Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Octamethylcyclotetrasiloxane; [D4]

EC Number: 209-136-7
CAS Number: 556-67-2

CLH-O-0000001412-86-192/F

Adopted

9 March 2018

9 March 2018

CLH-O-0000001412-86-192/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Octamethylcyclotetrasiloxane; [D4]**

EC Number: **209-136-7**

CAS Number: **556-67-2**

The proposal was submitted by Germany and received by RAC on **9 January 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **21 February 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 April 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Riitta LEINONEN**

Co-Rapporteur, appointed by RAC: **Stephka CHANKOVA-PETROVA**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 March 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	014-018-00-1	Octamethylcyclotetrasiloxane; [D4]	209-136-7	556-67-2	Repr. 2 Aquatic Chronic 4	H361f*** H413	GHS08 Wng	H361f*** H413			
Dossier submitters proposal					**Retain** Repr. 2 and **Modify** Aquatic Chronic 1	**Retain** H361f*** and **Modify** H410	**Retain** GHS08 Wng and **Add** GHS09	**Retain** H361f*** and **Modify** H410		**Add** M=10	
RAC opinion					**Retain** Repr. 2 and **Modify** Aquatic Chronic 1	**Retain** H361f*** and **Modify** H410	**Retain** GHS08 Wng and **Add** GHS09	**Retain** H361f*** and **Modify** H410		**Add** M=10	
Resulting Annex VI entry if agreed by COM					Repr. 2 Aquatic Chronic 1	H361f*** H410	GHS08 GHS09 Wng	H361f*** H410		M=10	

GROUNDS FOR ADOPTION OF THE OPINION

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Octamethylcyclotetrasiloxane (D4) is classified as Aquatic Chronic 4 in Annex VI of the CLP Regulation. The Dossier Submitter (DS) proposed to classify D4 as Aquatic Chronic 1; H410, based on its high potential for bioaccumulation, no rapid degradation and chronic NOEC values ≤ 0.1 mg/L. An M-factor of 10 was also proposed by the DS due to the NOEC value of 0.0044 mg/L for fish and 0.0079 mg/L for aquatic invertebrates, respectively.

Degradation

There was one ready biodegradability test available on D4 (OECD TG 310, GLP) using 10 mg solids/L inoculum (activated sludge, sewage, soil) and 10 mg/L (based on DOC) test substance. After 29 days, 3.7 % CO_2 evolution was observed indicating that the substance is not readily biodegradable. No microbial toxicity of D4 was indicated by CO_2 evolution of 54 % after 29 days from toxicity control (degradation was > 25% after 14 days) (REACH Registration dossier).

The hydrolysis of D4 was tested at 10, 25 and 35 °C and at pH 4, 7, and 9 (OECD TG 111, GLP). The average half-life at pH 7 and 25 °C was calculated to be 3.9 days. At pH 4 the half-life was 1.77 hours and at pH 9 0.902 hours at 24.6 °C. At pH 6.99 and 9.5 °C the half-life was 542 hours (~23 days) and at pH 7 and 12 °C the estimated half-life was 16.7 days. The DS also reported a half-life of 2.9 days at pH 8 and 9 °C in marine water in the CLH Report. According to the DS, one of the degradation products was dimethylsilanediol (CAS 1066-42-8) which has not been self-classified for environmental hazard by any of the 49 notifiers in the ECHA C&L Inventory. The full study report of the above mentioned OECD TG 111 study was provided after the Public Consultation. In addition to half-lives, the study also analysed kinetic data. It has been demonstrated that, at concentrations below the limit of solubility, D4 was readily transformed via hydrolysis to a smaller, more polar compound, likely dimethylsilanediol ($Me_2Si(OH)_2$). This probably occurs through a series of consecutive irreversible (pseudo) first-order reactions that follows formation of tetramer diol.

Based on an OECD TG 308 (GLP) test, D4 had an estimated degradation half-life of 242 days in aerobic sediment at 24 °C which is expected to be longer at lower temperatures. The major degradation products were hydrolytic products, such as dimethylsilanediol and non-extractable silanols while $^{14}CO_2$ generation was minimal, indicating that complete mineralisation of D4 or its degradation products is very slow. D4 degradation in non-sterilised samples was significantly faster than that in the chemically sterilised samples, suggesting that the degradation of D4 in the sediment might not be purely abiotic (REACH Registration dossier). There was no data on photodegradation available in the CLH Report because they were not seen as relevant.

