. A minimal MOS of 50 is considered appropriate for this effect. The latter is established by taking into account an interspecies factor of 10 (4 for metabolic size differences * 2.5 for remaining differences) and an intraspecies factor of 5. Comparison of the calculated MOS values with the minimal MOS value leads to **conclusion ii** (no concern).

4.1.3.2.3 Occupational limit values

At the moment, occupational limit values for HHCB have not been established. The health risk assessments of inhalation exposure dose not give reasons to establish occupational exposure limit values.

4.1.3.2.4 Summary of risk characterisation for workers

For workers for all relevant endpoints **conclusion ii** was reached.

4.1.3.3 Consumers

4.1.3.3.1 Introduction

Consumer exposure occurs from consumer products to which HHCB is added intentionally as a component of the fragrance that enhances the product. It is used as an ingredient in commercial preparations (fragrance oils) intended to be used to fragrance a wide variety of consumer products such as perfumes, creams, toiletries, soaps and shampoos. It is also used in household and laundry cleaning products and air fresheners. The exposure is of a repeated nature and the main exposure route is dermal, with some inhalation exposure. There is no oral exposure from consumer products.

The starting point for the risk characterisation is the external dermal exposure level of 0.85 mg/kg bw/day together with the inhalatory exposure level of 0.0085 mg/kg bw/day. Because the absorption of HHCB through human skin is 5.2% (worst-case assumption) this external exposure level results in an internal exposure level of 0.044 mg/kg bw/day. For inhalation, 100% absorption is assumed, so the internal exposure level is 0.0085 mg/kg bw/day. The total internal exposure amounts 0.053 mg/kg bw/day.

4.1.3.3.2 Comparison of exposure and effects

Irritation

Based on the available data, HHCB is judged not be a skin irritant. Hence, there is no concern for consumers (**conclusion ii)**.

In available tests for photoirritation, HHCB was not identified as a photoirritating substance. Hence, there is no concern for consumers for photoirritation (**conclusion ii)**.

There is no concern for consumers for eye irritation, because HHCB is not an eye irritant. (**conclusion ii)**.

No data are available on local effects in the respiratory tract. However, given the lack of skin and eye irritation potential, no significant respiratory irritation potential is expected (**conclusion ii**).

Sensitisation

HHCB is not a skin sensitiser in a guinea pig maximization test or in humans in concentrations up to 100%. Consumers are thus not at risk after (repeated) dermal exposure. (**conclusion ii)**.

In available tests for photosensitisation, HHCB was not identified as a photosensitiser. Hence, there is no concern for consumers (**conclusion ii)**.

Repeated dose toxicity

The starting point for the risk assessment is the oral NOAEL of ≥150 mg/kg bw/day from the oral 90-day repeated dose study with rats. Assuming an oral absorption value of 50% for rats, this NOAEL corresponds to an internal no-effect dose of 75 mg/kg bw/day.

Comparing this internal no-effect dose with the calculated human systemic exposure level of 0.053 mg/kg bw/day, a margin of safety (MOS) of ≥1400 can be calculated.

Taking into account intra- and interspecies differences and the use of a NOAEL from a semi-chronic study in which no adverse effects were observed up to and including the highest dose tested, this MOS indicates no concern for consumers following repeated dermal exposure. (**conclusion ii)** based on comparison with a minimal MOS of 200 (factors of 10 for intra- and 10 (4*2.5) for interspecies differences, a factor of 2 for duration extrapolation and a factor of 1 for dose-response).

Mutagenicity

HHCB is a non-genotoxic substance. (**conclusion ii)**

Carcinogenicity

There are no data available on the carcinogenic potential of HHCB. The mutagenicity data on HHCB do not indicate a concern with regard to carcinogenicity nor does HHCB possess any structural features that would raise a concern. (**conclusion ii)**

Reproductive toxicity

There are no indications for effects on fertility in the oral 90-day study with rats although in this study investigation was limited to histological examination of the reproductive organs, and no adverse effects were reported up to the highest dose tested (NOAEL ≥150 mg/kg bw/day).

Dermal developmental studies are lacking.

