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Development of predicted no effect concentration (PNEC) for TCS to terrestrial species

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HIGHLIGHTS

- Chronic toxicity tests of TCS to 6 terrestrial species were conducted.
- The PNEC value for TCS to terrestrial species ranged from 0.04 to 0.21 mg kg^{-1}.
- Species from different taxonomic levels are required in deriving the PNEC values.

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ABSTRACT

Triclosan (TCS) is an important broad-spectrum antimicrobial agent widely utilized in a range of personal care products, and is therefore commonly found in the environment. A few studies have been conducted to investigate predicted no effect concentration (PNEC) for TCS on terrestrial organisms. This could be due to lack of toxicity data especially chronic toxicity data for species on various taxonomic levels. In the present study, chronic toxicity of TCS on 6 terrestrial species (3 dicotyledonous plants, 2 monocotyledonous plants and 1 terrestrial invertebrate) were tested. PNEC values of TCS based on toxicity data of 14 terrestrial species (5 dicotyledonous plants, 4 monocotyledonous plants and 5 terrestrial invertebrates) from 4 Phyla and 11 Families were calculated using the log–logistic species sensitivity distribution (SSD) method. The result of our toxicity tests showed that the dicotyledonous plant *Lactuca sativa* was the most sensitive species to TCS exposure. The PNEC value for TCS was derived to be 0.04–0.21 mg kg^{-1} when using the log–logistic SSD method. The use of toxicity data from various taxonomic levels is recommended in deriving the PNEC value in the terrestrial environment.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are broadly used in everyday life, and are therefore commonly detected in all environmental media (Ternes et al., 2004; Amorim et al., 2010; Schnug et al., 2014). The presence of PPCPs in the environment and their potential effect on nontarget organisms has drawn greater attention worldwide (Daughton and Ternes, 1999; Kümmerer, 2004; Veldhoen et al., 2006). One group of PPCPs that has received increasing attention is antimicrobials because of their highly toxic to microbe and alga (Ying and Kookana, 2007). Previous study reported that triclosan (TCS, CAS#3380-34-5) could inhibit lipid biosynthesis (McMurry et al., 1998; Levy et al., 1999), and is therefore a broad spectrum antimicrobial agent utilized throughout the world in a range of personal care products including soaps, toothpaste, and so on (McAvoy et al., 2009; Price et al., 2010). As a result, TCS is widespread in the environment. TCS has already been concerned in scientific research and has been found in surface water, wastewater, soil, sediment worldwide in the recent years (Benotti et al., 2008; Kasprzyk-Hordern et al., 2008; Cha and Cupples, 2009; Zhao et al., 2010; Ramaswamy et al., 2011; Katz et al., 2013; Huang et al., 2014; Macherius et al., 2014). TCS was also detected in field-collected earthworms (non-identified taxa) two years after application of biosolids (Pannu et al., 2012). As for the terrestrial environment, TCS usually enter soil through the application of sewage sludge. Once in the soil, it mostly remains in the upper 10–20 cm layer (Reiss et al., 2009). The final soil concentration of TCS depends on the initial biosolid concentrations, soil types, and other factors.

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Once transferred to the environment, TCS may pose potential risk on organisms. Previous toxicity research showed that TCS is toxic to aquatic organisms (Ishibashi et al., 2004; Orvos et al., 2009; Yang et al., 2009; Palenske et al., 2010). As for terrestrial species, the adverse effects of TCS to soil microbial processes (Liu et al., 2009), plants (*Triticum aestivum*, *Brassica rapa*), worms (*Eisenia andrei*, *Enchytraeus albidus*), and collembolans (*Folsomia candida*) (Amorim et al., 2010) were tested. Our previous study reported the chronic toxicity on growth and toxic effects on antioxidative enzyme of snail (*Achatina fulica*) (Wang et al., 2014a). So, TCS could impose toxic effects on the terrestrial environment. However, toxicity data of TCS, especially chronic toxicity on terrestrial organisms, are limited. And, the PNEC (predicted no effect concentration) value based on terrestrial species from a variety of taxonomic levels is limited.

In this work, the toxicity of TCS was assessed using a series of toxicity tests of historically used terrestrial organisms (OECD, 2004, 2006; Wang et al., 2015). Standard test organisms from various taxonomic levels (2 Phyla and 6 Families) were tested, including 5 terrestrial plants (*Zea mays* (Poaceae); *Allium tuberosum* (Alliaceae); *Solanum lycopersicum* (Solanaceae); *Lactuca sativa* (Asteraceae); *Glycine max* (Fabaceae)) and 1 terrestrial animal (*Eisenia fetida* (Lumbricidae)). Moreover, the PNEC value of TCS was derived based on the toxicity data of our present study and precious studies (Liu et al., 2009; Amorim et al., 2010; Wang et al., 2014a).

