TC NES SUBGROUP ON IDENTIFICATION OF PBT AND VPVP SUBSTANCES

RESULTS OF THE EVALUATION OF THE PBT/VPVB PROPERTIES OF:

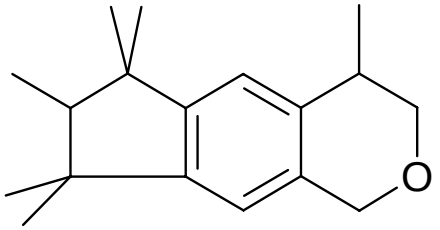
Substance name: 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylindeno(5,6-c)pyran

EC number: 214-946-9

CAS number: 1222-05-5

Molecular formula: C18H26O

Structural formula:



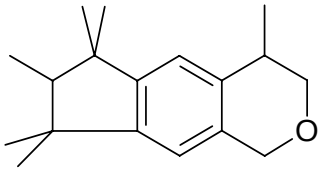
Summary of the evaluation:

1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylindeno(5,6-c)pyran (HHCB) is not considered to be a PBT substance. It does not meet the P, B and T-criteria. The biodegradation products of HHCB were not assessed for persistency but they do not fulfil the B-screening criterion.

JUSTIFICATION

1 IDENTIFICATION OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

Name:	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylindeno(5,6-c)pyran
EC Number:	214-946-9
CAS Number:	1222-05-5
IUPAC Name:	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran
Molecular Formula:	C18H26O
Structural Formula:	

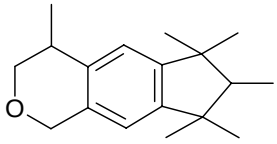


Molecular Weight:	258.41
Synonyms:	**HHCB** Abbalide Chromanolide Pearlide Galaxolide

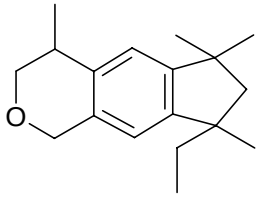
1.1 Purity/Impurities/Additives

According to IFF (2001), the sum of the isomers illustrated below in typical composition of a technical product is ≥ 95% w/w.

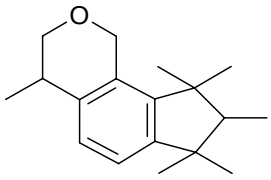
CAS No 1222-05-5 Main isomer	74-76%	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-γ-2-benzopyran



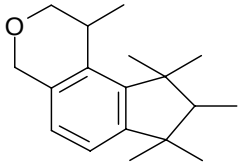
CAS Nos 78448-48-3 and 78448-49-4:	6-10%	1,3,4,6,7,8-hexahydro-4,6,6,8-tetramethyl-(6 or 8)-ethylcyclopenta- γ -2-benzopyran



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%	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%	β,1,1,2,3,3-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,

Impurities: The PBT properties of the impurities were not assessed by the working group, except for CAS No 1217-08-9. For this substance it was concluded that it does not meet the PBT criteria (Rapporteur Sweden, ECB website), based on read-across with Galaxolide.

1.2 Physico-Chemical properties

Table 1 Summary of physico-chemical properties. For details and references, see European Commission (2008).

REACH ref Annex, §	Property	Value	Comments
V, 5.1	Physical state at 20 ºC and 101.3 Kpa	viscous liquid	IFF(2001)
V, 5.2	Melting / freezing point	-10 - 0 ºC	IFF (2001)
V, 5.3	Boiling point	160 ºC (at 4 mmHg), corresponds 330 ºC at 760 mmHg	IFF (2001)
V, 5.5	Vapour pressure	0.0727 Pa at 25 °C	MacGillivray, 1966
V, 5.7	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)	Edwards, 1996 (the value chosen for the risk assessment; other values available)
V, 5.8	Partition coefficient n-octanol/water (log value)	5.3	Artola-Garicano, 2002 (the value chosen for the risk assessment; other values available)
VII, 5.19	Dissociation constant	-	

2 MANUFACTURE AND USES

HHCB is produced at one site in Europe with a production volume of 1000 - 5000 tpa (year 2000). Approximately 63% of the production volume is exported outside Europe. The use volume in the year 2000 was 1,427 t (used for the calculations) and the use volume for the year 2004 is 1,307 t. The use volume has been declining since the early nineties (European Commission, 2008).

