Annex XV dossier

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE AS A CMR CAT 1 OR 2, PBT, vPvB OR A SUBSTANCE OF AN EQUIVALENT LEVEL OF CONCERN

Proposal for identification of Hexabromocyclododecane as a SVHC

Substance Name: Hexabromocyclododecane

EC Number: 247-148-4

CAS Number: 25637-99-4

- *It is proposed to identify the substance as a PBT according to Article 57 (d).*

7 ENVIRONMENTAL HAZARD ASSESSMENT

The results of ecotoxicity tests, which have been considered reliable by EU RAR, 2008, are presented in Table 7.1.

Table7-1 Acute and chronic ecotoxicity data, which are considered reliable according to EU RAR (2008)

Compartment/Species	Method	Results	Remark and reference
AQUATIC COMPARTMENT			
FISH			
Onchorhyncus mykiss	OECD 203 and TSCA 40/797/1400, and ASTM Standard E729-88a	No mortalities or other effects around 2.5 µg/l.	Graves and Swigert (1997a)
Onchorhyncus mykiss	Flow-through OECD 210 and OPPTS 850.1400	NOEC: Hatching success ≥3.7 µg/l Swim-up ≥3.7 µg/l Larvae and fry survival ≥3.7 µg/l Growth ≥3.7 µg/l	Drottar et al. (2001)
INVERTEBRATES			
Daphnia magna	OECD 202. Static immobilisation test, and TSCA 40/797/1300, and ASTM Standard E729-88a	48 h EC_{50} >3.2 µg/l	Graves and Swigert (1997b)
Daphnia magna	TSCA , OECD Flow through 21 day test.	NOEC 3.1 µg/l LOEC length 5.6 µg/l	Drottar and Krueger (1998)
ALGAE			
Selenastrum capricornutum	OECD 201 and TSCA40/797/1050	96 h EC_{50} >2.5 µg/l	Roberts and Swigert (1997)
Skeletonema costatum *Thallassiosira pseudonana* Chlorella sp.	Marine algal bioassay method, different marine growth media	72 h EC_{50} = 9 µg/l (lowest value) 72 h EC_{50} = 40 µg/l (lowest value) 96h EC_{50} >water solubility	Walsh et al. (1987) Not according to guidelines, results only used as supportive
Skeletonema costatum	OECD 201, ISO 10253:1995 and EU Directive 92/69/EEC – Method C.3. One test concentration at the limit of respective water solubilites of each diastereomer.	NOEC <40.6 µg/l EC_{50} >40.6	Desjardins et al. (2004)
Skeletonema costatum	OECD 201. EC50 obtained from a limit test with one test concentration (54.5 µg/l) at the limit of respective water solubilites of each diastereomer.	NOEC >10 µg/l EC_{50} = 52 µg/l	Desjardins et al. (2005)
SEWAGE TREATMENT PLANT, MICRO-ORGANISMS			
Acivated sludge	Respiration inhibition OECD 209	EC_{50} = 15 mg/l	Limit test with one test concentration, EC_{50} is an estimated value. Schaefer and Siddiqui (2003)
SEDIMENT COMPARTMENT			
INVERTEBRATES			
Hyalella azteca	Sediment toxicity test 28-day	LOEC >1000 mg/kg	Thomas et al.

