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Aquatic toxicity testing of liquid hydrophobic chemicals – Passive dosing exactly at the saturation limit

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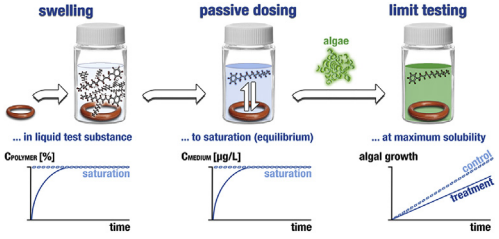
HIGHLIGHTS

- Aquatic toxicity testing of highly hydrophobic liquids exactly at saturation.
- Passive dosing from silicone O-rings established and maintained constant exposure.
- Loading of silicone by swelling: direct immersion in the pure liquid test substance.
- Precise and repeated algal toxicity test shows moderate toxicity of dodecylbenzene.

GRAPHICAL ABSTRACT



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ABSTRACT

The aims of the present study were (1) to develop a passive dosing approach for aquatic toxicity testing of liquid substances with very high K_{ow} values and (2) to apply this approach to the model substance dodecylbenzene (DDB, Log K_{ow} = 8.65). The first step was to design a new passive dosing format for testing DDB exactly at its saturation limit. Silicone O-rings were saturated by direct immersion in pure liquid DDB, which resulted in swelling of >14%. These saturated O-rings were used to establish and maintain DDB exposure exactly at the saturation limit throughout 72-h algal growth inhibition tests with green algae *Raphidocelis subcapitata*. Growth rate inhibition at DDB solubility was 13 ± 5% (95% CI) in a first and 8 ± 3% (95% CI) in a repeated test, which demonstrated that improved exposure control can lead to good precision and repeatability of toxicity tests. This moderate toxicity at chemical activity of unity was higher than expected relative to a reported hydrophobicity cut-off in toxicity, but lower than expected relative to a reported chemical activity range for baseline toxicity. The present study introduces a new effective approach for toxicity testing of an important group of challenging chemicals, while providing a basis for investigating toxicity cut-off theories.

1. Introduction

Highly hydrophobic chemicals are used in a broad range of applications. Currently, more than 850 chemicals with a Log $K_{ow} \geq 5.5$ are registered in the European Chemicals Agency (ECHA) database within the European REACH regulation (ECHA, 2015; OECD, 2015). Reliable assessments of their environmental fate, exposure, and

Abbreviations: CD, cross section; DAD, diode array detection; DDB, dodecylbenzene; DIN, German Institute for Standardization; EC, effective concentration; FLD, fluorescence detection; OD, outer diameter; PAR, photosynthetically active range; PTFE, polytetrafluoroethylene; SIM, selected-ion monitoring.

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effects are urgently needed. However, the experimental determination of, e.g., their biodegradability, bioaccumulation, and toxicity is associated with scientific, conceptual, and technical challenges; particularly when it comes to aquatic testing of highly hydrophobic organic chemicals. The present study addresses several of these challenges and provides new concepts and methods related to the aquatic toxicity testing of highly hydrophobic chemicals that are liquids at ambient temperature, amounting to more than 300 chemicals currently registered in the ECHA database (ECHA, 2015; OECD, 2015).

Effective concentrations (e.g., EC_{50}) for baseline toxicity, also referred to as non-polar and polar narcosis, generally decrease with increasing Log K_{ow} due to increasing partitioning from water into the lipid membranes of the test organism. This was already shown in the classical fish toxicity study by Könemann (Könemann, 1981) and forms the basis for a wide range of quantitative structure activity relationships (QSARs) (Wong et al., 1984; Veith et al., 1983; Qin et al., 2010; Fu et al., 2015; Aruoja et al., 2014; Hsieh et al., 2006). While such relationships are well established within the moderate Log K_{ow} range of 2–5, the situation is less clear for highly hydrophobic chemicals with Log K_{ow} values above 5 and very low aqueous solubility, e.g., below 10 μg L^{-1}. Can such highly hydrophobic chemicals cause toxic effects on aquatic organisms at very low concentrations or are they largely non-toxic even at their aqueous solubility limit? When addressing this question, it is important to distinguish between solid and liquid chemicals for two reasons: (1) For solids, the absence of toxicity at the solubility limit can be due to the so called melting point cut-off in toxicity, when chemicals crystallize at concentrations below the levels required for toxicity (Mayer and Reichenberg, 2006; Mayer and Holmstrup, 2008). The maximum chemical activity of these chemicals is then below the chemical activity required for baseline toxicity, which is typically in the range of 0.01–0.1 (Mayer and Holmstrup, 2008; Reichenberg and Mayer, 2006; Schmidt and Mayer, 2015). From a physicochemical point of view, the situation is fundamentally different for liquids. Their maximum chemical activity is by definition 1, when using the pure liquid state as reference (Reichenberg and Mayer, 2006), and they are thus by default expected to have the potential for exerting baseline toxicity at their respective solubility limit. (2) The absence of toxicity for a solid chemical does not imply that it is not contributing to mixture toxicity, since solid non-toxic chemicals may still form toxic mixtures (Smith et al., 2013b; Sugatt et al., 1984). The physicochemical basis for this is that aqueous solubilities of solid hydrophobic organics generally are additive in a mixture (Banerjee, 1984), which leads to higher exposure in a mixture. Such solubility additivity is not expected for liquid chemicals (Banerjee, 1984), which means that the extrapolation of non-toxicity data from single substance testing to complex mixtures is more straightforward for liquid than for solid chemicals.