The substance is used as an ingredient in fragrance oils for a wide variety of consumer products like cleaning agents, cosmetics and fabric conditioners. HHCB is used in diluted form due to its viscosity. Dilution to an organic solvent (to 65 % w/w) is carried out mainly at a separate site after production. Two following industrial life-cycle steps are generally necessary for the manufacture of end products. HHCB is first compounded into a fragrance oil after which the fragrance oil is mixed in a formulation step to the end products (European Commission, 2008).

3 CLASSIFICATION AND LABELLING

HHCB is not classified in the Annex I of Directive 67/548/EEC:

Proposal in the draft risk assessment (European Commission, 2008):

N; R50-53 Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation (P)

4.1.1 Abiotic degradation

Indirect photochemical degradation in the atmosphere is considered to be fast based on the estimated rate constant for the reaction with OH-radicals using AOP v1.91 of $3.8 \cdot 10^{-11}$ cm^3 $molecule^{-1}s^{-1}$. The experimentally obtained reaction rate is $2.6 \pm 0.6 \cdot 10^{-11}$ cm^3 $molecule^{-1}s^{-1}$. With this rate the half-life was 3.7 h (12 h day^{-1}; $1.5*10^6$ OH cm^3, Aschmann et al, 2001). With the TGD defaults (24 h day^{-1}; $5*10^5$ OH cm^{-3}) this corresponds with half-lives of 15 and 10 h, respectively.

Buerge et al. (2003) observed a degradation rate of 0.15 d^{-1} (DT50 of 109 h) for lake Zürichsee water and 0.12 d^{-1} (DT50 of 135 h) for distilled water illuminated with actinic lamps, comparable to 24h-averaged sunlight in July under clear sky radiation conditions (50 ºN). Degradation was concluded to be mainly due to direct photodegradation. The author derived an average photolysis rate range of 3.3 to 6.6 * 10^{-5} d^{-1} 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). In summer, considering only the epilimnion, they estimated a rate about 2 orders of magnitude higher than in winter for the whole lake: 3.2 – 6.5 * 10^{-3} d^{-1}. Due to very varying attenuation conditions of European water bodies, the results cannot be used as representative for photodegradation rate in general.

Based on a preliminary identification of photodegradation products in a laboratory study under radiation of 248 nm wavelength, Sanchez-Prado et al. (2004) concluded that HHCB does not photodegrade from 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.

Environmentally relevant exposure occurs in the whole water column and, in the case of HHCB, especially in sediment. Photodegradation of HHCB can be expected to be a relevant removal pathway in the environment only in shallow clear waters and in the first few centimetres layer of the water column with some exceptions. Therefore aquatic photodegradation is not considered to have relevant impact on the overall persistency of HHCB in the environment.

4.1.2 Biotic degradation

No mineralization was observed in the two ready biodegradability tests (King, 1994, a modification of OECD 301B, adapted sludge, isopropyl myristate as dispersant; Jenkins, 1991, OECD 301B, non-adapted sludge).

Degradation of ^{14}C-HHCB spiked to activated sludge samples from three STPs (test conc. 25 µg l^{-1}) and to river water (test conc. 0.5 µg l^{-1}) was followed by Federle et al. (2002). The same study was also reported with slightly different results by Langworthy et al. (2000). The first order rates for the disappearance of HHCB in activated sludge were 0.010 and 0.021 h^{-1} with 5 and 25 µg l^{-1}, respectively (or DT_{50} of 69 and 33 hours). No significant volatilization was observed. For river water test, the disappearance rate constant was 0.016 h^{-1} (DT_{50} = 43 h). After 300 hours (12.5 d) 40 % of initial radioactivity was volatilized from water.

