Chemosphere 156 (2016) 95–100

Contents lists available at ScienceDirect

Chemosphere

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

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

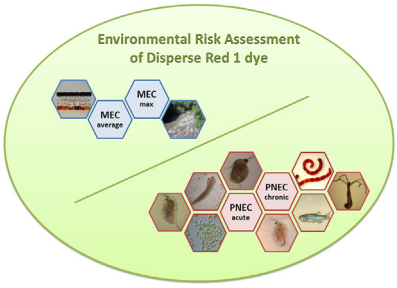
Francine Inforçato Vacchi [a, b], Peter Carsten Von der Ohe [c], Anjaína Fernandes de Albuquerque [b], Josiane Aparecida de Souza Vendemiatti [b], Carina Cristina Jesus Azevedo [b], Jaqueline Gonçalves Honório [b], Bianca Ferreira da Silva [d], Maria Valnice Boldrin Zanoni [d], Theodore B. Henry [e], Antonio J. Nogueira [f], Gisela de Aragão Umbuzeiro [a, b, *]

[a] Faculty of Pharmaceutical Sciences, University of São Paulo, USP, São Paulo, Brazil
[b] School of Technology, State University of Campinas, UNICAMP, Limeira, SP, Brazil
[c] Amalex Environmental Solutions, Leipzig, Germany
[d] Chemical Institute, State University of São Paulo UNESP, Araraquara, SP, Brazil
[e] School of Life Sciences, Heriot-Watt University, Edinburgh, United Kingdom
[f] Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

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



ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form
4 April 2016
Accepted 29 April 2016
Available online 9 May 2016

Handling Editor: Jim Lazorchak

Keywords:
Daphnia
Algae
Fish
Dyes

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

* Corresponding author. School of Technology, State University of Campinas, Limeira, SP, Brazil.
E-mail address: giselau@ft.unicamp.br (G.A. Umbuzeiro).

http://dx.doi.org/10.1016/j.chemosphere.2016.04.121

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

measures the chronic toxicity, using ten neonates (less than 24 h-old) for each concentration during seven days, in a static renewal test. The effect measurement was reproduction inhibition. Significant differences among concentrations were analyzed with the paired T-test to determine the NOEC.

The chronic toxicity test was done with *Daphnia similis* according to OECD guideline 211 (OECD, 2012) and the exposure time was modified to 14 days based on the study of Lameira (2008). This method measures the chronic toxicity, using ten neonates (less than 24 h-old) for each concentration in a total renewal test. The effect measurement was reproduction inhibition. Significant differences among concentrations were analyzed with the paired T-test to determine the NOEC.

The acute toxicity test with *Hydra attenuata* was conducted in 12-well microplates, with 3 replicate wells for each concentration (Trottier et al., 1997). 5 mL of sample and 3 organisms were put in each well. Organisms were exposed to the test solution for 96 h. The results were statistically analyzed using the Trimmed Spearman–Karber method for estimating the median lethal concentration (Hamilton et al., 1977).

The chronic toxicity test using *Hydra attenuata* was performed to detect adverse effects on reproduction on cnidarians (Holdway, 2005). The test format involves immersing test animals over 7 days in a range of concentrations of the test solution with 100% daily solution renewal. Fifteen budding hydras were randomly assigned to each concentration and fed daily with alive brine shrimp nauplii (*Artemia salina*). After feeding, test solutions were changed and the number of organisms was observed. A one-way ANOVA followed by the Dunnet test was used to detect differences between the treatments and the control as well as to determine the NOEC.

The acute toxicity test using larvae of *Danio rerio* was performed according to OECD guideline 210 (OECD, 2013). 72 h-old larvae were exposed to the dye test solutions for 96 h. Thirty organisms were used per replicate, in duplicates per concentration. The acute toxicity was also tested with juveniles of *Danio rerio* according to OECD guideline 203 (OECD, 1992). We exposed juveniles with 2.0 ± 1.0 cm of body length for 96 h. We used 3 replicates per concentration, with 5 organisms each. The effect measurement was mortality in both tests and the endpoint of EC50 was calculated using the Trimmed Spearman–Karber method (Hamilton et al., 1977).

