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Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (*Triticum aestivum* L.)

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ABSTRACT

Biochemical responses of wheat (*Triticum aestivum* L.) seedlings stressed by two typical personal care products (PCPs) – triclosan (TCS) and galaxolide (HHCB) were experimentally investigated to assess their ecological risks. The results showed that wheat shoot and root elongation was significantly inhibited by 50–250 mg L^{-1} TCS and HHCB. Wheat roots were sensitive to TCS, while shoots were sensitive to HHCB. The median effect concentration (EC_{50}) of TCS and HHCB based on the inhibition of their sensitive sites were 147.8 and 143.4 mg L^{-1}, respectively. Moreover, the damage of wheat seedlings treated by low concentration of TCS and HHCB during a long period cannot be neglected. After a 21-d exposure, 0.2–3.0 mg L^{-1} TCS and HHCB treatment caused the damage to the accumulation of chlorophyll (CHL), the synthesis of soluble protein (SP), and the activity of peroxidase (POD) and superoxide dismutases (SOD) in different degree. However, different changing trends of these physiological indexes treated by different PCPs were observed after 7-d to 14-d exposures, especially the activity of POD and SOD. The activity of POD and SOD in wheat leaves and roots decreased with an increase in the concentration of TCS and the exposure time. However, the enzyme activities in wheat leaves treated by 0.2–3.0 mg L^{-1} HHCB increased after a 14-d exposure, and with the prolongation of exposure time, the enzyme activities significantly decreased. The variations in these physiological indexes of wheat could be considered as good biomarkers of serious stress by TCS and HHCB in the environment.

1. Introduction

Personal care products (PCPs) containing a diverse group of chemicals have recently been recognized as particular contaminants in the environment (Daughton and Ternes, 1999). Among the numerous varieties of PCPs, triclosan (TCS) and galaxolide (HHCB) are the most frequently used in many contemporary consumers and professional health care products including hand soaps, body lotions, cosmetics, laundry detergents, toothpastes, and mouthwashes (Schmeiser et al., 2001; Jones et al., 2000). As a broad spectrum antimicrobial and preservative agent, there are obviously a great deal of production and usage of TCS and HHCB. In Europe countries, approximately 350 tons of TCS have been produced annually for commercial applications (Singer et al., 2002), and more than 2000 tons of HHCB and tonalide are used annually (Balk and Ford, 1999) due to their aroma, stability, and cheapness (Bester, 2004).

Because of the ceaseless input, low removal efficiency and the large amount of TCS and HHCB consumed in developed societies, significant concentrations of these two compounds have recently been detected in sewage effluents (McAvoy et al., 2002; Ricking et al., 2003), surface and ground water (Kolpin et al., 2002), and soils and sediments (Singer et al., 2002). The concentration of TCS in effluents was found ranging from 23 ng L^{-1} to 2700 ng L^{-1} (Halden and Paul, 2005; Ying and Kookana, 2007). Significant amounts of TCS in wastewater streams remain in the sludge due to its hydrophobic nature, with concentrations high up to 55 mg kg^{-1} (Bester, 2003; Heidler and Halden, 2007). The residual levels of HHCB in the environment are also remarkable (Carballa et al., 2004), especially in the sludge. The concentration of HHCB in sludge was detected from 6.1 mg L^{-1} to 61.5 mg L^{-1} (Kupper et al., 2004; Shek et al., 2008).

The increasing residual concentrations of TCS and HHCB in the environment inevitably threaten the health of ecosystems. More and more researches concerning PCPs have been presented due to their toxicity on the aquatics, especially concerning on their possible subtle effects on non-target organisms (Smital et al., 2004). For examples, Binelli et al. (2009) found that TCS had genotoxic

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and cytotoxic effect on Zebra mussel hemocytes, connected with both oxidative stress and/or a direct effect on DNA. TCS also has toxicity to *Daphnia magna* with a 48-h median effective concentration of 390 μg L^{-1} and fish with a 96-h lethal concentration of 260 μg L^{-1} (Orvos et al., 2002); Yamauchi et al. (2008) found that HHCB had high acute toxicity on the early life stages of medaka. However, there were little documents published about the phytotoxicity of TCS and HHCB on crops and the different toxic effects of TCS and HHCB on the same crop.

Generally speaking, phytotoxicity of a chemical can be assayed using seed germination and plant growth test, which has been used to examine the toxic effects of antibiotics and heavy metals (Migliore et al., 2003; Liu et al., 2007). The purpose of this study was to investigate the effects of TCS and HHCB on seed germination and the antioxidant enzymes in seedlings of wheat (*Triticum aestivum* L.), which is one of the most important agricultural crops in China and other countries in the world. It is easily cultured and maintained in a laboratory and has been used in toxicity tests to investigate the toxic effects of pollutants such as chlorimuron-ethyl and cadmium (Wang and Zhou, 2006).

2. Materials and methods

2.1. Materials

TCS and HHCB (99% purity) were purchased from the Wuhan Yuancheng Technology Development Co. Ltd., China, and used without further purification. The molecular formula of TCS and HHCB are $C_8H_9NO_2$ and $C_{18}H_{26}O$, respectively. All the reagents used in the study were purchased from the Tianjin Kermel Reagents & Instruments Co. Ltd., China, and they were analytical grade.