Bioaccumulation

A Log K_{ow} of 6.488 was determined at 25.1 °C in an OECD TG 123 study (GLP). There were three fish bioconcentration studies available. In the first study the bioconcentration factor (BCF) was measured based on ^{14}C measurements for *Pimephales promelas* following EPA OTS 797.1520 (GLP). The study included a preliminary test with 6 days exposure followed by 14 days of depuration and a definitive test with 28 days of exposure and 14 days of depuration. A steady-

state BCF of 12 400 L/kg after 28 days and a kinetic BCF of 13 400 L/kg was reported. The data of the study were re-analysed to take the variable exposure concentrations during the tests into account (concentration in the range of 0.2 to 0.5 µg/L. The re-analysis resulted in a kinetic lipid-normalised BCF of 14,900 L/kg (lipid content = 6.4 %).

The two other studies were carried out according to OECD TG 305 (GLP) with *Cyprinus carpio* and had an exposure period of 60 days followed by a depuration period of 15 days in the first study and 12 days in the second. The lipid content in both studies was close to 5 %. The first study was carried out using two exposure concentrations (0.22 µg/L and 2.52 µg/L) in a continuous-flow system. The concentration in the fish was found to reach steady state within 39 days. The steady-state BCF values were 3,129 L/kg for the higher exposure level and 3,000 L/kg for the lower exposure level. The second study with *Cyprinus carpio* was carried out using 0.23 µg/L and 2.4 µg/L of D4. Steady state was reached by day 46. The mean BCF at steady state was 3,329 L/kg at the higher exposure level and 3,967 L/kg at the lower. A kinetic BCF of 4,106 L/kg was estimated for the higher treatment level and 5,540 L/kg for the lower.

Acute toxicity

The measured water solubility of D4 was 0.0562 mg/L at 23 °C and pH ca. 7. The Henry's law constant of 1.21 × 10^6 Pa×m^3/mol at 21.7 °C indicated a high potential for volatilization from water surface.

Table : Summary of relevant information on acute aquatic toxicity (including also data from the most recently updated REACH Registration dossier (October 2017))

Method	Species	Test conditions	Results	Remarks	Reference
EPA 797.1400, GLP	*Oncorhynchus mykiss*	flow-through sealed system, freshwater, 12 °C, pH 6.5-7.2	96 h LC_{50} > 22 µg/L (mm) no effects; 14 d LC_{50} = 10 µg/L mm [(1)] 14 d NOEC = 4.4 µg/L (mm)	Prolonged acute tox. study.	Anonymous, 1995
EPA 797.1400, GLP	*Cyprinodon variegatus*	flow-through sealed system, seawater (salinity 20 ppm), 25±2°C, pH 7.9-8.1	96 h LC_{50} > 6.3 µg/L (mm) 14 d LC_{50} > 6.3 µg/L mm, no effects 14 d NOEC ≥ 6.3 µg/L (mm)	Prolonged acute tox. study	REACH Registration dossier 10/2017
EPA 797.1300, GLP	*Daphnia magna*	flow-through, freshwater, 20±2°C, pH 7.3-7.9	48 h EC_{50} > 15 µg/L (mm) no effects		Sousa *et al*. 1995
EPA 797.1930, GLP	*Americamysis bahia*	flow-through, salt water (salinity 20 ppm), 25±2°C, pH 7.4-8.1	96 h LC_{50} > 9.1 µg/L (mm) no effects		REACH Registration dossier 10/2017
EPA 797.1050, GLP	*Selenastrum capricornutum* (new: *Pseudokirchneriella subcapitata)*	limit test; freshwater; sealed, no headspace; 23-24°C, pH 7.5-10	96 h E_rC_{50} > 6 µg/L (mm, corresponding 22 µg/L im)		ECHA 2016a

[(1)] Data from REACH Registration dossier 10/2017
im = initially measured
mm = mean measured

There were two reliable prolonged acute toxicity tests to fish, one with *Oncorhynchus mykiss* and one with *Cyprinodon variegatus*. The *Oncorhynchus mykiss* test was performed according to EPA Guideline 797.1400 at 12 ± 2 °C. Sealed glass vessels were used as D4 is volatile. Five concentrations (2.9, 4.4, 6.9, 12 and 22 µg/L mean measured) were used with two replicates each and ten organisms per vessel (flow-through). No vehicle was used. In contrast to the OECD TG 203 (average fish size: 5 ± 1 cm), the fish had an average size of only 3.7 cm. The biomass loading rate was 0.17 g/L. Up to day 7, no effects were observed. Therefore, the 96 h EC_{50} was > 22 µg/L (mean measured). At day 14, 20 % of the organisms in test concentration 6.9 µg/L died. Therefore, the 14 d NOEC for survival was 4.4 µg/L. In the flow-through test (EPA 797.1400) with *Cyprinodon variegatus*, concentrations up to 6.3 µg/L (maximum achievable concentration) were tested in the prolonged acute toxicity test and no effects were observed. Therefore, the 14 d NOEC was 6.3 µg/L. The test was performed in salt water (salinity 20 ppt), at 25 °C, and at pH 7.9-8.1 (REACH Registration dossier).

