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Species-dependent toxicity, accumulation, and subcellular partitioning of cadmium in combination with tetrabromobisphenol A in earthworms

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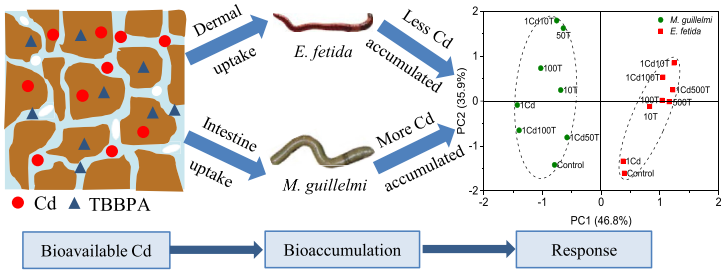
HIGHLIGHTS

- *M. guillelmi* showed higher sensitivity than *E. fetida* to Cd and TBBPA co-exposure.
- The two earthworm species differed in the pattern of oxidative stress responses.
- TBBPA addition inhibited Cd uptake in *E. fetida* due to Cd bioavailability decrease.
- More Cd accumulated in *M. guillelmi* in the presence of TBBPA.
- The high dose of TBBPA altered Cd subcellular partitioning in earthworms.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd) and tetrabromobisphenol A (TBBPA) are two ubiquitous pollutants in soils and are often found together at electronic waste recycling sites. In this study, their toxicity as well as the accumulation and subcellular partitioning of Cd were determined in two ecologically different earthworms *Eisenia fetida* and *Metaphire guillelmi* exposed for 14 days to Cd (1 mg kg^{-1}) and TBBPA (10, 50, 100, and 500 mg kg^{-1}) alone and in combination. In general, Cd-TBBPA co-exposure resulted in synergistic effects in terms of acute toxicity, growth inhibition, histopathological changes in body walls, and oxidative stress responses to earthworms, moreover, *M. guillelmi* showed higher sensitivity than *E. fetida*. Principal component analysis showed that the two earthworm species differed in their biomarker expression patterns. In addition, Cd accumulation was significantly ($P < 0.01$) reduced in *E. fetida* co-exposed to TBBPA but significantly ($P < 0.05$ and 0.01) enhanced in *M. guillelmi*. The difference in bioaccumulation between the two earthworm species may be made by their different exposure routes despite the decrease of Cd bioavailability (assessed by the diffusive gradients in thin films technique) in the soils. High doses of TBBPA also altered the subcellular distribution of Cd in the earthworms. These findings

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demonstrate the need to include more ecologically relevant earthworm species, represented in this study by *M. guillelmi*, in soil risk assessments of Cd and TBBPA co-exposure.

1. Introduction

Environmentally polluted sites are generally contaminated by multiple pollutants which different physicochemical properties and toxic mechanisms interact to elicit potentially complicated toxic effects on the biota. However, most risk assessments have focused on single-pollutant exposure, ignoring the effect of additional exposures and thus potentially underestimating the environmental risk (Barata et al., 2006; Chen et al., 2015). The few studies thus far have concentrated on the co-toxicity of organic pollutants, especially pesticides and polycyclic aromatic hydrocarbons, and metals to terrestrial organisms (van der Geest et al., 2000; Zhu et al., 2006; Lister et al., 2011; Wang et al., 2012, 2015a; Zhou et al., 2013; Lu et al., 2014; Chen et al., 2015). Moreover, most combined toxic effects are not simple addition of individual toxicity, but synergistic or antagonistic (Gauthier et al., 2014).

Electronic waste (e-waste) has become one of the fastest-growing waste fractions, constituting 8% of the municipal waste as early as 2005 (Ramesh Babu et al., 2007). As a heterogeneous mixture of metals, plastics, glass, and ceramics, e-waste contains considerable amounts of hazardous matter, including brominated flame retardants (BFRs) and heavy metals. An analysis of the soil of an informal e-waste dismantling site in China showed that cadmium (Cd) was one of the primary metal contaminants, with a mean concentration of 6.33 mg kg^{-1} (Wang et al., 2015b). The amounts reported in three major e-waste disposal and recycling locations in China were similar (Zhang et al., 2012). Tetrabromobisphenol A (TBBPA) is the predominant BFR in e-waste (Morf et al., 2005), and high levels of TBBPA, in the range of ppb–ppm, have thus been detected in soils (Wang et al., 2015b; Liu et al., 2016), commonly together with Cd at e-waste-contaminated sites. While TBBPA seems to be of very low acute toxicity, it can induce oxidative stress responses in the earthworm *Eisenia fetida* at relatively low exposure doses (Xue et al., 2009a, 2009b; Shi et al., 2015). Nonetheless, the toxicity of TBBPA to *E. fetida* is greater than that of other two major BFRs, hexabromocyclododecane (HBCD) and decabrominated diphenyl ether (BDE209) (Shi et al., 2015). In addition, while heavy metals have been intensively studied with respect to their toxicity to earthworms (Bernard et al., 2015), there is little knowledge about their co-toxicity with BFRs. In *E. fetida*, the combination of BDE209 and lead (Pb) induced more serious oxidative stress than did either pollutant alone (Liu et al., 2015; Zhang et al., 2015a, 2015b; Hu et al., 2016) and inhibited growth and reproduction, but exhibited an antagonistic effect on DNA damage (Li et al., 2015). A histological examination revealed a damaged epidermis in *E. fetida* exposed to both BDE209 and Pb, resulting in their enhanced uptake (Zhang et al., 2014a, 2015a). In the case of Cd, the above studies demonstrate the importance of determining its biological effects in the presence of TBBPA.

