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Derivation of aquatic predicted no-effect concentration (PNEC) for 2,4-dichlorophenol: Comparing native species data with non-native species data

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ABSTRACT

2,4-Dichlorophenol (2,4-DCP) is known as an important chemical intermediate and an environmental endocrine disruptor. There is no paper dealing with the predicted no-effect concentration (PNEC) of 2,4-DCP, mainly due to shortage of chronic and site-specific toxicity data. In the present study, toxicity data was obtained from the tests using six Chinese native aquatic species. The HC₅ (hazardous concentration for 5% of species) was derived based on the constructed species sensitivity distribution (SSD), which was compared with that derived from literature toxicity data of non-native species. For invertebrates, the survival no-observed effect concentrations (NOECs) were 0.05 and 1.00 mg L⁻¹ for *Macrobrachium superbum* and *Corbicula fluminea*, respectively. NOECs based on fishes' growth were 0.10, 0.20 and 0.40 mg L⁻¹ for *Mylopharyngodon piceus*, *Plagiognathops microlepis* and *Erythroculter ilishaeformis*, respectively. For aquatic plant *Soirodela polyrhiza*, NOEC based on concentration of chlorophyll was ranged between 0.008 and 0.045 mg L⁻¹. There is no significant difference between HC₅ derived from native and that from non-native taxa.

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1. Introduction

Chlorophenols are widely used synthetic organic compounds either used as synthesis intermediates in dyestuffs and pesticides or as biocides themselves. Chlorophenols commonly occur in industrial wastes and as direct pollutants in the water environment, which have been frequently detected (Czaplicka, 2004; Gao et al., 2008). Among them, 2,4-dichlorophenol (2,4-DCP) is the most abundant chlorophenol in aquatic environment (House et al., 1997). 2,4-DCP is usually used as a mothproofing agent, germicide, antiseptic and precursor in the production of herbicide 2,4dichlorophenoxyacetate (Zhang et al., 2008). Although 2,4-DCP presently has no direct commercial application, it is used as an important chemical intermediate, it is also synthesized from dilute aqueous solutions, and released into the environment as an intermediate compound from paper mills and chemical industries. 2,4-DCP is recognized as a priority pollutant in the aquatic environment in the USA as well as in China due to their high toxicity to aquatic life, resistance to degradation, and potential to be bioaccumulated (USEPA, 1979; Yin et al., 2003). It is also been reported that 2,4-DCP is an endocrine disruptor (Zhang et al., 2008). In addition, permanent impairment of vision or blindness of the eyes and severe injury of the upper respiratory tract were observed while human and animals were exposed to 2,4-DCP (USEPA, 2000). The concentrations of 2,4-DCP in rivers were less than 1 μ g L⁻¹ in United Kingdom (House et al., 1997) and ranged from 1.1 to 19 960 ng L⁻¹ in China (Gao et al., 2008). Therefore, the deleterious effects and ecological risk of 2,4-DCP on estuarine and coastal ecosystems have raised considerable concern.

An important step in ecological risk assessment of chemicals is the determination of the maximum concentration at which the ecosystem is protected, i.e., the predicted no-effect concentration (PNEC). PNECs are usually derived from laboratory-based toxicity test (especially for chronic) using well-defined protocols on a limited number of species. Despite the numerous toxicity data of 2,4-DCP available on fish, *Daphnia* and algae, few have been tested for its adverse effects on the environment on the basis of chronic tests owing to the high financial investment required, especially for local species in China (Yin et al., 2003). So no final decision was made



Abbreviations: 2,4-DCP, 2,4-dichlorophenol; PNEC, predicted no-effect concentration; SSD, species sensitivity distribution; NOEC, no-observed effect concentration; LOEC, lowest observed effect concentration; MATC, maximum allowable toxicant concentration; CCC, criterion continuous concentration; ACRs, acute to chronic ratios; AF, application factor.

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regarding PNEC derivation for 2,4-DCP. Yin et al. (2003) have derived a criterion continuous concentration (CCC) of 0.212 mg L⁻¹ for protection of aquatic life in China using acute to chronic ratios (ACRs) (also called application factors, AFs). However, the use of ACRs has been criticized (Chapman et al., 1998; Crane and Newman, 2000; Roex et al., 2000; Isnard et al., 2001). In some cases, average ACRs may be inadequate to extrapolate accurately from acute to chronic value (Brix et al., 2001; Besser et al., 2005).

The present paper focuses on the derivation of PNECs using the species sensitivity distribution (SSD) method (Garay et al., 2000; Hampel et al., 2007; Caldwell et al., 2008; Amorim et al., 2010). Usually a point estimate known as the HC₅ (hazardous concentration for 5% of species) is calculated. This is a concentration that will exceed no more than 5% of species effect levels. For this purpose, SSDs are generally constructed by fitting cumulative probability distributions to a plot of species toxicity data against rankassigned percentile (Van Straalen and Denneman, 1989; Aldenberg and Slob, 1993; Wheeler et al., 2002). The SSD method may result in more robust PNECs, but only a substantial amount of chronic data for several taxonomic groups is available, for most new and existing substances, this type data is lacking (Sijm et al., 2001). Furthermore, in most countries, SSD curves and HC₅ values are being used to derive PNECs for toxicants based on local species data or site-specific data (USEPA, 1985; ANZECC&ARMCANZ, 2000; Yin et al., 2003). The potential use of non-native toxicity data for description of local problems is controversial, and leaves one to question whether criteria based on species from one geographical region provide appropriate protection for species in a different region (Davies et al., 1994). However, this argument could not be resolved previously in large part due to the paucity of toxicity data applicable for local species.