Two reliable tests with aquatic invertebrates (freshwater: *Daphnia magna* and marine: *Mysidopsis bahia*, new name: *Americamysis bahia*) were conducted. They were performed according to EPA 797.1300 and EPA 797.1930. The *Daphnia magna* test was a flow-through test with a duration of 48 hours and analytical monitoring. The test concentrations were 1.7, 2.9, 3.7, 7.8 and 15 µg/L (mean measured). No vehicle was used. There was no effect observed up to the highest test concentration. Therefore, the 48 h EC_{50} was > 15 µg/L. With *Americamysis bahia* the test was conducted 96 hours without vehicle with test concentrations of 1.3, 2.2, 3.7, 6.9 and 9.1 µg/L (mean measured) in salt water (20 ppt solubility). No effects up to the highest test concentration were observed. Therefore, the 96 h LC_{50} was > 9.1 µg/L.

There is an algae test available. The test was performed according to EPA Guideline 797.1050 with *Selenastrum capricornutum* (new: *Pseudokirchneriella subcapitata*). It was a limit test with sealed test vessels without headspace. The limit concentration was 22 µg/L (initial measured) (corresponds to 6 µg/L mean measured concentration). No vehicle was used. Despite the fact being performed according to EPA standards, the validity criteria of OECD TG 201 were fulfilled. The cell density was decreased 18 % in the treatment compared with the control group. The cell density in both (test system and control system) was lower than expected. In the test also an open-system reference control system was included, which demonstrated that the restricted gaseous exchange in the sealed system caused a reduction in growth rate.

The Dossier Submitter concluded that no short-term (acute) aquatic hazard classification is necessary for D4 because no effects were shown at the highest concentrations tested in the acute toxicity test.

Chronic toxicity

Table. Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
40 CFR 797.1600, GLP	*Oncorhynchus mykiss*	flow-through, closed system, freshwater, 12 °C, pH 6.8-7.5	93 d NOEC ≥ 4.4 µg/L (mm)	No effects at highest test concentration.	ECHA 2016a; Anonymous, 1995
EPA 797.1400, GLP	*Oncorhynchus mykiss*	flow-through sealed system, freshwater, 12 °C, pH 6.5-7.2	14 d NOEC = 4.4 µg/L (mm) 14 d LOEC = 6.9 µg/L (mm)	Prolonged acute tox. study.	Anonymous, 1995
OECD 204 [3] GLP	*Oncorhynchus mykiss*	flow through, fresh water, 11.5-12.5 °C, pH 7.3-7.7	14 d LC_{50} = 17 µg/L (mm) 14 d NOEC = 6.8 µg/L (mm)	Prolonged acute tox. study Fish average wet weight 0.12 g	REACH Registration dossier 10/2017
EPA 797.1330, GLP	*Daphnia magna*	flow-through, freshwater, 19-22 °C, pH 6.6-7.6	21 d NOEC = 7.9 µg/L (mm) (survival); 21 d NOEC ≥ 15 µg/L (growth and reproduction)[1]		Sousa *et al*. 1995
EPA 797.1050, GLP	*Selenastrum capricornutum* (new: *Pseudokirchneriella subcapitata*)	limit test; freshwater; sealed, no headspace; 23-24 °C, pH 7.5-10	96 h NOE_rC > 6 µg/L (mm, corresponding 22 µg/L as im) recalculated: E_rC_{10} > 22 µg/L[2] im		ECHA 2016a

[1] Data from REACH Registration dossier 10/2017

[2] Public Consultation

[3] Study received after the Public Consultation, also included in the REACH Registration dossier 10/2017

im = initially measured

mm = mean measured

In addition to the prolonged acute toxicity study with *Oncorhynchus mykiss*, described in relation to acute toxicity, a full study report on fish prolonged acute toxicity was submitted during the RAC evaluation. This GLP study was performed according to OECD 204 Guideline with *Oncorhynchus mykiss* under flow-through test conditions. Five concentrations were tested (1.9, 3.4, 6.8, 13 and 29 µg/L, mean measured representing 54, 49, 49, 46 and 52 % of the nominal) at pH 7.3-7.7 and 11.5-12.5 °C. Mortality in the 13 and 29 µg/L was 25 % and 90 %, respectively. The 14-day LC_{50} value was calculated to be 17 µg/L and the NOEC was 6.8 µg/L.

