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Uptake and subcellular distribution of triclosan in typical hydrophytes under hydroponic conditions☆

Yupeng He, Enguang Nie, Chengming Li, Qingfu Ye, Haiyan Wang*

Institute of Nuclear Agricultural Sciences, Key Laboratory of Nuclear Agricultural Sciences of Ministry of Agriculture and Zhejiang Province, Zhejiang University, Hangzhou 310029, China

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ABSTRACT

The increasing discharge of pharmaceuticals and personal care products (PPCPs) into the environment has generated serious public concern. The recent awareness of the environmental impact of this emerging class of pollutants and their potential adverse effects on human health have been documented in many reports. However, information regarding uptake and intracellular distribution of PPCPs in hydrophytes under hydroponic conditions, and potential human exposure is very limited. A laboratory experiment was conducted using ^{14}C-labeled triclosan (TCS) to investigate uptake and distribution of TCS in six aquatic plants (water spinach, purple perilla, cress, penny grass, cane shoot, and rice), and the subcellular distribution of ^{14}C-TCS was determined in these plants. The results showed that the uptake and removal rate of TCS from nutrient solution by hydrophytes followed the order of cress (96%) > water spinach (94%) > penny grass (87%) > cane shoot (84%) > purple perilla (78%) > rice (63%) at the end of incubation period (192 h). The range of ^{14}C-TCS content in the roots was 94.3%–99.0% of the added ^{14}C-TCS, and the concentrations in roots were 2–3 orders of magnitude greater than those in shoots. Furthermore, the subcellular fraction-concentration factor (3.6×10^2–2.6×10^3 mL g^{-1}), concentration (0.58–4.47 μg g^{-1}), and percentage (30%–61%) of ^{14}C-TCS in organelles were found predominantly greater than those in cell walls and/or cytoplasm. These results indicate that for these plants, the roots are the primary storage for TCS, and within plant cells organelles are the major domains for TCS accumulation. These findings provide a better understanding of translocation and accumulation of TCS in aquatic plants at the cellular level, which is valuable for environmental and human health assessments of TCS.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) include diverse groups of compounds that are used extensively in modern societies (Stamatis et al., 2014). The recent awareness of the environmental impact of this emerging class of pollutants (Kolpin et al., 2002) and their potential adverse effects on ecosystem and human health have been documented in many reports (Stamatis et al., 2014; Wu et al., 2012). Triclosan (TCS), a widely used antimicrobial additive, has been extensively found with concentrations ranging from 338 to 2300 ng L^{-1} in rivers and lakes (Sapkota et al., 2007; Miller et al., 2008; Zhao et al., 2013; Halden and Paull, 2004; Wu et al., 2009) and from 3800 to 16600 ng L^{-1} in influent wastewater (McAvoy et al., 2002; Zhao et al., 2013), urine (Pycke et al., 2014; Meeker et al., 2013), blood plasma (Pycke et al., 2014), and breast milk of pregnant women (Arbuckle et al., 2015). Meanwhile, TCS was also detected in many edible vegetables irrigated with wastewater under field conditions, such as carrot (Wu et al., 2014), and *Typha latifolia* (Zarate et al., 2012). This represents a possible exposure pathway of TCS to animals and humans via their consumptions of TCS-contaminated vegetables.

Plants irrigated with reclaimed water or cultivated in contaminated water could accumulate TCS from the water. The uptake and translocation of TCS in plants has been documented in several studies. For example, Wu et al. (2013) showed that TCS could accumulate in the roots of lettuce, spinach, cucumber and pepper with concentration range from 3.2 to 560 μg kg^{-1}, but only 0–1.3 μg kg^{-1} in leaves of these plants after 21-d growth in a nutrient solution containing mixed PPCPs (including TCS) at

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* Corresponding author.
E-mail address: wanghaiyan@zju.edu.cn (H. Wang).

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5 μg L^{-1} for each chemical. Mathews et al. (2014) found that less than 3.7% of TCS was translocated from roots to shoots in cucumber, tomato, cabbage, okra, pepper, and potato after 30-d growth in nutrient solution with TCS at 500 μg L^{-1}. Macherius et al. (2012) reported TCS concentration of 2.82 μg g^{-1} in the peels of carrot roots, but 0.23 μg g^{-1} in carrot leaves after 60-d growth in soil with TCS at 10 mg kg^{-1}. These studies revealed that TCS concentrations in plant roots were generally greater than that in other plant tissues. However, most these studies were conducted using xerophytes, few studies were performed on uptake of TCS by aquatic plants.

Recently, Reiss et al. (2009) found that TCS can attach to numerous intracellular and cytoplasmic sites within microorganism cells. Subcellular distribution of heavy metals (Liu et al., 2009), polycyclic aromatic hydrocarbons (Gao et al., 2013; Kang et al., 2010), and organic solutes (Büssis and Heineke, 1998; Kuzniak and Sklodowska, 2004) in plants have been investigated extensively. For example, more than 80% of phenanthrene and pyrene has been shown to be distributed in cell walls and organelles in ryegrass roots (Kang et al., 2010). Little information is available regarding the distributions of PPCPs (especially TCS) within the intracellular tissues of plants.

