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April 7, 2017

To Whom It May Concern,

I am writing in response to the public consultation on the hazard classification for octamethylcyclotetrasiloxane (CAS 556-67-2). I note in the "Proposal for Harmonised Classification and Labelling" (Proposal) submitted by Germany, that there is a proposed chronic aquatic hazard classification of 1, with an M-factor of 10. This hazard classification is based on the results of a 21-day reproduction study with *Daphnia magna*, supported by the results of a 14-day prolonged acute study with rainbow trout. I believe that this proposal is not based on a thorough evaluation of the science and should be reconsidered.

D4 is an extremely volatile compound with low water solubility and, as such, aquatic testing is a challenge. The studies described in the paper by Sousa et al. (1995)[1], referenced in the Proposal, were conducted in completely enclosed systems in order to prevent loss of test material. This is not an environmentally-relevant test system; however, it was necessary in order to maintain measurable test concentrations. In the *Daphnia magna* reproduction study, there was a statistically significant increase in mortality at the highest measured test concentration of 15 µg/L. However, there was no impact on reproduction, and in fact, reproduction increased with increasing test concentrations. Thus, the no observable effect concentration (NOEC) for reproduction was $\geq$ 15 µg/L. Reproduction is the population-relevant endpoint and should take precedence in the scientific evaluation of the study, especially given the extreme measures required just to keep D4 in solution.

The Proposal also points to the results of a 14-day prolonged acute rainbow trout study, with an acute NOEC for mortality of 4.4 µg/L, in support of the chronic hazard classification. It is unclear why an acute study is being used to support a chronic classification. Sousa et al. (1995) reported on the results of a 93-day early life-stage (ELS) study with rainbow trout that had no effects up to the highest dose tested of 4.4 µg/L. In order to evaluate the likely reliability of the results of the 14-day prolonged acute study, given the apparent inconsistency in the results of the two rainbow trout studies, modelling was employed to determine the critical body burden (CBB) required to elicit an adverse effect in both of the two studies using procedures described in Mackay et al. (2015)[2]. The CBB is defined as the lowest observed total body concentration of a chemical in an organism which is associated with the occurrence of adverse toxic effects. In the work of Mackay et al. (2015), a simple first order pharmacokinetic model ($C_{fish} = K_1/k_2*C_w*(1-\exp-(k_2+k_m)*time)$) is used to estimate fish CBB levels and compare those CBB levels to those associated with a narcotic mode of action, under which materials like D4 are proposed to operate (Redman et al., 2012)[3].

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[1] Sousa et al. 1995. Environ Toxicol Chem 14:1639-1647
[2] Mackay et al. 2015. Environ Sci Technol 49:11913-11922
[3] Redman et al. 2012. Environ Toxicol Chem 31:1911-1919

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The results of the 93-day trout ELS study at 12°C with a NOEC of 4.4 µg/L indicate that no adverse effects on embryos and larvae were noted at this dose/exposure time combination. As shown in Figure 1, these empirical 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. Five other dose regimes were modeled in addition to 4.4 µg/L: 6.9, 11, 12, 22, and 27 µg/L; the last modeled dose is the functional water solubility for D4 at 13°C (calculated). The graph in Figure 1 indicates that only at dose concentrations of 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 (Mackay et al., 2015). This suggests 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 occurring.

The pharmacokinetic model of Mackay et al. (2015) was also used to examine the unexplained mortality noted in the D4 14-day prolonged acute study. As shown in Table 1, the Mackay et al. (2015) model was used to calculate the time to achieve CBB at a given D4 dose concentration of 6.9, 12, and 22 µg/L, concentrations that elicited mortality in the acute study. The 4.4 µg/L empirical dose level in the 93-day trout ELS study is also presented. The results show that the experimental results from the D4 14-day prolonged acute study are anomalous compared to the pharmacokinetic modeled results, which indicate that dose concentrations as high as 22 µg/L should not have produced the observed trout mortality in 14 days or less. It is unknown why the mortality occurred in the prolonged acute study. It is possible that since the water accommodated fraction was established at room temperature (approximately 21°C) but the study was performed at 12°C, the decrease in temperature resulted in a super-saturated solution that might have introduced a precipitate into the test system causing a physical effect. Further work would be needed to confirm this hypothesis.

