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Effects of benthic organism *Tubifex tubifex* on hexachlorocyclohexane isomers transfer and distribution into freshwater sediment

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ABSTRACT

In this study, bioaccumulation and elimination of HCHs in tubifex, and the distribution of HCHs in overlying water and sediment, were studied during a 10-d experiment. A sensitive method was developed for the determination of HCHs in samples based on gas chromatograph (GC) equipped with a nickel-63 electron capture detector (μECD). The limit of detection (LOD) was 0.35 μg/kg for α-HCH and 0.82 μg/kg for β-HCH. Tubifex accumulated HCHs rapidly, and the curves were approximately M-type. The highest level was reached on the 7th day, with 0.34 mg/kg$_{wwt}$ for α-HCH and 0.87 mg/kg$_{wwt}$ for β-HCH in worms. The AFs of β-HCH in tubifex were higher than those of α-HCH. Moreover, the existence of tubifex significantly reduced β-HCH fluxes from the overlying water to sediment by uptake or degradation and decreased the concentrations of β-HCH in the sediment, but it had little influence on α-HCH fluxes. Moreover, enantioselectivity of α-HCH enantiomers was not observed in tubifex, whether in the bioaccumulation or elimination experiments. At the end of the elimination experiment, approximately 80% and 70% of α-HCH and β-HCH were eliminated, and the depuration half-lives were 4.43 and 5.39 days, respectively.

1. Introduction

Organochlorine pesticides (OCPs) have attracted wide attention for decades due to their large production and usage, their nature of persistence, bioaccumulation and harmful effects in the environment (Ali and Jain, 1998; Hussain et al., 2015; Jones and De Voogt, 1999; Nakata et al., 2002; Zhou et al., 2013). Although most of them have been officially banned for many years, OCPs residues are still detectable in various environmental media, even in remote high latitude or high altitude regions, such as polar regions, Tibetan Plateau and high mountains (Zhang et al., 2013; Zheng et al., 2009).

Most organochlorine pesticides are categorized as persistent organic pollutants (POPs), and hexachlorocyclohexanes (HCHs) were recognized as POPs by the Stockholm Convention in 2009 (UNEP). HCHs were used as worldwide insecticides during the 1950s to 1980s (Ali and Ali, 2004; Niu et al., 2013). Two types of HCHs products were used around the world: technical HCHs (mainly containing α-HCH, β-HCH, and γ-HCH) and lindane (γ-HCH > 99%) (Zhang et al., 2011). These HCH isomers have different physicochemical properties, which cause different partitioning in the environment (Wu et al., 2013). α–HCH has a high vapor pressure and is prone to evaporation to the atmosphere. In contrast, β-HCH, with all chlorine atoms in the equatorial conformation, is in the most energetically favorable configuration, therefore it is the most stable molecule among the HCH isomers (Chessells et al., 1988). If there were no fresh inputs of technical HCH, β-HCH would be the predominant isomer in most sediments (Wu et al., 2013).

OCPs have a strong affinity for suspended particulates and sediments, on account of their low-water solubility and high *n*-octanol/water partitioning coefficient values (K_{ow}). Thus, sediments can serve as reservoirs, or "sinks" for OCPs (Yang et al., 2011). Once disturbed, the sediments can be resuspended and the contaminants may re-enter the aquatic environment, resulting in a second contamination (Yang et al., 2011; Zeng and Venkatesan, 1999). Benthic organisms, living at the sediment-water interface, transfer the sediment-associated contaminants to mix back into the aqueous phase by bioturbation, and/or accumulate sedimentary pollutants and subsequently transfer them into higher trophic levels through the food web (Egeler. et al., 2001; Liu et al., 2015).

Sediment-associated POPs are known to exhibit narcotic effects in benthic organisms, and they also have been implicated in the development of tumors, malformation, loss of fertility, or immune deficiency in many organisms (Liu et al., 2009; Lu et al., 2013). Cosmopolitan aquatic oligochaete, tubifex worms have intimate

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contact with the aqueous and solid phase, burrowing the anterior part into the sediment and undulating the posterior part in the overlying water to allow cutaneous respiration (Liu et al., 2015). Tubifex can be found in both unpolluted and highly polluted waters and is often the last to disappear in a contaminated site (Reynoldson et al., 1996). Previous studies have shown that the autotomy and regeneration of their caudal part may participate in efficient detoxification of tubifex, which make them survive in highly contaminated media (Lagauzere et al., 2009; Meller et al., 1998). Moreover, *T. tubifex* plays an important role in self-purification of water bodies, and has been designated as a representative freshwater test organism to assess sediment toxicity and accumulation of organic compounds (Fangtong et al., 2011; Lagauzere et al., 2009; Liu et al., 2015; Vidal and Horne, 2003). This study aims to better understand how tubifex worms affect HCHs fluxes from overlying water to the sediment and assess the bioaccumulation and elimination of HCH isomers in *Tubifex tubifex*.

Four experimental treatments were studied. For the bioaccumulation test, three experimental conditions were analyzed: contaminated water column with or without worms and uncontaminated water column with worms. The last one was the poisonous worms eliminated HCHs treatment. This research investigates the effects of tubifex on fluxes of HCHs between the water column and sediment compartments. The different bioaccumulation and elimination behaviors of α-HCH and β-HCH in tubifex were observed in the present study. Moreover, the enantioselectivity of α-HCH was also assessed.