In an oral developmental toxicity study with rats, developmental toxicity only occurred at maternal toxic dose levels ($NOAEL_{developmental\ toxicity}$ 150 mg/kg bw/day, $NOAEL_{maternal\ toxicity}$ 50 mg/kg bw/day). A peri/postnatal study with rats, including endpoints such as pup weight, pup survival and postnatal death, resulted in a NOAEL for developmental toxicity of ≥20 mg/kg bw/day (highest dose tested). Assuming an oral absorption value of 50% for rats, this $NOAEL_{developmental\ toxicity}$ corresponds to an internal no-effect dose of ≥10 mg/kg bw/day.

Comparing this internal no-effect dose with the calculated human systemic exposure level of 0.053 mg/kg bw/day, a MOS of ≥189 can be calculated. Taking into account intra- and interspecies differences and the lack of effect at the highest dose tested, this MOS indicates no concern for consumers for developmental toxicity (**conclusion ii),** based on comparison with a minimal MOS of 100 (factors of 10 for intra- and 10 (4*2.5) for interspecies differences and a factor of 1 for dose-response).

4.1.3.3.3 Summary of risk characterisation for consumers

For consumers, for all relevant endpoints a **conclusion ii** was reached.

4.1.3.4 Humans exposed via the environment

4.1.3.4.1 Introduction

For man exposed via the environment the inhalation and oral route are applicable. The contribution of the inhalation of HHCB via air is neglible compared to other uptake routes, hence only the main oral exposure route via fish and root crops is taken into account. Because of the occurence of HHCB in mother's milk, a separate risk characterization is necessary for breast-fed babies.

4.1.3.4.2 Exposure via food and water

In the EUSES calculations the local total daily intake (external exposure) is estimated at 2.6 µg/kg bw/day following production, whereas the regional total daily intake is 0.097 µg/kg bw/day.

Repeated dose toxicityThe starting point for the risk assessment is the oral NOAEL of ≥150 mg/kg bw/day from the oral 90-day repeated dose study with rats. Assuming an oral absorption value of 50% for rats, this NOAEL corresponds to an internal no-effect dose of 75 mg/kg bw/day. Taking into account intra- and interspecies differences and the use of a NOAEL from a semi-chronic study in which no adverse effects were observed up to and including the highest dose tested, a minimal MOS of 200 (factors of 10 for intra- and 10 (4*2.5) for interspecies differences, a factor of 2 for duration extrapolation and a factor of 1 for dose-response) is applicable.

A margin of safety (MOS) of >3E+4 can be calculated for the local production scenario. Because the estimated human daily intake doses via food, water and air are comparable for the other local scenarios it can be concluded that HHCB is of negligible risk for man exposed indirectly via the environment (**conclusion ii**). For the regional scale the MOS is even higher (>8E+5), and also a conclusion ii can be drawn.

Mutagenicity

HHCB is a non-genotoxic substance. (**conclusion ii)**

Carcinogenicity

There are no data available on the carcinogenic potential of HHCB. The mutagenicity data on HHCB do not indicate a concern with regard to carcinogenicity nor does HHCB possess any structural features that would raise a concern. (**conclusion ii)**

Reproductive toxicity

There are no indications for effects on fertility in the oral 90-day study with rats although in this study investigation for reproductive toxicity was limited to histological examination of the reproductive organs, and no adverse effects were reported up to the highest dose tested (NOAEL ≥150 mg/kg bw/day).

In an oral developmental toxicity study with rats, developmental toxicity only occurred at maternal toxic dose levels ($NOAEL_{developmental\ toxicity}$ 150 mg/kg bw/day, $NOAEL_{maternal\ toxicity}$ 50 mg/kg bw/day). A peri/postnatal study with rats, including endpoints such as pup weight,

pup survival and postnatal death, resulted in a NOAEL for developmental toxicity of ≥20 mg/kg bw/day (highest dose tested). Assuming an oral absorption value of 50% for rats, this $NOAEL_{developmental\ toxicity}$ corresponds to an internal no-effect dose of ≥10 mg/kg bw/day.

Comparing this internal no-effect dose with the the local and regional values, MOSses of 3846 and 1E+5 respectively can be calculated. These MOSses indicate no concern for humans exposed indirectly via the environment for developmental toxicity (**conclusion ii)**, based on comparison with a minimal MOS of 100, taking into account intra- (factor of 10) and interspecies differences (factor of 10 (2.5 x 4)) and the lack of effect at the highest dose tested (factor of 1 for dose-response).

