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Toxic effects of pentachlorophenol and 2,2',4,4'-tetrabromodiphenyl ether on two generations of *Folsomia candida*

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ABSTRACT

The standard *Folsomia candida* test (ISO 11267), in which only the survival and reproduction of the parental generation (F0) were determined, is insufficient to assess the toxicity of chemicals, like endocrine disrupting chemicals (EDCs), since the effects of EDCs could last for several generations and sometimes can be transgenerational. It's necessary to assess the effects on subsequent generations to address the long-term consequences of these chemicals exposure. In this study, the effects of pentachlorophenol (PCP) and 2,2',4,4'-tetrabromodiphenyl (BDE47) were assessed on F0 and the first filial generation (F1) of *F. candida* after 28-day or 10-day exposure of F0. In the 28-day exposure method, F0 was exposed to PCP or BD47 for 28 days and F1 was exposed for about 21 days. In the 10-day exposure method, F0 was exposed for 10 days and F1 was not exposed. The *F. candida* reproduction of F0 and F1 can be assessed in both methods, while transgenerational effects can further be evaluated in the 10-day exposure method. The numbers of F1 and F2 (second filial generation) juveniles in the 28-day exposure method and F1 juveniles in the 10-day exposure method decreased significantly for the PCP treatment. For BDE47, only the number of F1 juveniles in the 28-day exposure method significantly decreased. The EC50 values of F0 reproduction (the number of F1 juveniles) in the 28-day exposure method were 89 and 306 mg/kg dry soil for PCP and BDE47, respectively. Results suggested that PCP could affect *F. candida* egg hatching or juvenile survival and adult reproductive capacity, while BDE47 was more likely to affect egg hatchability or juvenile survival rather than adult reproductive capacity. It also indicated that *F. candida* exposed to PCP or BDE47 could recover in clean soil. Transgenerational effects were not observed for neither PCP nor BDE47 in this study.

1. Introduction

Endocrine disrupting chemicals (EDCs) interfere with the endocrine system of animals, thus, influencing growth, development, and reproduction (Park and Kwak, 2010). Pentachlorophenol (PCP) and polybrominated diphenyl ethers (PBDEs) are widely used and have potential endocrine disrupting effects accrording to many reports (Hamers, 2006; Orton et al., 2009; Guo and Zhou, 2013).

PCP and its sodium salt have been used as wood presertive, pesticide, herbicide, bactericide and fungicide since the 1930s (Geyer et al., 1987). In China, PCP was mainly used for schistosomiasis control and wood preservation (Zheng et al., 2012). The use of PCP in agriculture was banned but is still allowed in some restricted applications in China (Ge et al., 2007). Due to its extensive use in the past decades and its persistence, PCP can be detected in various organisms and environment media (Zheng et al., 2012). In China, the concentration of PCP was up to 48.3 mg/kg (dry weight) in sediment and 103.7 μg/L in water from Dongting Lake (Zheng et al., 2000). In dairy farms near Nanjing Chemical Industry Park, the mean concentrations of PCP in soil were ranged from 0.2 to 2.9 mg/kg dry weight (Sun et al., 2015). Zheng et al. (2011) found that PCP levels decreased over time in water and sediment with half-lives ranging from 2.7 to 5.9 years in western countries. The mean half-lives of PCP in the arable soils were 30 days under flooded condition and 50 days under upland condition (Kuwatsuka and Igarashi, 1975). The adverse effects of PCP on health and environment have been reported extensively in recent decades. For example, PCP can repress the reproduction (EC20: 41.7 mg/kg, EC50: 87 mg/kg) and growth (EC20: 94.6 mg/kg) of the springtail *Folsomia candida* in artificial soil (Crouau et al., 1999; Crouau and Moïa, 2006). The plasma thyroid hormone levels of zebrafish *Danio rerio* were altered after

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exposure to PCP (27 μg/L) for 70 days (Yu et al., 2014). Morales et al. (2014) indicated that PCP can cause significant overexpression of the estrogen-related receptor gene (at 25 μg/L), the early ecdysone-inducible E74 gene (at 25 μg/L) and the ecdysone receptor gene (at 250 μg/L) in *Chironomus* larvae.

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants for decades in many products, such as electronics, textiles, furnitures and building materials (Wang et al., 2005). Penta-BDE, octa-BDE and deca-BDE mixtures are three typically commercial products (La Guardia et al., 2006). In China, the use of penta-BDE and octa-BDE was banned but the use of deca-BDE is still allowed (Wang et al., 2017). PBDEs have become widespread pollutants of concern because of their wide use and their persistent and toxic nature (Lee and Kim, 2015). In China, the recycling of electronic waste has been an important source of PBDEs pollution. The total concentrations of 21 PBDEs congeners ranged from 5.3 to 22,110 μg/kg with an average of 2283 μg/kg in soils from an electronic waste burning site in South China (Nie et al., 2015). Wong et al. (2012) found that three PBDE congeners (BDE-28, 47, 99) did not degrade in an urban soil (5.6% organic carbon) after incubating for 360 days under room temperature and darkness. Among 209 PBDE congeners, 2,2',4,4'-tetrabromodiphenyl ether (BDE47) is one of the predominant congeners detected in soil (McGrath et al., 2017). Various studies have reported the adverse effects on organisms caused by BDE47. For example, the thyroid hormone homeostasis of adult manila clam *Ruditapes philippinarum* was interfered after exposure to 1 μg/L BDE47 for 15 days (Song et al., 2016). BDE47 repressed molting and metamorphosis of the copepod *Tigriopus japonicus* and influenced molting and metamorphosis-related genes during a 20-day exposure at concentration of 50, 100 and 200 μg/L (Hwang et al., 2016). An *in vitro* study using epidermis tissues of the blue crab *Callinectes sapidus* showed that BDE47 (100 nM) can significantly induce the *N*-acetyl-β-glucosaminidase gene, which is involved in the ecdysteroid signaling (Booth and Zou, 2016).

