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# pH-dependent aquatic criteria for 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol

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## HIGHLIGHTS

► The effects of pH on toxicity of CPs to *Daphnia magna* and *Scenedesmus obliquus* were studied.
► There were strong correlations between the logarithmic $EC_{50}$s and pH values for both *D. magna* and *S. obliquus*.
► The manipulation of data (intra-species variation or/and proportions of taxonomic groups) is important to the result of WQC.

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## ABSTRACT

Due to their agricultural as well as industrial uses, 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP) are ubiquitous in the environment and recognized as priority pollutants in many countries. In this study, effects of pH on toxicity to the crustacean *Daphnia magna* and the alga *Scenedesmus obliquus* were investigated. Combined published toxicity data of the three chlorophenols along with; relationships between toxicity and pH reported here were used to establish pH-dependent water quality criteria (WQC). The WQC expressed as a function of pH, also considered intra-species variation and proportions of taxonomic groups. At pH 7.8, the recommended acute exposure water quality criteria (WQC) were 286.2 μg 2,4-DCP/l, 341.5 μg 2,4,6-TCP/l and 11.4 μg PCP/l. The recommended chronic exposure WQC were 16.3 μg 2,4-DCP/l, 54.6 μg 2,4,6-TCP/land 3.9 μg PCP/l.



## 1. Introduction

In China water quality criteria (WQC) refer to concentrations of individual pollutants that are developed for specified uses of surface waters, including drinking water, recreation, production of fish, propagation of other aquatic life, and industry and agriculture. WQC are used in environmental management and pollution control. Several methods have been developed for derivation of WQC (ANZECC and ARMCANZ, 2000; CCME, 2007; EU, 2003; Stephan et al., 1985). In China, WQC have been established for some chemicals; but they are mainly derived from environmental quality standards or criteria of more developed countries, which may be over- or under-protected aquatic organisms due to differences between hydrographic conditions and species in China and those in other countries (Wu et al., 2010; Yan et al., 2012). The Major State Basic Research Development Program and the National Major Project of Science & Technology Ministry of China on the Development of China were initiated in 2008. Until now, studies on WQC in China have been based on effects of chemicals on several native species, but the methods used to derive WQC were those of more developed countries, such as those of the United States Environmental Protection Agency (USEPA) (USEPA-FAV method), or Canada, Australia and New Zealand (mainly species sensitivity distributions (SSDs) method). SSDs have some advantages compared to USEPA approaches. The SSD approach considers all available toxicity data and uses the entire species distribution to calculate the protection level with a graphical component that allows visualization of the SSD. Another criticism of the EPA-FAV method is that the procedure can give poor extrapolations from small datasets or with datasets with severe outliers in toxicity values (Stephan et al., 1985). SSD methods are increasingly used in derivation of water quality criteria by many countries including China. In China, historically WQC had not considered intra-species variation or/and proportions of taxonomic groups, and/or physical–chemical properties of water.

Due to their use in agriculture and industry as pesticides, wood preservatives, personal care formulations, and in the production of other products chlorophenols (CPs) are ubiquitous globally in

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surface waters, groundwater, wastewater, sludge and drinking waters (Czaplicka, 2004; Davì and Gnudi, 1999; Gao et al., 2008; Olaniran and Igbinosa, 2011; Zheng et al., 2011). CPs have received worldwide attention due to their toxicity to aquatic life, persistence, and potential to bioaccumulate (Ge et al., 2007; Olaniran and Igbinosa, 2011; Xing et al., 2012a; Zheng et al., 2011; Zheng et al., 2012). In China among CPs, 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP) are ubiquitous at concentrations of as much as 103.70 μg/l (Gao et al., 2008; Zheng et al., 2012; Zhong et al., 2010). Such concentrations pose risks to humans and aquatic organisms (Cooper and Jones, 2008; Ma et al., 2011; Ruder and Yiin, 2011; Xing et al., 2012a). For these reasons, CPs are classified as priority pollutants in the United States (USEPA, 1991) and China (Zhou et al., 1990).

Recently, WQC for 2,4-DCP, 2,4,6-TCP and PCP for protection of aquatic life based on resident aquatic biota have been derived in China (Jin et al., 2012a; Jin et al., 2012b; Jin et al., 2011; Yin et al., 2003a; Yin et al., 2003b). However, these WQC did not consider effects of water characteristics such as pH on toxicity of CPs. Because chlorophenols have an OH functional group depending on pH they can exist as protonated or ionic forms (Kishino and Kobayashi, 1995). The proportion of the CPs in each form is governed by their pKa (Erickson et al., 2006a,b). The degree of dissociation of weak acids, such as phenols and specifically CPs is thus a function of pH. The fraction of weakly acidic organic compounds is directly proportional to acidity and thus inversely proportional to pH (Kishino and Kobayashi, 1995; Saarikoski and Viluksela, 1981). Unionized molecules are more soluble in lipids and can diffuse more easily across membranes to exert their effects. This effect is important because the toxicity of some compounds is affected by the degree of dissociation (USEPA, 1991). In the case of CPs, the protonated form, which is unionized, is more accumulated and thus more toxic than the unionized form. For this reason, in 1995, the USEPA developed criteria for protection of aquatic life from the effects of CPs in ambient water that were dependent on pH. WQC for PCP are expressed as functions of pH (http://www.epa.gov/ost/criteria/wqctable/).