One reliable long-term fish early life stage toxicity (FELS) study was available. The test was performed according to Guideline 40 CFR 797.1600 with analytical monitoring and without the use of a vehicle. Test temperature was 12 ± 2°C. It was a flow-through test with five concentrations (0.25, 0.53, 1.1, 1.9 and 4.4 µg/L, measured). The resulting NOEC from the FELS test was ≥ 4.4 µg/L (mean measured), the highest concentration tested for embryo viability, hatching success, larval survival and growth (REACH Registration dossier). On the other hand in a prolonged acute toxicity study with *Oncorhynchus mykiss,* mortality occurred at the next highest concentration of 6.9 µg/L. It cannot be ruled out that effects might have been observed at higher concentrations than tested in the FELS test. For the purpose of chronic toxicity assessment a long-term test with early life stages is strongly prefered over a prolonged acute test. However, the two studies did not overlap in test concentration, so the true level of toxicity to fish over the long-term is unclear. Overall, the long-term NOEC for fish is assumed to be around 4-6 µg/L. The prolonged acute toxicity test submitted after the Public Consultation gives results of the same magnitude as the one presented in the CLH Report.

A reliable 21 day reproduction study with *Daphnia magna* using a flow-through system with no head space was carried out. The D4 tested was > 99 % pure and stock solutions of the substance were prepared by slow-stirring dilution water with a floating layer (approximately 6 mm thick) of D4. This method of stock-solution preparation gives reproducible results and can achieve a maximum concentration of ca. 15 µg/L in hard freshwater. Five exposure concentrations were used (measured concentrations were 1.7, 1.8, 4.2, 7.9, and 15 µg/L). This study showed a statistically significant reduction in the survival at the highest concentration tested (survival in the 15 µg/L was 77 %) compared with the control population (survival was 93 %) after 21 days. The 21-day $NOEC_{survival}$ was therefore 7.9 µg/L. For the reproduction endpoint, the mean cumulative number of offspring per female daphnid was 111 in the control, 107, 92, 123, 151, and 167 in the 1.7, 1.8, 4.2, 7.9 and 15 µg/L treatment groups, respectively. There were no statistically significant differences between the control response and the treatment response in the 1.7, 1.8, and 4.2 µg/L groups, but the mean cumulative number of offspring per female was significantly higher in the 7.9 µg/L treatment group than in the control groups (the data for the 15 µg/L treatment group were not included in the statistical analysis as a reduction in daphnid survival occurred in this group). Therefore, it was concluded that concentrations of D4 ≤ 7.9 µg/L do not adversely affect the reproduction of *Daphnia magna*.

As described above, there was one toxicity study with algae available. As it is a limit test, the validity of the study was restricted. The resulting NOEC is > 6 µg/L (mean measured) (corresponding to 22 µg/L initially measured). During the Public Consultation, industry informed that they had re-calculated the result to a scientifically more precise E_rC_{10} of > 22 µg/L (initial measured) (the maximum water solubility level in the test medium).

For the long-term (chronic) aquatic hazard, the Dossier Submitter concluded that the lowest chronic toxicity values are 14 d NOEC = 0.0044 mg/L for fish, 21d NOEC = 0.0079 mg/L for invertebrates and 96 h NOE_rC < 0.022 mg/L for algae.

The Dossier Submitter proposed to classify D4 as Aquatic Chronic 1; H410 based on high potential for bioaccumulation, no rapid degradation and a 21d NOEC for Daphnia = 0.0079 mg/L supported by a 14 d $NOEC_{fish}$ = 0.0044 mg/L. An M-factor of 10 was also proposed because the lowest NOEC value is in the range 0.01 < NOEC ≤ 0.001 and the substance is not rapidly degradable.

Comments received during public consultation

Sixteen comments were received during the Public Consultation (PC). Three Member States supported the Dossier Submitters proposal to modify the D4 classification to Aquatic Chronic 1, M-factor = 10. In addition, comments were provided by individuals (UK, US and Canada). One company and two industry associations also gave comments. 'Reconsile REACH Consortium' informed that they had changed the self-classification of D4 to a weight of evidence Aquatic Chronic 2 classification based on chronic NOECs ≥15µg/L and rapid degradability or high bioaccumulation potential. This classification was supported by another industry organisation.

