TC NES SUBGROUP ON IDENTIFICATION OF PBT AND VPVP SUBSTANCES

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

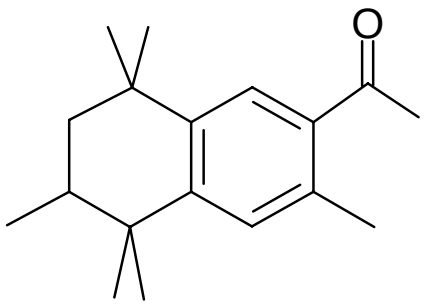
Substance name: 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one

EC number: 216-133-4 or 244-240-6

CAS number: 1506-02-1 or 21145-77-7

Molecular formula: C18H26O

Structural formula:



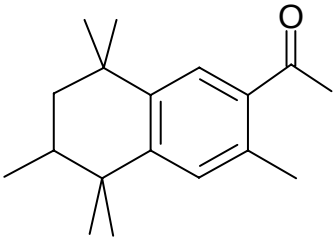
Summary of the evaluation:

1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one (AHTN) is not considered to be a PBT substance. It does not meet the P, B and T criteria. The biodegradation products 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-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one
EC Number: 216-133-4 or 244-240-6
CAS Number: 1506-02-1 or 21145-77-7
IUPAC Name: 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline
Molecular Formula: C18H26O
Structural Formula:



Molecular Weight: 258.41
Synonyms: 1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-napthyl)ethan-1-one
2' –Acetonaphtone, 5',6',7',8'-tetrahydro-3',5',5',6',8',8'-hexamethyl
6-Acetyl-1,1,2,4,4,7-hexamethyl-1,2,3,4-tetrahydronaphtalene
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline
7-Aceto-1,1,3,4,4,6-hexamethyltetraline
7-Aceto-1,2,3,4-tetrahydro-1,1,3,4,4,6-hexamethylnaphtalene
7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphtalene
AHMT
AHTN

1.1 Purity/Impurities/Additives

According to PFW (2001), the purity of 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one (AHTN) is ≥ 98% w/w. Technical grade AHTN contains two optical enantiomers in a ratio of 1:1.

Impurities: 6-Acetyl-3-isopropyl-1,1,3,5-tetramethylindane, ca. 0.35% w/w
1,1,2,3,3,6-Hexamethylindan-5-yl methyl ketone, ca. 0.18% w/w (CAS-No 15323-35-0)
7-Acetyl-1,1,3,4,4,6-hexamethyltetraline, ca. 0.08% w/w

Separation of the enantiomers is only possible with very special enantioselective (chiral) GC or HPLC columns and therefore normal environmental analyses do not distinguish between the enantiomers. Four studies (Franke et al. 1999; Gatermann et al., 2002b; Berset et al., 2003; Bester et al., 2002) have investigated environmental samples (fish, shellfish, stp sludge, SPMD, stp effluent) for both enantiomers. European Commission (2008) concludes that the enantiomer ratio in environmental samples is the same as in the reference AHTN sample.

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 C and 101.3 Kpa	solid	European Commission (2008)
V, 5.2	Melting / freezing point	> 54 ºC	Janssen, 2004a
V, 5.3	Boiling point	326 ºC (at 1013 hPa)	Janssen, 2004b
V, 5.5	Vapour pressure	0.0682 Pa at 25 °C	MacGillivray, 1996
V, 5.7	Water solubility	1.25 mg/l at 25 °C (1.31 mg/l at pH 5; 1.22 mg/l at pH 7 and 9)	Edwards, 1996 (the value chosen for the risk assessment; other values available)
V, 5.8	Partition coefficient n-octanol/water (log value)	5.4	Artola-Garicano, 2002 (the value chosen for the risk assessment; other values available)
VII, 5.19	Dissociation constant		

2 MANUFACTURE AND USES

AHTN is produced at one site in Europe with a production volume of 1000 - 5000 tpa. Approximately 62% of the production volume is exported outside Europe. The European use volume in the year 2000 was 358 t (used for the calculations) and based on a recent survey for 2004, the total use volume has declined with 30% between the year 2000 and 2004 to 247 t. The use volume has been declining steadily 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. Two separate industrial life-cycle steps are generally necessary for the manufacture of the end products. AHTN is first compounded into the fragrance oil after which the fragrance oil is mixed in a formulation step to the end products (European Commission, 2008).

