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Improving the reliability of aquatic toxicity testing of hydrophobic chemicals via equilibrium passive dosing – A multiple trophic level case study on bromochlorophene

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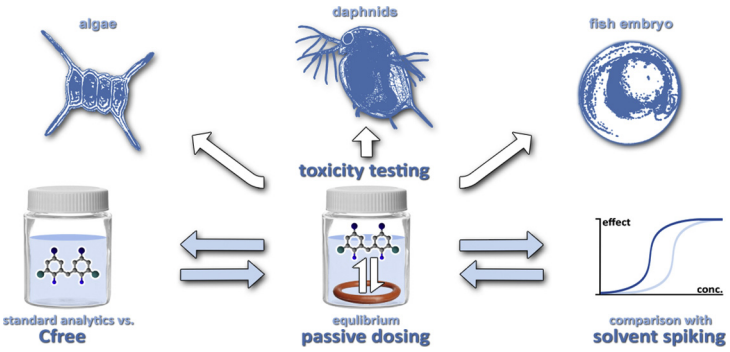
HIGHLIGHTS

- Aquatic toxicity testing of a hydrophobic chemical by equilibrium passive dosing
- Bromochlorophene affects multiple trophic levels (algae, daphnids, fish embryos).
- Streamlining simultaneous ecotoxicity testing
- Passive dosing reveals higher toxicity compared to aquatic standard tests.

GRAPHICAL ABSTRACT



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ABSTRACT

The main objective of the present study was to improve the reliability and practicability of aquatic toxicity testing of hydrophobic chemicals based upon the model substance bromochlorophene (BCP). Therefore, we adapted a passive dosing format to test the toxicity of BCP at different concentrations and in multiple test systems with aquatic organisms of various trophic levels. At the same time, the method allowed for the accurate determination of exposure concentrations (i.e., in the presence of exposed organisms; C_{test}) and freely dissolved concentrations (i.e., without organisms present; C_{free}) of BCP in all tested media. We report on the joint adaptation of three ecotoxicity tests – algal growth inhibition, *Daphnia magna* immobilization, and fish-embryo toxicity – to a silicone O-ring based equilibrium passive dosing format. Effect concentrations derived by passive dosing methods were compared with corresponding effect concentrations derived by standard co-solvent setups. The passive dosing format led to EC_{50}-values in the lower μg L^{-1} range for algae, daphnids, and fish embryos, whereas increased effect concentrations were measured in the co-solvent setups for algae and daphnids. This effect once more shows that passive dosing might offer advantages over standard methods like co-solvent setups when it comes to a reliable risk assessment of hydrophobic substances. The presented passive dosing setup offers a facilitated, practical, and repeatable way to test hydrophobic chemicals on their toxicity to aquatic organisms, and is an ideal basis for the detailed investigation of this important group of chemicals.

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1. Introduction

Hydrophobic chemicals, which have high octanol water partition coefficients (i.e. log $K_{OW} > 5$) are used in a broad range of applications. Currently, >850 chemicals with a log $K_{OW} \geq 5.5$ are registered in the European Chemicals Agency (ECHA) database within the EU Chemical Legislation regulation REACh (OECD, 2015, ECHA, 2015). In the field of cosmetics (also referred to as personal care products; PCP), a high amount of ingredients is estimated to be hydrophobic and, e.g., applied as emulsifiers or preservatives (Tolls et al., 2009). A high production volume and the typical way of 'rinse off' application promotes the potential transfer of substantial amounts of these chemicals from wastewater to the aquatic environment (Brooks et al., 2009, Daughton, 2002, Damme et al., 2011). Additionally, these chemicals are of high environmental concern because of their unfavorable environmental properties, i.e., persistence, bioaccumulation potential, and toxicity (PBT).

Reliable assessments of environmental fate, exposure, and effects of these compounds are urgently needed. However, the experimental measurements are associated with scientific, conceptual, and technical challenges. Maintenance of constant exposure concentrations during aquatic toxicity testing is difficult due to extensive sorption of the substances to surfaces such as test vessels, micro particles, and organisms (Mayer et al., 1999, Smith et al., 2010a, Smith et al., 2010b). Also, analysis of the freely dissolved concentration in the test medium (C_{free}) over time is by no means straightforward. Several publications have discussed the importance of substance quantification by appropriate methods (e.g., solid phase micro extraction - SPME), especially in case of hydrophobic chemicals (Birch et al., 2010, Jonker and van der Heijden, 2007).

Knowledge of the water solubility and lipophilicity (log K_{OW}) is mandatory for developing suitable ecotoxicity test regimes. However, reliable data on the water solubility of hydrophobic chemicals are often not readily available in literature. Also experimentally determined solubility values according to standard guidelines (OECD 105, OECD, 1995) are scarce and prognostic tools using available structure analysis software are not always reliable for describing the substance properties.