Compartment/Species	Method	Results	Remark and reference
(Amphipod)	exposure period under flow-through conditions.	dw of sediment NOEC 1000 mg/kg dw of sediment.	(2003b)
Lumbriculus variegatus (Worm)	28-day sediment bioassay	LOEC = 28.7 mg/kg dw NOEC = 3.1 mg/kg dw Normalized: NOEC = 8.61 mg/kg dw	Oetken et al. (2001)
Chironomus riparius (Mosquito)	28-day sediment bioassay Egg production of F generation	LOEC = 159 mg/kg dw NOEC = 13.6 mg/kg dw Normalized: NOEC = 37.8 mg/kg dw	Oetken et al. (2001)
TERRESTRIAL COMPARTMENT			
PLANTS			
Plants: corn (*Zea mays*), cucumber (*Cucumis sativa*), onion (*Allium cepa*), ryegrass, (*Lolium perenne*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*)	Seedling emergence, suvival, height 21 days OECD 308 (proposal for revision), 850.4100 and 850.4225 (public drafts)	NOEC >5000 mg/kg dry soil	Porch et al. (2002)
INVERTEBRATES			
Eisenia fetida (Earthworm)	Survival and reproduction, 56 days OECD prosal and 207 and OPPTS 850.6200	NOEC 128 mg/kg dry soil Normalized: NOEC 59 mg/kg dry soil (EC_{50} 771 mg/kg dry soil)	Aufderheide et al. (2003)

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

The acute toxicity of HBCDD to rainbow trout, *Oncorhynchus mykiss*, was studied in a 96 h flow through test by Graves and Swigert, 1997b.

The acute toxicity of the substance was studied in five nominal test concentrations (1.5, 2.2, 3.2, 4.6 and 6.8 µg HBCDD/l) and compared to control and solvent control.

No mortalities or other effects were observed throughout the test. The results indicate that HBCDD is not acutely toxic to fish at a nominal concentration of about 6.8 µg/l (mean measured concentration 2.5 µg/l).

Long-term toxicity to fish

An early life-stage toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) (Drottar *et al.*, 2001). Endpoints examined were: hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival.

The test was performed with newly-fertilised eggs. The nominal test concentrations were 0.43, 0.85, 1.7, 3.4 and 6.8 µg/l. Test concentrations were measured every 7th day from day 0 to day 84 and also day 88 resulting in the following mean measured test concentrations: 0.25, 0.47, 0.83, 1.8, and 3.7 µg/l. A negative control and a solvent control were also run. The total exposure period was 88 days, including a 27-day hatching period and a 61-day post-hatch period.

The hatching success ≥83 % in the exposed groups was not statistically different (p >0.05) from the pooled controls. There were no statistically significant reductions in the numbers of fish swimming up in any HBCDD treatment group compared to the pooled control groups. There was no significant difference in survival between the different groups. There was no significant difference in growth between the different groups.

Hence, NOEC was ≥3.7 µg/l.

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

An acute flow through toxicity study on *Daphnia magna* (neonates) was performed with duplicates for each test concentration with 10 animals per replicate, at 20±2 °C (Graves and Swigert, 1997a).

The nominal HBCDD concentrations were: 1.5, 2.2, 3.2, 4.6, and 6.8 µg/1, solvent control, and negative (dilution water) control. The measured test concentrations day 0 were: 2.17/2.26, 1.74/1.85, 2.16/1.55, 2.73/2.47, 2.99/3.33 µg/l; and at day 2 they were: 2.48/2.50, 1.75/1.70, 2.48/2.27, 1.55, 3.41 µg/l.

The EC_{50} (48h) was >3.2 µg/l, which is the mean of the measured values at the highest nominal test concentration.

Long-term toxicity to aquatic invertebrates

A flow-through 21 day life-cycle toxicity test was performed with the cladoceran *Daphnia magna* (Drottar and Krueger, 1998). Survival of the first and second generation daphnids, the number of young produced per reproductive day, and the length and dry weight of surviving first-generation daphnids were evaluated.

The nominal test concentrations were: 0.85, 1.7, 3.4, 6.8 and 13.6 µg HBCDD/l, solvent control, and negative (dilution water) control. Test concentrations were measured day 0, 7, 14 and 21 resulting in the following mean measured test concentrations (range): negative control <LOQ, solvent control <LOQ, 0.87 (0.72-1.02), 1.6 (1.34-1.85), 3.1 (2.69-3.63), 5.6 (4.75-6.38), and 11 (9.82-12.3) µg/l.