3 CLASSIFICATION AND LABELLING

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

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

Xn; R22	Harmful if swallowed
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 relatively fast based on the estimated half-life of 7.3 hours for the reaction with OH-radicals (12 h day^{-1}; $1.5*10^6$ OH cm^3) using AOP v1.91 as reported by Aschmann et al. (2001). Using the TGD defaults (24 h day^{-1}; $5*10^5$ OH cm^{-3}) the estimated half-life is 22 hours.

Buerge et al. (2003) observed degradation half-lives of approximately 4 hours for AHTN in lake Zürichsee water and 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 due to direct photodegradation. The author derived an average photolysis rate range of 1.0 to 2.0 * 10^{-3} 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: 0.10 – 0.19 d^{-1}. Due to very varying attenuation conditions of European water bodies, the results cannot be used as representative for photodegradation rate in general.

Environmentally relevant exposure occurs in the whole water column and, in the case of AHTN, especially in sediment. Photodegradation of AHTN can be expected to be a relevant removal pathway in the environment only in very 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 AHTN in the environment.

4.1.2 Biotic degradation

In two ready biodegradability tests (Jenkins, 1991, OECD 301B, non-adapted sludge and King, 1994, a modification of OECD 301B, adapted sludge) 0 % mineralization was observed, despite the use of a dispersant in the last mentioned study.

An inherent biodegradability test conducted by Rudio (1993b) according to OECD 302C, resulted in no degradation (measured as BOD; adapted sludge). In a two-phase closed bottle test of Boersma and Hagens (1991) using 22 mg l^{-1} AHTN and repetitive additions after 4 and 5 weeks of exposure, a degradation of 21 % after 3 weeks and 12 % after 7 weeks was observed.

Lee et al. 2001 described a sludge die-away test using test concentrations of 5 and 50 µg l^{-1} ^{14}C-AHTN. Several polar metabolites were detected after 3 days, and after 20 days AHTN was largely biotransformed. Half-life of the parent AHTN was 12-24 h. This half-life refers to disappearance of the parent compound. A similar die-away test was conducted in river water (Schaefer and Koper 2006). AHTN was tested at a concentration of 5 µg l^{-1} and sludge inoculum 10 mg TSS l^{-1} in 1-gallon (3.8 l) test vessels. AHTN steadily disappeared with an overall half-life of 200 hours, by a combination of processes, including also volatilisation. By correction for volatilisation and combining with the data of the abiotic control, it was shown that the loss of parent material due solely to biodegradation (therefore primary biodegradation) was 42% after 28 days. The extracts from the sludge study were combined and analyzed for Kow by HPLC using a linear methanol/water gradient on a C_{18} column with on-line radioactivity detection. The log Kow values for AHTN and biotransformation products were estimated from their LC retention compared to the LC retention of a set of standards with know Kow using ACD logD suite software. The abiotic control sample contained only AHTN confirming that no chemical changes occurred during the test. In this study the log Kow of AHTN was 5.88. Biotransformation products eluted in two major groups. The log Kow value of the first group is 0.34 - 0.73 and of the second 3.92. Thus it is concluded that the metabolites are much more polar than the parent material.

Lee et al. (2001) carried out also a CAS -test with 10 μg l^{-1} ^{14}C-labelled AHTN in realistic STP operation conditions (addition of waste water, sludge retention time 10 d, hydraulic retention time 6 h). A complete mass balance could be derived and it showed that the total removal of the parent AHTN was 87.5% of which a half (42.5%) was caused by biotransformation and a half by sorption (44.3%). Volatilisation played a minor role (3.3%) (Lee et al. 2001, Federle et al. 2002).

In another study, concentrations of AHTN in activated sludge samples were followed for 2 days. The samples were not additionally spiked and the initial dissolved and total concentrations were 1.15 and 5.25 μg l^{-1}, respectively. Loss due to volatilisation was also determined. The 'true biodegradation rate constant' based on the dissolved concentration was 0.023 ± 0.010 h^{-1}. The rate constant based on total concentrations was 0.0075 h^{-1} (Artola 2002).

No experimental data on degradation in sediment and marine environment are available.

