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Diverse impacts of a step and repeated BDE209-Pb exposures on accumulation and metabolism of BDE209 in earthworms

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HIGHLIGHTS

- The presence of high-level Pb could promote the bioaccumulation of BDE209 in earthworms.
- A step persistent exposure aggravated the damage in earthworms more than a repeated exposure.
- A step exposure decreased the capability of earthworms to metabolize BDE209 in the presence of Pb.
- BDE209 accumulation in post-clitellum was higher than that in pre-clitellum.

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ABSTRACT

Decabromodiphenyl ether (BDE209) and lead (Pb) are the two common contaminants at e-waste recycling sites (EWRSs). A laboratory incubation study was conducted to explore the impacts of a step and repeated BDE209-Pb exposures on accumulation and metabolism of BDE209 in earthworms *Eisenia fetida* for the first time. The results indicated that BDE209 concentrations in repetitively-polluted soils were clearly higher. And the existence of high-level Pb could promote the bioaccumulation of BDE209 in earthworms along the exposure time. The post-clitellum contents of BDE209 were more than the pre-clitellum during the entire incubation. Additionally, GC/MS analysis results demonstrated that BDE206, BDE208, BDE153, BDE99, BDE47 and BDE28 could be detected in *Eisenia fetida* throughout 28-d experiment, and BDE206 and BDE208 were predominant metabolic products. A step exposure decreased the capability to metabolize BDE209 in the presence of Pb. Average bioaccumulation factor (BAF) for a step treatment was 0.525, as well as it was more than 1.1 times that of repeated exposure (BAF = 0.48). SEM observations suggested that a step exposure mode aggravated the damage in earthworms than repeated exposure. The results and related findings will establish a useful scientific basis for soil ecological risk assessment at EWRSs.

1. Introduction

In recent decades, there has been a growing concern in waste electrical and electronic equipment (e-waste) pollution events in China (Song and Li, 2014). According to a newly scientific publication (Balde et al., 2015), in 2014 the domestic quantity of e-waste generation was around 6.0 million metric tonnes in China, only second to the United States (7.1 million). E-waste contains high amounts of toxic materials such as polybrominated diphenyl ethers (PBDEs) and heavy metals. Due to rough recycling techniques, these pollutants may have the chance to release into the environment and cause potential risks to human and organisms (Robinson, 2009; Chen et al., 2011) during the primitive recycling activities such as open-burning actions, acid stripping and dismantling.

PBDEs are highly hydrophobic compounds with high log octanol/water partition coefficients (logKow), such as 9.97 for decabromodiphenyl ether (BDE209) (Tittlemier et al., 2002; Braekevelt et al., 2003), which often appears in soil, sediment, and organisms (Nie et al., 2015). An extremely high concentration of PBDEs (average = 2,283 ng g^{-1} dw (dry weight)) was detected in soil from an area surrounding the e-waste recycling site in Qingyuan, Guangdong Province, China, and BDE209 accounted for 93% of

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$\sum_{21}$PBDEs (Nie et al., 2015). Pb is regarded as one of the most common co-existing heavy metals at e-waste dismantling site (Li et al., 2014). An investigation indicated that average Pb concentration was 6,082.9 mg kg^{-1} in Taizhou, Zhejiang Province, China, much higher than the 500 mg kg^{-1} limit for Grade III soil of the Chinese environmental quality standard (Zhang et al., 2014a).

Earthworm *Eisenia fetida* (*E. fetida*) has the ability to accumulate large quantities of contaminants and is considered as a typical organism for knowing the effects of toxicants (OECD, 2004). Our previous studies reported that exposure to BDE209 and/or Pb had detrimental influences on earthworms (Zhang et al., 2014b, 2014c, 2015d, 2015e; Li et al., 2015). However, these reports were basically depended on a step rather than repetitive treatment. Actually, the real soil environmental pollution is usually generated from repeated exposure events (Burges et al., 2015; Chen et al., 2015; Pardo et al., 2016).

Additionally, present reports on the accumulation of the two contaminants were generally based on the entire body of earthworms, ignoring the differences between pre-clitellum and post-clitellum (Honeycutt et al., 1995; Edwards and Bohlen, 1996; Tewatia, 2007; Li et al., 2009a; Shi et al., 2013). Therefore, it would be urgent to explore BDE209 and/or Pb distributions in diverse regions of earthworms.

This study was conducted primarily to explore the concentrations of BDE209 in soils as well as its distribution and transformation in *E. fetida* after a step and repeated exposures to the two chemicals. The results of these observations will provide a basic understanding of the potential eco-toxicological effects of joint PBDEs and heavy metal exposures on terrestrial invertebrates.

2. Materials and methods

2.1. Experimental chemicals

BDE209 (purity > 98%) was obtained from J&K Scientific Ltd., Shanghai, China. Lead nitrate ($Pb(NO_3)_2$), acetone, *n*-hexane, dichloromethane, granular anhydrous sodium sulfate, concentrated sulfuric acid, and neutral alumina were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Granular anhydrous sodium sulfate, silica gel, and neutral alumina were baked at 400 °C for over 6 h before use.