The first metabolite formed in the sludge experiments was co-eluting in Rad-TLC analysis with lactone (HHCB-lactone, i.e., Galaxolidone, was the suggested identity of the metabolite) and accounted for about 40% of the original radioactivity between day 1 and 8. Gradually a second

metabolite emerged making up to 45% of the radioactivity after 650 hours. This metabolite had a similar elution time as hydroxycarboxylic acid. A third, highly polar metabolite made up to 15% of the radioactivity after 150 hours (Langworthy et al., 2000). Following termination of the test (68 days) the metabolites in the supernatant were characterised using reversed phase HPLC and the authors observed that the degradation products had even lower adsorption potential than the standards HHCB-lactone (log K_{ow} 4.0) and -hydroxycarboxylic acid (log K_{ow} 0.5). A log K_{ow} between 0.1 and 3.1 was suggested for the metabolites. It was concluded, that further oxidation of metabolites had occurred .

The emergence of Galaxolidone in waste water was observed also in a study by Brändli (2002) during the transport in the sewer and sewage treatment. Bester (2003) showed that this structure is produced during sewage treatment.

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

Schaefer et al. (2005) described a series of die-away tests for ^{14}C-HHCB using test concentrations of 17.4 μg l^{-1} in activated sludge (2.5 g TSS l^{-1}) and 5 μg l^{-1} in river water (with 10 mg TSS l^{-1}) performed in 1-gallon (3.8 l) test vessels. They derived overall half-lives of 10 - 15 h and 100 h in sludge and in river water, respectively. These half-lives refer to the parent compound disappearance. No mineralization to CO_2 was observed in the river die-away test. A variety of metabolites was formed at different rates. Degradation products made ca. 62% of added radioactivity at the end of the test (day 28) in solvent extract and 15.9% had volatilized. The biological degradation after 28 days was circa 60%. The polarity of degradation products increased during the test.

No experimental data on degradation in marine environment are available.

PFW (1996 and 1997) reported to have isolated from soils collected in the Netherlands several cultures of the fungus *Phanerochaete chrysosporium* which were capable of degrading HHCB. Approximately 40% of the 64 soil samples showed a positive degrading potential towards one or both of the polycyclic musks AHTN and HHCB (17 % of soils degrading HHCB). In cultures of *P. chrysosporium* (ATCC 32629) HHCB disappeared in 3 days.

Degradation of HHCB ^{14}C-labelled in the aromatic ring was investigated using two *P. chrysosporium* strains and two unknown fungus strains growing in culture media at pH 4. All strains were observed to degrade HHCB to more polar, non-volatile metabolites but mineralization was not observed (Envirogen 1997).

The most viable strain (identified as *Cladosporium cladosporiodes*) was investigated additionally in a culture, where the growth medium was after 4 weeks adjusted to pH 7 and amended with additional nutrients and an inoculum from mixed soil and sludge samples. Transformation of 81 and 95% of the HHCB followed 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. Amounts of polar metabolites increased during the test. Mineralization 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 percent 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 degradable by other common soil organisms (bacteria or fungi) (Envirogen 1997).

Envirogen (1998) followed the fate of ^{14}C-HHCB for one year in flasks containing oak forest soil, agricultural soil (no sludge), domestic sludge amended agricultural soil and sediment of the Delaware River (NJ). The samples were spiked with 10 µg HHCB g^{-1} soil (10 mg kg^{-1}) and incubated at laboratory temperature. Only 4, 7 and 9% of the initial HHCB concentration remained after one year in the river sediment, the forest soil and the sludge amended soil, respectively. In non-amended agricultural soil 35% remained after one year. A TLC analysis showed that HHCB had been degraded to various fractions which were more polar than HHCB. Estimated primary degradation 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 ([1]) and 0.0088 d^{-1} and 79 days for river sediment.

4.1.3 Other information [2]

The available temporal series of monitoring data indicate that HHCB would not be persistent in sediment, which can be considered based on the physical-chemical properties to be the most relevant compartment of distribution together with soil. The decrease of concentrations in sewage sludge, water and suspended mater, which reflect the reduction of the input of HHCB to sewers over the years seem to be followed by a similar decrease of concentrations in sediment. This phenomenon is illustrated by Figure 1 and Table 2. Other available monitoring data show a similar trend (European Commission, 2008).