4.1.3.4.3 Exposure via mother's milk

HHCB has been detected in human milk samples. The source of HHCB in these samples is not entirely clear. Maternal exposure to consumer products, intake via food, water or air and occasionally also occupational exposure may contribute to the HHCB level in milk. However, from the point of view of the child, HHCB in milk is an indirect environmental exposure. Therefore this exposure is dealt with in this section, rather than the sections on consumer or combined exposure.

An analysis of the milk from 107 nursing mothers revealed levels of HHCB with a mean value of 80 µg/kg milk fat and a standard deviation of 149. The minimum and maximum values found were close to zero and 1316 µg/kg milk fat, respectively. A mean and a median fat content of 3.7 and 3.4%, respectively, were also reported. Based on the mean fat content, human milk contains 2.9 µg/kg whole milk (mean) or 48 µg/kg whole milk (maximum). In an oral peri/post natal study in which female rats were exposed orally to HHCB from day 14 of gestation through weaning, there were no effects on the dams at maternal doses of up to 20 mg/kg bw/day nor on the pups which were exposed via the milk during nursing. Measurements of levels of HHCB (17.6 and 5.0 µg/ml at 4 or 8 hr post dosing, respectively; parent HHCB only) in the milk of the dams dosed at 20 mg/kg bw/day compared to the levels found in human milk samples indicate that the pups in the high dose group were exposed to levels approximately 1700 to 6000 times the mean levels. This corresponds to approximately 100 to 360 times the maximum level found in human milk samples (2.9 and 48 µg HHCB/kg whole milk, respectively).

Even for the highest concentration in human milk samples, compared to the highest concentration in rat milk, a sufficiently high MOS can be calculated (~100). Taking into account that at the top maternal dose no effects were observed at all (i.e. the real NOAEL is at least equal but probably above this top dose), a ***conclusion ii*** is reached.

Additional to the assessment above, which is only based on concentrations in human *versus* rat milk, an assessment is carried out which also takes into account, the amount of milk that is consumed by infants and rat pups, in a way similar to the assessment applied in the Risk Assessment Report on MCCP (ECB, 2004). For the mentioned references see this RAR.

It is assumed that an infant breast feeds for 1 year, and this year of life is subdivided into two periods – 0 to 3 months and 3 to 12 months – reflecting the changing feeding demands of the infant. It is assumed that over the first 3 months the infant has an average weight of 6 kg (data taken from the UK growth charts, published by the Child Growth Foundation, 1995; Freeman *et al*, 1995 and Cole, 1994), that the infant ingests 0.8 kg of milk per day, that 50% of the ingested HHCB is absorbed and that the breast milk has an average fat content of 3.7% (see

above). From 3 to 12 months, it is assumed that the infant has an average weight of 10 kg (data taken from same source as above), that the infant ingests 0.5 kg of milk per day, that 50% of the ingested HHCB is absorbed and that the breast milk has a fat content of 3.7% (see above). It is also assumed that the content of HHCB remains constant during the breast-feeding period.

Using the following equation and the assumptions detailed above, the average daily uptake of the breast-feeding infant (ADU_{infant}) is estimated for both the 0-3 month and 3-12 month periods of infant life. The resultant uptakes are then summed to generate an average uptake for the infant in mg/kg/day.

$$ADU_{\text{inf } ant} = \frac{C_{milk-fat} xf\,3 xf\,4 xIR_{milk}}{BW_{\text{inf } ant}}$$

where:

$C_{milk\text{-}fat}$	is the concentration of HHCB in mg/kg fat in breast milk
f3	is the fraction of fat in breast milk (0.037 kg fat/kg milk)
f4	is the absorbed fraction of ingested HHCB (0.5)
IR_{milk}	is the ingestion rate of milk (kg/day)
BW_{infant}	is the average infant body weight over the exposure period (kg)

HHCB uptake during 0-3 months, assuming a concentration of HHCB in human breast milk of 1316 µg/kg (maximum measured value):

$$ADU_{\text{inf } ant} = \frac{1.316x0.037x0.5x0.8}{6} = 3.2x10^{-3}\, mg/kg/day$$

HHCB uptake during 3-12 months, assuming a concentration of HHCB in human breast milk of 1316 µg/kg:

$$ADU_{\text{inf } ant} = \frac{1.316x0.037x0.5x0.5}{10} = 1.2x10^{-3}\, mg/kg/day$$

Based on these estimates, the time-weighted year-average uptake of HHCB for the first 12 months of life is 1.7 x 10^{-3} mg/kg/day.