In the current study, chronic toxicity tests were conducted for six Chinese native species, including three fish species, two invertebrate species and one hygrophyte species. Then, the experimental chronic toxicity data for 2,4-DCP combined with data reported on native species in the literature were compared with non-native taxa using HC_5 and values which was calculated by fitting SSD curves. The aims to this study are (1) a supplement to 2,4-DCP chronic toxicity database, (2) derivation of PNEC for 2,4-DCP, and (3) comparison of the difference between native species and nonnative species for the establishment of PNECs for site-specific ecological risk assessment.

2. Materials and methods

2.1. Test species and conditions

Six Chinese local species of two benthic invertebrates (*Corbicula* fluminea and *Macrobrachium superbum*); three species of fish (*Mylopharyngodon piceus*, *Plagiognathops microlepis* and *Erythroculter ilishaeformis*) and one hygrophyte (*Soirodela polyrhiza*) were selected primarily based on their wide distribution, economic significance and adaptability to laboratory conditions. These test species were provided by the Huazhong Agricultural University (Wuhan, China), and were acclimated to test conditions ($24 \pm 1 \,^{\circ}$ C, pH 7.24 ± 0.16) for more than 2 weeks prior to the experiments.

In the experiment, the lowest average dissolved oxygen concentration for all the test species were approximately 80% of saturation. The pH ranged from 7.4 to 7.9. Conductivity (mmhos cm⁻¹) and hardness (as mg L⁻¹ CaCO3) averaged 512 and 100, respectively during the freshwater tests. Strip chart records of temperature showed that an average temperature of 24 ± 1 °C was maintained for all tests.

2.2. Test chemical

Analytical grade 2,4-DCP (CAS RN: 120-83-2) with 99.0% purity was purchased from Sigma (Deisenhofen, Germany). Tap water, dechlorinated with activated carbon, was used for all tests. The water quality parameters were measured as follows: pH: 7.24 \pm 0.16; dissolved oxygen concentration (DO): 8.43 \pm 0.24 mg L⁻¹, total organic carbon (TOC) content: 0.017 mg L⁻¹, and total hardness: 100 mg L⁻¹.

2.3. Exposure of organisms

Chronic exposures of 2,4-DCP to six native species were conducted using daily replaced static-renewal diluters. Test solutions were maintained by renewal of 90% every 24-h. There were five treatments (nominal concentration) of test chemical plus a control and three replicates of each treatment, each beaker containing 10 test organisms. Test concentrations were chosen based upon the results of preliminary acute toxicity tests (data not shown). Dissolved oxygen, conductivity, temperature, pH, and salinity were measured every 2 d with a multiparameter water quality meter (YSI Model 85 m; Yellow Springs, OH).

2.3.1. Invertebrates

Three week survival tests using *M. superbum* (39.63 ± 0.47 mm, 0.87 ± 0.08 g) and C. fluminea (20.80 ± 0.20 mm, 3.66 ± 0.40 g) were conducted in glass container containing 4000 mL and 1000 mL test solution, respectively. The nominal concentrations for C. fluminea and *M. superbum* used in the study were 0, 1.00, 2.00, 4.00, 6.00, 8.00 mg L⁻¹ and 0, 0.05, 0.10, 0.20, 0.30, 0.40 mg L⁻¹ 2,4-DCP, respectively. Test organisms were fed daily with a solution of microalgae concentrate prepared from instant algae shellfish diet and nannochloropsis concentrate according to standard guidelines for conducting chronic tests with macro invertebrates (ASTM, 1993). During the exposure, beakers were kept in an incubator at 24 ± 1 °C with 16 L: 8 d photoperiod. Mortality and abnormal behavior were monitored daily and dead organisms were removed immediately. At the end of test, the 21 d no-observed effect concentrations (NOEC) and the lowest observed effect concentrations (LOEC) were derived by analyzing survival rate and behavioral effects of test organisms.

2.3.2. Fish

Twenty-eight days chronic growth inhibition toxicity test using early life stages of *M. piceus* $(17.65 \pm 0.40 \text{ mm}, 3.80 \pm 0.22 \times$ 10^{-2} g), P. microlepis (16.40 \pm 0.37 mm, 2.67 \pm 0.19 \times 10^{-2} g) and *E. ilishaeformis* $(23.59 \pm 0.29 \text{ mm}, 5.50 \pm 0.20 \times 10^{-2} \text{ g})$ were done in glass container containing 1000 mL test solution. The nominal concentrations used in these studies were 0, 0.10, 0.20, 0.40, 0.60 and 0.80 mg L^{-1} 2,4-DCP for both *P. microlepis* and *E. ilishaeformis*, and 0, 0.10, 0.20, 0.40, 0.80, 1.60 mg L^{-1} 2,4-DCP for *M. piceus*. During the exposure, beakers were kept in an incubator at 24 ± 1 °C with 16 L: 8 d photoperiod, and juvenile fishes were fed with brine shrimp at a rate of 0.1% body weight twice daily. At the end of the test, length and weight of all tested fish were measured and survival rate was calculated at each concentration. from which NOEC and LOEC were derived. For fry growth, the specific growth rate (SGR) was chosen because it is less dependent on the initial size of the fish and on the time between measurements than the other endpoint such as relative growth rate (RGR) (Mallett et al., 1997). The SGR was calculated as ((In(final mass) – $\ln(\text{initial mass})$ × 100)/d of exposure (Crossland, 1985). At the end of the chronic toxicity test, all animals survived in the control.

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