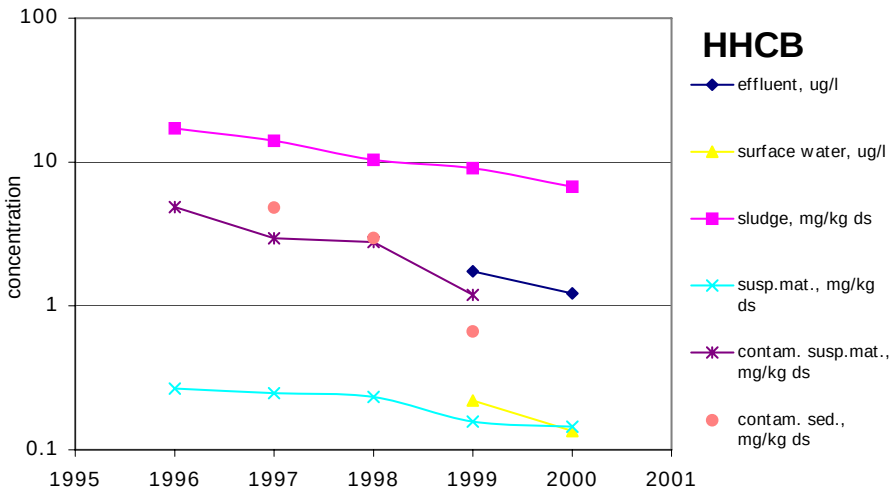


Figure 1. Trend in the median concentrations of HHCB in various environmental compartments, Hessen, Germany (based on data from HLUG 2001) (Remark: Contaminated suspended matter: mean for 3 sites; contaminated sediment: only one site. Logarithmic scale).

[1] 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)

[2] For example, half life from field studies or monitoring data

Table 2. 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	median	6.7		1.6	4
µg/l	90th-perc.	10.8			
	max	13.3		2.1	
Sludge	median		8.3	10.2	1
mg/kg dwt	90th-perc.				
	max		11.5	12	
Sediment	median	0.9		0.26	3.5
mg/kg dwt	90th-perc.	1.9			
	max	2.2		1.1	

It must be noted that monitoring data on degradation products of HHCB has not been reviewed.

DiFrancesco et al. (2004) conducted in mesocosm scale a 1-year die-away study of fragrance materials in four different soils. Soil samples were amended with sludge and they contained initially HHCB between 0.1 and 0.27 mg kg^{-1} dwt. A parallel set of soils was amended with sludge and in addition spiked with HHCB resulting initial concentration of 6 and 13 mg kg^{-1} dwt. The test trays containing 24 liter soil and mixed with 1 liter sludge were placed outdoors. After 3 months, the concentrations were 8 to 60% of the initial concentrations. 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. Leachate was collected during first 3 to 5 months. The leached amount was 0.03 to 0.18% of the initial amount in the spiked soils and in the leachate from the unspiked soils HHCB was not detected. Hence the influence of leaching on the disappearance was negligible. No relation of concentrations in leachate and the organic matter content of soils could be found. The share of volatilization and degradation of the disappearance were not determined. According to HLC of 36.9 Pa m^3 mol^{-1} determined by Artola-Garicano (2002), HHCB is volatile from moist surfaces and hence the influence of volatilization on the disappeareance may have been considerable.

AHTN and HHCB concentrations in 13 field locations in Baden-Württemberg, Germany, were measured in 2002 and compared to estimated concentrations after last sludge application. Different, recorded quantities of sludge had been applied in different periods to each field and the time from the last sludge application varied from field to field. Reference fields were also included. The remaining AHTN+HHCB concentration after the last application was calculated to be 0.2 % after 13 years, 0.5 % after 7 years, < 0.4 % after 4 years, 1.9 % after 3 years and < 0.3 % after 3 years, 1,8 after 2 years and 1.3 % after 1 year (LfU-BW 2003). It must be noted that the estimated disappearance reflect the influence of all losses. On the basis of physical-chemical properties of AHTN and HHCB, loss via leaching can be considered as very low, whereas volatilization can be expected to have caused a relevant part of disappearance.

4.1.4 Summary and discussion of persistence

Indirect photochemical degradation half-life for the atmosphere of 10 hours for the reaction with OH-radicals have been predicted using AOP v1.91 (24 h day^{-1}; $5*10^5$ OH^- cm^{-3}).

Degradation simulation tests are not available for marine water or marine sediment. Evidence of fast primary degradation is provided from the available experiments with sludge, river water, soil and sediment. However, the studies reviewed detected negligible or very low mineralization rates of HHCB. Instead, polar metabolites were formed readily and polarity increased over time.