PFW (1996 and 1997) reported to have isolated from soils collected in the Netherlands several pure cultures of the fungi *Aureobasidium pullulans* and *Phanerochaete chrysosporium* which were capable of degrading AHTN and AHTN-alcohol. Approximately 40% of the 64 soil samples showed a positive degrading potential towards one or both of the polycyclic musks AHTN and HHCB (28% of soils degrading AHTN). In cultures of the fungus *A. pullulans* (ATCC 66657) about 80% of AHTN disappeared in 3 weeks. GC-MS analysis of ethyl acetate extracts indicated reduction of the acetyl-group. In cultures of the white rot fungus *P. chrysosporium* (ATCC 32629) AHTN disappeared within 3 days. GC-MS analysis of the metabolites of AHTN in ethyl acetate extracts showed a temporary presence of small amounts of AHTN + O and AHTN + 2 O. It is suggested that later stages of degradation were too polar to be extracted by ethyl acetate.

4.1.3 Other information [1]

The available temporal series of monitoring data indicate that AHTN 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 AHTN to sewers over the years seem be followed by a similar decrease of concentrations in sediment. This phenomenon is illustrated by Figure 1 and Table 2. Other available sediment monitoring data show a similar trend (European Commission, 2008).

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

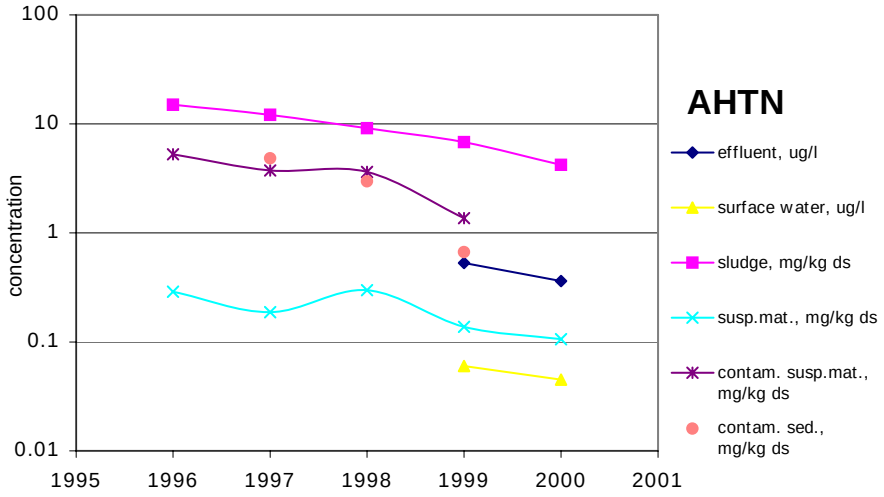


Figure 1. Trend in the median concentrations of AHTN in various environmental compartments, Hessen, Germany (based on data from HLUG 2001) (Remark: Contaminated suspended matter: mean for 3 sites; contaminated sediment: only one site. Logarithmic scale).

Table 2. Decrease of the AHTN concentrations in samples from STPs along Teltow Canal and sediment, Berlin (compiled from: Heberer 2002, Fromme et al. 2000, Fromme et al. 2001a, Blok et al. 2005).

		1996/1997	2000	2004	Reduction factor
Effluent (µg/l)	median	2.2		0.23	10
	90-P.	3.4			
	max	4.4		0.29	
Sludge (mg/kg dwt)	median		3.6	2.9	1
	90-P				
	max		5.1	3.8	
Sediment (mg/kg dwt)	median	0.9		0.22	4
	90-P	2.2			
	max	2.6		0.46	

It must be noted that monitoring data on degradation products of AHTN 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 contained initially AHTN between 0.1 and 0.27 mg kg^{-1} dwt. A parallel set of soils was amended with sludge and in addition spiked with AHTN 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. In the spiked soils, concentrations rapidly decreased during the first month and then decreased steadily. In the non-spiked soils this phenomenon was not observed. After 3 months, the concentrations in unspiked soil were 65 to 80% of the initial concentrations. During the next three months period the soil was frozen and the concentrations of all test materials remained stable. After one year, the concentrations of AHTN in unspiked soils ranged from 42 to 61% of the initial concentration. In spiked soils the concentrations were slightly more variable. Leachate was collected during the first 3 to 5 months. The leached amount was 0.04 to 0.18% of the initial amount in the spiked soils and in the leachate from the unspiked soils AHTN was not detected. Hence the influence of leaching was negligible on the disappearance. 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 37.1 Pa m^3 mol^{-1} determined by Artola-Garicano (2002), AHTN is volatile from moist surfaces and hence the influence of volatilization on the disappearance 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 (LfU-BW 2003). Using the data in the report, 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. It must be noted that the estimated disappearance reflects 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 22 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 sediment. Evidence of relatively fast primary degradation is provided from the available experiments with sludge, river water and soil. However, the studies reviewed detected negligible or very low mineralization rates of AHTN. Instead, polar metabolites were formed readily and polarity increased over time.

