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Occurrence and risk assessment of an azo dye – The case of Disperse Red 1

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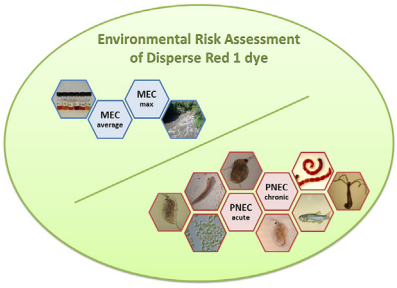
HIGHLIGHTS

- Disperse Red 1 dye was found in river waters in concentrations above the PNEC.
- The PNEC was based on toxicity endpoints for a commercial dye and its purified form.
- The CRED method was used to evaluate the quality of endpoints used in PNEC derivation.

GRAPHICAL ABSTRACT



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ABSTRACT

Water quality criteria to protect aquatic life are not available for most disperse dyes which are often used as commercial mixtures in textile coloration. In this study, the acute and chronic toxicity of the commercial dye Disperse Red 1 (DR1) to eight aquatic organisms from four trophic levels was evaluated. A safety threshold, i.e. Predicted No-Effect Concentration (PNEC), was derived based on the toxicity information of the commercial product and the purified dye. This approach was possible because the toxicity of DR1 was accounting for most of the toxicity of the commercial mixture. A long-term PNEC of 60 ng L^{-1} was proposed, based on the most sensitive chronic endpoint for *Daphnia similis*. A short-term PNEC of 1800 ng L^{-1} was proposed based on the most sensitive acute endpoint also for *Daphnia similis*. Both key studies have been evaluated with the new "Criteria for Reporting and Evaluating ecotoxicity Data" (CRED) methodology, applying more objective criteria to assess the quality of toxicity tests, resulting in two reliable and relevant endpoints with only minor restrictions. HPLC-MS/MS was used to quantify the occurrence of DR1 in river waters of three sites, influenced by textile industry discharges, resulting in a concentration range of 50–500 ng L^{-1}. The risk quotients for DR1 obtained in this work

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Mixture
PNEC

suggest that this dye can pose a potential risk to freshwater biota. To reduce uncertainty of the derived PNEC, a fish partial or full lifecycle study should be performed.

1. Introduction

Disperse dyes are synthetic colorants for hydrophobic substrates and are commonly applied as commercial mixtures in textile coloration. They are often used in great quantities and due to the huge amount of water involved in the associated dyeing processes and the high proportion of the dye that remains in the water bath, large volumes of wastewater can be generated (Hunger, 2003). It is also known that the conventional treatment of these wastewaters, involving aerobic lagoons or activated sludge, are not efficient in the removal or biological degradation of these dyes and alternative treatment process are necessary to achieve this removal (da Silva Leite et al., 2016; USEPA, 1990). Therefore, the unreacted dyes are often still present in the wastewaters and sludges from textile plants (Umbuzeiro et al., 2005). Although disperse azo dyes are poorly water-soluble compounds, they become dispersed in water because their commercial formulations contain surfactants needed for the dyeing process. One of the main surfactants used for disperse dyes is sulfonated lignin, because of its low cost and easy availability (Tehrani-Bagha and Holmberg, 2013).

Disperse dyes have been found in rivers and sediments worldwide (Maguire, 1992; Zocolo et al., 2015) and mutagenic activity of surface waters and sediments were attributed to the presence of these compounds (Oliveira et al., 2006; Umbuzeiro et al., 2005). Moreover, synthetic dyes are assumed to be toxic to aquatic organisms (Ribeiro and Umbuzeiro, 2014). For example, Disperse Red 1 (DR1) is representative for phenylazoaniline dyes and there are more than 50 commercial products on the market (Colour Index, 2011). This dye also has previously been shown to have a high acute toxicity to the water flea *Daphnia similis*, both when it was tested as pure compound as well as commercial formulation (Ferraz et al., 2011; Vacchi et al., 2013). More recently, it was shown that DR1 affects the regeneration and fecundity of the freshwater planarian *Girardia tigrina* (Ribeiro and Umbuzeiro, 2014). This dye also showed genotoxic potentials in the *Salmonella*/microsome assay, the comet assay using HepG2 cells, as well as the micronucleus assay involving human lymphocytes and HepG2 cells (Chequer et al., 2009; Ferraz et al., 2011; Oliveira et al., 2010). Another study showed that DR1 induces cytotoxic and genotoxic effects in mouse germ cells, indicating the harmful activity of this dye (Fernandes et al., 2015). Nevertheless, currently no regulatory thresholds exist for DR1 to ensure the protection of aquatic biota or human from this important group of compounds (Ribeiro and Umbuzeiro, 2014).