Intra-species variation or/and proportions of taxonomic groups is also an important consideration in the derivation of WQC. The effects of the species considered in deriving the WQC are more important than the statistical methods employed (Duboudin et al., 2004; Maltby et al., 2005; Wheeler et al., 2002). WQC for the three CPs were derived by the use of a log-logistic model, a typical species sensitivity distribution (SSD) approach (CCME, 2007; Duboudin et al., 2004; Maltby et al., 2005; Wheeler et al., 2002) and corrected for the effects of pH. The effects of pH on toxicity of the three CPs, including 2,4-DCP, 2,4,6-TCP and PCP, to the crustacean, *Daphnia magna* and green alga, *Scenedesmus obliquus* were investigated. According to Duboudin et al. (2004), four cases in SSD analyses should be considered when deriving a criterion (Fig. A.1 of the Supplementary information): (1) intra-species variation weighted by the use of geometric means, and unweighted proportions of taxonomic groups; (2) intra-species variation weighted by each data to give each species the same weight, and unweighted proportions of taxonomic groups; (3) intra-species variation weighted by geometric mean, and weighted proportions of taxonomic groups; (4) intra-species variation weighted by each data to give each species the same weight, and weighted proportions of taxonomic groups.

## 2. Materials and methods

### 2.1. Test chemicals and culture of daphnids and alga

2,4-DCP (CAS No. 120-83-2, 99% purity) and 2,4,6-TCP (CAS No. 88-06-2, 98% purity) were purchased from Acros Organics (Morris Plains, NJ, USA), and PCP (CAS No. 87-86-5, 98% purity) was purchased from Sigma-Aldrich (St Louis, MO, USA). All chemicals were used as supplied and prepared in HPLC-grade dimethyl sulfoxide (DMSO) and were kept in thoroughly cleaned glass containers and stored at −20 °C. Final concentrations of DMSO in experimental media were equal to or less than 0.05% for *D. magna* and 0.1% for *S. obliquus*, respectively. *S. obliquus* was cultured in 250 ml Erlenmeyer flasks containing 150 ml of WC medium (Kilham et al., 1998) under continuous light conditions (illuminance 5000 lx) in incubator at 25 ± 1 °C. Cells during exponential phase were used for experiments. *D. magna* were cultured and maintained following previously published methods (Xing et al., 2012b). Tap water, aerated for more than three days, was used as culture medium, which had a pH of 8.12 ± 0.11, dissolved oxygen concentration of 6.07 ± 0.24 mg/l, conductivity of 319 ± 9.1 μs/cm, alkalinity of 95.48 ± 4.64 mg/l as $CaCO_3$, and hardness of 125.5 ± 4.95 mg/l as $CaCO_3$. Neonates (<24 h) were used for the experiments.

### 2.2. Experimental design

Three different pH values adjusted by the use of buffers (MES (2-(N-morpholino) ethanesulfonic acid, CAS No. 4432-31-9) for pH 6.5, MOPS (3-(N-morpholino) propanesulfonic acid, CAS No. 1132-61-2) for pH 7.5 and CHES (2-(cyclohexylamino) ethanesulfonic acid, CAS No. 103-47-9) for pH 9.0). Buffers with purities of ≥98% and 0.1 mol/l NaOH were used to maintain constant nominal pH (Neuwoehner and Escher, 2011). Concentrations of buffers in media were 5 mmol/l for *D. magna*, 20 mmol/l for *S. obliquus*, respectively. Static non-renewal tests were conducted for all the experiments and the pH values were measured by pH meter (Thermo Scientific Orion 5-Star Plus) at the beginning and end of the experiments.

Studies of *S. obliquus* were initiated with a cell density approximately $10^5$ cells/ml in 50 ml Erlenmeyer flasks containing 20 ml sterilized WC medium for 72 h (OECD, 2002; Yeesang and Cheirsilp, 2011). The other conditions were the same as those during cultivation. For each chemical and each pH, algae were exposed to seven concentrations, one solvent control and one medium control with three replicates. Algae were pre-cultured in medium at various designed pH values for at least 3 days before the experiment in order to allow for adaptation. Algae cell numbers were determined every 24 h from 0 to 72 h.

Studies of *D. magna* experiments were conducted in 6-well Costar® plates with 10 ml medium according to OECD method 202 (OECD, 2004). The observed endpoint, immobilization, was judged by the inability to move during 10 s after exposure for 24 and 48 h. Experimental conditions were the same as those used during culture. For each chemical and each pH, seven concentration groups, solvent control and medium control with five repeats containing five neonates were carried out. Simultaneously, tests with the reference chemical $K_2Cr_2O_7$ were conducted to ensure that the test organisms exhibited constant sensitivities to this reference toxicant as an indicator of the physiological status of the organisms. *D. magna* were pre-cultured in medium at designed pHs for at least 2 weeks before the experiment in order to allow for adaptation.

### 2.3. Collection and selection of toxicity data

Values for toxicities to fish, amphibians, molluscs, crustaceans, algae for the three CPs considered here were collected from the ECOTOX database (http://cfpub.epa.gov/ecotox/) or published literature. The acceptability of toxicity data was assessed according to the principles of aquatic life criteria with accuracy, relevance and reliability (CCME, 2007; Klimisch et al., 1997; Stephan et al., 1985). If endpoints were extrapolated beyond the range of concentrations tested or greater than the limit of solubility, the studies were not included in the database. Briefly, only toxicity data for species existing broadly or cultivated widely in freshwaters of China were considered and characterized by a specific endpoint, duration time and pH value. In