2. Materials and methods

2.1. Chemicals and reagents

α-, β-hexachlorocyclohexane (HCH) pesticide standards (purity > 99.0%) and recovery surrogate 2,4,5,6-tetrachloro-m-xylene (TCmX) were obtained from J&K Scientific Ltd (Beijing, China). All test chemicals were initially dissolved in n-hexane. Acetone, n-hexane, and petroleum ether (60–90 °C) were HPLC grade and purchased from commercial sources (Beijing Chemical Reagent Co., China).

2.2. Test worms and sediment

T. tubifex was obtained from a commercial breeder (Da Senlin Flower Market Beijing, China). Worms were maintained in 2 L plastic tanks containing uncontaminated sediment and dechlorinated tap water at 20 ± 1 °C, with 12 h light and 12 h darkness per day for one week to acclimate to the environment. The worms were fed TetraMin Flakes (Tetra Werke, Melle, Germany) weekly. For the experiments, adult *T. tubifex* (aged five to seven weeks) were used, and the organisms were not fed during the experimental period.

The sediment was collected from Ulla Gail Lake (Inner Mongolia, China) from the 0–10 cm surface layer of sediment. No HCHs were found at detectable levels in the sediment. After freeze-drying, sediment samples were homogenized and sieved through a 2 mm sieve, and kept in the dark. The physicochemical properties of the sediment were as follows: clay, 1.81 ± 0.04%; silt, 7.39 ± 2.22%; sand, 90.80 ± 2.26%; organic matter, 5.31 ± 0.21%; and pH, 9.56 ± 0.05.

2.3. Bioaccumulation and elimination experiment

Experimental design. The experiments were conducted with four scenarios, which were denoted as +TSE, −TSE, TSE and EE.

The experiments were conducted in 500 mL beakers. Sediment 150 g (water content 35%) was put into the bottom of each beaker with a height of 2–3 cm, and 100 mL dechlorinated tap water with HCHs (0.1 mg/L) was added slowly. The scenario with acclimated tubifex worms (10 g) were placed into beakers (18 beakers, 6 sampling points, triplicates for one sampling point). After an exposure period (1, 2, 3, 5, 7, and 10 days), overlying water was gently poured and sampled firstly. The alive worms were collected by heating the beakers in a water bath (40 °C, 30–45 min), which caused them rising to the surface of sediment (Lagauzere et al., 2009; OECD, 2005). Removed the worms from the beakers and rinsed with dechlorinated tap water, then dried the peripheral water using absorbent paper. Three replicates including sediments, overlying water and worms were sampled on the days of 1, 2, 3, 5, 7, and 10 during the 10-d exposure period, weighed and stored at −20 °C. The scenario including contaminated water column with tubifex and uncontaminated sediment was denoted as +TSE.

To investigate the bioturbation of tubifex, a separate experiment (negative control) with spiked water and sediment was denoted as −TSE, and this treatment was without tubifex. The other control scenario, including tubifex, water and sediment without HCHs, was denoted as TSE. Sample modes were consistent with +TSE treatments.

The elimination experiment (EE) was conducted after three days of bioaccumulation experimentation; worms were removed to beakers with contaminant-free overlying water and sediment. Sampling was conducted at 0.5, 1, 3, 5, and 7 days. Overlying water, sediment and worms were collected and stored at −20 °C. The treatments had three replicates.

For the four scenarios, the test beakers were weighed daily, and the loss of water resulting from evaporation was compensated by adding dechlorinated tap water. All of the beakers were cultured with a randomized block in the climate chamber at 20 ± 1 °C and a light-dark cycle of 12 h. Water quality parameters including pH, ionic potential, conductivity, total dissolved solids, salinity and resistivity in the overlying water were measured before sampling, using a SX736 portable pH/conductivity/dissolved oxygen meter (Shanghai San-Xin Instrumentation, Inc.).

2.4. Samples extraction and purification

All the samples were thawed at room temperature. The overlying water samples (20 g) were extracted with 20 mL of petroleum ether in a 50 mL polypropylene centrifuge tube. After stirring for 3 min on a vortex mixer, the extraction was repeated twice using fresh solvent. The combined solvent phase was filtered through 5 g of anhydrous sodium sulfate for dehydration, transferred to a pear-shaped flask, and then evaporated to dryness at 35 °C by vacuum rotary evaporator (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China). The extractive was redissolved with 500 μL n-hexane after passing through a filter membrane (pore size, 0.45 μm).

Microwave-assisted solvent extraction (MAE) was applied to extract the tubifex and sediment samples. MAE was carried out with a Mars 6 Xpress (maximum power: 1800 W) microwave extraction system (CEM, Matthews, NC, USA). Tubifex samples were homogenized with an Ultra-Turrax T18 homogenizer for 30 s, and 3 g homogenate was transferred to the microwave extraction vessels (TCmX was added as recovery surrogate), adding 20 mL extraction solution (acetone: petroleum ether=1:1, V:V). The vessels were covered with pressure-resistant holders and placed into the rotary base. The extraction temperature program was as follows: ramped to 100 °C in 5 min, held for 10 min and cooled to room temperature. The extraction solvent was filtered through 5 g of anhydrous sodium sulfate for dehydration and transferred to a pear-shaped flask, then concentrated to dryness. The Florisil-SPE