Half-life for primary degradation of HHCB in activated sludge was less than 3 days (Federle et al. 2002, Langworthy et al. 2000, Artola-Garicano 2002). Schaefer et al. (2005) observed in die-away tests disappearance half-lives of 10 - 15 h in activated sludge and 100 h in river water. With 10 mg/l suspended solids from activated sludge, the conditions in the river die-away test simulate the situation after release of test substance in effluent into river with respect to the concentration and origin of suspended solids in the receiving river. The sediment downstream of a STP is formed by settlement of suspended solids that will originate at least partly from the solids discharged with the effluent (30 mg/l). Therefore the conditions are environmentally relevant but do not correspond with standard biodegradation simulation tests in surface water, where an amendment with sediment from the same site is allowed, but not with suspended solids derived directly from an STP. With 5 µg/l is the HHCB concentration in the test still relatively high.

In addition, a primary degradation half-life of 78 days was observed in sediment as reported by Envirogen (1998).

The available monitoring data from sludge, surface water, suspended matter and sediment provide evidence that HHCB degrades in the aquatic compartment. Measured concentrations in these compartments have been decreasing over time and follow hence the diminishing use volume. It must be noted, however, that no monitoring data on metabolites have been reviewed.

Three soil studies (LfU-BW, 2003; DiFrancesco et al., 2004; Envirogen, 1998) have measured the degradation rate of HHCB in soil. These studies give evidence that HHCB is degraded by soil organisms in a moderate to fast rate. The environmentally most relevant study (LfU-BW, 2003) measured disappearance of AHTN and HHCB over several years in 13 field locations, where the substances had been applied with STP sludge to soil. On the basis of this study, HHCB is not expected to have long residence time in soils. The study does not distinguish between different dissipation routes and volatilisation may have caused a large part of disappearance. However, HHCB is fast degraded in air by indirect photochemical reaction with OH –molecules and hence the part volatilised is not distributed further.

Langworthy, et al. (2000), in their biodegradation test using activated sludge, observed during the degradation process the formation of metabolites of HHCB, from a lactone-derivative of HHCB (Galaxolidone) and a hydroxycarboxylic acid –derivative, which are more polar than HHCB to even more polar metabolites (unidentified) in relevant amounts showing log Kow < 1. These metabolites are assumed to be the most relevant metabolites also in water and soil. HHCB-lactone has been identified also by other authors.

4.2 Environmental distribution

Data not reviewed for this report.

4.2.1 Adsorption

4.2.2 Volatilisation

4.2.3 Long-range environmental transport

4.3 Bioaccumulation (B)

4.3.1 Screening data[3]

A predicted BCF of 6,383 was obtained using the QSAR according to the TGD (EC 2003) and log Kow of 5.3. BCFWIN v2.15 calculates a BCF of 2,404 using the same logKow -value. BCFWIN takes in addition to logKow –value also the molecular structure into account in its estimation.

The main biodegradation metabolites were observed by Langworthy et al. (2000) to be eluted in TLC together with HHCB-lactone (logKow 4) and HHCB-hydroxycarboxylic acid (logKow 0.5). Reverse phase HPLC analysis showed that 70% of the metabolites had log Kow < 0.1, 25% had log Kow 2.0 and for the remaining 5% log Kow was 3.3.

4.3.2 Measured bioaccumulation data[4]

Van Dijk (1996) carried out a flow through bioconcentration test according to the former OECD Guideline 305E. *Lepomis macrochirus* were exposed to two concentrations of radio-labelled HHCB. A solubiliser (DMF, Tween 80) was used in a concentration of 0.001% w/v to prepare a solution. Nominal exposure concentrations were 1 and 10 µg l^{-1}. The fish were exposed for 28 days followed by an elimination period of 28 days. BCF was derived from actual concentrations of parent compound in water and the steady-state concentration in fish (days 21 and 28). BCF for the whole fish was 1,584.