Physical-chemical properties and degradation

It was indicated by Industry, that test criteria and procedures developed for hydrocarbon-based chemicals are not appropriate to use for D4 due to its low water solubility, relatively high vapour pressure, very high Henry's law constant and high Log K_{ow}. Air is the final compartment of residence in the environment. Environmental exposures to D4 were expected to be minimal due to the lack of reliable evidence that D4 in air could be re-deposited into soil or water or absorbed by biota. This is also due to D4 having a relatively short half-life in air through degradation by hydroxyl radicals to silanols, which are not of toxicological concern. Both the very low solubility of D4 in water (56 µg/L) and its lower solubility in test media likely compromised the results of the hydrolysis and the ready biodegradation tests. Given that the recommended concentration of 10 mg D4/L in OECD TG 310 exceeded the maximum solubility of D4 more that 170-fold, the bioavailability of undissolved material is questionable.

The DS agreed that the test concentrations in degradation studies were well above the water solubility but a sediment simulation study supports the conclusion that the substance is not rapidly degradable. The DS agreed to the fact that substances with a low water solubility show a different solubility in the test media than in the water solubility test.

Hydrolysis

Comments were made related to the abiotic hydrolysis of D4 in water which is seen as a key degradation process in the environment. Both the rate of hydrolysis of D4 and intermediates are considered as a function of pH and temperature. The siloxanediol intermediates are not expected to require classification based on their own rapid hydrolysis and the hydrolysis product DMSD is not toxic to aquatic organisms, therefore not fulfilling the criteria for classification as hazardous to the aquatic environment. Thus, D4 meets the 16 day criterion for rapid degradability. It was argued that temperature correction for the OECD TG 111 hydrolysis test data is risk assessment rather than hazard assessment and should not be used in classification. By correcting for the mean temperature of European surface waters of 12 °C and the respective median pH value of 7.94 the hydrolysis half-life of D4 is far below 16 days. Another comment argued that when correcting to 12 °C, the slight excess of the threshold of 16 days (half-life 16.7 days at pH 7) should not be considered as being specific concern, as the study report noted that the reported hydrolysis rate constants based on linear regression analysis of the earlier time points probably underestimate the actual rate by at least 10 %. Two comments referred to Annex 9 of GHS (UN, 2013; A9.4.1.1) and Annex II of the CLP Guidance (ECHA, 2015) which suggest that classification of substances should be based on consideration of both intrinsic properties of the substance and the prevailing environmental conditions, including pH and temperature.

The DS agreed that both the relevant temperature and pH values should be considered. The DS presented a study were the pH varies from 7.0 to 8.5 and can be considered as representative for Europe. D4 should be considered as not rapidly degradable because at this pH range at 12 °C, the half-life ≤ 16.7 days.

Bioaccumulation

The $LogK_{ow}$ and BCF values used to evaluate bioaccumulation were not contested by Industry.

Aquatic toxicity

In many comments ecotoxicity tests were claimed not to be realistic because of using sealed exposure systems.

The DS responded that it is recommended in the CLP Guidance, in the OECD test guidelines and in the OECD Guidance No. 23 to minimise losses from test systems.

Toxicity to algae

There were also comments regarding the algae test. As it is a limit test, the validity of the study for use in chronic classification was questioned. Growth in controls was reduced similarly to that of the treated flask (one treatment level at the functional solubility of 22 µg/L). Cell density essentially remained unchanged in all flasks suggesting that D4 is not acutely toxic to the algae. The NOEC for algae > 22 µg/L (initial measured, 6 µg/L as mean measured) was based on yield/biomass but arguments were provided that OECD TG 201 and the CLP Guidance clearly indicate that the growth rate is preferred. As raw data had been reported in the study report, a re-analysis had been conducted revealing an inhibition of the average specific growth rate in the treatment group by less than 7 % after 72 h and 96 h, respectively, compared to the control. Therefore, the E_rC_{10} > 22 µg/L (initial measured), the maximum water solubility level in the test medium.

The DS agreed and informed that the study record reported only mean cell densities but not the cell counts per replicate and that recalculation was therefore not possible.