The effects of EDCs on aquatic organisms have been widely studied in the past few decades, while rather few studies are available for soil invertebrates. Springtails are soil invertebrates that play an important role in the soil ecosystem. The springtail *Folsomia candida* has been employed as a model species in soil ecotoxicology. In the ISO 11,267 (2014) standard springtail reproduction test, 10–12 day old juveniles (F0) are exposed to polluted soil and produce the first batch of eggs (F1) after about one week, which hatch after about 9–12 days. Twenty-eight days after the test begins, the survival of F0 adults and the number of F1 juveniles are determined. The standard test allowes assessing the effects of chemicals on the adult phase as well as the egg and juvenile phase of *F. candida* (Fig. S1). However, it is insufficient for evaluating the effects of chemicals, especially for EDCs, because the effects of EDCs could last for several generations and sometimes can be transgenerational. For instance, when only the F0 generation of *F. candida* was exposed to methoprene (160, 190 and 220 mg/kg) and teflubenzuron (0.036 and 0.12 mg/kg) for 10 days and the F1 generation was not exposed, the reproduction of the F1 generation was repressed, suggesting that the effects of the two chemicals are transgenerational (Campiche et al., 2007). Therefore, it's necessary to assess the effects on subsequent generations to address long-term effects of chemical exposure. Only a few studies have reported the effects of chemicals on springtails over multiple generations (Campiche et al., 2007; Paumen et al., 2008; van Gestel et al., 2017; Oliveira et al., 2018).

This study aims to assess the effects of PCP and BDE47 on two generations of *F. candida* after long-term (28-day) or short-term (10-day) exposure of the F0 generation using methods developed by Campiche et al. (2007). The affected endpoints and possible recovery of *F. candida* in clean soil were evaluated by comparing the results of the two methods. Furthermore, transgenerational effects can also be detected in the 10-day exposure method.

2. Materials and methods

2.1. Test organism

F. candida usually becomes adult at the age of 17–20 days and then produces the first batch of eggs, which hatches about 9–12 days later. *F. candida* continues to oviposit during its life. The second oviposition often occurs about one week after the first oviposition. The interval between oviposition usually lengthens with the increased age of the adult *F. candida*.

F. candida was cultured in plastic Petri dishes containing a substrate of plaster of Paris, activated carbon, and distilled water in a mass ratio of 9:1:7. The animals were kept in constant darkness at 20 ± 1 °C with a relative humidity of 70 ± 5% and fed dry yeast. Distilled water was added weekly to keep the substrate wet. To obtain synchronized juveniles, a number of adults were transferred to new substrate to lay eggs and then the adults were removed. The eggs hatched about 9–11 days later. Two days after egg hatching, the juveniles were transferred to new substrate and cultured. The 10–12 days old juveniles were used for the test.

2.2. Test soil and chemicals

All tests were performed in artificial soil, consisting of 70% quartz sand, 20% kaolinite clay, and 10% ground sphagnum peat sieved to 2 mm and the pH was adjusted to 6.0 ± 0.5 by adding $CaCO_3$. The pH of the soil measured in KCl solution (1 mol/L) was 5.6 and the organic matter content was 6.1%. The soil was spiked with PCP (Chem Service, 99.1% purity) or BDE47 (Toronto Research Chemicals, 98.2% purity) using acetone as the carrier solvent. The acetone solution of PCP or BDE47 was added into 10% of the test soil and they were kept in a closed glass container for 24 h. Then the container was opened and placed in the fume hood for 24 h to allow the acetone to evaporate. The remaining soil (90%) was added and mixed thoroughly. The solvent control soil was spiked with the same amount of acetone as all the test soils. The soil was moistened to 50% of the maximum water holding capacity by adding distilled water. Five nominal concentrations of each chemical were tested (PCP: 30, 60, 90, 120, and 150 mg/kg dry soil; BDE47: 50, 100, 200, 400, and 800 mg/kg dry soil).

2.3. Experimental design

2.3.1. General principle

The two different test methods developed by Campiche et al. (2007) were used with minor modifications (Fig. 1). Both methods contained two stages. In the first stage, the F0 generation of springtail was exposed to polluted artificial soil for different time and produced the F1 generation. In the second stage, the F1 generation was cultured in unpolluted artificial soil and produced the F2 generation. The two methods were named method A and method B respectively. The main difference of the two methods was the exposure duration of F0 and F1. In method A, F0 was exposed for 28 days from 10 to 12 day old (juvenile) to 38–40 day old (adult) and F1 was exposed from egg phase to juvenile phase. In method B, only F0 was exposed for 10 days.

Tests were conducted in 100 mL glass beakers filled with 30 g of moist soil. Four replicates were used for each exposure concentration. All beakers were sealed with Para film and opened for aeration once per week. Dry yeast was added each week as a food supply. The water content of the soil was checked weekly by weighing and distilled water was added if there was water loss. All beakers were kept under the same conditions as the springtail cultures (constant darkness, 20 ± 1 °C, 70 ± 5% relative humidity).

For PCP, the control group (without acetone) and solvent control group (with acetone) were both performed in parallel with the treatment groups (PCP spiked) and no significant difference was found between the control group and solvent control group. Therefore, only the