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Bioaccumulation, distribution and elimination of lindane in *Eisenia foetida*: The aging effect

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HIGHLIGHTS

- Aging decreased the bioavailability of lindane.
- Lindane could be taken up through both skin diffusion and ingesting soil particles.
- The soil-bound lindane entered the earthworm mainly through dietary route.
- The soil-bound lindane took longer to depurate from the organisms than free lindane.

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ABSTRACT

Soil aging will influence the chemical speciation of pesticides, thus affecting the uptake route to be bioavailable to the organism. So far, studies on the possible effects of the uptake route on the distribution and elimination of pesticides in the organism that also considers effects of aging are limited. In our study, *Eisenia fetida* was exposed to 4.5 mg kg^{-1} lindane aging for 0, 30 and 180 d, and the accumulation, distribution and elimination of lindane in the earthworms were analyzed. The results showed that the 6 h Tenax-extracted fraction exhibited a good linear correlation with the lindane accumulated in the earthworms. With aging time increasing, the bioaccumulation of lindane decreased and the accumulative balance was more easily reached in the earthworms. Lindane distributions were found in the whole earthworm and the proportions of lindane content at sub-organism level and the mass distribution of each fraction were similar for 0 d and 180 d aged groups. The foregut accumulated the highest content of lindane (20%) relative to its low mass distribution proportion (10%). The elimination rate of lindane in the earthworms decreased with aging time extending. Our conclusion was that the 6 h Tenax extraction could be used to assess the bioavailability of aging lindane. Although soil aging decreased the bioavailability of lindane, the soil-bound lindane entered the earthworm through dietary route would take longer to depurate from the organisms than free lindane, which implied the potential ecological risk of bound pesticide residues.

1. Introduction

Pesticides are a large group of important environmental pollutants that are characterized by large usage, high toxicity and thus extensive impact on the environment. Lindane (γ-Hexachlorocyclohexane, γ-HCH) was ubiquitously used as an insecticide due to its well-known high insecticidal property during the 1950s–1980s before it was officially banned worldwide and listed as a persistent organic pollutant at the Stockholm Convention (Vijgen et al., 2011). The structural persistence of lindane leads to its inconvenient residues in especially soil environment (Prakash et al., 2004; Shi et al., 2009; Niu et al., 2013), which raises great concerns about the continuing risk of lindane.

Soils as complex matrices can provide a wide variety of binding sites for pesticides entered into them, which causes pesticides existing in soils in both aqueous-phase dissolved and soil-bound residues (Gevao et al., 2000; Kästner et al., 2014). Increased soil-pesticide contact time, which is called aging process, will change the proportion of free and bound pesticide residues (Gevao et al.,

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2000). Existing theory asserts that only aqueous-phase pesticides are bioavailable and therefore possess toxic effects. However, a lot of studies have been carried out about the bioavailability and release of bound residues and potential toxic impacts to living organisms (Craven, 2000; Gevao et al., 2001; Han et al., 2009; Liu et al., 2015). In general, pesticides in the aqueous phase are more likely to be bioavailable to organisms, while the bound residues have relatively low bioavailability (Gevao et al., 2000; Qi and Chen, 2009). The hazard level of organic pollutants in ecosystems depends on their bioavailability (Šmídová, 2013), while studies about the potential impacts of differences in bioavailability of free and bound residues on organisms are limited.

Earthworms, as sentinel species in soil, have been widely used to assess the risks of chemicals to soil ecosystem (Sanchez-Hernandez, 2006). Earthworms may take up organic compounds through skin diffusion as well as from ingesting soil particles, and this is related to the hydrophobicity of the chemicals (Jager et al., 2003), and the difficulty of chemicals desorption from soil particles (Qi and Chen, 2009). The compounds absorbed through skin diffusion distribute in the body wall first, while those absorbed through ingesting soil particles intend to distribute in the digestive system first, and the compounds distribution finally achieve a steady state in the whole body under the blood circulation (Li et al., 2017). For different earthworm species and chemicals, the proportions of these two ways of absorption are also different, which may influence the time to reach accumulative equilibrium and the distribution or elimination of the chemicals in earthworms. So far, studies on the bioavailability of organic compounds taking account of aging factor that use earthworms are mostly focused on uptake or elimination kinetic studies (Amorim et al., 2002; Regitano et al., 2006; Šmídová, 2013). Reports about possible aging effects on the uptake route and thus the influences on the distribution and elimination of pesticides in the earthworms are limited.