The acute toxicity test using larvae of *Chironomus xanthus* was performed according to Novelli et al. (2012). *Chironomus xanthus* larvae (IV instar – 7/8 days age) were exposed to dye solutions at 23 °C ± 2 temperature and photoperiod of 12:12 h light/dark. Six larvae were added in chambers containing 240 mL of test solution and 50 g of sediment composed of sterilized fine sand (550 °C for 2 h), in four replicates. After 96 h, the living organisms were counted and the EC50 was calculated using the Trimmed Spearman–Karber method (Hamilton et al., 1977).

2.3. Evaluating the quality of the ecotoxicity tests

To date, the evaluation of the reliability and relevance of the ecotoxicity test that is finally used for the derivation of quality criteria (i.e. often referred to as key study) is often based on the established Klimisch method (Klimisch et al., 1997). This method is commonly used in the regulatory context and favors highly standardized tests conducted from GLP-certified laboratories. It therefore penalizes test results that for example are produced in a scientific context with greater time and budget restraints. In order to address this bias, the "Criteria for Reporting and Evaluating ecotoxicity Data" (CRED) method was recently introduced (Kase et al., 2016; Moermond et al., 2016), applying a set of 20 reliability and 13 relevance criteria (Supplementary Material I). CRED evaluates ecotoxicity tests according to transparent questions that allow the objective assessment of whether the test was conducted under appropriate conditions, as well as whether it is suitable for the given regulatory context (i.e. the derivation of Quality Criteria for surface waters). It aims at improving the reproducibility, transparency and consistency of aquatic ecotoxicity study evaluations amongst regulatory frameworks, countries, institutes and individual assessors. For this purpose, the assessor has to answers each question as to whether the criteria is i) fulfilled, ii) not fulfilled, iii) not applicable or iv) not reported. In general, a study should only be assigned to being "reliable without restrictions" when all important information is provided, and the study has no critical flaws in experimental design and results. Similarly, "reliable with restrictions" can be assigned to studies for which not all details are given, and/or raw data is not provided, and/or in which there are some minor flaws in experimental design, but for which it can still be assumed with reasonable certainty that the results are still reliable. A properly performed and reported peer-reviewed study (whether GLP or not) may be evaluated as "reliable without restrictions", just as a poorly designed and/or performed guideline and/or GLP study should be assigned as "not reliable", if relevant criteria are not fulfilled. "Not assignable" should be assigned to studies that lack the details necessary to evaluate their reliability. Please note that these studies cannot *per se* be regarded as "not reliable". In a similar way, the relevance of a study is assigned.

2.4. PNEC derivation and risk assessment

The PNECs values for Disperse Red 1 dye were derived according to European guidelines (European Commission, 2011) by using the deterministic approach. Essentially the deterministic approach takes the most sensitive reliable and relevant toxicity endpoint of the available set of test results and applies a respective assessment factor (AF) to extrapolate to an environmentally protective concentration. In order to cover both long- and short-term effects resulting from heterogeneous environmental exposures (i.e. average and peak concentrations), two PNECs are normally recommended by these guidelines: a long-term quality standard based on chronic toxicity data; and a short-term quality standard based on acute toxicity data.

In the first step of any chemical risk assessment for the aquatic environment, the risk quotient (RQ) compares the measured environmental concentration level (MEC, i.e. based on the maximum or average concentration) to the respective PNEC. In case the MEC/PNEC ratio exceeds 1, an ecological risk cannot be excluded (European Commission, 2003). This usually triggers further actions, such as extensive monitoring obligations or direct risk mitigation measures.