Polar metabolites became apparent in water from day 3 of the exposure period. They accounted for 10 to 19 % of total radioactivity in water. Also polar metabolites in fish were detected at the level of 9 to 24 % of total radioactivity in tissue (for edibles and non-edibles, respectively). The polar metabolites in water and fish were identical. BCF based on total radioactivity (parent and metabolites) in fish was 1,624. Van de Plassche and Balk (1997) and Balk and Ford (1999a) conclude that during exposure and depuration HHCB is metabolised and the metabolites are excreted with a turnover rate of about 38 – 50% per day.

In another study according to OECD 305E, *Brachydanio rerio* was exposed to a test concentration of 7.3 µg l^{-1}. Methanol (0.05 %) was used as solvent and the test was conducted in tap water at pH of ca. 8. Depuration half-life was determined to be less than three days. Bioconcentration factor was determined to be 624 (Butte and Ewald, 1999).

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[3] For example, log K_{ow} values, predicted BCFs

[4] For example, fish bioconcentration factor

A semi-static test with *Brachydanio rerio* used nominal test concentrations of 25.8 and 258 μg l^{-1} (Schreurs et al, 2004). The estimated BCF from this study is 2,400-2,500. These values are calculated from the average of fluctuating exposure concentrations. The study has several deficiencies, i.a., too large fluctuation of test concentrations (down to 5 % of nominal before test media renewals), too high exposure concentrations and a low number of fish. In addition, accumulation via feeding cannot be excluded.

Artola-Garicano (2002) reported on an HHCB accumulation test with fourth instar midge larvae (*Chironomus riparius*) and the worm *Lumbriculus variegatus* using a flow-through system. The organisms were not fed during the 12 d exposure period. In parallel to the bioconcentration experiment, a similar experiment was run with the addition of 5 mg l^{-1} of the cytochrome P-450 inhibitor piperonyl butoxide (PBO). The aqueous HHCB concentrations in the test with *C. riparius* were stable at 9.8 ± 1.4 μg l^{-1}. The concentrations in the larvae increased to a maximum level between day 1 and 3 and then the level decreased to a new steady state. A BCF of 85 – 138 could be calculated from the test. With the addition of PBO, BCF was 525, and hence Artola-Garicano (2002) concludes that HHCB is likely to be biotransformed in midge larvae. The exposure concentration in the experiment with *L. variegatus* was 4.6 ± 0.6 μg l^{-1}. The uptake of HHCB in worms reached a plateau level after 3 days. Log BCF was determined at 3.43 indicating that in this organism biotransformation does not take place.

On the basis of a plant uptake study of Müller et al. (2002) using lettuce and carrots, it was concluded that transfer of HHCB from soil to plants is not a relevant accumulation pathway (European Commission, 2008).

4.3.3 Other supporting information[5]

Field bioaccumulation factors have been derived for HHCB based on measured concentrations in water and fish.

Table 3. Field derived ratios of C_{fish} and C_{water} expressed as BAF for fish. For details and references, see European Commission (2008).

BAF	Source
Eel BCF_{wwt} = 150 to 600 non-eel BCF_{wwt} = 49 to 188	Balk and Ford (1999a) from data in Eschke et al. (1995), Rijs and Schäfer (1998), Rimkus (1997)
Eel BCF_{wwt} = 862 (range 201 – 1561)	Fromme et al. (2001b)
Eel BCF_{wwt} = 995	Heberer (2002) from data of Fromme et al. (2001b)
Rudd BCF_{wwt} = 20 Tench BCF_{wwt} = 510 Crucian carp BCF_{wwt} = 580 Eel BCF_{wwt} = 290 Zebra mussel BCF_{wwt} = 620	Gatermann et al. (2002a) *

* Species differences related to fat content

[5] For example, measured concentrations in biota

4.3.4 Summary and discussion of bioaccumulation

Bioconcentration in *Lepomis macrochirus* was studied according to OECD Guideline 305E. The BCF of 1,584 was determined. Polar metabolites were found in tissue as well as in the water. The depuration of HHCB metabolites was observed to be fast (Van Dijk 1996). This study was carried out under GLP. In another bioconcentration study with *Brachydanio rerio* according to OECD Guideline 305E the measured BCF was 624 (Butte and Ewald, 1999). BAF -values determined from actual measurements in fish and surface water are available and they range from 20 to 1,561 depending on the species.