A similar calculation can be performed for the rat. Pup body weight at birth is around 6 g and at weaning is about 40-50 g; an average weight of about 20 g will be assumed for the purposes of this calculation. Milk production in the lactating rat varies over the lactation period. Sampson and Jansen (1984) derived a model to estimate daily milk yield in the lactating rat. Based on this model, on day 10 of lactation, milk yield was estimated to be 29.5 ml for a dam nursing 8 pups. This equates to about 3.7 ml (or 3.7 g) milk per pup, and will be used as the average daily milk consumption for this calculation.

Based on these assumptions, and using the value of 17.6 mg/l milk for HHCB content in rat milk, estimated daily pup uptake is about 1.6 mg/kg/day (i.e. level of HHCB per kg whole milk x daily milk consumption (l) x absorption fraction / pup body weight (kg) = 17.6 mg/l x 3.7 x 10^{-3} x 0.5 / 20 x 10^{-3} mg/kg/day).

Comparing these two estimates of uptake, there is a difference of approximately 3 orders of magnitude between the levels of HHCB exposure in the rat study (in which no adverse effects were found) and human infant exposure. This large Margin of Safety (MOS) leads to little cause for concern and thus a ***conclusion (ii)***.

4.1.3.4.4 Summary of risk characterisation for exposure via the environment

A **conclusion ii** was reached for man exposed indirectly via the environment at the local scale as well as at the regional scale, and also for breast-fed babies.

4.1.3.5 Combined exposure

A worst case estimate for the combined (internal) exposure to HHCB would be the sum of the worst case estimates for the three individual populations, i.e. 0.038 mg/kg bw/day (dermal and inhalation, scenario 2 "compounding" for workers) + 0.053 mg/kg bw/day (dermal and inhalation, consumers) + 0.0026 mg/kg bw/day (oral and inhalation, locally via the environment). This results in a total internal (worst case) combined exposure estimate of 0.094 mg/kg bw/day. The contribution of the exposure via the environment attributes only about 3%. The contribution to the total exposure as worker or as consumer is about equal. This value is compared to the two relevant chronic endpoints, namely repeated dose toxicity and reproductive toxicity.

Comparing this value to an internal no-effect dose of ≥75 mg/kg bw/day from the repeated dose toxicity study, a MOS of 798 can be derived. Based on a comparison with a minimal MOS of 100 (established by taking into account an interspecies factor of 10 (4 for metabolic size differences * 2.5 for remaining differences), an intraspecies factor of 5 and a factor of 2 for semichronic to chronic exposure extrapolation, no additional risks are expected for repeated dose toxicity upon combined exposure (**conclusion ii**).

Comparing this value to an internal no-effect dose of ≥10 mg/kg bw per day for maternal toxicity, a MOS of ≥106 can be derived. A minimal MOS of 50 is considered appropriate for this effect. The latter is established by taking into account an interspecies factor of 10 (4 for metabolic size differences * 2,5 for remaining differences) and an intraspecies factor of 5. Comparison of the calculated MOS values with the minimal MOS value leads to **conclusion ii** for workers after total combined exposure (no concern).

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

As HHCB is not explosive, flammable and has no oxidising potential, this exposure assessment is not filled in.

4.2.1.1 Workers

4.2.1.2 Consumers

4.2.1.3 Humans exposed via the environment

4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

HHCB is not explosive.

4.2.2.2 Flammability

HHCB is not flammable.

4.2.2.3 Oxidizing potential

HHCB is not oxidizing, as indicated by its structure and by experience.

4.2.3 Risk characterisation

Based on the absence of physico-chemical hazards, there is no reason for concern. **Conclusion (ii)** applies.

4.2.3.1 Workers

4.2.3.2 Consumers

4.2.3.3 Humans exposed via the environment

5 RESULTS [14]

5.1 ENVIRONMENT

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all compartments and all scenarios.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.1.4 Combined exposure

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

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[14] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

5.2.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
dwt	dry weight / dwt
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT_{50}	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC_{50}	Effect Concentration measured as 50% reduction in biomass growth in algae tests

EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC_{50}	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC_{50}	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EU-15	European Union, 15 member states
EU-15+2	EU-15 plus Norway and Switzerland
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC_{50}	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues

Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
$L(E)C_{50}$	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC_{50}	median Lethal Concentration
LD_{50}	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling

PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum

UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

Appendix 1

Evaluation of the aquatic toxicity test with *Nitocra spinipes*

Nitocra spinipes test (Breitholtz et al. 2003)

The test was carried out in 10 ml of test medium to which salmon food was added at a level of 75 mg per liter. Every other day 70% of the overlying water was replaced with fresh medium and new food was added (38 mg/L). The organic residues are not removed, so an increasing amount of organic matter was available for sorption.