2.5. Chemical analysis of Disperse Red 1 in river water samples

A total of fourteen samples were collected at three river sites (i.e. one sample every 2–3 month during one year) located in São Paulo State, Brazil, that are influenced by textile discharges. Five samples were taken at the first site, which is located at Piracicaba river upstream of Quilombo river discharge (Fig. 1, site A). Another five samples were taken at the second site, which lays downstream of this confluence (Fig. 1, site B) while four samples were taken at the third site at the Quilombo river (Fig. 1, site C), which is an affluent of Piracicaba river.

The extraction of the environmental samples was done by liquid-liquid extraction, using dichloromethane and methanol (2.5:1, v/v) as organic phase. The extracts were dried under a gentle

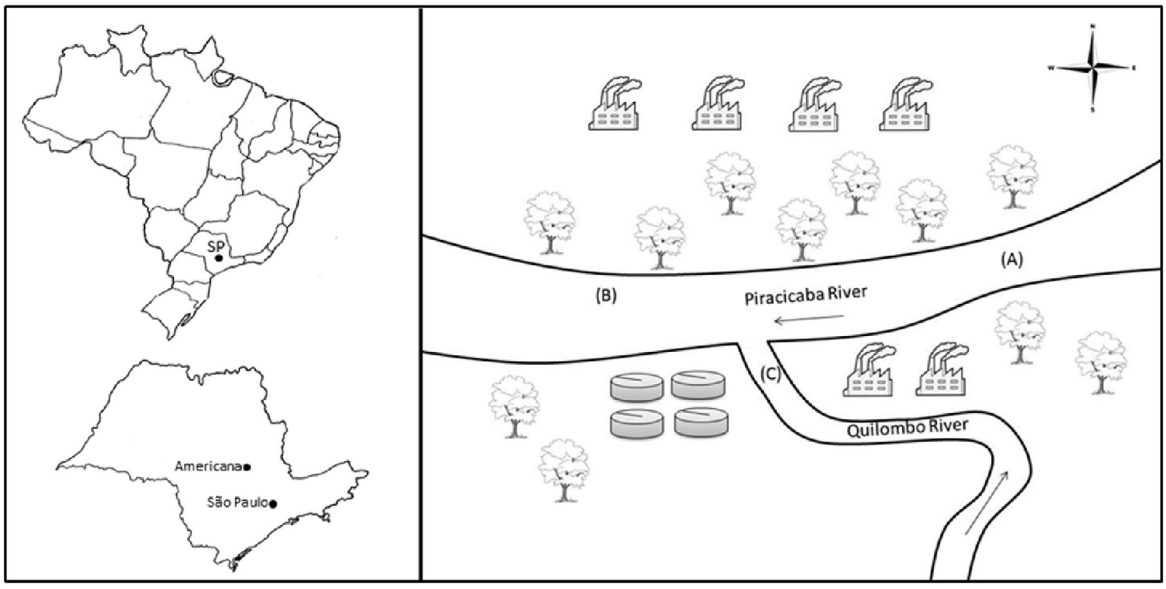


Fig. 1. Sites of sampling at Piracicaba river upstream (A), Piracicaba river downstream (B), and Quilombo river (C) in Americana city, Sao Paulo State, Brazil.