Half-life for primary degradation of AHTN in activated sludge was less than 1 day (Lee et al. 2001, Federle et al. 2002, Artola 2002). The overall half-life of AHTN in a river water die-away test (Schaefer et al. 2006) was shown to be 200 hours and the biological degradation after 28 days was 42%. With 10 mg 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 AHTN concentration in the test still relatively high.

Lee et al. (2001) observed that during the degradation process over time three different metabolites were formed with an increasing polarity with log Kow going down from 3.9 to circa 0.5. This observation was confirmed in later studies by Schaefer and Koper (2006).

The available monitoring data from sludge, surface water, suspended matter and sediment provide evidence that AHTN 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.

The available mesocosm scale soil dissipation study of DiFrancesco et al. (2004) resulted in a very slow disappearance of AHTN as 42 to 61 % of the initial concentration was left in soil after one year. Opposite results have been gained in the large German study (LfU-BW, 2003) measuring 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, AHTN 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, AHTN is fast degraded in

air by indirect photochemical reaction with OH –molecules and hence the part volatilised is not distributed further.

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[2]

A predicted BCF of 7,762 was obtained using the QSAR according to the TGD (EC 2003) and log Kow of 5.4. BCFWIN v2.15 calculates a BCF of 415 using the same logKow value. BCFWIN takes in addition to the logKow value also the molecular structure into account in its estimation.

Lee et al. (2001) observed that the metabolites formed during biodegradation were more polar than the parent material, with a group with log Kow of 3.9 and a second group with log Kow 0.34 - 0.73.

4.3.2 Measured bioaccumulation data[3]

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 AHTN. 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 597.

Water soluble metabolites became apparent in water on day 3 of the exposure period. During the period between day 3 and day 28 the daily amount of water-soluble metabolites was 38 – 50% of the average level of radioactivity present in the fish tissue. The main metabolite fractions in water and in tissue proved to be identical based on TLC and HPLC retention times. Smaller fractions of metabolites with intermediate polarity occurred both in tissue and in water but were not clearly identical. A BCF of 1,320 was determined based on total radioactivity. AHTN made 91-93 % and metabolites 2-8 % of total radioactivity in water, for fish edibles the fractions are 41-49 % and 33-

2 For example, log K_{ow} values, predicted BCFs

3 For example, fish bioconcentration factor

48 % and for fish non-edibles 31-42 % and 44-60 %, respectively. Van de Plassche and Balk (1997) and Balk and Ford (1999a) conclude that during exposure and depuration AHTN 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 10.5 µg l^{-1}. Methanol (0.05 %) was used as solvent. Depuration half-life was determined to be less than three days. Bioconcentration factor was determined to be 600 (Butte and Ewald, 1999).

A semistatic 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 2,300-3,200. However, 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, low number of fish and accumulation via feed cannot be excluded.

Artola-Garicano (2002) and Artola-Garicano et al. (2003) reported on an AHTN 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 AHTN concentrations in the test with *C. riparius* were stable at 5.8 ± 0.6 µg l^{-1}. The concentrations in the larvae increased to a maximum level between day 1 and 4 and then the level decreased to a new steady state. A BCF of 50 – 112 can be calculated from the test. With the addition of PBO, BCF was 7,943, and Artola-Garicano (2002) concludes that AHTN is likely to be relatively fast biotransformed in midge larvae. The exposure concentration in the experiment with *L. variegatus* was 3.6 ± 1.6 µg l^{-1}. The uptake of AHTN in worms reached a plateau level after 3 days. After 8 days a new plateau seemed to be reached, although with some outliers. Including these outliers, log BCF was 3.84 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 AHTN from soil to plants is not relevant (European Commission, 2008).

4.3.3 Other supporting information[4]

Field bioaccumulation factors have been derived for AHTN based on measured concentrations in water and biota.