Daphnids exposed to 11 µg/l for 21 days had statistically significant reduced lengths, dry weight and fewer young. Daphnids exposed to 5.6 µg/l for 21 days had statistically significant reduced mean lengths. The used test concentrations are below the maximum water solubility of HBCDD. Thus, the LOEC was determined to 5.6 µg/l.

No statistical effects on survival, reproduction or growth were observed in *Daphnia magna* exposed for 21 days to 3.1 µg/l, and hence, the NOEC was 3.1 µg/l.

7.1.1.3 Algae and aquatic plants

Data are available from four reliable algal growth inhibition studies.

The toxicity of HBCDD to the freshwater alga, *Selenastrum capricornutum,* was studied in a static 96 h growth inhibition test (Roberts and Swigert, 1997).

The effects on growth rate and biomass were studied in five nominal test concentrations (1.5, 2.2, 3.2, 4.6 and 6.8 µg HBCDD/l). The measured test concentrations (corrected for a mean procedural recovery of 113 %) on day 0 were: 1.30, 2.25, 3.38, 4.28 and 6.44 µg/l, and on day 4 (in the abiotic test solution): <0.571 (detection limit), 1.20, 1.90, 1.64 and 2.47 µg/l.

No effects were seen at the highest tested concentration, i.e NOEC =2.5 µg/l (day 4). Thus, the 72-hour EC_{50} is >2.5 µg/l and the LOEC is >2.5 µg/l.

The algal growth inhibition of HBCDD was also studied in six marine media (Walsh *et al.*, 1987). The studied test organisms were *Skeletonema costatum, Thalassiosira pseudonana* and *Chlorella sp.* Population density was estimated by cell counts on a haemocytometer. Toxicity, EC_{50}, was based upon cell numbers after incubation for 72 hr for *S. costatum* and *T. pseudonana* and for 96 h for *C. sp*.

The EC_{50}s:

*Skeletonema costatum**	EC_{50} (72h) 9-12.2 µg/l
Thalassiosira pseudonana	EC_{50} (72h) 40-380 µg/l
Chlorella sp.	EC_{50} (96h) >1500 µg/l

* Only results from tests in five different media

No NOEC was determined in the test.

There are some question marks regarding the methodology used in this study. For instance, it is not shown that the growth rate is calculated during exponential growth. Since this study appears to deviate from standard methods, the results will only be used as supportive to more recent studies, performed more in line with standard methods.

A 72 hours growth inhibition study was performed with *Skeletonema costatum* (Desjardins *et al.*, 2004). The test was performed to study effects on algal growth of the mixed diastereomers of HBCDD at the limit of their respective water solubility.

Passing saltwater algal medium through a generator column saturated with HBCDD produced the single test concentration (40.6 µg/l). In this way the composition of HBCDD in the saltwater algal medium became 74.6 % α-, 21.5 % β- and 3.97 % γ- diastereomer which is different from that of the technical product.

There was a 10 % inhibition of the growth rate at the measured test concentration of HBCDD 40.6 µg/l. NOEC is <40.6 µg HBCDD/l and EC_{50} >40.6 µg HBCDD/l.

Desjardins *et al.*, 2005 performed a 72 hours study with HBCDD on the marine diatom alga *Skeletonema costatum* using (i) a co-solvent, and (ii) a saturated solution. Both the biomass and the growth rate were derived.

i) Study with a co-solvent

Nominal test concentrations of 0.64, 1.6, 4.0 and 10 µg HBCDD/l, were prepared by diluting a stock solution in dimethylformamide (DMF) with saltwater medium. The analytical results performed at the beginning of the test corresponded to 332, 131, 94 and 108 % of the nominal concentration, respectively. The solvent concentration in the solvent control and treatment groups was 0.1 ml/l.