stream of nitrogen gas. Chemical analysis was performed in the extracts using a high performance liquid chromatography (HPLC) Agilent 1200 system (Waldbronn, Germany) coupled to an AB Sciex 3200 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer (MS). The extracts were diluted with methanol:water (50:50, v/v) containing 0.1% formic acid. Chromatographic separation was accomplished using a Kinetex PFP analytical column (150 mm × 4.6 mm; 5 μm, Phenomenex). The mobile phase constituted of water (A) and acetonitrile (B), both spiked with 0.1% formic acid, at flow rate of 1.5 mL min^{-1} with the following gradient program for water/acetonitrile: 0–1 min, 5% B; 1–5.5 min, 5–9% B; 5.5–6.5 min, 9–25% B; 6.5–9.5 min, 25–40% B; 9.5–11.5 min, 40–45.5% B; 11.5–16.5 min, 45.5–60.5% B; 16.5–18.5 min, 60.5–100% B, 18.5–23 min, 100% B and re-established by 5% B over 7 min, total run length was 30 min. Column temperature was set to 40 °C and injection volume was 20 μL. The chromatography system was coupled to the 3200 QTRAP via an electrospray ionization (ESI) source, operating in positive ion mode with the following ionization parameters: spray voltage, 5500 V; capillary temperature, 650 °C; the nebulizing gas (Nitrogen, 45 psi); the heating gas (Nitrogen, 45 psi) and the curtain gas, 15 psi. Identification of the compound was performed in the selected reaction monitoring (SRM) mode, where two SRM transitions were selected to eliminate any false result. The Limit of Detection (LOD) of the dye was 0.3 ng L^{-1} and the Limit of Quantification (LOQ) was 1 ng L^{-1}.

3. Results & discussion

3.1. Ecotoxicity tests and their evaluation

Tests with eight aquatic organisms were carried out to evaluate the ecotoxicity of the commercial dye Disperse Red 1 (Table 1). Results of the acute toxicity of a commercial formulation containing Disperse Red 1 and the purified dye for *Daphnia similis* (Vacchi et al., 2013), as well as the results of commercial dye on the planarian *Girardia tigrina* (Ribeiro and Umbuzeiro, 2014), were previously published. The other tests have been conducted exclusively for this paper to allow for a more sound risk evaluation of DR1 (Table 1).

Since *Daphnia similis* was by far the most sensitive species in the acute toxicity tests for the commercial product, we decided to test only *D. similis* with the purified dye. The respective acute and chronic endpoints for the purified dye were a NOEC of 3 μg L^{-1}, and an EC50 of 180 μg L^{-1}, respectively (Table 2). Both tests were also the most sensitive endpoints with regard to the commercial product data and were therefore selected for PNEC derivation.

The CRED evaluation method has been applied for acute and chronic studies used in the PNEC derivation (Supplementary Material II for CRED of chronic study; Supplementary Material III for CRED of acute study; and Supplementary Material IV for raw data). Both studies fulfilled 18 of the 20 reliability criteria (i.e. being strictly no GLP study and using nominal concentrations) and 11 of the 13 relevance criteria (the other two not being applicable) and have been assigned to be reliable and relevant to derive a sound PNEC.

3.2. PNEC derivation

Using the deterministic approach, two PNECs were derived for the dye Disperse Red 1, a short term $PNEC_{acute}$ and a long term $PNEC_{chronic}$, both based on the toxicity of the purified dye. Nevertheless, the tests with the commercial formulation were also considered, due to the fact that Disperse Red 1 is responsible for most of its toxicity (Vacchi et al., 2013).

A long-term $PNEC_{chronic}$ of 60 ng L^{-1} was derived based on the NOEC for *D. similis* (3 μg L^{-1}) divided by an assessment factor of 50. This AF was selected because although four long-term NOECs (i.e. Algae, Cladocerans, Cnidarians and Platyhelminthes) are available, no valid NOEC for fish (secondary consumers) was available. The latter taxonomic group is requested for the base set of species (i.e. Algae, Daphnia and Fish), and its being missing justifies a higher AF.

A short-term $PNEC_{acute}$ of 1800 ng L^{-1} was proposed for Disperse Red 1, based on the lowest LC50 (i.e. *D. similis* 180 μg L^{-1}) divided by an assessment factor of 100, considering the availability of at least one short-term L(E)C50 from each of the three trophic levels of the base set.

With information on the sensitivity of species representing four different trophic levels towards the commercial dye as well as data on the purified compound (for *D. similis* only), it was possible to derive short and long-term PNECs for the azo dye Disperse Red 1. We used the approach of combining all available effect data, because disregarding the data on the commercial formulation the

Table 1
Aquatic toxicity data for a commercial product containing Disperse Red 1.