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	Reference
Eel BAFwwt = 200 to 650 non-eel BAFwwt = 50 to 145	Balk and Ford 1999a from data in Eschke et al. 1995, Rijs and Schäfer 1998, Rimkus 1997
Eel BAFwwt = 1069 (range 250 – 1791)	Fromme et al 2001b
Eel BAF_{wwt} = 1421	Heberer 2002 from data of Fromme et al. 2001b

[4]For example, measured concentrations in biota

BAF	Reference
Rudd BAFwwt = 40 Tench BAFwwt = 280 Crucian carp BAFwwt = 670 Eel BAFwwt = 400 Zebra mussel BAFwwt = 570	Gatermann et al. 2002

4.3.4 Summary and discussion of bioaccumulation

Van Dijk (1996) and Butte and Ewald (1999) have reported a BCF of ca. 600 for fish from reliable bioconcentration studies according to the former OECD 305E. Van Dijk (1996) has also followed the shares of metabolites in the test system and identified a BCF of 1,320 for the parent compound and metabolites in total. In addition, depuration of AHTN metabolites has been observed to be fast for fish. Bioaccumulation factors derived from concentrations in biota and water in the field are between 50 and 1,421 for the parent compound. Bioconcentration in benthic midge larvae *Chironomus riparius* was measured to be low (BCF 50-112; parent compound) and the midge was assumed to biotransform AHTN effectively (Artola, 2002 and Artola et al., 2003). However, a test of the same authors with *Lumbriculus variegatus* gave indication that AHTN is not biotransformed but accumulated by the species.

On the basis of the available experimental and field data, AHTN is considered to have a moderate bioaccumulation potential. In the EU risk assessment of AHTN (European Commisson, 2008), the BCF of 597 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 from log Kow 3.9 to values between 0.3 and 0.7. Hence, the transformation products will have a low 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, 45, 46, 48, 60, 61, 62, 63, 64, 68. All toxicological tests performed on mammals only justify the classification harmful when swallowed (R22).

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; AHTN 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 AHTN is expected to photodegrade at radiation wavelengths below 325 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 AHTN (European Commission, 2008). Table 4 gives 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 AHTN (GLP and completely documented). For details and reference, see European Commission (2008).

Test and reference	Results [1] [mg/l]	Remarks [2]
Pseudokirchneriella	*Test A*	Van Dijk 1997
subcapitata	72h-NOECr = 0.438	carrier: 0.005% DMF and 0.005% Tween 80
72-h static	NOECb = 0.204	n=5,
	LOECr = 0.797	HPLC identification
	LOECb[4] = 0.438	*Test A*
	ErC50 > 0.797	start conc. 81-90% of nominal
	EbC50 = 0.468 <0.434 - 0.508>	end conc. 31-85% of nominal
	Test B	*Test B*
	72h-NOECr = 0.374	start conc. 77-90% of nominal
	LOECr[4] = 0.835	end conc. 53-142% of nominal
	ErC50 > 0.835	geometric mean NOEC = 0.276
	EbC50 ≈ 0.835	valid without restrictions
Daphnia magna	21d-NOECrep = 0.196, LOEC[4] = 0.401	Wüthrich 1996a
21-d semi-static	ErC50 = 0.244 <0.239 - 0.249>	carrier: 0.008% DMF and 0.002% Tween 80
Wüthrich 1996a	IC50 = 0.341 <0.243 - 0.433>	n=5
		HPLC identification
		conc.fresh 84-103% of nominal
		conc.used 70-85% of nominal
		valid without restrictions
Marine copepod	6d-NOECdevelop. = 0.022	Bjørnestad (2007)
Acartia tonsa	LOECdevelop[4]. = 0.044	Radiolabelled ^{14}C-AHTN solved in ethanol
6d-static, daily feeding	EC10develop. = 0.0282 <0.017 - 0.037>	(< 0.01%)
	EC50develop. = 0.072 <0.061 - 0.087>	n=7
		LSC identification
		conc. > 80% of nominal
		valid without restrictions
Bluegill sunfish	21d-NOECgrowth = 0.089, LOEC[4] = 0.184	Wüthrich 1996b
Lepomis	LC50 = 0.314 <0.226 - 0.448>	carrier: 0.005% DMF and 0.005% Tween 80
macrochirus		n=5
21-d flow-through		HPLC identification
		conc. 57-111% of nominal
		valid without restrictions

Test and reference	Results [1] [mg/l]	Remarks [2]
Fathead minnow *Pimephales promelas* flow-through 32 days post hatch, 36 days overall	LOEChatch > 0.140 NOECsurv. = 0.067, LOECsurv. = 0.140 LC50 = 0.100 <0.097 - 0.100> NOECgrowth = 0.035, LOECgrowth[5] = 0.067 NOECdevelop.= 0.035, LOECdevelop.= 0.067	Croudace 1997 solvent triethylene glycol n= 5 GC identification conc. 55-108% of nominal valid without restrictions
Zebrafish *Brachydanio rerio* 34 d intermittent flow-through	LOEChatch > 0.075 LOECsurv. > 0.075 NOECgrowth = 0.035 (length) LOECgrowth = 0.050 NOECdevelop. = 0.035 LOECdevelop. = 0.050	Hooftman 1999 solvent triethylene glycol or generator column n= 5 HPLC conc. 104 – 110% 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 Dunnet's test (p=0.05)