There were no statistically significant effects at any of the test concentrations. It is probable that the actual test concentrations were almost equal, i.e. about the solubility of γ-HBCDD at all four nominal test concentrations. The other diastereomers would still not have reached significant concentrations at these nominal concentrations of technical HBCDD. Hence, it can be concluded that there are no significant effects at the solubility of γ-HBCDD, and that the NOEC of technical HBCDD in this study was >10 µg/l.

ii) Study at saturated solution

The test was performed to study effects on algal growth of the mixed diastereomers of HBCDD at the limit of their respective water solubility. Only one test concentration was used. The test solution used in this study corresponded to the saturated solution of HBCDD in saltwater. The mean measured HBCDD concentration as a sum of the diastereomers was 54.5 µg/l.

The growth rate inhibition rose during the study and was 17% compared to the column control after 24 hours, 29 % after 48 hours and 51% after 72 hours. The authors of the study used non-linear regression fitting to cumulative normal distribution to calculate EC_{50}. The 72-hr EC_{50} for biomass and growth rate was calculated to be 27 and 52 µg/l respectively. The relevance of calculating an EC_{50} from a study where only one test concentration has been used can be questioned. However, as the growth rate inhibition (0-72 h) was 51% at a test concentration of 54.5 µg HBCDD/l, the calculated EC_{50}-value of 52 µg/l seems adequate. Furthermore, this EC_{50}-value is in line with the result obtained with the saturated solution where EC_{10} was around 40.6 µg/l (Desjardins *et al.*, 2004).

Summary of algal toxicity

Based on the most reliable algal toxicity study (Desjardins *et al.*, 2005) the EC_{50} for algae based on growth rate, is concluded to be 52 µg HBCDD/l. The 72-hr NOEC is determined to be between 10µg/l and 40 µg/l (EU RAR, 2008).

7.1.1.4 Sediment organisms

Two toxicity tests have been performed on the amphipod *Hyalella azteca* (Thomas *et al.*, 2003a-b). Groups of amphipods were exposed to six test concentrations and a control in each study. Eight replicate test compartments were maintained in each treatment and control group, with 10 amphipods in each test compartment. Additional replicates were added in the control group, low and high treatment groups for analytical sampling of water and sediment at day 0, 7 and at the end of the test. Nominal test concentrations were 31, 63, 125, 250 500 and 1000 HBCDD mg/kg of sediment based on dry weight of sediment. Results of "the analytical replicates" were used to confirm the lowest and the highest test concentration. The results of the studies are based on the nominal test concentrations. The measured endpoints were survival and growth as determined by dry weight measurements.

In both studies LOEC was concluded to be >1000 mg/kg dwt of sediment and NOEC was concluded to be 1000 mg/kg dwt of sediment.

Chronic tests (28 days, static) were also performed with *Lumbriculus variegatus* and *Chironomus riparius* in spiked sediment with an organic matter content of about 1.8 % (Oetken *et al.*, 2001). For *L. variegatus*, different endpoints resulted in different NOECs. The lowest NOEC, 8.6 mg/kg dwt (normalized to standard organic carbon content, *i.e*. 5 %), was obtained for the total number of worms.

Most of the results from the test with *C. riparius* are considered invalid. However, based on the endpoint number of eggs from the F1 generation a NOEC of 13.6 mg/kg dwt was determined for *C. riparius.*

7.1.1.5 Other aquatic organisms

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

7.1.2.1 PNEC water

Long term studies are in general considered more relevant than short term studies particularly for substances with low water solubility. Reliable long term studies are available for all three trophic levels, but all studies, except the 21d-study with *Daphnia magna*, resulted in larger-than values. None of the larger-than values, is below the 3.1 μg/l NOEC-value for *Daphnia*, except for the LOEC-value of >2.5 μg/l from the 72(96) h growth inhibition test with *Selenastrum capricornutum*. This may indicate that the NOEC for algae could be <3.1 μg/l, i.e. the NOEC-value for *Daphnia*. The lowest NOEC, the 21d-NOEC 3.1 μg/l for *Daphnia magna*, will be used for derivation of PNEC.

