Ecotoxicology and Environmental Safety 166 (2018) 26–34

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to polystyrene microplastics

Alice A. Horton[a,b,*], Martina G. Vijver[b], Elma Lahive[a], David J. Spurgeon[a], Claus Svendsen[a], Roel Heutink[b], Peter M. van Bodegom[b], Jan Baas[a,b]

[a] Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Wallingford Oxfordshire OX10 8BB, UK
[b] Institute of Environmental Sciences, University of Leiden, P.O. Box 9518, 2300 RA Leiden, the Netherlands

ARTICLE INFO

Keywords:
Ecotoxicology
Microbeads
Log Kow
Insecticide
Dimethoate
Deltamethrin

ABSTRACT

Daphnia magna were exposed to two pesticides in the presence or absence of microplastics (300 000 particles ml^{-1} 1 μm polystyrene spheres) and to microplastics alone. The pesticides were dimethoate, an organophosphate insecticide with a low log Kow, and deltamethrin, a pyrethroid insecticide with a high log Kow. *Daphnia* were exposed to a nominal concentration range of 0.15, 0.31, 0.63, 1.25, 2.5, 5 mg l^{-1} dimethoate and 0.016, 0.08, 0.4, 2, 5 and 10 μg l^{-1} deltamethrin. Exposure to polystyrene microplastics alone showed no effects on *Daphnia magna* survival and mobility over a 72 h exposure. In the dimethoate exposures, mobility and survival were both affected from a concentration of 1.25 mg l^{-1}, with effects were seen on mobility from 28 h and survival from 48 h, with greater effects seen with increasing concentration and exposure time. In deltamethrin exposures, survival was affected from a concentration of 0.4 μg l^{-1} and mobility from a concentration of 0.08 μg l^{-1}. Effects of deltamethrin on mobility were seen from 5 h and on survival from 28 h, with greater effects on survival and mobility seen with increasing concentration and exposure time. Contrary to expectations, pesticide toxicity to *Daphnia magna* was not affected by the presence of microplastics, regardless of chemical binding affinity (log Kow). This therefore suggests that polystyrene microplastics are unlikely to act as a significant sink, nor as a vector for increased uptake of pesticides by aquatic organisms.

Capsule: Polystyrene microplastics are unlikely to act as vector for increased uptake of pesticides by aquatic organisms

1. Introduction

Microplastics are a pollutant of increasing environmental concern based on their ubiquitous and persistent nature. It is widely recognised that microplastics will form biological and chemical associations within the environment. For example microplastics may become associated with algae or bacteria (biofilms) (Hoellein et al., 2016; McCormick et al., 2014) or may sorb organic chemicals due to their hydrophobic nature (Bakir et al., 2012; Koelmans et al., 2016; Mato et al., 2001). The potential for association of hydrophobic organic chemicals (HOCs) with microplastics has been recognised and has prompted studies on whether this association will affect the bioavailability of HOCs, and thus their toxicity to organisms. Studies have shown that microplastics can make HOCs either more bioavailable, by acting as a vector for uptake following ingestion (Avio et al., 2015; Chen et al., 2017; Rochman et al., 2013b), or less bioavailable due to strong irreversible binding of HOCs to microplastics, removing HOCs from solution and remaining bound even if ingested (Beckingham and Ghosh, 2016). It has even been suggested that microplastics may lead to the removal of HOCs from body tissues following the ingestion of clean plastics by a previously contaminated organism (Koelmans et al., 2013). The majority of studies on microplastics and chemical associations to date have focussed on the marine environment. However, concentrations of HOCs and microplastics in continental terrestrial and freshwater environments are expected to be higher than marine environments due to their proximity to the sources combined with limited dispersal and dilution, thus highlighting the importance of studying terrestrial and freshwater systems (Dris et al., 2015; Horton et al., 2017).