Phylum	Specie	Observed effect	Exposure	Chronic NOEC (mg L^{-1})	Acute E(L)C50 (mg L^{-1})
Chlorophyta	*Raphidocelis subcapitata*	Growth inhibition	72 h	25	102
Crustacea	*Ceriodaphnia dubia*	Reproduction inhibition	7 days	0.1	–
		Acute lethality	48 h	–	0.55
	Ceriodaphnia silvestrii	Acute lethality	48 h	–	0.80
	Daphnia magna	Acute lethality	48 h	–	0.58
	Daphnia similis	Reproduction inhibition	14 days	0.003	–
		Acute lethality[a]	48 h	–	0.13
Cnidaria	*Hydra attenuata*	Reproduction inhibition	7 days	1	–
		Acute lethality	96 h	–	48
Plathyhelminthes	*Girardia tigrina*[b]	Fecundity	5 weeks	0.1	–
		Newborn acute lethality	96 h	–	79
		Adult acute lethality	96 h	–	154
Chordata	*Danio rerio*	Larvae acute lethality	96 h	–	>50
		Adult acute lethality	96 h	–	>100
Arthropoda	*Chironomus xanthus*	Larvae acute lethality	96 h	–	>100

[a] Vacchi et al. (2013).
[b] Ribeiro and Umbuzeiro (2014).

Table 2
Ecotoxicity data of purified dye in chronic and acute tests with *Daphnia similis*.

Concentration purified dye (μg L^{-1})	Chronic data (% of reproduction inhibition)	Acute data (% of organisms immobilised)
0	0	0
1	5	–
3	6	–
10	45	0
30	55	–
50	–	5
75	–	0
100	65	10
150	–	25
250	–	85
500	–	100
750	–	100
1000	–	100
Endpoint	NOEC 3 μg L^{-1}	EC50 180 μg L^{-1}

– not tested.

PNEC would have become overestimated, due to a respectively higher AF. Another reason is that it would have been highly laborious to obtain reliable data for the purified compound with all test organisms, since it is very difficult to produce a high quantity of the purified dye, which is poorly water soluble (Vighi et al., 2003). Nevertheless, we recommend that more chronic data should be generated to complement the current data set, i.e. a chronic fish assay should be performed to allow for the reduction of the assessment factor to 10 and a more accurate PNEC.

3.3. Occurrence and risk of Disperse Red 1 in river waters

Samples of Piracicaba River upstream (A), Piracicaba River downstream (B) and Quilombo River (C) were analyzed after organic extraction. Disperse Red 1 dye was detected in six of the 14 samples, in a concentration range of about 50–500 ng L^{-1} (Fig. 2).

Risk quotients (RQs) were calculated for both PNECs, considering the highest concentration of the dye in the river water samples for the $PNEC_{acute}$ and the average concentration for $PNEC_{chronic}$. A chronic RQ_1 of 1.7 was determined based on the average MEC (100 ng L^{-1}) and a long term PNEC of 60 ng L^{-1}, indicating potential adverse effects at all observed river sites. Please note that even a somewhat higher $PNEC_{chronic}$ of 300 ng L^{-1}, based on an AF of 10 instead of 50 would have been exceeded at least once, usually triggering a country-wide screening study in Europe. An acute RQ_2 of 0.3 was found for the maximum MEC (500 ng L^{-1}), suggesting rather chronic effects due to exposure to this dye. However, please note that the maximum concentration measured in this study is most likely underestimating the real exposure level; given the fact that only a few random grab samples were taken. A much higher variability is actually assumed for the dyeing process, since a batch process is the most common method used for dyeing in textile industries (Hunger, 2003). So, the dye concentration in the effluent and, consequently, in the river may vary along with the stage of the dyeing process.