The BCF for fish can be considered to be between 600 and 1,600 and thus it is concluded that HHCB has a moderate to high bioaccumulation potential. However, there is an indication that it may accumulate in *Lumbriculus variegatus,* which is not capable of metabolising the substance.

On the basis of the available experimental and field data, HHCB is considered to have a moderate to high bioaccumulation potential. In the EU risk assessment of HHCB (European Commisson, 2008), the BCF of 1,584 derived by Van Dijk (1996) is used as a representative value for bioaccumulation.

The biodegradation studies showed the consecutive formation of increasingly more polar metabolites, starting from log Kow < 4 but with the majority at log Kow < 1 (Langworthy et al. 2000). Hence, the biodegradation products are expected to have low to moderate bioaccumulation potential.

5 HUMAN HEALTH HAZARD 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.

6 ENVIRONMENTAL HAZARD ASSESSMENT

6.1 Aquatic compartment (including sediment)

Based on its UV/Vis spectrum, HHCB is expected to photodegrade at radiation wavelengths below 300 nm. Therefore the radiation in laboratory conditions under fluorescent lamps in the ecotoxicity studies (> 400 nm) is not assumed to have caused photodegradation.

6.1.1 Toxicity test results

Available aquatic ecotoxicity test results have been reviewed in the risk assessment of HHCB (European Commission, 2008). Table 4 provides an overview of the results from long-term and prolonged tests with most sensitive results.

L(E)C50-values for acute tests are above 0.1 mg l^{-1} and results from chronic and prolonged studies are above 0.01 mg l^{-1} .

Table 4. Aquatic toxicity of HHCB from long-term and prolonged tests with lowest results (GLP and completely documented). For details and reference, see European Commission (2008).

Test organisms	Results[1] [mg l^{-1}]	Remarks[2]
Pseudokirchneriella subcapitata[3] 72-h static	72h-NOEC = 0.201 LOEC[5] = 0.466 ErC_{50} > 0.854 EbC_{50} = 0.723 <0.678-0.778>	Van Dijk (1997) 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 valid without restrictions
Daphnia magna 21-d semi-static	21d-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]	Wüthrich (1996a) 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 valid without restrictions
Acartia tonsa 6d-static, daily feeding	6d-NOECdevelop. = 0.038 LOECdevelop[5].= 0.075 6d-EC10develop.= 0.044 <0.030-0.055> EC50develop.= 0.115 <0.131-0.153>	Bjørnestad (2007) Radiolabelled ^{14}C-HHCB solved in ethanol (< 0.01%) n=5 LSC identification conc. > 80% of nominal valid without restrictions
Bluegill sunfish *Lepomis macrochirus* 21-d flow-through	21d-NOECclinical signs = 0.093, LOEC[5] = 0.182 NOECgrowth = 0.182 LC_{50} = 0.452 <0.316-0.911>	Wüthrich (1996b) carrier: 0.005% DMF and 0.005% Tween 80 n=5 HPLC identification conc. 66-86% of nominal valid without restrictions
Fathead minnow *Pimephales promelas* 32 days post hatch, 36 days overall	LOEChatch > 0.140 NOECsurv. = 0.068 LOECsurv. = 0.140 LC50 > 0.140 NOECgrowth = 0.068, LOECgrowth[6] = 0.140 NOECdevelop.= 0.068, LOECdevelop.= 0.140	Croudace (1997) solvent triethylene glycol GC identification conc. 50-104% of nominal valid without restrictions

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)

6.1.2 Sediment organisms

Three reliable sediment ecotoxicity studies are available (see table 5) and they have been reviewed in the risk assessment of HHCB (European Commission, 2008). The lowest NOEC was found for *Hyalella azteca* (NOEC = 19.7 mg/kg dwt, normalized to 5 % OC) in the study of Egeler (2004).

Table 5. Summary of sediment toxicity data. For details and references, see European Commission (2008)

	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

6.1.3 Other aquatic organisms

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).

6.2 Terrestrial compartment

Long term ecotoxicity test results are available for *Eisenia fetida* and *Folsomia candida* (see table 6*)*. The studies have been reviewed in the risk assessment of HHCB (European Commission, 2008). No data on ecotoxicity to plants or soil micro-organisms are available.

Table 6. Long-term toxicity data for soil organisms. For details and references, see European Commission (2008).