There is intensive discussion about the aquatic ecotoxicity testing of hydrophobic chemicals and the modification of standard procedures (Mayer and Reichenberg, 2006, Mayer et al., 1999, Smith et al., 2010b, Rufli et al., 1998, Kwon et al., 2016, Stibany et al., 2017). One method regarded as suitable for the testing of hydrophobic chemicals in particular is passive dosing, where a polymer (e.g., silicone) is loaded with the test substance at different concentrations up to saturation and acts as a reservoir (Mayer and Holmstrup, 2008, Mayer et al., 1999, Smith et al., 2010a, Smith et al., 2010b, Birch et al., 2010). The silicone polymer is able to take up considerable amounts of the hydrophobic test substance, and can provide constant exposure concentrations in the test medium by equilibrium partitioning. Silicone O-rings have proven versatile for the passive dosing of hydrophobic chemicals in environmental and toxicological testing and research (Smith et al., 2010b, Bougeard et al., 2011, Smith et al., 2012, Schmidt et al., 2013, Gilbert et al., 2014, Vergauwen et al., 2015, Seiler et al., 2014, Butler et al., 2013, Smith et al., 2013). Additionally, O-rings are practicable, compatible with many different aquatic toxicity test systems, and commercially available in high quality and multiple standardized sizes.

In the current study, different aquatic toxicity tests (according to OECD) have been adapted to a passive dosing format: the algal growth inhibition test with the green algae *Desmodesmus subspicatus* (OECD, 2011, Eisentraeger et al., 2008), the *Daphnia magna* immobilization test (OECD, 2004, Eisentraeger et al., 2008), and the fish embryo toxicity test with the zebrafish *Danio rerio* (OECD, 2013, Johann et al., 2016). The preservative agent bromochlorophene (BCP; also referred to as 2,2′-methylenebis(6-bromo-4-chlorophenol)) was selected as a model compound. With predicted log K_{OW} values of 6.12 (EPI Suite, US EPA, 2016) and >5.82 (pH < 7.4; ACD/I-Lab, 2016) its hydrophobicity is high, with a correspondingly low solubility in water. Due to its anti-microbial, germicidal, and preserving properties, it is used as an antimicrobial agent in personal care products like deodorants, antiseptic soaps, mouthwashes, and toothpaste (Courant et al., 1995, Moran et al., 2005, Ash and Ash, 2004). Similar substances like dichlorophene are in widespread use as antimicrobial agents, e.g., in household products (Ash and Ash, 2004, Lone et al., 2017, Kwon et al., 2013). However, experimental data on the aquatic toxicity of BCP as well as predicted environmental concentrations are currently not available. With the current study we provide first experimental data on this unevaluated chemical.

The main technical and scientific objectives of this study were: (1) the application of a reliable dosing method for the aquatic toxicity testing of hydrophobic substances, while improving practicability by the conduction of three different test systems and simultaneous measurement of freely dissolved concentrations (C_{free}) in the respective media over time, (2) the achievement of a comprehensive series of aquatic toxicity test data with a chemical of unknown toxicity and organisms of different trophic levels by passive dosing methodologies, and (3) the comparison of the derived effect data with effects measured by standard methods, such as co-solvent spiking.

2. Material and methods

2.1. Chemicals and materials

Analytical grade bromochlorophene was used as model compound (CAS 15435-29-7; >95.0%; TCI Europe N.V., Belgium, product code *M1940*). Food-grade silicone O-rings were used as partitioning donors in the passive dosing experiments (Altec Products Ltd., United Kingdom). The O-rings differed in cross section (CS) and (outer) diameter (OD), i.e., in mass. Depending on the size of the test vessels and the amount of medium applied in the different experiments, the following sizes of O-rings made from the same polymer material were used: (i) algal growth inhibition test: Altec ORS-BS316, CS of 5.33 mm, OD of 32.25 mm, and mass of 2460 mg, (ii) *Daphnia magna* immobilization test: Altec ORS-BS309, CS of 5.33 mm, OD of 21.13 mm, and mass of 1453 mg, and (iii) fish-embryo toxicity test: Altec ORS-BS131, CS of 2.62 mm, OD of 47.76 mm, and mass of 991 mg (Altec Products Ltd., United Kingdom). Algal growth inhibition tests were conducted in 100-mL Erlenmeyer flasks (VWR International GmbH, Germany), *Daphnia magna* immobilization tests were conducted in 40-mL glass vials (VWR International GmbH, Germany), and fish-embryo toxicity tests were conducted in 50-mL crystallizing dishes (VWR International GmbH, Germany). To minimize sorption, only inert materials (e.g. glass, stainless steel, and PTFE) were in contact with the test media. All solvents were purchased from VWR (HPLC grade, VWR International GmbH, Germany). Milli-Q water (Milli-Q-Reagent Grade Water System, Merck Millipore, Germany) and lint-free tissues (Karl Hecht GmbH, Germany) were used for rinsing and drying, respectively.

2.2. Loading the silicone O-rings with BCP

Before use, glassware was rinsed twice with ethanol and Milli-Q water, respectively. The silicone O-rings were carefully pre-cleaned before loading in order to remove potential impurities. First, O-rings were soaked for 2 h in excess methanol (shaking at 150 rpm). The cleaning step was then repeated overnight in a fresh portion of excess methanol, before the O-rings were soaked in methanol for 2 h again and finally rinsed in Milli-Q water over night. The O-rings were left to dry for at least 24 h at room temperature.

A saturated BCP stock solution was created by the addition of excess BCP to pure methanol, followed by constant shaking for at least 48 h (Smith et al., 2010a). The solid phase of pure BCP was then given another 24 h to settle, separating it from the overlying saturated BCP methanol stock solution. Loading solutions with different concentrations were afterwards prepared by dilution of this BCP methanol stock solution. All silicone O-rings were loaded together in the same closed glass vial,