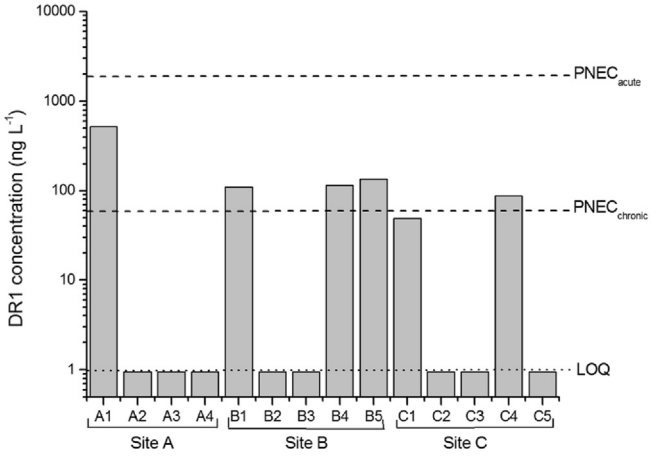


Fig. 2. Occurrence of Disperse Red 1 (DR1) in river waters from four samples of site A (Piracicaba River upstream), five samples of site B (Piracicaba River downstream) and five samples of site C (Quilombo River).

Moreover, the low number of sites and samples is not representative for Brazilian surface waters. Therefore, a monitoring study should be considered for this dye, considering its confirmed occurrence and potential risks, using a much larger number of sampling sites and a longer period of sampling with shorter frequency. The monitoring seems also indicated due to its genotoxicity potential for humans, which has been demonstrated not only *in vitro* but also by *in vivo* testing with mammals (Fernandes et al., 2015). Sediment samples should be analyzed as well, because Disperse Red 1 itself is poorly water soluble due to its relatively high log K_{ow} (4.2) and low water solubility (0.8 mg L^{-1}) and therefore, it is expected to easily adsorb to sediment.

4. Conclusions

This work presents a practical approach to derive PNECs for a disperse dye with a limited set of data on the purified compound. Testing of the commercial product helped to characterize the toxicity of the dye needed for risk assessment. The RQs obtained in this work suggest a potential risk to freshwater biota of Brazil. Therefore, a broader investigation study should be considered for this dye to improve the limited occurrence data. Moreover, additional chronic tests data (i.e. a fish partial or full lifecycle study) would be eligible to complement and improve PNEC derivation and preliminary risk assessment.

Acknowledgement

The authors thank FAPESP (2008/10449-7, 2009/12739-5, 2012/13344-7 and 2014/13157-8) for financial support and Department of Water & Sewerage of City Americana/SP, Brazil, especially to Guilherme Thiago Maziviero for helping with sampling.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.04.121.

Conflict of interest

The authors declare no conflict of interest.

References

Chequer, F.M.D., Angeli, J.P.F., Ferraz, E.R.A., Tsuboy, M.S., Marcarini, J.C., Mantovani, M.S., de Oliveira, D.P., 2009. The azo dyes Disperse Red 1 and Disperse Orange 1 increase the micronuclei frequencies in human lymphocytes and in HepG2 cells. Mutat. Res. 676, 83–86. http://dx.doi.org/10.1016/j.mrgentox.2009.04.004.

Colour Index International, 2011. Society of Dyers and Colourists and American Association of Textile Chemists and Colorists. Online Edition.

da Silva Leite, L., de Souza Maselli, B., de Aragão Umbuzeiro, G., Pupo Nogueira, R.F., 2016. Monitoring ecotoxicity of disperse red 1 dye during photo-Fenton degradation. Chemosphere 148, 511–517. http://dx.doi.org/10.1016/j.chemosphere.2016.01.053.

European Commission, 2003. Technical Guidance Document on Risk Assessment - Part II - Environmental Risk Assessment. European Union, 337 p.

European Commission, 2011. Technical Guidance for Deriving Environmental Quality Standards. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 27. European Union. 204 p.