Breitholtz et al. (2003) characterize the test organism *Nitocra spinipes*: 'it lives mainly on sandy bottoms, feeding on bacteria or particles.' In their discussion it is stated that 'it is likely that a significant fraction of the synthetic musks were adsorbed to particulate organic material and to the glass…. . Ingestion of synthetic musks adsorbed to particles could therefore be an equally or more significant route of exposure than uptake through the water for the bottom dwelling *N. spinipes* which feeds on bacteria and particles'.

Potential routes of disappearance of the test substance include sorption to organic matter, to the vessel glass wall, degradation and evaporation. Breitholtz et al. (2003) refer to the presence of various sinks for hydrophobic test substances, as was shown by Breitholtz and Wollenberger (2003)[15] on brominated flame retardants (their figure 2). In this study the walls of the test vials and especially particulate matter were clearly the most important targets of the test substances. At day 8 about 35% - 45% of the compounds was sorbed to the glass wall and 50 – 60% to the particulate matter. After another week the percentage found on particulate matter had increased to 60 – 80%, illustrating indeed that this test design led to increased accumulation of the material on the particulate matter in time. Although sorption to organic material can explain the decrease in concentrations of the two studied polybrominated diphenyl ether (PBDE) congeners (PBDE 47 and PBDE 99), this may be only partly valid for the polycyclic musk compounds AHTN and HHCB, which are less hydrophobic than the PBDE congeners. The log K_{ow} of AHTN and HHCB is about 1 to 2 log units lower than of the two PBDEs, on basis of accurate slow-stirring values[16]. The estimated log K_{oc} values of PBDEs are therefore higher as well.

With the lower log Koc values for AHTN and HHCB, sorption to organic matter may not be a sufficient explanation of the loss of test material. Methodological studies on the further development of a suitable test system for *Acartia tonsa* showed that up to 50% of AHTN and HHCB are lost from open test vessels after 1 day (Egeler et al 2007) [17]. The results of Kroon once more illustrate the relevance of sorption of polycyclic musks to algae cells (see below).

For substances with high log K_{ow} (>5) oral exposure is considered to be a significant route of exposure. In the EU-TGD (EC 2003) this exposure is taken into account by lowering PEC/PNECsediment by a factor of 10 when PNEC is based on equilibrium partitioning. In true sediment toxicity tests, oral exposure is inherent in the test system. In their discussion on

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[15] Breitholtz M, L Wollenberger (2003) Effects of three PBDEs on development, reproduction and population growth rate of the harpactticoid copepod *Nitocra spinipes*. Aquat. Toxicol. **64**, 85-96.

[16] Braekevelt E, SA Titlemier, GT Tomy (2003) Direct measurement of octanol–water partition coefficients of some environmentally relevant brominated diphenyl ether congeners. Chemosphere 51: 563-567.

[17] Egeler P, JP Ferreira, D. Gilberg (2007). AHTN and HHCB: Preliminary studies on the toxicity to the larval development of the marine calanoid copepod *Acartia tonsa.* Report to PFW Aroma Chemicals and to IFF, ECT Oekotoxikologie GmbH, April 2007.

the toxicity of PBDEs, Breitholtz and Wollenberger (2003) draw the attention to the importance of oral ingestion of the sorbed fraction to the toxicity:

'The test organism used in the present study, *Nitocra*, feeds on bacteria and small particles.' and 'We are convinced that the main route of exposure in Nitocra in our test system is via ingestion of particle-adsorbed PBDEs.

As cited already above, Breitholtz et al. (2003) recognize the importance of the sorbed fraction for both polycyclic musk compounds AHTN and HHCB as well.

Sensitivy of *Nitocra* as a sediment organism

For comparison to the results of the sediment toxicity tests, the estimated concentration sorbed to the particulate matter (expressed per kg organic carbon) can be related to a concentration in sediment. The concentration on organic carbon (f_{oc}) was normalised to a sediment containing 5% organic carbon:

$$\textit{Estimated conc. in normalised sediment} = \textit{Conc in on particulate matter}_{[OC]} * \textit{foc}$$

The results of the calculation for HHCB and AHTN are shown in table A2-1 and figure A2-1 and A2-2.