The capacity for a chemical to bind to microplastics is, among other factors, determined by its hydrophobicity, usually expressed as the log Kow value. Kow represents the partition coefficient between octanol and water (Brooke, 2014). A chemical with a high log Kow will have a lower water solubility than less hydrophobic substances (with a lower log Kow), meaning that it will preferentially bind to organic particulate

* Corresponding author at: Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Wallingford Oxfordshire OX10 8BB, UK.
E-mail address: alihort@ceh.ac.uk (A.A. Horton).

https://doi.org/10.1016/j.ecoenv.2018.09.052
Received 15 June 2018; Received in revised form 7 September 2018; Accepted 10 September 2018

matter within the system rather than remaining within solution (Lee et al., 2014; Mackay et al., 1980). It is therefore expected that a chemical with a high log Kow (high hydrophobicity) will also have a higher affinity for binding to microplastics in an aqueous system than a chemical with a lower log Kow (higher hydrophilicity) (Wang et al., 2018). Such interactions can potentially remove the chemical from solution and concentrate it on the surface of the plastic, thereby changing bioavailability (Gouin et al., 2011; Lee et al., 2014; Velzeboer et al., 2014). The aim of this study was therefore to investigate how the presence of microplastics would affect the toxicity of high and low log Kow organic pesticides to a relevant freshwater organism, the cladoceran *Daphnia magna*. Pesticides were chosen as their toxicity is well-documented. The starting hypothesis was that the presence of microplastics within an aquatic solution would reduce the toxicity of a pesticide with a high log Kow, due to its high binding capacity to the microplastics making it less bioavailable (Beckingham and Ghosh, 2016; Koelmans et al., 2013), whereas the toxicity of a low log Kow pesticide would be less affected by the presence of microplastics.

2. Materials and methods

2.1. The test chemicals

We chose two pesticides to represent chemicals with high and low log Kow, both with known toxicity to *Daphnia magna*. Dimethoate and deltamethrin were chosen both for their differing chemical properties (specifically log Kow) and because they are environmentally relevant, being representative of two widely used classes of insecticides. Both pesticides target receptors associated with nervous system function to cause neurotoxicity. Dimethoate is an organophosphate insecticide with a low log Kow (0.704) (Pesticide Properties Database, 2017b). It is relatively soluble in water (between 23.5 and 39.8 $g\,l^{-1}$ at 25 °C) (Pesticide Properties Database, 2017b; Sigma-Aldrich, 2017). It was first registered for use in 1962 and is still widely applied to agricultural land worldwide (Van Scoy et al., 2016). Deltamethrin is a pyrethroid insecticide also widely used in agriculture (Ren et al., 2009) and aquaculture (Ernst et al., 2014). Deltamethrin is very poorly soluble in water, with a solubility between 0.2 and 2 $\mu g\,l^{-1}$ at 25 °C (Mestres and Mestres, 1992; Pesticide Properties Database, 2017a). Due to this hydrophobic nature, with a log Kow reported between 4.6 (Kaneko, 2010) and 6.2 (PubChem Compound Database, 2017), deltamethrin entering a water body would be expected to adsorb readily to particulate matter such as microplastics, in addition to sediment and organic matter (Lee et al., 2014, 2002).

2.2. The test organism

Daphnia magna is commonly used for ecotoxicological testing and as such, toxicity data are readily available for *D. magna* for both deltamethrin and dimethoate toxicity (Andersen et al., 2006; Toumi et al., 2013), as well as information on microplastic uptake and toxicity (Besseling et al., 2014; Jemec et al., 2016; Rehse et al., 2016). This makes them an ideal species for investigating how toxicity may be influenced by the interaction of these pesticides with microplastics.

D. magna were taken from the Leiden University culture which has been continuously maintained for over six years in the laboratory. According to the OECD guideline 202, *D. magna* were cultured in glass containers with Artificial ElendtM4 medium at a density of 1 individual/10 ml of ElendtM4 medium (OECD, 2004). The culture medium was refreshed twice a week. The test organisms were fed *ad libitum* with *Raphidocelis subcapitata* algae and maintained inside a temperature controlled chamber (20 ± 1 °C) under a 16:8 light-dark cycle. Throughout the duration of culturing, sensitivity of the test species was checked every six months using the standardized toxicity test conducted with $K_2Cr_2O_7$ as a reference compound (OECD, 2004).