Fernandes, F.H., Bustos-Obregon, E., Salvadori, D.M.F., 2015. Disperse Red 1 (textile dye) induces cytotoxic and genotoxic effects in mouse germ cells. Reprod. Toxicol. 53, 75–81. http://dx.doi.org/10.1016/j.reprotox.2015.04.002.

Ferraz, E.R.A., Umbuzeiro, G.A., De-Almeida, G., Caloto-Oliveira, A., Chequer, F.M.D., Zanoni, M.V.B., Dorta, D.J., Oliveira, D.P., 2011. Differential toxicity of Disperse Red 1 and Disperse Red 13 in the Ames test, HepG2 cytotoxicity assay, and Daphnia acute toxicity test. Environ. Toxicol. 26, 489–497. http://dx.doi.org/10.1002/tox.20576.

Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11, 714–719. http://dx.doi.org/10.1021/es60130a004.

Holdway, D.A., 2005. Hydra population reproduction toxicity test method. In: Blaise, C., Férard, J.-F. (Eds.), Small-scale Freshwater Toxicity Investigations. Springer-Verlag, pp. 395–411. http://dx.doi.org/10.1007/1-4020-3120-3.

Hunger, K., 2003. Industrial dyes: chemistry, properties. Applications. Wiley. http://dx.doi.org/10.1002/3527602011, 648 pp.

Kase, R., Korkaric, M., Werner, I., Ågerstrand, M., 2016. Criteria for Reporting and Evaluating ecotoxicity Data (CRED): comparison and perception of the Klimisch and CRED methods for evaluating reliability and relevance of ecotoxicity studies. Environ. Sci. Eur. 28, 7. http://dx.doi.org/10.1186/s12302-016-0073-x n/a–n/a.

Klimisch, H.J., Andreae, M., Tillmann, U., 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25, 1–5. http://dx.doi.org/10.1006/rtph.1996.1076.

Lameira, V., 2008. Estudos dos efeitos letais e subletais (reprodução e teratogênese) do farmáco triclosan para Daphnia similis, Ceriodaphnia dubia, Ceriodaphnia silvestrii (Cladocera, crustacea). Dissertação de Mestrado. Instituto de Pesquisas Energéticas e Nucleares - USP.

Maguire, R.J., 1992. Occurrence and persistence of dyes in a canadian river. War. Sci. Tech. 25, 265–270.

Moermond, C.T., Kase, R., Korkaric, M., Ågerstrand, M., 2016. CRED: criteria for reporting and evaluating ecotoxicity data. Environ. Toxicol. Chem. 35 (5), 1297–1309. http://dx.doi.org/10.1002/etc.3259.

Novelli, A., Vieira, B.H., Cordeiro, D., Cappelini, L.T.D., Vieira, E.M., Espíndola, E.L.G., 2012. Lethal effects of abamectin on the aquatic organisms Daphnia similis, Chironomus xanthus and Danio rerio. Chemosphere 86, 36–40. http://dx.doi.org/10.1016/j.chemosphere.2011.08.047.

OECD, 1992. Test No. 203: fish, acute toxicity test. OECD Guidel. Test. Chem. http://dx.doi.org/10.1787/9789264069961-en, 29 pp.

OECD, 2004. Test No. 202: Daphnia sp. Acute immobilisation test. OECD Guidel. Test. Chem. http://dx.doi.org/10.1787/9789264069947-en, 12 pp.

OECD, 2006. Test No. 201: freshwater alga and cyanobacteria, growth inhibition test. OECD Guidel. Test. Chem. http://dx.doi.org/10.1787/9789264069923-en, 25 pp.

OECD, 2012. Test No. 211: Daphnia magna reproduction test. OECD Guid. Test. Chem., OECD Guidel. Test. Chem. http://dx.doi.org/10.1787/9789264185203-en. Section 2 25 p.