From this table it is clear that with the present measured concentrations in overlying water and log K_{oc}, the concentrations in particulate matter are similar to those in the sediment toxicity test with the sensitive benthic crustacean, *Hyallela azteca*. For *Nitocra spinipes* the variation in the parameter LDR seems relatively large. The LOEC for HHCB lies at 21-26%, while a similar positive effect ('stimulation') was found at equal concentration of AHTN. However, given the uncertainty in the calculation of the corresponding sediment concentrations, it can not be excluded that *Nitocra* is indeed the most sensitive of the tested benthic species.

Table A1-1: Calculated HHCB and AHTN 'sediment concentrations' based on log K_{oc} values.

	Calculated concentration in sediment with 5% OC in mg/kg dwt
HHCB	
Lumbriculus sediment test	NOEC (foc 5%) 38.6 (-13% biomass)
Hyalella sediment toxicity test	NOEC (foc 5%) 19.7 (-3% length, 0% biomass)
Nitocra 'aquatic toxicity test'	0.31 (-3% LDR) 1.1 (-20% LDR) 5.6 (-26% LDR) 20 (-21% LDR) 54 (-51% LDR)
AHTN	
Hyalella sediment toxicity test	NOEC (foc 5%) 42 (-3.5% length, -15% biomass)
Nitocra 'aquatic toxicity test'	0.77 (+ 10% LDR) 38 (-6% LDR) NOEC ≥38
Lumbriculus sediment toxicity test	NOEC (foc 5%) 17.2 (-13% biomass)

Therefore, a comparison of the acute toxicity studies, in which the organimsms are only exposed via the aqueous phase, is necessary. From the comparison with other acute toxicity data for benthic organisms tested in a water-only system it appears that *Nitocra spinipes* is not particularly sensitive for the polycyclic musk compounds.

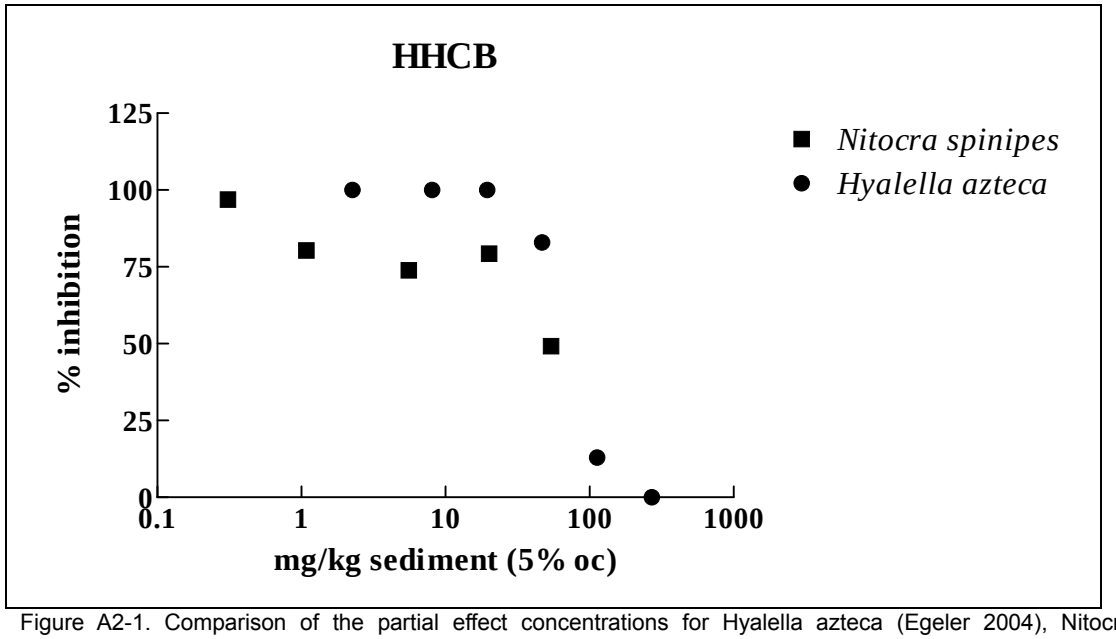


Figure A2-1. Comparison of the partial effect concentrations for Hyalella azteca (Egeler 2004), Nitocra spinipes (Breitholz et al. 2003) and Acartia tonsa (Wollenberger et al. (2003) for HHCB