Chronic toxicity studies

Comments were made related to the fact that the stock test media were prepared at ambient temperatures in the most reliable long term studies although the test itself had been performed in colder temperature. The stock concentration, whether prepared by slow stirring or via solvent addition methods, was typically at saturation around 25 µg/L. The slow-stiring method to produce solutions used an excess of substance and the possibility for particulates to 'break off' and be present in the media could not be excluded. The use of solvent addition could also produce over-saturation in the vicinity of the added concentrate. The stock was then taken to a test system operating at the lower temperature of around 12 °C, and used in flow through systems with dilution. It could be anticipated that the solubility of D4 would be significantly lower at 12 °C than it was under standard conditions. However, there is no information on the solubility in the test system at 12 °C. Therefore, it was possible that the aqueous media had (before dilution) an amount of substance present that exceeded the saturated solubility at the test temperature. The analytical methods used would have analysed all the D4 present, whether dissolved or undissolved. Through a personal communication with the study director of the 14 d prolonged acute toxicity study with fish, it was confirmed that the stock solution was prepared at room temperature whereas the test solution temperature was 12 °C. It was thought possible that a super-saturated solution was active during the study leading to mortality not relevant for classification.

The Dossier Submitter noted that the test media were prepared according to the OECD guidance and the test concentrations were analytically confirmed. There was no indication of any undissolved material. The measured concentration was lower than the maximum water solubility in the standard OECD TG which is not unusual. Taking the results from the tests and also the measured concentrations together, the tests were considered conclusive by the DS. Concerning

the prolonged acute toxicity fish study (Anonymous, 1995), the DS was of the opinion that the proposed effect of the decrease in temperature is conjecture. During the analytical confirmation of the test concentration, nothing unusual was seen.

Daphnia study (Sousa et al., 1995)

Comments were made suggesting that the reproduction endpoint should be preferred to the mortality endpoint in the chronic *Daphnia* study (Sousa *et al*., 1995), OECD TG 211). A statistically significant difference in reproduction ($p \leq 0.05$) observed at the 7.9 µg/L compared to the control was not an actual effect since the number of offspring per daphnid did not decrease depending on the concentration (15 µg/L), but increased cumulatively from 111 (control) to 167 (15 µg/L) offspring/daphnid. A significant difference ($p \leq 0.05$) was also observed in the survival rate at the 7.9 µg/L treatment level in comparison with the control. The survival rate of parent daphnia changed from 93 % in the controls to 87 % and 77 % respectively at 7.9 µg/L and 15 µg/L. Some variability must also be noted; from two replicates used for statistical analysis only one was affected at 15 µg/L - a slight reduction in survival, and ultimately D4 exposure at 15 µg/L did not affect *Daphnia* reproduction or neonate size. Therefore, the overall chronic daphnia NOEC in this study should be considered ≥ 15 µg/L.

The DS stated that in the OECD TG 211, the survival of adults is also an endpoint to be documented as well as reproduction. In the same Guideline it is stated that parental mortality can also be used as an effect and that if parental mortality occurs in exposed replicates, it should be considered whether or not the mortality follows a concentration-response pattern. As the mortality occurred at the highest test concentration this could not be excluded. Therefore, the NOEC for long-term toxicity to *Daphnia* is taken as 7.9 µg/L.

Fish studies

Narcotic mode of action

The comments state that it was not surprising that D4 has no toxicity or has a low level of toxicity in most aquatic species. Like most hydrophobic chemicals, D4 acts via a narcotic mode of action, which requires the accumulation of chemical in the tissues to achieve a critical (toxic) body burden. Thus, the concepts of narcotic mode of action and chemical activity explain the apparent lack of toxicity of D4 to water column species under environmentally realistic conditions.

Some comments were made related to the results of a 14 day prolonged acute *Oncorhynchus mykiss* study, with an acute NOEC for mortality of 4.4 µg/L in support of the chronic hazard classification. The results of a 93 day early life-stage (ELS) study with *Oncorhynchus mykiss* had no effects up to the highest dose tested of 4.4 µg/L. Given the apparent inconsistency between the results of these two studies modelling was employed to determine the critical body burden (CBB) defined as the lowest body concentration of a chemical in an organism associated with adverse toxic effects. No adverse effects on embryos and larvae were noted when a 93 day trout ELS study was conducted at 12 °C up to the highest tested dose of 4.4 µg/L. These results are consistent with the results of the Mackay *et al*. (2015) simulation of the exposure, where fish averaged 1.6 g in weight by the end of the study. Additionally to 4.4 µg/L, five dose regimes were modelled: 6.9, 11, 12, 22, and 27 µg/L; the last of which is the functional water solubility for D4 at 13 °C (calculated). Only at 22 and 27 µg/L would the small trout accumulate sufficient D4 by day 90 of the simulation to exceed the CBB for a narcotic mode of action of 3 mmol/kg. This suggested that concentrations of D4 up to and including 12 µg/L could have been used in the 93 day trout study without any adverse effects. The combination of fish size/dose concentration indicates that CBB will not be achieved for 9 months (269 days). The results in the 14 day D4 prolonged acute study were inconsistent with the narcotic mode of action and

pharmacokinetic modelled results, which indicated that dose concentrations as high as 22 µg/L should not have produced mortality in day 14 or less.