OECD, 2013. Test No. 210: fish, early-life stage toxicity test. OECD Guidel. Test. Chem. http://dx.doi.org/10.1787/9789264203709-en, 24 pp.

Oliveira, D.P., Carneiro, P.A., Rech, C.M., Zanoni, M.V.B., Claxton, L.D., Umbuzeiro, G.A., 2006. Mutagenic compounds generated from the chlorination of disperse azo-dyes and their presence in drinking water. Environ. Sci. Technol. 40, 6682–6689. http://dx.doi.org/10.1021/es061020p.

Oliveira, G.A.R., Ferraz, E.R.A., Chequer, F.M.D., Grando, M.D., Angeli, J.P.F., Tsuboy, M.S., Marcarini, J.C., Mantovani, M.S., Osugi, M.E., Lizier, T.M., Zanoni, M.V.B., Oliveira, D.P., 2010. Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. Mutat. Res. 703, 200–208. http://dx.doi.org/10.1016/j.mrgentox.2010.09.001.

Ribeiro, A., Umbuzeiro, G., 2014. Effects of a textile azo dye on mortality, regeneration, and reproductive performance of the planarian, Girardia tigrina. Environ. Sci. Eur. 26, 22. http://dx.doi.org/10.1186/s12302-014-0022-5.

Tehrani-Bagha, A., Holmberg, K., 2013. Solubilization of hydrophobic dyes in surfactant solutions. Mater. (Basel) 6, 580–608. http://dx.doi.org/10.3390/ma6020580.

Trottier, S., Blaise, C., Kusui, T., Johnson, E.M., 1997. Acute toxicity assessment of aqueous samples using a microplate-based Hydra attenuata assay. Environ. Toxicol. Water Qual. 12, 265–271. http://dx.doi.org/10.1002/(SICI)1098-2256(1997)12:3<265::AID-TOX10>3.0.CO;2–9.

Umbuzeiro, G.A., Freeman, H.S., Warren, S.H., de Oliveira, D.P., Terao, Y., Watanabe, T., Claxton, L.D., 2005. The contribution of azo dyes to the mutagenic activity of the Cristais River. Chemosphere 60, 55–64. http://dx.doi.org/10.1016/j.chemosphere.2004.11.100.

USEPA, 1990. Aerobic and Anaerobic Treatment of C.I. Disperse Blue 79. United States Environmental Protection Agency. United States Environmental Protection Agency.

USEPA, 2002. Method 1002.0-Daphnid, Ceriodaphnia dubia, survival and reproduction test. In: Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. United States Environmental Protection Agency, Washington, DC, pp. 141–196.

Vacchi, F.I., Albuquerque, A.F., Vendemiatti, J.A., Morales, D.A., Ormond, A.B., Freeman, H.S., Zocolo, G.J., Zanoni, M.V.B., Umbuzeiro, G., 2013. Chlorine disinfection of dye wastewater: implications for a commercial azo dye mixture. Sci. Total Environ. 442, 302–309. http://dx.doi.org/10.1016/j.scitotenv.2012.10.019.

Vighi, M., Altenburger, R., Arrhenius, A., Backhaus, T., Bödeker, W., Blanck, H., Consolaro, F., Faust, M., Finizio, A., Froehner, K., Gramatica, P., Grimme, L.H., Grönvall, F., Hamer, V., Scholze, M., Walter, H., 2003. Water quality objectives for mixtures of toxic chemicals: problems and perspectives. Ecotoxicol. Environ. Saf. 54, 139–150. http://dx.doi.org/10.1016/S0147-6513(02)00047-7.

Zocolo, G.J., Pilon dos Santos, G., Vendemiatti, J., Vacchi, F.I., Umbuzeiro, G. de A., Zanoni, M.V.B., 2015. Using SPE-LC-ESI-MS/MS analysis to assess disperse dyes in environmental water samples. J. Chromatogr. Sci. 53, 1257–1264. http://dx.doi.org/10.1093/chromsci/bmu221.