According to the revised TGD (Table 20) an assessment factor of 10 can be applied on the lowest NOEC, when reliable NOEC values are available for three trophic levels to derive the $PNEC_{aquatic}$.

Thus, the predicted no effect concentration for the aquatic compartment is 3.1/10 = 0.31 μg/l.

For intermittent releases to the aquatic environment the lowest $L(EC)_{50}$ of at least three short-term tests from three trophic levels is recommended in the revised TGD with applying an assessment factor of 100 for calculation of PNEC. The lowest EC_{50} is the one from the algae growth inhibition test with *Skeletonema costatum,* which is 52 μg/l.

Thus the PNEC for intermittent releases in the water phase is 52/100 = 0.52 μg/l.

7.1.2.2 PNEC sediment

Two toxicity tests have been performed on the amphipod *Hyalella azteca* to determine the effects of sediment-incorporated HBCDD during a 28-day exposure period under flow-through conditions. The results from the two tests were similar and the NOEC for Hyalella was 1000 mg/kg dwt.

Chronic tests (28 days, static) were also performed with *Lumbriculus variegatus* and *Chironomus riparius* in spiked sediment (organic matter content about 1.8 %). The lowest NOEC, 8.6 mg/kg dwt (normalized to standard organic carbon content, *i.e.* 5 %), was obtained for the total number of worms.

Most of the results from the test with *C. riparius* are considered invalid. However, based on the endpoint number of eggs from the F1 generation a NOEC of 13.6 mg/kg dwt was determined for *C. riparius.*

According to the revised TGD an assessment factor can be used on the lowest NOEC for the calculation of $PNEC_{sed}$. In this case there are chronic results from three species with different feeding regimes. Therefore, an assessment factor of 10 is used on the lowest NOEC above (Table 19, revised TGD).

Thus $PNEC_{sed}$, based on chronic test data, is 8.6/10 = 0.86 mg/kg dwt.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

7.2.1.1 Toxicity to soil macro organisms

Acute toxicity

There are no studies on the acute toxicity of HBCDD to earthworms available.

Long term toxicity

A test on the survival and reproduction of earthworm was performed by Aufderheide *et al.*, 2003. The test species was earthworm, *Eisenia fetida* (clitellate adults). Control worms had an initial mean weight of 433.2 mg/worm and the weight of the test worms ranged from 354.0 to 502.6 mg/worm.

The NOEC was estimated to 128 mg HBCDD/kg dry soil and the LOEC to 235 mg HBCDD/kg dw.

In the study the weight fraction of organic matter content was 7.4 %, whereas in a standard soil the organic matter content is 3.4 %, according to the TGD. The NOEC (NOEC = 128 mg HBCDD/kg dry soil) is therefore normalized with the equation 71 in TGD:

$NOEC_{standard} = NOEC_{exp} \times (Fom_{soil(standard)}/Fom_{soil(exp)})$

where Fom is fraction of organic matter.

The normalized NOEC is 59 mg/kg dry soil.

7.2.1.2 Toxicity to terrestrial plants

Porch *et al.*, 2002 performed a seedling emergence test with six plant species.

The test species were corn (*Zea mays*), cucumber (*Cucumis sativa*), onion (*Allium cepa*), ryegrass, (*Lolium perenne*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*).

For the onion seedlings there were seemingly a decrease in dry weight and height at 725 mg/kg and above. The decrease was however not significant according to the Dunnett's test. With a post-test for trends it should be possible to show the decreasing trend. Since there is another terrestrial test, survival and reproduction of earthworm, with a lower PNEC there is no need to make any further effort with a trend test.

No NOEC could be determined for the tested plants.