In this study, we investigated the uptake and translocation of TCS in six common hydrophytes using ^{14}C-labeled TCS and radioautographic imaging technology. Radioautography offers the visualization of radioactive compounds in biological tissues, which is used to examine the distribution of radioactive TCS in plants. The concentration of ^{14}C-TCS in plant fractions and subcellular tissues was quantified by liquid scintillation counter. This information is used to elucidate the uptake and distribution of TCS in aquatic plants at the subcellular level, and will provide useful information that can be incorporated into assessments of TCS in food items and the environment.

2. Materials and methods

2.1. Chemicals

A supply of [dichlorophenyl-U-^{14}C]-labeled TCS (radiochemical and chemical purity > 99%, specific activity 77 mCi $mmol^{-1}$; see Fig. 1 for structure and ^{14}C labeling positions) was purchased from American Radiolabeled Chemicals (St Louis, MO, USA). The radioactivity was quantified using a liquid scintillation counter after mixing with 15 mL of scintillation cocktail I, which was prepared by dissolving 0.5 g of 1,4-bis-(5-phenyloxazol-2-yl)-benzene (POPOP, Arcos Organics, Geel, Belgium) and 5.0 g of 2, 5-diphenyloxazole (PPO, Arcos Organics) in 400 mL of 2-methoxyethanol (Sinopharm Chemical Reagent Co., Ltd.) and 600 mL of dimethylbenzene (Sinopharm Chemical Reagent Co., Ltd.).

2.2. Plants

The hydrophyte plants used in the study included water spinach (*Ipomoea aquatica* Forsk), purple perilla (*Prilla frutescens* (L.) Britt.), cress (*Oenanthe javanica* (Blume) DC), penny grass (*Hydrocotyle vulgaris*), cane shoot (*Zizania latifolia* (Griseb.) Stapf), and rice (*Oryza sativa*). Plants were germinated from seeds (water spinach, cress, rice), old roots (cane shoot) in vermiculite, or were intact plants (penny grass, purple perilla) purchased from Fengqi Birdflower Market in Hangzhou city, China. The plants were rinsed with distilled water and transplanted to nutrient solution when the seedlings were grown after 3–4 weeks, and cultivated in a growth chamber at 20–25 °C (night-day), with a humidity of 80% and a day/night cycle of 12 h/12 h and constant aeration for an acclimatization of one week (Wang et al., 2013a).

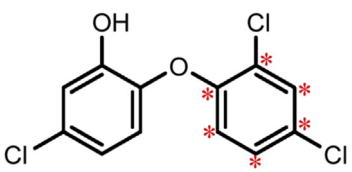

Fig. 1. [Dichlorophenyl-U-^{14}C]-labeled Triclosan (^{14}C atoms marked with *).

2.3. Plant uptake

Each plant was cultivated in a 30-mL amber glass bottle with 20 mL-nutrient solution mixed with ^{14}C-TCS. ^{14}C-TCS concentration (0.042 μg mL^{-1}, 30.19 Bq mL^{-1}, calculated by equation (1)) in the nutrient solution were generally greater than those found in rivers and lakes in the environment; the purpose of the high concentration of TCS was to ensure that sufficient ^{14}C-TCS could be taken up for measurement (Herklotz et al., 2010). To reduce the photolysis of TCS, all of the experimental bottles were wrapped with aluminum foil to minimize light penetration the roots and nutrient solutions. Seedling samples were taken at 6, 12, 24, 48, 72, 120, and 192 h after treatment. After sampling, each seedling was washed with distilled water and divided into shoots and roots. The collected plant roots and shoots were oven-dried at 110 °C for 1 h to deactivate enzymes, and kept in the oven at 70 °C to reach nearly constant weight. The weighed samples were then combusted in a biological oxidizer (OX-501 Biological Oxidizer, RJ Harvey Instrument Co., NJ, USA), and the released $^{14}CO_2$ was trapped in 15.0 mL of scintillation cocktail II composed of 0.5 g POPOP and 7.0 g PPO in 600 mL dimethylbenzene, 175 mL ethanolamine, and 275 mL glycol ether. The radioactivity was quantified using a TriCarb-2910 liquid scintillation counter (LSC, Perkin-Elmer Inc., Downers Grove, IL, USA) (Wang et al., 2013a).

$$\text{Concentration of } {}^{14}C-TCS = \frac{R \cdot M}{V \cdot C \cdot S \cdot 1000} \quad (1)$$

In which R (dpm) is the ^{14}C radiation value of nutrition solution measured by liquid scintillation counter, M (mg $mmol^{-1}$) is the molar mass of ^{14}C-TCS, V (mL) is the volume of nutrition solution, C is a convert unit 1mCi = 2.22×10^9 dpm, and S (mCi $mmol^{-1}$) is the specific activity of ^{14}C-TCS (77 mCi $mmol^{-1}$).

2.4. Radioautographic imaging

Six additional seedlings that had exposed to ^{14}C-TCS solution were sampled at 192 h after treatment. After being rinsed with deionized water, the six plants were oven-dried at 110 °C for 1 h to deactivate enzymes (Wang et al., 2013a). The distribution of ^{14}C within the six seedlings was recorded using a Bioimaging Analyzer System (Fuji BAS 1800; Fuji Photo Film, Tokyo, Japan). The intact plants were exposed to a sensitive imaging plate in the dark for three months, which was then scanned with a laser beam and the images were recorded into a computer. The imaging plate was tightly wrapped in a thin transparent plastic film to minimize contamination from the plate during image recording (Wang et al., 2013a).

2.5. Subcellular distributions of TSC

Five plants of same species were cultivated in 100 mL beaker to ensure that the separated subcellular fractions could be detected.

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