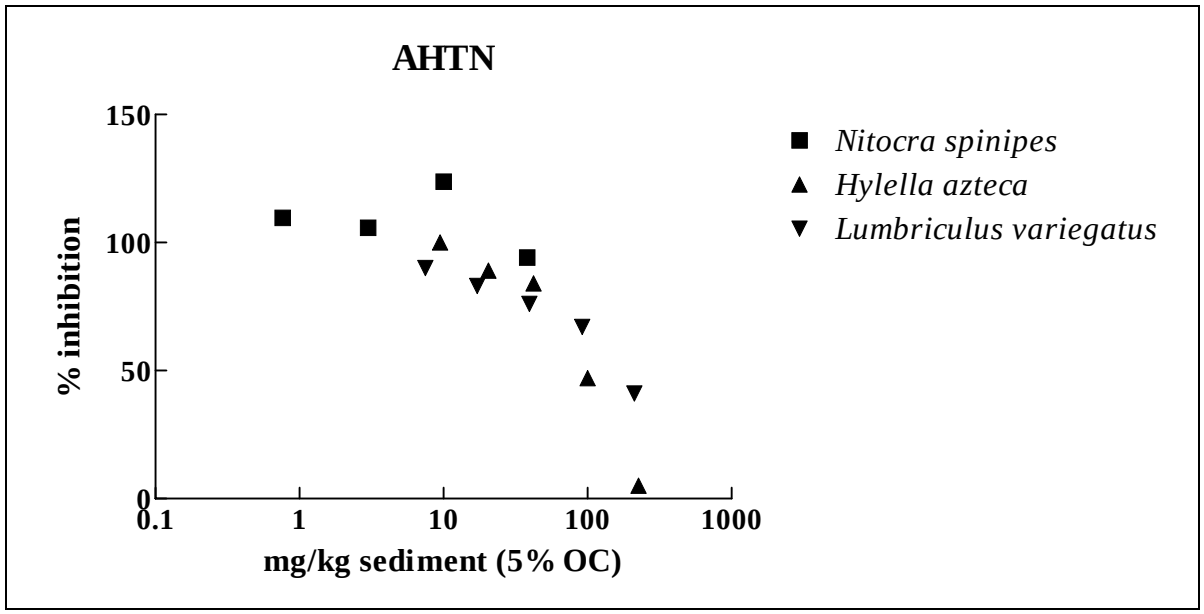


Figure A2-2. Comparison of the partial effect concentrations for Hyalella azteca (Egeler 2004), Lumbriculus variegatus (Egeler and Gilberg 2004b), Nitocra spinipes (Breitholz et al. 2003) for AHTN

For AHTN the 5-d EC50 for *Lumbriculus variegatus* was 0.397 mg/l (measured concentration). No acute toxic effects were observed for *Chironomus riparius* up to 0.460 mg/l (measured concentration). The 96-h LC50 for *Nitocra spinipes* was 0.61 mg/l (nominal concentration). For HHCB the 5-d EC50 for *Lumbriculus variegatus* was 0.394 mg/l (measured concentration). For *Chironomus riparius* the 96-h LC50 was 0.288 mg/l (measured concentration). The 96-h LC50 for *Nitocra spinipes* was 1.9 mg/l (nominal concentration).

In conclusion both for AHTN and HHCB at least the acute toxicity to *Lumbriculus variegatus* resulted in lower EC50s than the LC50 for *Nitocra spinipes*. For HHCB the LC50 for *Chironomus variegatus* was lower too, while for AHTN this could not be established, because the highest tested concentration was lower than the LC50 for *Nitocra spinipes*.

Conclusion

1. The decrease of the test substance concentration is probably caused by a combination of volatilisation and sorption to organic residues. This has caused a lower exposure through the water phasse as well as an additional exposure by oral uptake. Therefore the actual exposure durng the test cannot be established.