7.2.1.3 Toxicity to soil micro-organisms

A study on the effects of HBCDD on micro-organisms in soil has been performed by Förster, 2007). HBCDD was dissolved in acetone and mixed into quartz sand. After evaporation of the acetone the sand was mixed into sieved (2 mm) field soil (Lufa standard soil 2.3 containing 1.02% organic carbon and 61% sand based on dry weight) that was amended with ground Lucerne meal (5 g/kg soil). The water content of the soil was adjusted to 50% of the maximum water holding capacity. The nominal concentrations of HBCDD were 10.0, 31.6, 100.0, 316.2 and 1000 mg/kg soil dw. Three replicates were set up for each test concentration and control (including a solvent control). The soil was incubated in glass jars in the dark for 28 days at 20 ± 2°C. Soil nitrate concentration was measured day 0 and day 28. The concentration of HBCDD was measured in the 10, 100 and 1000 mg/kg test concentrations and was 104%, 83.1% and 75% of the nominal concentrations, respectively.

No statistically significant differences in nitrate production between the controls and HBCDD treated soil samples were detected. (ANOVA, $p \leq 0.05$).

Thus the NOEC from this study was ≥750 mg HBCDD/kg dw.

7.2.1.4 Toxicity to other terrestrial organisms

Toxicity to birds

Toxicity to other above ground organisms

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

There are studies on terrestrial organisms from three trophic levels available. Thus an assessment factor of 10 can be applied (revised TGD Table 20). The normalized NOEC value for reproduction of earthworms is used to calculate the PNEC for the terrestrial environment.

Applying an assessment factor of 10 results in a predicted no effect concentration for the terrestrial compartment $PNEC_{soil}$ of 59/10 = 5.9 mg/kg dry soil.

7.3 Atmospheric compartment

There are no effect data available for the atmospheric environment and therefore it is not possible to calculate a $PNEC_{air}$.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

An oxygen consumption test using *Pseudomonas putida* was carried out by Siebel-Sauer (1990). The nominal test concentrations were between 1250-10000 mg/l. No toxic effects compared to control were observed at the maximum nominal concentration of 10000 mg/l. The results from this study indicate that HBCDD has a low toxicity to micro-organisms.

However, the nominal test concentrations were much above the water solubility of HBCDD. Furthermore, the study was shortly described which makes the reliability difficult to assess. According to the TGD tests on individual bacterial populations are considered less relevant. It has therefore not been considered relevant to base a $PNEC_{STP}$ on the results from this study.

An activated sludge respiration inhibition test has been performed (Schaefer and Siddiqui, 2003).

The test substance was a composite sample from three manufacturers of hexabromocyclododecane and had a purity of 95.86 %. The activated sludge used in the test was from a wastewater treatment plant that receives mainly domestic sewage. The test was carried out at 20-21 °C and the sludge used had a total suspended solids content of 4213 mg/l and a pH of 7.8. The test substance, HBCDD, was dosed at a limit concentration of 15 mg/l being tested in triplicate. Two controls were run and a reference substance (3,5-dichlorophenol) was also tested at concentrations of 3, 15 and 50 mg/l. The respiration rate after 3 hours in the three replicate HBCDD treatments were 42.4, 41.0 and 40.0 mg O_2/l/hour, which was equivalent to approximately 29.1 % inhibition when compared to the controls. Thus only an approximate EC_{30} value of 15 mg/l can be estimated.

The study is considered reliable. However, due to the use of a limit concentration no inhibition curve can be obtained and a true EC_{50} cannot be calculated. The test concentration 15 mg HBCDD/l activated sludge is above the water solubility of HBCDD. Activated sludge is however not pure water and the test concentration is therefore considered acceptable.

The EC_{30} of 15 mg/l will be used for calculation of PNEC.