In this context, the derivation of Predicted No-Effect Concentrations (PNEC), i.e. the concentrations at which no adverse effects on the ecosystem are expected, is an important step in the risk assessment of these chemicals. PNECs are commonly derived from standard toxicity tests, using well-defined protocols for a limited number of reference species, as well as an assessment factor to account for the uncertainty related to laboratory-field extrapolations. To derive sound PNECs, it is advantageous to use various chronic tests from species covering different trophic levels, representing a wide variety of taxonomic groups and sensitive species, although acute tests can also be used (European Commission, 2011). Thereby, it is most crucial that the endpoints used to derive the respective PNECs are reliable (Moermond et al., 2016). To adequately test chemical substances, it is necessary to have the pure compound dissolved in the testing media. This can be challenging for pure disperse dyes because of their poor water solubility. Therefore testing the commercial formulation is easier, but this approach does generally provide less reliable information. Nevertheless, a PNEC for the main compound can be derived when this is considered.

The main objective of this study was therefore to derive short and long-term PNECs for the protection of freshwater biota towards Disperse Red 1, based on a set of relevant and reliable ecotoxicity tests on the commercial dye. Furthermore, we assessed the risk of Disperse Red 1 for aquatic life due to its occurrence in freshwaters of São Paulo State that are influenced by textile industries discharges.

2. Methods

2.1. Commercial dye Disperse Red 1

The commercial dye used in this study was previously characterized and the main dye Disperse Red 1 was purified (>99%) (Vacchi et al., 2013). It contains 60% of Disperse Red 1 (i.e. *N*-Ethyl-*N*-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline; CAS number 2872-52-8), another six similar dye components (20%) and one unknown surfactant (20%). All ecotoxicity tests performed using the commercial dye was dissolved in the appropriate test medium for each organism without any solvent; except for tests with *Daphnia similis* performed with the purified Disperse Red 1, which was dissolved in water containing 1% of methanol for acute test and 0.01% of dimethyl sulfoxide (DMSO) for chronic test.

2.2. Ecotoxicity testing

Chronic toxicity was tested with the freshwater algae *Raphidocelis subcapitata* (former *Pseudokirchneriella subcapitata*) according to OECD guideline 201 (OECD, 2006). The inoculum was composed of algae cells harvested from a liquid stock algal culture that was 3 days old and in a logarithmic phase of growth. The initial cell density was 10,000 ± 1000 cells/mL. The final volume was 45 mL (test sample, algal inoculum and enrichment medium). The test was performed under static conditions for 72 h without media renewal, at 24 ± 2 °C under continuous fluorescent light (4000 ± 400 lux). The effect measurement was the growth inhibition rate, for which the endpoint IC50 (median inhibition concentration) was determined.

Acute toxicity tests with *Daphnia similis*, *Daphnia magna*, *Ceriodaphnia silvestrii* and *Ceriodaphnia dubia* were performed according to OECD guideline 202 (OECD, 2004). Twenty neonates (<24 h old) from 2 to 3 week-old mothers were placed in 4 replicates for each concentration (5 organisms/replicate). Tests were performed at 21 ± 1 °C under a photoperiod of 16 h light and 8 h darkness. After 48 h, the number of immobile daphnids was recorded. The results were statistically analyzed using the Trimmed Spearman–Karber method for estimating the endpoint EC50 (median effect concentration) according to Hamilton et al. (1977).

The chronic toxicity test was done with *Ceriodaphnia dubia* according to USEPA method 1002.0 (USEPA, 2002). This method