Recently, new passive dosing formats were developed for the toxicity testing of solid hydrophobic chemicals in various test systems, which then were applied to gain a better understanding of toxicity, toxicity cut-offs, and also mixture toxicity (Mayer and Holmstrup, 2008; Smith et al., 2010a, 2013b; Butler et al., 2013). The scientific aim of the present study is to make progress in testing and understanding the toxicity of hydrophobic liquid substances. The null hypothesis is that a highly hydrophobic liquid substance can exert aquatic toxicity when tested at the saturation limit and thus the maximum exposure level (i.e., at chemical activity of unity). The technical aim of the study is to develop and apply a practical dosing method for aquatic toxicity testing of liquid hydrophobic chemicals exactly at the saturation limit, while at the same time avoiding the presence of free liquid substance (undissolved) and co-solvent in the test, which both can lead to effects that are not directly related to the toxicity of the chemical. Although it can be questioned whether the solubility limit of highly hydrophobic substances actually will be reached in the environment, the importance of suitable ecotoxicological methods is strongly supported by two facts. First, the general possibility of assessing the environmental impact of highly hydrophobic compounds is essential, therefore suitable methods are strongly needed. Second, for substances with very low aqueous solubility it is of particular interest to know whether the substance can exert toxicity at exposures up to the saturation level, while avoiding physical effects of the pure form (e.g., film formed at surfaces, droplets, and emulsions) on the test organism. Here, we introduce a new passive dosing approach, where a silicone polymer is loaded to saturation by directly immersing it in the liquid test substance. The saturated silicone polymer is then used to control exposure exactly at the saturation limit by equilibrium partitioning in an aquatic toxicity test. Silicone O-rings were used as passive dosing format, since they have proven versatile for passive dosing of hydrophobic organic chemicals in environmental and toxicological testing and research (Smith et al. 2010b, 2012, 2013a Bougeard et al. 2011; Gilbert et al., 2014; Vergauwen et al., 2015). Additionally, we also tried to saturate test medium by partitioning from silicone microtubes that were filled with the liquid test substance. However, this did not work as well and is thus only briefly described.

The algal growth inhibition test was selected for the present study because it (1) is required for the Environmental Hazard and Risk Assessment of chemicals (European Union, 2006), (2) provides information about acute and chronic toxicity within a short test duration, and (3) is particularly prone to test substance losses by evaporation and/or binding and therefore especially appropriate for the application of passive dosing. Losses can further be minimized by conducting the test in a closed (headspace) system (Brack and Rottler, 1994; Halling-Sorensen et al., 1996; Chen et al., 2009; Mayer et al., 2000; Tsai and Chen, 2007). Additionally, unicellular green algae have much higher surface to volume ratios when compared to for instance Daphnia and fish, which leads to much faster bioconcentration kinetics and allows steady state or equilibrium concentrations to be reached in the test organism within a shorter time span (Sijm et al., 1998). The fast equilibration is further promoted by the rapid mixing, which is essential to keep algae in suspension. The liquid hydrophobic substance dodecylbenzene (DDB) was selected as the model compound. It has an experimentally determined Log K_{ow} of 8.65 (Sherblom et al., 1992), and only few aquatic toxicity data were found in the literature for this substance (Gledhill et al., 1991). DDB is among other chemicals used in cooling lubricants for machining processes in the metalworking industry (Baumann and Herberg-Liedtke, 1995) and as a precursor substance of anionic surfactants like sodium dodecylbenzene sulfonate, which are widely used in cleaning agents, laundry detergents, and personal care products (Kosswig, 2000).

2. Materials and methods

2.1. Chemicals and materials

Analytical grade dodecylbenzene was used as model compound (DDB; CAS 123-01-3; >98.0%; liquid at standard conditions; TCI Europe N.V., Belgium, product code *D1074*). Food-grade silicone O-rings and medical-grade silicone tubes were used as partitioning donors in the initial passive dosing experiments and the subsequent algal growth inhibition tests. The O-rings had a cross section (CS) of 2.40 mm, outer diameter (OD) of 14.40 mm, and mass of 223.6 ± 1.1 mg (n = 13), while the tubes had a length of 17 cm, CS of 2.00 mm, bore of 1.00 mm, and mass of 452.9 ± 5.0 mg (n = 10) (Altec Products Ltd, United Kingdom, product code *ORS-0096-24* for