7.4.2 PNEC for sewage treatment plant

The EC_{30} obtained at 15 mg/l in the respiration inhibition test (Schaefer and Siddiqui, 2003) discussed above, is taken as an estimate for the EC_{50} for the PNEC derivation. When deriving a PNEC for micro-organisms from an EC_{50} value an assessment factor of 100 should be used according to the revised TGD. Thus $PNEC_{STP}$ is 0.15 mg/l.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

For the assessment of secondary poisoning, the results have to be expressed as the highest concentration in food causing no effects. Equations and factors for the conversion from NOAEL to NOEC are given by TGD. In addition an extra assessment factor, accounting for interspecies variation, lab-to-field extrapolation and acute/subchronic to chronic extrapolation should be applied to derive a PNEC. According to the human health risk assessment, two effects could be relevant for the derivation of this PNEC, i.e., repeated dose toxicity on liver and the thyroid with an oral NOAEL of 22.9 mg/kg/day from a 28 days study in rats, and reproductive toxicity with a diet NOAEC of 150 ppm HBCDD dry weight (corresponding to a dose of 10 mg/kg/day).

According to TGD, an assessment factor of 300 can be applied to the NOEC for a 28 day repeated dose test on mammalian species. However, in this case a factor of 30 is chosen because there is no need to use an assessment factor for subchronic to chronic extrapolation. In the human risk assessment a factor of 1 is chosen to account for the differences between the 28 day study and chronic exposure. The reason for this is that there is no indication that the liver weight will increase more with time of exposure (similar liver weight increases are observed after 28 days and 90 days exposure). In addition, if assuming that enzyme induction is the primary event triggering the other effects, enzyme induction is neither likely to increase with time. There is some uncertainty as to whether the thyroid effects could become more severe after chronic exposure, but on balance, it is decided not to use an extra assessment factor for subchronic to chronic extrapolation.

A 2-generation study has recently been performed according to OECD TG 416 (Ema et al, 2008). HBCDD was administered via the diet by mixing HBCDD-particles with ground dry feed, at concentrations of 150, 1.500, and 15.000 ppm (dry weight). Because of the dosing of HBCDD particles, with the bioavailability likely being dependent on particle size and dose, there is some uncertainty regarding the actual systemic doses obtained especially in the higher dose groups. A significantly reduced number of primordial follicles in the mid and high dose groups was evident (30 %, only measured in F1). A dose-dependent decrease (8-14%) in fertility index was indicated in both generations, although statistically significant only in F0. In addition, a high and dose-dependent pup mortality during lactation was observed in the F2 generation (increased by 35 % in the high dose group and 15 % in the mid dose group), although only being statistically significant in the high dose group. Overall, a NOAEL of 150 ppm dry weight (10 mg/kg/day) can be deduced based on ecologically relevant effects at 1.500 ppm. As no assessment factor is needed for duration correction when the data come from a 2-generation study, the total assessment factor to be used is 30.

As reproductive toxicity may be more ecologically relevant than liver and thyroid effects, and also give the lowest NOAEC/NOAEL, the PNEC will be calculated based on the reproductive toxicity NOAEC of 150 ppm.

However, the derived PNEC is considered to be uncertain. There are indications that HBCDD may have developmental neurotoxicity effects at lower exposure levels than those cited above, although this needs to be confirmed. Consequently, the results from the neurotoxicity study cannot be used to derive a PNEC for secondary poisoning. The uncertainties in the mammalian toxicity database are also acknowledged in the human health risk characterization where a conclusion (i) on hold (awaiting results from ongoing studies) is drawn with regards to the need for a developmental neurotoxicity study in rodents.

7.6 Conclusion on the environmental classification and labelling

The proposed classification for the environment is:

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

Concentration limits:

According to the proposal on specific concentration limits for very toxic substances (ECBI/65/99 Add.10), the reported L(E)C50 range of 10-100 µg/l will give rise to the following concentration limits of preparations:

Concentration limits of substance	*Classification of preparation*
C ≥2.5 %	N; R50-53
C ≥0.25 %	N; R51-53
C ≥0.025 %	R52-53

The proposal is based on the toxic effects seen in a 72-hour study on the marine algae *Skeletonema costatum* (EC_{50} 52 µg/l), the lack of biodegradation seen in a standard test and the very high bioconcentration factor (18 100) determined in a BCF study on fish. The proposed classification is supported by the results from a 21-day life cycle test on *Daphnia magna,* in which the LOEC, based on reduced mean lengths, was determined to 5.6 µg/l. The proposed classification is further supported by the results from two other 72-hour studies on the marine algae *Skeletonema costatum*: In one study an EC_{50} of about 10 µg/l is obtained, however this study is older and appears to deviate from standard methods and therefore the results are only used as supportive to the result above. In the other study a NOEC <40.6 µg/l and EC_{50} >40.6 µg/l is obtained for HBCDD.