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

Test and reference	Results for HHCB (nominal concentrations)	Remarks
Springtail *Folsomia candida*	4wk-NOEC = 45 mg/kg, LOEC[2] = 105 mg/kg, mortality and reproduction	Klepka 1997 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)

6.3 Atmospheric compartment

No experimental data available.

7 PBT AND vPvB

7.1 PBT, vPvB assessment

Persistence: HHCB does not meet the P criterion due to relatively fast primary degradation. No standard degradation simulation test data are available for HHCB. Evidence of inherent biodegradability from tests with activated sludge, river water, sediment and soil is available. Rapid disappearance was observed in tests with activated sludge, river water, river sediment and soil. Polar metabolites were readily formed in activated sludge and river water. In experiments with soil and river water, volatilisation may have contributed significantly to the disappearance, but due to fast degradation of HHCB in air, possible volatilisation is considered to enhance the overall degradation rate. In a series of die-away tests on HHCB, disappearance half-lives of 10 - 15 h in activated sludge and 100 h in river water were determined. In the river water simulation test, amended with 10 mg/l activated sludge, the loss of HHCB solely due to biodegradation was 60% in 28 days. The river die-away test half-life of 100 hours and other half-lives from available experiments are not directly comparable to half-lives derived from standard biodegradation simulation tests, but the observed half-lives are so short, that P criteria for all compartments are considered not fulfilled for primary degradation.

However, it must be noted that negligible or zero mineralization has been observed in the reported experiments. During the degradation process the formation of metabolites has been observed with an increasing polarity, from HHCB-lactone, a hydroxycarboxylic acid product of HHCB and resulting in a majority of metabolites showing log Kow < 1. A detailed evaluation of persistence of metabolites was not feasible based on data available.

Monitoring data from several years show decreasing trend of concentrations in sludge, water, suspended matter, biota and sediment and provide hence additional evidence on that HHCB degrades in the aquatic environment.

A study investigating the residence of HHCB in 13 soils with different histories of sludge application showed that the substance had disappeared almost completely regardless of the time

elapsed since the last sludge application. Volatilisation may have contributed to the disappearance but this distribution route is not considered relevant, because HHCB is rapidly degraded in the atmosphere.

Bioaccumulation: the substance does not meet the B criterion. Based on reliable experimental BCFs for *Brachydanio rerio* and *Lepomis macrochirus* and BAFs derived from concentrations measured in field, BCF between 600 and 1,600 (parent compound) is considered representative for fish. In addition, metabolites have been followed in the study with *Lepomis macrochirus*. Fish was observed to biotransform HHCB and excrete the metabolites fast. BCF below 2,000 for the parent and metabolites together was determined.

For *Chironomus riparius* larvae a BCF of 85-138 (parent compound) was derived and the larvae were responding to blocking the receptor P450. Hence, the midge larvae can be assumed to biotransform HHCB. However, a similar test conducted with *Lumbriculus variegatus* resulted a BCF of 2,692 and it was concluded that the species could not biotransform HHCB but was accumulating it.

The primary biodegradation products have $\log K_{ow}$ below 4 and further degradation products are increasing more polar to log Kow < 1. Hence, biodegradation products do not meet the B screening criterion.

Toxicity: the substance does not meet the T criterion. Long-term and prolonged aquatic ecotoxicity studies are available for algae, three fish species, *Daphnia magna* and *Acartia tonsa*. In addition, several prolonged studies for invertebrates have been reported. NOECs from all tests are > 0.01 mg l^{-1}. There are no indications for an assignment of T on basis of human-toxicological data. It is concluded that the T criterion is not met.

Summary: HHCB does not meet the P criterion due to fast primary degradation. In addition, it neither fulfils the B criterion nor the T criterion. The biodegradation products do not fulfil the B screening criterion.

It is concluded that HHCB is not considered as a PBT-substance.

INFORMATION ON USE AND EXPOSURE

Not relevant as the substance is not identified as a PBT.

OTHER INFORMATION

The information and references used in this report were taken from the following source:

European Commission, 2008. European Risk Assessment Report, Final draft for submission to SCHER of January 2008, HHCB, CAS No: 1222-05-5, EINECS No: 214-946-9.