5 Wilcoxon rank sum test (p=0.05)

6.1.2 Sediment organisms

Three sediment ecotoxicity studies are available (see table 5) and they have been reviewed in the risk assessment of AHTN (European Commission, 2008). The lowest NOEC was found for *Lumbriculus variegatus* (NOEC = 17.2 mg/kg dwt, normalized to 5 % OC) in the study of Egeler and Gilberg (2004b).

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, 5% OC [mg/kg dwt],
Insecta	*Chironomus riparius*	28d-NOECdevelopment = 101 (OC 2.4%)	28d-NOECdevelopment = 210
Crustaceans Amphipoda	*Hyalella azteca*	28d-NOECgrowth = 18.2 (OC 2.15%)	28d-NOECgrowth = 42.3
Worms Oligochaeta	*Lumbriculus variegatus*	28d-NOECgrowth = 7.1 (OC 2.06%)	28d-NOECgrowth = 17.2

6.1.3 Other aquatic organisms

The acute toxicity of AHTN was tested on the South African clawed frog larvae (*Xenopus laevis*) in a procedure analogous to ASTM guideline E 1439-91. The 96h-LC50 for embryo-adult was > 2.0 mg/l, the 96h-EC50 was > 1.0 mg/l for embryo growth and > 4.0 mg/l for embryo malformation (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 AHTN (European Commission, 2008). No data on ecotoxicity to plants or soil micro-organisms are available.

Table 6. Toxicity data for soil organisms. For details and references, see European Commission (2008).

Test and reference	Results for AHTN (nominal concentrations)	Remarks
Earthworm *Eisenia foetida*	8wk-NOEC = 105 mg/kg, LOEC[2] = 250 mg/kg, reproduction and food consumption 4wk-NOEC ≥ 250 mg/kg, mortality and growth	Gossmann1997 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
Springtail *Folsomia candida*	4wk-NOEC = 45 mg/kg, LOEC[3] = 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: AHTN does not meet the P criterion. Evidence of inherent biodegradability from tests with activated sludge is available. Rapid primary biodegradation was observed and polar

metabolites were readily formed. It must be noted that only negligible mineralization has been observed. In an activated sludge die-away test a primary degradation half-life of 12-24 h was found and in a CAS test 42.5 % of parent compound was degraded to its metabolites. In a river water die-away test 42% was degraded after 28 days.

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 resulting in metabolites showing log Kow from 3.9 to circa 0.5. A detailed evaluation of the persistence of metabolites was not feasible based on data available.

Monitoring data from several years show a decreasing trend of concentrations in sludge, water, suspended matter, biota and sediment which is evidence that AHTN degrades in the aquatic environment.

A study investigating the residence of AHTN 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 AHTN is rapidly degraded in the atmosphere.

Bioaccumulation: the substance does not meet the B criterion. Experimental BCF for fish (*Brachydanio rerio* and *Lepomis macrochirus*) is ca. 600 for the parent compound based on two reliable studies according to OECD 305E. In addition, metabolites have been followed in the study with *Lepomis macrochirus*. Fish was observed to biotransform AHTN and excrete the metabolites fast. BCF below 2,000 for the parent and metabolites together was determined.

For *Chironomus riparius* larvae a BCF of 50-112 (parent compound) was derived and the larvae were responding to blocking the receptor P450. Hence, the midge larvae can be assumed to biotransform AHTN. However, a similar test conducted with *Lumbriculus variegatus* indicated that the species could not biotransform AHTN but was accumulating it.

Bioaccumulation factors estimated based on a rather large set of measured data in fish and shellfish in field studies range between 50 and 1,421 and hence indicate that AHTN does not have a high bioaccumulation potential.

The log Kow of the biodegradation products of AHTN formed in the sludge die-away study were shown to be below 4 and therefore 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 other invertebates 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.

Summary: AHTN does not meet the P, B and T criteria.

It is concluded that the substance 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, AHTN, CAS No: 1506-02-1 or 21145-77-7, EINECS No: 216-133-4 or 244-240-6.