8 PBT, VPVB AND EQUIVALENT LEVEL OF CONCERN ASSESSMENT

8.1 Comparison with criteria from annex XIII

Persistence: Hexabromocyclododecane (HBCDD) fulfils the P-criterion. Based on a standard degradation simulation study, HBCDD seems to be persistent in aerobic soil. No firm conclusion can be drawn solely from the performed simulation degradation studies regarding whether or not HBCDD fulfils the P-criterion for sediment. The assessment is complicated by the fact that available data indicate that the different diastereomers have different degradability. For α-HBCDD, which seems to be the least degradable, an aerobic DT_{50} of approximately 210 days in sediment at 12°C was determined, which is above the P-criterion of 120 days. For γ-HBCDD the available data indicate very different half-lives depending on test concentration. When tested at a concentration similar to what is measured close to polluted areas, the DT_{50} was 190 days (12°C).

The measured data available from dated sediment cores indicate, that HBCDD has degraded in these sediments more slowly than what would be expected based on some of the available experimental sediment degradation half-lives. Furthermore, HBCDD is found to be ubiquitously present in remote areas in abiotic samples and biota providing evidence, that the substance is persistent in the environment. Also the temporally increasing concentrations found in biota support the picture of HBCDD as a persistent substance.

Bioaccumulation: HBCDD meets the vB criterion based on reliable experimental BCFs from two flow-through bioconcentration tests with fish. A BCF of 18 100 was chosen as a representative value in the EU risk assessment (European Commission, 2007). Furthermore, a large set of measured data in biota in the field indicate, that HBCDD is biomagnified in the environment. No diastereomer specific BCFs are available. However, the concentration of α-HBCDD in biota is generally much higher than the concentration of the other two main diastereomers despite it being present in commercial HBCDD in a relatively low concentration. Several reasons may have lead to this difference in diastereomeric accumulation

Toxicity: HBCDD fulfils the T criterion. A 21d-NOEC of 3.1 µg l^{-1} has been derived for *Daphnia magna* in a flow-through test. It is noted, that ecotoxicity testing of HBCDD is highly complicated due to its very low water solubility.

Other: HBCDD has a high potential for long-range environmental transport. Its half-life in the atmosphere is > 2 days and it has been found in remote areas in abiotic samples (air, deposition, sediment) and biota (polar bears, bird eggs, seals) in the majority of samples of the last years. Additionally, a study comparing long-range transport potential of "existing" POPs and HBCDD with the help of tuna fish samples, found HBCDD to have a very high potential for long-range environmental transport.

8.2 Assessment of substances of an equivalent level of concern

8.3 Emission characterisation

8.4 Conclusion of PBT and vPvB or equivalent level of concern assessment

Hexabromocyclododecane (HBCDD) fulfils the vB-criterion based on experimental data (BCF=18100) and measured data from biota. With a NOEC of 3.1 µg/l for daphnia, the T-criterion is also met. The available soil degradation simulation tests indicate that the half-life of HBCDD in aerobic soil is > 120 d and thus the P-criterion in soil is met. The experimental data regarding persistence in sediment are varying. According to some of the sediment degradation simulation studies available the P-criterion is met, whereas other studies substance indicates that the substance is degradable in certain experimental conditions. However, data from dated sediment cores gives support to HBCDD being persistent also in sediment. Furthermore, HBCDD has potential for long-range environmental transport based on environmental monitoring data and modelling. Overall it is concluded, that HBCDD is a PBT substance.