2.2. Soil and earthworm collection

The soil (0–20 cm) was collected from East China University of Science and Technology, Shanghai, China. At the laboratory, large plant residues and pebbles were removed, and then the soil was air-dried and sieved by a 2-mm mesh. Soil pH was measured in $CaCl_2$ solution (0.01 M) at a soil to solution ratio of 1:5. Organic matter content was determined using the Walkley-Black wet oxidation method. The soil was characterized by silty clay loam, pH = 7.3 and 6.5% organic matter.

Mature earthworms were purchased from Yonghe Earthworm Culture Farm, Shanghai, China. The average weight of earthworms was approximately 0.5 g. All earthworms were allowed to acclimatize to laboratory conditions for about 28 days before use.

2.3. Preparation of the contaminated soils

Lead stock solution was prepared by dissolving $Pb(NO_3)_2$ in deionized water, and BDE209 stock solution was prepared by dissolving its powder in methylbenzene. In order to determine exposure concentrations, based on previous toxicity evaluation about the two chemicals, we had carried out some preliminary experiments (Zhang et al., 2014b, 2014c, 2015d, 2015e; Li et al., 2015).

Experiments were conducted in 800-mL glass beakers. Fig. 1 illustrates the procedures of a step and repeated exposures. (1) For a step treatment, BDE209 concentration was directly applied as 100 mg kg^{-1} dry soil (abbreviated to 100B), and three different levels of Pb concentrations were applied as the low- (50 mg kg^{-1} – abbreviated to 50 Pb), moderate- (250 mg kg^{-1} – abbreviated to 250 Pb), and high- (500 mg kg^{-1} – abbreviated to 500 Pb) levels. Hence, four serial treatments were performed as 100B0Pb, 100B50 Pb, 100B250 Pb, and 100B500 Pb, respectively. (2) For repetitive treatment, on day 1, BDE209 solution was thoroughly mixed with 300 g soil; After methylbenzene was completely volatilized, the soils containing BDE209 were treated by $Pb(NO_3)_2$ solution and then one hundred acclimatized earthworms were added into each beaker; After 5 and 10 days of incubation, another 100 g spiked soils were added to each beaker and remixed as second and third exposures; the controls received the same amount of clean soil. The beakers were kept in the HPG-280HX artificial climate chamber with 20 ± 1 °C, 75% humidity and 12/12 h day/dark cycling period, and we regularly supplemented deionized water to bring the moisture concentration of the soil to 65% of the total WHC (water holding capacity).

After the completion of pre-exposure operation, we restarted sampling time. We can easily find the actual day 0 in Fig. 1. After 2, 7, 14 and 28 days of incubation, twenty earthworms were randomly removed from each treated group, and then they were washed with normal saline and allowed to purge for 24 h on clean filter paper, then frozen using liquid nitrogen. According to previous reports (Tewatia, 2007; Shi et al., 2013), we conducted the experiment with slight modifications, and the earthworms were grouped into two fractions: pre-clitellum (from head to clitellum) and post-clitellum (from clitellum to posterior). The body segments were stored at −70 °C until further analysis.

2.4. Sample extraction and clean up for BDE209 determination

The sampled earthworms were freeze-dried and then pulverized. About 0.2 g dried sample was weighed and then extracted using ETHOS ONE-41 microwave-aided extraction. The instrumental conditions were as follows: the solvent was *n*-hexane/acetone (1:1, v:v, 25 mL), the temperature was 110 °C, and the power was 1500 W with 15 min heating and 20 min in static state. The extract was filtered through filter paper packed with anhydrous sodium sulfate and then approximately reduced to 1 mL with a rotary evaporator. The concentrated solution was transferred with hexane to a 10-mL centrifugal tube. The volume was diluted to 10 mL, 1 mL of which was used for determination of the lipid contents. The other 9 mL was blown down to 1 mL with gentle N_2 flow. After that, the solution was concentrated and loaded onto a multilayer silica column (10-mL SPE cartridge packed from the bottom up with degreasing cotton, 2 cm neutral alumina, 2 cm activated silica gel, 2 cm acid silica gel (44% of sulfuric acid), 2 cm alkaline silica gel (30% of sodium hydroxide), and 1 cm anhydrous sodium sulfate, activated by 10 mL *n*-hexane). The column was washed twice with 2 mL *n*-hexane and finally eluted with 8 mL *n*-hexane/DCM (1:1, v:v) to obtain the PBDEs solution. The eluent was blown to dryness by gentle nitrogen flow. The concentrated solution was diluted by *n*-hexane to a constant volume of 1 mL and then transferred to a vessel for detection.

2.5. Instrumental analysis of BDE209

PBDEs were analyzed using gas chromatography coupled with mass spectrometry (GC/MS, Agilent, USA). The GC is an Agilent 7890A with a 7693 autosampler, and the MSD mass spectrometer is