2.3. Preparation of the microplastic beads

Microplastics as fluorescent polystyrene beads were purchased from Phosphorex (USA) with a nominal size of 1 μm, as a solution containing DI water, an anti-microbial agent (sodium azide) and a surfactant (Tween 20). The size of particles was confirmed by TEM as being 1.2 ± 0.2 μm (mean ± SD) (Fig S1). Previous experimental studies have shown that microplastics within the size range 20 nm – 5 μm are commonly ingested by *D. magna*, as they represent a similar size range as their common algal food sources (Besseling et al., 2014; Ogonowski et al., 2016; Rehse et al., 2016; Rist et al., 2017; Rosenkranz et al., 2009). Both sodium azide and Tween 20 may act as toxicants and so the beads were washed in order to remove these from the solution used for microplastic spiking. For washing, the supplied stock of beads (1 ml) was diluted to approximately 12 ml with Milli-Q water, vortexed to mix and then centrifuged at 5180 g (5000 rpm) (Beckman Coulter Avanti J-E centrifuge, USA) for 5 min. The supernatant was then carefully pipetted leaving approximately 1 ml of solution containing the particles at the bottom. These cleaning steps of dilution and centrifuging were then repeated twice more to ensure maximum removal of the sodium azide and Tween20. Following the final cleaning step the solution was diluted with Milli-Q water to give a total stock solution volume of 10 ml. The number of beads per ml of this new bead stock was measured using a flow cytometer (BD Accuri C6, BD Biosciences, USA). This bead stock was used for spiking the test medium to a nominal concentration of 300,000 particles ml^{-1}. This concentration is roughly equivalent to the number of algal cells that daphnids would be exposed to in an excess food situation (*i.e.* under culture conditions) and equates to approximately 0.29 $\mu g\,ml^{-1}$ (287.7 $\mu g\,l^{-1}$, calculations in SI).

2.4. Preparation of the test solutions

A dimethoate (PESTANAL®, analytical standard, Sigma Aldrich Ltd, UK) stock solution of 1 $g\,l^{-1}$ was prepared directly in Elendt artificial freshwater. In order to produce the required concentrations, the appropriate amount of stock solution was made up to 250 ml with Elendt artificial freshwater. Based on toxicity values of dimethoate to *D. magna*, with 48 h LC_{50} ranging from 0.86 to 2 $mg\,l^{-1}$ (Beusen and Neven, 1989; Syberg et al., 2008), exposure concentrations were made in the range 0.156, 0.313, 0.625, 1.25, 2.5, 5 $mg\,l^{-1}$ (0.68, 1.36, 2.73, 5.45, 10.9, 21.8 μM).

To spike the test medium with deltamethrin it was necessary to dissolve it in a solvent carrier due to its low solubility in water. Deltamethrin (certified reference material, Sigma-Aldrich Ltd, UK) was dissolved in acetone to prepare a stock solution of 10 000 $\mu g\,l^{-1}$. A serial dilution of this stock, was made by further dilution in acetone to create a deltamethrin concentration series for spiking into artificial freshwater. A volume of 375 μl of the relevant stock was added to 250 ml Elendt artificial freshwater (giving an acetone concentration of 0.15% within the exposure solution) in order to give the required exposure concentration range: 0.016, 0.08, 0.4, 2, 5 and 10 $\mu g\,l^{-1}$ (0.03, 0.16, 0.79, 3.96, 9.9, 19.79 nM). These exposure concentrations were based on literature toxicity data for *D. magna* with 48 h LC_{50}s ranging from 0.038 to 0.45 $\mu g\,l^{-1}$ (Ren et al., 2009; Xiu et al., 1989) and 24 h LC_{50}s ranging from 0.113 to 9.4 $\mu g\,l^{-1}$ (Toumi et al., 2013; Xiu et al., 1989).

For both pesticides, treatments were prepared with and without microplastics. For the microplastic treatments, the polystyrene bead stock solution was added to the exposure solutions after the artificial freshwater had been spiked with the chemicals. The appropriate volume of stock solution (as determined using the flow cytometer) was added to a volume of 250 ml of spiked solution to give a nominal concentration of 300 000 particles ml^{-1}. Four replicates of 40 ml exposure solution held in 50 ml glass jars were prepared for each treatment. With an average particle size of 1.2 μm ± 0.2 μm, the average surface area of the microplastics within 40 ml was calculated as approx.