The DS did not see the differences between the critical body burden (CBB) calculations and test result in the 93-day fish study as significant because the calculations refer to LC_{50} and the test result is expressed as a NOEC. The fish size was also different in the estimatate and the test. The DS was of the opinion that the calculation presented in the comment fits very well to the results of the prolonged acute fish toxicity study and the results of the FELS test.

Several arguments were weighed up by the DS to come to the conclusion that the differences between the Mackay *et al.* (2015) calculations and the results of the experimental study are to be expected. Most notablly that the calculations refered to LC_{50} values, whilst the experimental study referred to NOECs.

RAC agrees with the DS. Mackay *et al*. (2015) employ the conventional equation for dynamic uptake from water by respiration as it applies to standardized flow-through bioconcentrations tests. RAC is of the opinion that the Mackay *et al*. (2015) modelling does not explain the inconsistency between results of the 14 day study and the results of the 93 day study. The fish size used is 5 g and 0.42 g in modelling and in the 14 day study, respectively. In the 93 day study different life stages were exposed (fertilized eggs, embryos, larvae and juvenile fish). In addition, the results are expressed as NOEC in the tests and the modelling refers to LC_{50}. In Fairbrother & Woodburn (2016) it is stated that "Although the early life stage *Oncorhynchus mykiss* study conducted by Sousa *et al.* (1995) lasted 93 days, the rapid growth of the larval fish likely resulted in growth dilution such that critical body residues were not achieved even at the functional water solubility concentration".

Prolonged Toxicity Test – Long-term hazard

Comments were made concerning the use of the OECD 204 to assess chronic toxicity. According to the CLP guidance, Appendix I, I.2.1.2, tests consistent with OECD Test Guideline 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can be used in the classification scheme. The REACH IR/CSA Guidance R.7b, R.7.8.4.1 states that tests performed according to OECD 204 (Fish, Prolonged Toxicity Test: 14-Day Study (OECD 1984)) or similar guidelines cannot be considered suitable long-term tests. Therefore, the EPA 797.1400 study is not relevant for chronic toxicity and should not be used for the evaluation of chronic aquatic toxicity.

The DS agreed that OECD 204 is not considered suitable as a long-term toxicity test as only adults are exposed and maybe sensitive life stages are missed. But the OECD 204 test with D4 showed effects and this information should be used.

Assessment and comparison with the classification criteria

Degradation

Only 3.7 % of D4 degraded in 29 days in a ready biodegradability test following OECD TG 310, indicating that the substance is not readily biodegradable. RAC agrees with the comments received under Public Consultation suggesting that test concentration in the ready biodegradation study was well above the water solubility of D4. However, the OECD TG 310 is also applicable to insoluble test substances, though good dispersion of the substance should be ensured. According to the test report that was submitted to RAC, the test vessels were placed upside down on rotary shaker tables to ensure dispersion. RAC concluded that D4 is not readily biodegradable.

The average hydrolysis half-life for D4 under OECD TG 111 was calculated to be 3.9 days at pH 7 and 25 °C. At pH 4 the half-life was 1.77 hours and at pH 9 0.902 hours at 24.6 °C. However, at pH 6.99 and 9.5 °C, the half-life was 542 hours (~ 23 days). Consequently, an estimated half-life of 16.7 days at pH 7 and 12 °C was derived for freshwater. One of the degradation products was dimethylsilanediol which has not been classified for environmental hazard in the ECHA Classification and Labelling Inventory. There is no information on the environmental hazard of other intermediates or final degradation products.

The hydrolysis of D4 is dependent on both pH and temperature. Furthermore, the longest hydrolysis half-life determined within the pH range of 4-9 that is shorter than 16 days should be considered (Guidance on the Application of the CLP Criteria (Annex II.2.3.8).

D4 is not readily biodegradable, the hydrolysis half-life is longer than 16 days in environmentally realistic conditions (CLP Regulation 4.1.2.9.2.) and there is no information on environmental hazard of all degradation products. Therefore, it cannot be demonstrated that the hydrolysis products do not fulfil the criteria for classification as hazardous for the aquatic compartment. RAC is of the opinion that D4 is not rapidly degradable.

Bioaccumulation

The CLH report contains three fish bioconcentration (BCF) studies:

1. 28 day kinetic BCF for fish of 13 400 L/kg re-analysed to BCF of 14 900 L/kg (lipid content=6.4 %).