The aims of this study are (1) a supplement to TCS toxicity database for terrestrial species, and (2) derivation of PNEC value to terrestrial species using multiple methods and toxicity data from various taxonomic levels. This study provides valuable information for environmental risk assessment and pollution management imposed by TCS in terrestrial environment.

2. Materials and methods

2.1. Test chemicals

TCS, $C_{12}H_7Cl_3O_2$, ⩾97% purity (HPLC), was purchased from Sigma Aldrich. The acetone was bought from the Beijing Guoyao Chemical Co. Ltd. (Beijing, China) and was of analytical grade with chemical purity of 97–99%.

2.2. Test organisms

2.2.1. Plants

In the present study, 5 terrestrial species historically used in plant testing (OECD, 2006) were used. Seeds were obtained from Chinese academy of agricultural sciences (Beijing, China). Preliminary incubation showed that all the seeds used in this study had more than 90% germination rates.

2.2.2. Earthworm

Earthworm *E. fetida* was used in this study. The worms were obtained from in-house cultures at our key laboratory of Chinese Research Academy of Environmental Sciences. They were selected from a synchronized culture with a relatively homogeneous age structure. The selected worms were acclimatized for 7 d (days) with the same conditions as toxicity test (light/dark: 16/8 h, temperature: 20 ± 1 °C) (OECD, 2004). The earthworms were fed once a week with oatmeal.

2.3. Test soil

All the toxicity tests were carried out with natural soil. A surface (0–20 cm) soil was collected from farmland in Hebei province, China. The wastewater or the sewage sludge had not been applied to the farmland for more than 5 years, and it's far away from residential area. The main characteristics of the soil are: organic matter content of 23.19 ± 0.47 g kg^{-1}, pH of 8.05 ± 0.05, cation exchange capacity of 17.10 cmol kg^{-1}, 3.91% clay, 32.04% silt and 64.05% sand.

Appropriate TCS was dissolved in acetone, and was spiked into the test soil to obtain desired concentrations in treatment groups. Carefully mix the totality and let the acetone evaporate under darkness in a fume cupboard for 24 h. Moisture content was adjusted to 40–60% of the WHC (water hold capacity). The sub-samples of each batch were introduced into the test replicates. Details on the TCS concentrations used are given in the following test procedures.

2.4. Test procedures

2.4.1. Plants

The terrestrial plant tests (*Z. mays*, *A. tuberosum*, *G. max*, *S. lycopersicum* and *L. sativa*) were carried out according to the standard guideline OECD 208 (OECD, 2006). Additionally, controls (blank and solvent (acetone)) were tested at the same time. Four replicates for each treatment and control were used, and each replicate contained 400 g of the test soil and 10 seeds. The test duration was 21 d after germination of the 50% seeds in blank control. The duration of germination of the 50% seeds in blank control for the 5 species ranged from 1 d to 7 d. The following conditions were generally recommended for the plant tests: temperature of 22 ± 1 °C, humidity of 75 ± 10%, and light/dark of 16/8 h. The emergence and survival were recorded every day, and the growth and biomass of plants were measured at the end of the test.

2.4.2. Earthworm

The acute toxicity test was carried out according to the standard guideline OECD 207 (OECD, 1984). The final designed treatment concentrations were 200.0, 300.0, 450.0, 675.0 and 1012.5 mg kg^{-1}. Additionally, controls (blank and solvent (acetone)) were tested at the same time. Four replicates per concentration group and control were used, and each replicate contained 500 g of the test soil and 10 well-developed adults (300–400 mg). The acute test duration was 14 d. At the end of the test, the survival was recorded. The reproduction test was carried out according to the standard guideline OECD 222 (OECD, 2004). The final designed treatment concentrations were 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 mg kg^{-1}. The differences from the acute test were test duration (56 d) and food supply (5 g oatmeal was added weekly during the first 28 days). The adults were removed from the soil and were counted and weighted at day 28 of the test. A further 5 g of food was then administered to each test container. No further feeding took place during the remaining 28 days of the test. At the end of the second 28-d period, the number of juveniles was counted using a warm water bath method described in OECD 222 (OECD, 2004). The cocoon numbers were checked using a 0.5 mm mesh size sieves method described in OECD 222.

2.5. Statistical analysis

T-test was performed to check the differences between control and solvent control. Statistical analyses between control and treatment groups were carried out with one-way analysis of variance (ANOVA) followed by the least significant differences (LSD) test. The homogeneity of variance of control and the treatment groups were checked to be equal. The difference was considered to be significant when the p value was less than 0.05. The highest concentration that was not significantly different from the control was considered to be the no observed effect concentration (NOEC).