In summary, the 21-day *Daphnia magna* reproduction study resulted in a NOEC for reproduction of ≥ 15 µg/L, a concentration above the hazard classification 1 criteria. In addition, modeling of the pharmacokinetic behavior of D4 has shown that the empirically-observed top dose of 4.4 µg/L, the NOEC in the D4 93-day trout ELS study, could conceivably have been as high as 12 µg/L without adverse impact. However, the mortality observed in the prolonged acute study is inconsistent with a narcotic mode of action and the pharmacokinetic behavior of D4. In addition, information in the literature on possible environmental exposures suggests that D4 would not achieve a concentration in surface water that would cause toxicity to aquatic organisms. Thus, a hazard classification of 1 is inappropriate given the available data. Please let me know if you would like to discuss this further or have any questions.

Kind regards,

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Table 1. Simulation results using the Mackay et al. (2015) model to calculate time to time to achieve CBB at given D4 dose concentrations during both the 14-d prolonged acute study and 93-d trout ELS study.

D4 Concen. (µg/L)	Time (days) to achieve CBB at D4 Conc. and Fish Size
22 (14-d study)	27
12 (14-d study)	52
6.9 (14-d study)	96
4.4 (93-d ELS)	269
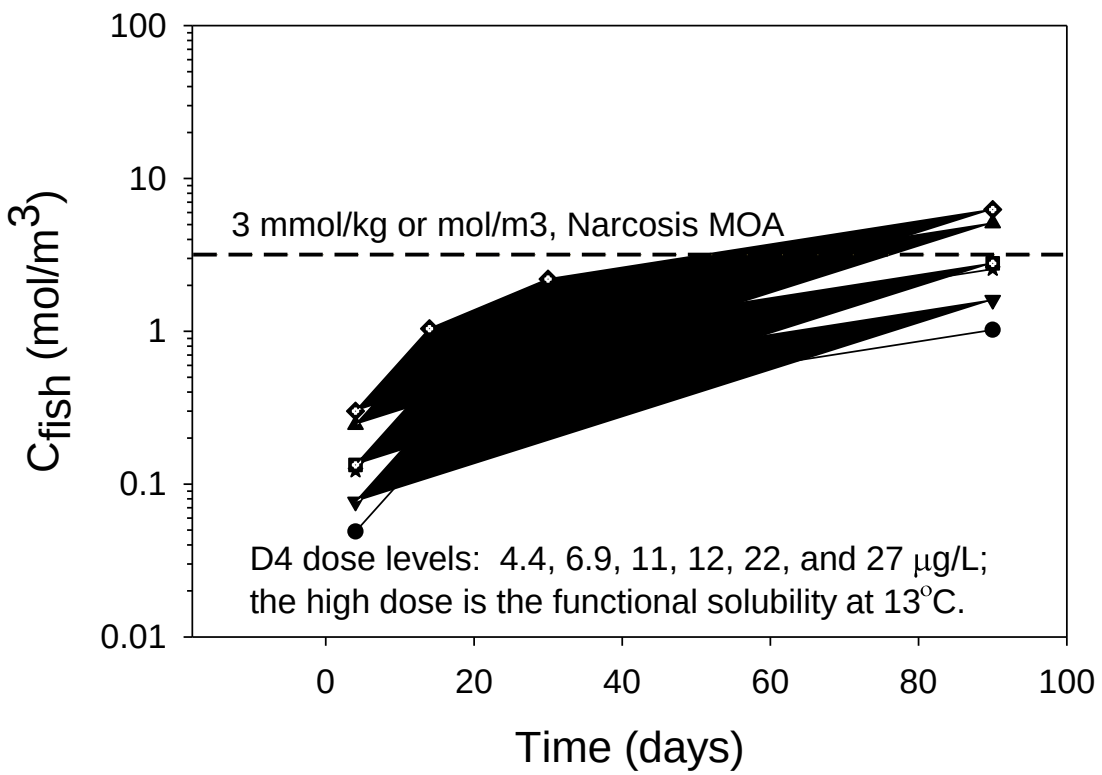
Figure 1. Pharmacokinetic modeling of D4 accumulation in fish tissue (C_{fish}) during up to a 90-d exposure of varying aqueous concentrations. At dose levels 4.4, 6.9, 11, and 12 µg/L, the C_{fish} is <3 mmol/kg (target narcosis MOA) at 90 days, while C_{fish} at 22 and 27 µg/L (the functional solubility of D4 at 13 °C) is >3mmol/kg after 90 days, suggested potential adverse impact.