2. Based on the nominal concentrations for the *Nitocra* test, it is concluded that *Nitocra*, *Lumbriculus* and *Hyallella* have 'sediment' effective concentrations in the same range, but *Nitocra* seems to be slightly more sensitive in the case of HHCB. In principle, *Nitocra spinipes* is a benthic organism, and therefore repeating the aquatic toxicity test is not very useful for the risk assessment of the water compartment. Moreover, the same problems of decreasing aquatic concentrations and oral exposure to substance sorbed to particulate matter will occur. The conclusion is that the results of this test with the benthic species *Nitocra* can not be used as an aquatic toxicity test in which exposure should take place only via the water phase. If a toxicity test with this benthic species should be performed this should be a sediment test in which exposure takes place directly via the sediment. However, considering the other tests with benthic organisms and the absence of a suitable test protocol, this has not much additional value compared with the already available tests with benthic organisms. Therefore, a new test with *Nitocra spinipes* is not considered necessary.

Adsorption and volatilisation of a polycyclic musk in an algae test

The study of Kroon with *Scendesmus subspicatus* (see figure 3) showed that:

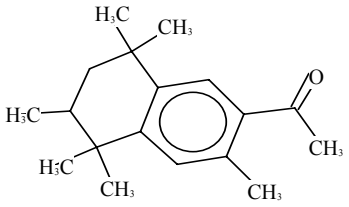
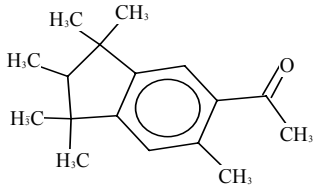
1. 20 – 25% of the test material is missing at the start of the test (other 'sinks' e.g., sorption to glass)
2. a considerable part (40% (= 1- (44/76)) of AHMI present in the algal suspension (10,000 cells/ml) was sorbed to the algal cells. Also when the total concentration in the vessel decreased in time, the fraction on the cells remained stable
3. the disappearance is linear in time. This could be explained by evaporation

If sorption is a partitioning process between the water phase and a lipid or organic matrix which is governed by log K_{ow}, the sorption of AHTN will probably be even higher than that of AHMI due to the higher log K_{ow} of AHTN. However, the results shown above would lead to bioconcentration factors by the algae, that would be much higher than would be predicted based on log K_{ow}.

The sorption in the test containing the larger alga *R. salina* at 50,000 cells/ml will be higher than the sorption in the test containing the smaller alga *S. subspicatus* at 10,000 cells/ml by an order of magnitude if sorption is based on log K_{ow} (cell volume ratio estimated to be about 20). However, if sorption is governed by the surface area available for sorption, the sorption

to the cell area will still be higher with *R. salina* compared to *S. subspicatus* by a factor of about 10.

Table A1-2. Structure of AHTN and AHMI

AHTN	**AHMI**
ClogP estimated log K_{ow} = 6.25	ClogP estimated log K_{ow} = 5.69
SRC estimated log K_{ow} = 6.35 measured log K_{ow} = 5.4	SRC estimated log K_{ow} = 5.85 with experimental value adjusted based on AHTN: estimated log K_{ow} = 4.91
	

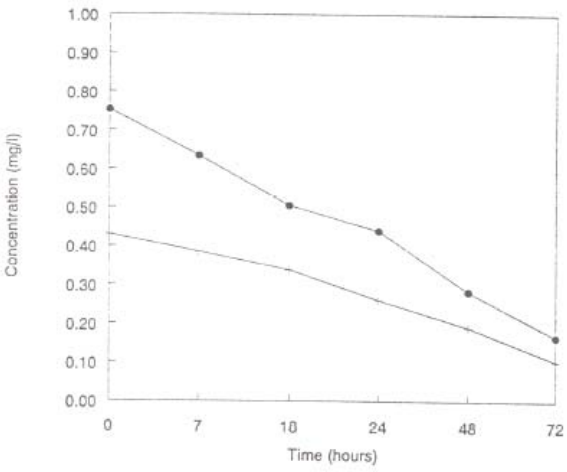


Fig. 2 Measured concentration of Phantolid® in aqueous solution incubated in the light with (+) or without (●) the addition of algae at the start of the test (1x10^4 cells/ml).

Figure A1-3. Adsorption of AHMI to algal cells (from Kroon 1998)

The report provides the comprehensive risk assessment of the substance 1,3,4,6,7,8-HEXAHYDRO-4,6,6,7,8,8-HEXAMETHYLCYCLOPENTA-g-2-BENZOPYRAN ((1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylin-deno[5,6-c]pyran - HHCB). It has been prepared by The Netherlands in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. The environmental risk assessment concludes that there is no concern for any of the environmental compartments.

For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified. The human health risk assessment concludes that there is no concern for any of these populations.