2. 39 day steady-state BCF for fish of 3 129 L/kg for the higher exposure level and 3 000 L/kg for the lower exposure level.

3. 46 day steady state BCF for fish was 3 329 L/kg at the higher exposure level and 3 967 L/kg at the lower. A kinetic BCF of 4 106 L/kg was estimated for the higher treatment level and 5, 40 L/kg for the lower.

Based on this evidence (supported by a Log Kow value of 6.488), RAC considers that D4 has a high potential to bioaccumulate.

Toxicity

Acute

No effects were seen within the accepted solubility limit of D4 in the acute toxicity studies presented in Table 1.

Chronic

The chronic tests are presented in Table 2.

RAC agrees with the DS on using sealed exposure systems to study the toxicity of D4 for classification purposes. The test methods used for D4 follow a tiered approach for selecting an appropriate exposure system suggested in OECD 23 step 4: Closed semi-static renewal or continuous flow-through system, with or without headspace, and analytically determined exposure concentrations.

In a chronic 93 day fish early life stage study, no effects were seen at the highest tested concentration of 4.4 µg/L. There are in addition two 14 day fish prolonged acute toxicity tests where effects were seen and NOECs of 4.4 and 6.8 µg/L were determined. RAC agrees with the DS's opinion that OECD TG 204 is not considered suitable as a long-term toxicity test, as only adults are exposed and sensitive life stages are missed. However, these 14 day studies with D4 showed effects and this information is useful as supportive evidence.

In a 21 day Daphnia test, a NOEC value of 7.9 µg/L was determined for survival and a NOEC greater or equal to 15 µg/L for growth and reproduction. RAC agrees to use the mortality endpoint in relation to this study. In OECD TG 211, the survival of adults is also an endpoint to be documented as well as reproduction and can be used as an endpoint.

In a 96 hour algae limit test an E_rC_{10} greater than 22 µg/L (initial measured, the limit of water solubility in the test media) was determined. The mean measured concentration of 6 µg/L as the algae E_rC_{50} is the correct interpretation of the test outcome according to RAC. Although the REACH Registration dossier states that the cell density was decreased 18% in the treatment group, cell densities in both the test and control systems were lower than expected. An additional open-system reference control demonstrated that the restricted gaseous exchange in the sealed system caused this apparent reduction in growth rate. During the test period, cell density in the control group grew by a factor of 18 and by a factor of 16.5 after 72 h, thereby fulfilling OECD TG 201 validity criteria, despite the fact that the actual test followed an EPA Guideline. RAC agrees that E_rC_{10} is preferred to $NOEC_{biomass}$.

Comparison with the criteria

According to the Guidance on the Application of the CLP Criteria, a substance is considered to be not rapidly degradable unless at least one of the following is fulfilled:

- the substance is demonstrated to be readily degradable in a 28 day test for ready degradability,
- the substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days,
- the substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life < 16 days and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

D4 does not fulfil any of the criteria above and is, thus, considered to be not rapidly degradable.

The BCF values are greater than the classification limit of 500 and the Log K_{ow} is also greater than the classification limit of 4. Consequently, RAC agrees with the DS that D4 is considered to be bioaccumulative for classification purposes.

In the available acute toxicity studies no effects were seen and **RAC is of the opinion that no acute classification is needed for D4**.

Altogether RAC agrees with the DS. The lowest chronic toxicity value was the NOEC of 0.0079 mg/L for aquatic invertebrates. Following the criteria for long-term (chronic) hazard, D4 warrants classification as **Aquatic Chronic 1**; **H410** with an **M-factor of 10** (not rapidly degradable and chronic toxicity in range of $0.01 < NOEC \leq 0.001$).

Additional references

MacKay, D. Powell, D.E. and Woodburn K.B. Bioconcentration and Aquatic Toxicity of Superhydrophobic Chemicals: A Modeling Case Study of Cyclic Volatile Methyl Siloxanes. Environ. Sci. Technol. 2015, 49, 11913-11922.

Fairbrother, A. and Woodburn K.B. Assessing the Aquatic Risks of the CYClic Volatile Methyl Siloxane D4. Environ. Sci. Technol. Lett. 2016, 3, 359-363.

Sousa, J.V., McNamara, P.C., Putt, A.E., Machado, M.W., Surprenant, D.C., Hamelink, J.L., Kent, D.J., Silberhorn, E.M., and Hobson, J.F. (1995). Effects of octamethylcyclotetrasiloxane (OMCTS) on freshwater and marine organisms. Environmental Toxicology and Chemistry *14*, 1639-1647.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).