The variety of tested wheat (*T. aestivum* L.) is Liaoning Spring No. 10. These wheat seeds were obtained from the Liaoning Dongya Seed Co., in Shenyang, China.

2.2. Seed germination experiment

According to 10–60% of the inhibitory rate of shoot and root elongation by the two PCPs in the preliminary experiment, the tested concentrations of TCS and HHCB in the formal solution culture were ascertained and equal to 0, 50, 100, 150, 200 and 250 mg L^{-1}, respectively.

Prior to their germination, the wheat seeds were surface-sterilized in 3% (v/v) H_2O_2 for 5 min and then rinsed with deionized water. Seed germination experiments were performed on filter papers placed in Petri dishes and moistened with 5.0 mL solution of the PCPs. Controls were obtained by moistening the filter papers with 5 mL deionized water. Twenty wheat seeds were placed in each dish, covered by the lid, and incubated in the darkness at 25 ± 2 °C in the culturing box (LRH-250-A, made in Guangdong Medical Instruments Factory, China). Seeds were considered to have germinated when both the plumule and radicle were over 2.0 mm. When the length of the growing roots cultured in the control reached 20.0 mm, the exposed experiment was finished. All treatments were replicated three times to minimize experimental errors. Shoot and root length of seedlings growing in the test solutions were expressed as percentage inhibition (%) compared with the control.

2.3. Seedling development experiment

Wheat seeds germinated in darkness at 25 ± 2 °C for 7 days. They then underwent further growth using solution hydroponics in a growth chamber operated with 12 h light: 12 h dark cycles at a constant temperature of 25 ± 2 °C. According to the median effect concentration (EC_{50}) of TCS and HHCB in the seed germination experiment, the concentrations of the PCPs were equal to 0.2, 0.4, 0.7, 1.5, and 3.0 mg L^{-1}, corresponding to EC_{50}/800, EC_{50}/400, EC_{50}/200, EC_{50}/100, and EC_{50}/50, respectively. The test solutions were renewed every day to avoid any change in the concentration and speciation of the two PCPs. After incubation for time intervals of 0, 7, 14, and 21 days, samples were taken and analyzed.

The content of chlorophyll (CHL) in wheat seedlings was determined in 80% acetone extract of 0.05 g leaf tissues as described by Hegedüs et al. (2001). About 0.1 g of leaf and root tissues were homogenized in 4 mL of an extraction buffer (50 mM $NaH_2PO_4{\cdot}Na_2HPO_4$ in 1% polyvinylpyrrolidone, pH 7.8). The filtered tissue extract was centrifuged at 12,857*g* for 30 min at 4 °C in the centrigufe (5804R, made in the Eppendorf Crop., Germany). The supernatant was used for the assay of soluble protein (SP) and enzyme activities. The details were described by Polle et al. (1997). The concentration of SP in the supernatant was determined using the dye-binding method of Coomassie Brilliant Blue according to Bradford (1976). All enzyme activity data were related to plant fresh weight. The activity of peroxidase (POD) and superoxide dismutases (SOD) was determined as described by Wu and von Tiedemann (2002). The activity of POD was determined with guaiacol by the spectrophotometry, and the activity of SOD was determined on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium.

2.4. Statistical analysis

All measurements were replicated three times in independent experiments and the determination of enzyme activities was performed with three parallel samples in all cases. All data were subjected to the analysis of variance (ANOVA) with factors of TCS and HHCB concentrations and three time intervals. Standard deviations (SD) were also calculated. When a significant ($p < 0.05$) difference was observed between treatments, multiple comparisons were made by the LSD test. All the values expressed in the work are mean ± SD, and the letters under *x*-axis refer to the difference at significance level $p < 0.05$ among different concentrations.

3. Results

3.1. Effects of TCS and HHCB on the germination of wheat seeds

As shown in Table 1, the treatments of TCS and HHCB both inhibited the shoot and root elongation of wheat seedlings. In comparison with the control, the shoot and root length significantly decreased with an increase in the concentration of TCS, resulting in 24.6–60.2% and 28.9–63.6% reduction, respectively. Similarly, the shoot elongation was significantly inhibited by 50–250 mg L^{-1} HHCB with the inhibitory rate from 32.7% to 67.8%. However, lower concentration of HHCB (⩽50 mg L^{-1}) could accelerate the growth of wheat roots. Compare with the control, the root length increased 13.0% treated by 50 mg L^{-1} HHCB. With an increase in the concentration of HHCB, the root length was decreased gradually. The inhibitory rate of root length treated by 100–250 mg L^{-1} HHCB was 4.3–22.6%. The statistical analysis showed that the difference of the inhibitory rate between shoots and roots treated by HHCB was significant ($p < 0.05$).

There were very significant ($p < 0.01$) positive linear correlations between the inhibitory rate (%) of shoot and root elongation and the concentrations of TCS or HHCB. The corresponding regression equations could be expressed as follows:

$$SI_{TCS} = 0.170X + 16.76 \quad (R^2 = 0.964,\ p < 0.01) \tag{1}$$

$$RI_{TCS} = 0.161X + 26.16 \quad (R^2 = 0.899,\ p < 0.01) \tag{2}$$