In our study, we selected lindane, a relatively persistent chemical in the environment that is suitable for aging studies, to investigate the effects of aging on lindane bioavailability using *Eisenia foetida*. Our objectives were to investigate the effects of aging on the bioaccumulation, distribution and depuration of lindane in *Eisenia foetida*, and to reveal the correlation between uptake route and distribution and depuration of lindane in the earthworms at different aging time.

2. Materials and methods

2.1. Chemicals and reagents

γ-Hexachlorocyclohexane (γ-HCH or lindane, 97% purity) was purchased from Sigma Aldrich, China. Chromatographic-grade dichloromethane (DCM), acetone, n-hexane, and methanol were obtained from Merck, China. Florisil (0.150–0.250 mm) was obtained from CNW Technologies Gmbh. Anhydrous sodium sulfate (99% purity) was purchased from Shanghai Titan Scientific Co. Ltd.

2.2. Earthworm and soil

Earthworms (*Eisenia foetida*) were purchased from an earthworm-culturing farm in Jurong, Jiangsu Province, China. The earthworms were acclimatized in the laboratory for at least 2 weeks prior to use. The animals were maintained in soil supplemented with cow dung, at 25 ± 1 °C and 60–80% humidity. Healthy adult earthworms (fresh weight of 400–800 mg with well-developed clitellum) were selected for the tests. Before the exposure experiments, the earthworms were carefully removed from the culture, rinsed with distilled water to remove litter particles, and put into the test soils.

Soils (0–20 cm) collected near the earthworm-culturing farm, with no pesticide residues detected, were used for the whole study. The soil was prepared by passing it through a 10-mesh (2 mm) sieve after being air-dried under shade, and stored at room temperature before use. The physical and chemical properties of the soil are as follows: pH 7.2, 7.94% TOC and a cation exchange capacity (CEC) of 12.574 cmol kg^{-1}.

2.3. Soil spiking and aging experiments

A total of 18 mg of lindane was dissolved in 100 mL of acetone and mixed well with 100 g of soil. After evaporation of the acetone, 10 g of the soil was mixed with 390 g of clean soil to obtain a concentration of 4.5 mg kg^{-1} dry weight (dw) (the calculation was given in **SI**), after which a specific amount of deionized water was added to obtain a final soil moisture content corresponding to 60% of the maximum water holding capacity (WHC). The soil was then blended thoroughly to ensure that the lindane was homogeneously distributed. Each treatment utilized six replicate jars. The jars were incubated in the dark at 25 ± 1 °C for 0, 7, 15, 30 and 180 d.

2.4. Desorption determination by consecutive and single-point Tenax extraction

The total amount of lindane in soil was performed with soxhlet extraction using 100 mL of n-hexane/DCM (1:1, v/v) for 24 h, followed by ultrasonic extraction using the same solvent 2 times (20 mL and 10 min each time). The extracts were combined, purified, and concentrated to 1 mL for GC analysis (the detailed procedure is given in 2.6).

Different aging time (0, 30 and 180 d) of the soils were used for the desorption kinetics study (400 h consecutive extraction). Briefly, 1 g of soil, 0.2 g of Tenax TA beads and 100 mL of deionized water (containing 200 mg L^{-1} sodium azide to prevent microbial degradation) were combined in a 250 mL conical flask. The flask was shaken horizontally on an oscillator at 250 rpm min^{-1} for 0.17, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 100, 200 and 400 h (25 °C). At selected time, the Tenax beads were refreshed, rinsed 2 times with deionized water, and subjected to ultrasonic extraction 3 times with 30 mL of n-hexane/acetone (1:1, v/v). The combined extracts were then cleaned and concentrated to 1 mL for GC analysis (the detailed procedure was given in 2.6).

2.5. Bioaccumulation and distribution experiments

After completion of aging (0, 30 and 180 d), ten *Eisenia foetida* (weighing 400–800 mg) were introduced into each jar. The jars were placed in the dark at 25 ± 1 °C with a 60% moisture content in the environment. At selected time-points (day-0, 3, 7, 10, 15, 20 and 25), one earthworm and approximately 1 g of soil were sampled from each jar. There were six replicates for each treatment. The soils were immediately stored at −20 °C. The earthworms were rinsed with distilled water and placed on moist filter paper for 24 h to allow for gut clearance. Afterwards, the earthworms were weighed and frozen at −20 °C. The frozen earthworms and soils were further freeze-dried under vacuum for lindane determination.

The earthworms exposure for 10 d to lindane that aged for 0 d and 180 d were used to investigate the distribution of lindane in earthworms. Each earthworm was divided into four parts according to the somites: foregut (13 somites), stomach plus intestine (12 somites), cecum (50 somites), and hindgut (25 somites). The stomach plus intestine and cecum together are called the midgut. The lindane concentrations in the different parts of the earthworms were determined.