While epigeic *E. fetida* is the standard earthworm species recommended by the OECD for toxicity and bioaccumulation studies, it is not a typical soil species (OECD, 1984, 2004; 2010). In fact, it has a much greater tolerance to many pollutants than is the case with other, more ecologically relevant species (Pelosi et al., 2013; Qiu et al., 2013, 2014; Velki and Hackenberger, 2013). In previous studies, we showed that the anecic *Metaphire guillelmi*, a true soil-inhabiting species, was more sensitive than *E. fetida* to single Cd and TBBPA exposures in soils, and attributed this difference to the species-dependent internal behaviors of the pollutants (Chen et al., 2017a, 2017b). Other studies also indicated that these two earthworm species differed in their accumulation, transformation and elimination of HBCD and atrazine (Wang et al., 2014; Li et al., 2016).

In this study, *E. fetida* as the standard tested species and *M. guillelmi* with greatly different ecophysiological traits from the former were used as the target organisms. We examined the biological effects in the earthworms exposed to TBBPA and Cd alone and in combination. Specifically, we evaluated (1) the lethality of the pollutants to the earthworms and the resulting biomass change; (2) the histopathological responses of the earthworms; (3) the changes in oxidative stress biomarkers, including reactive oxygen species (ROS), super oxidative dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione (GSH), and malonaldehyde (MDA); and (4) the accumulation and subcellular distribution of Cd in the earthworms. The results will contribute to the development of methods to accurately assess the soil risk of combined pollution such as TBBPA and Cd. They will also provide insights into the co-toxicity mechanism of TBBPA and Cd in earthworms.

2. Materials and methods

2.1. Soil and earthworm

The tested soil was sampled from the surface layer (0–20 cm) of a paddy rice field in Wujin, Changzhou, China and consisted of clay loam with 34.8% clay, 43.5% silt, and 21.7% sand. The pH was 7.72 (0.01 mol L^{-1} $CaCl_2$), the organic carbon content 0.30%, and the cation exchange capacity 25.8 cmol (+) kg^{-1}. Before its use, the soil was air-dried, gently ground, and sieved to 2 mm.

E. fetida and *M. guillelmi* were purchased from an earthworm breeding farm in Jurong, Zhenjiang, China. The earthworms were acclimated in non-spiked soil at 20 °C for at least 2 weeks before adults (with clitellum) with a fresh weight of 0.45–0.55 g $worm^{-1}$ (*E. fetida*) and 2.50–3.00 g $worm^{-1}$ (*M. guillelmi*) were depurated for 24 h in the dark on moist filter paper prior to the experiments.

2.2. Preparation of spiked soils and earthworm exposure

Soil containing 1 mg Cd kg^{-1} (referred to hereafter as 1Cd) was prepared by spiking an aqueous solution of $CdCl_2$ (99.99% purity, Sigma-Aldrich). The ultrapure water was added to obtain 40% of the maximum water holding capacity (MWHC). The mixture was aged for 30 days at 20 °C to achieve the equilibrium of Cd in the soil, and then prepared as the raw soil. For the co-contamination of Cd and TBBPA, the same volume of an acetone solution of TBBPA (>97% purity, Sigma-Aldrich) was thoroughly mixed into the Cd-spiked soil to obtain TBBPA concentrations of 10, 50, 100, and 500 mg kg^{-1} (abbreviated to 1Cd10T, 1Cd50T, 1Cd100T, and 1Cd500T, respectively). Meanwhile, for the single TBBPA pollution, the raw soil was amended with TBBPA as above, including the treatments of 10T, 50T, 100T and 500T. The solvent was evaporated overnight in a fume hood and the soils were moistened to 40% MWHC. Additionally, a control without Cd and TBBPA was designed. Each treatment was prepared in triplicate.