

# Use of a Dual-Labeled Bioaccumulation Method to Quantify Microplastic Vector Effects for Hydrophobic Organic Contaminants in Soil

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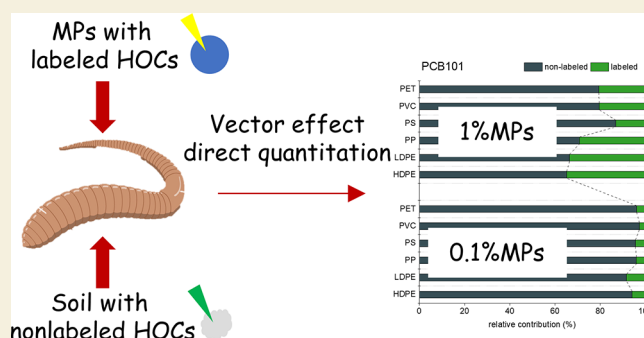
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**ABSTRACT:** Although *in vitro* simulation and *in vivo* feeding experiments are commonly used to evaluate the carrier role of microplastics in the bioaccumulation of toxic chemicals, there is no direct method for quantitatively determining their vector effect. In this study, we propose a dual-labeled method based on spiking unlabeled hydrophobic organic contaminants (HOCs) into soils and spiking their respective isotope-labeled reference compounds into microplastic particles. The bioaccumulation of the unlabeled and isotope-labeled HOCs in *Eisenia fetida* earthworms was compared. Earthworms can assimilate both unlabeled and isotope-labeled HOCs via three routes: dermal uptake, soil ingestion, and microplastic ingestion. After 28 days of exposure, the relative fractions of bioaccumulated isotope-labeled HOCs in the soil treated with 1% microplastics ranged from 15.5 to 55.8%, which were 2.9–47.6 times higher than those in the soils treated with 0.1% microplastics. Polyethylene microplastics were observed to have higher relative fractions of bioaccumulated isotope-labeled HOCs, potentially because of their surface hydrophobicity and amorphous rubbery state. The general linear models suggested that the vector effects were mainly due to the microplastic concentration, followed by polymer properties and HOC hydrophobicity. This proposed method and the derived empirical formula contribute to a more comprehensive understanding of the vector effects of microplastics for HOC bioaccumulation.

**KEYWORDS:** microplastics, earthworm bioaccumulation, isotope-labeled, vector effects, dual tracer, hydrophobic organic contaminants



## INTRODUCTION

Worldwide, approximately 360 million tonnes of plastic are produced annually.<sup>1</sup> Despite its remarkable benefits to human lives, awareness about its negative environmental impacts has increased because of the vast amount of plastic wastes. It has been estimated that the accumulation of plastic waste in landfills and/or in the natural environment would reach near 12,000 million tonnes globally by 2050.<sup>2</sup> Plastic wastes in the environment can progressively fragment into microplastics (<5 mm). Because of their small particle size, large specific surface areas, and inherent hydrophobicity, microplastics have been shown to sorb various hydrophobic organic contaminants (HOCs).<sup>3–5</sup> They can also contain up to several percent of multiple deliberate additives, such as plasticizers and flame retardants.<sup>6–9</sup> HOCs can be assimilated by organisms once microplastics are ingested as most of them are reversibly bound to the microplastics. This hypothesis, namely the “vector effect” of microplastics, has raised crucial concerns in the field of microplastic contamination.<sup>10–12</sup>

Experiments under simulated environmental conditions have frequently been used to study the vector effects of microplastics. Pioneering *in vitro* studies, which mimicked the gastrointestinal systems of aquatic or terrestrial fauna, have demonstrated that

leaching microplastic-associated HOCs under simulated gut conditions is faster than leaching them in water.<sup>13–16</sup> Nevertheless, these *in vitro* studies only used simple surfactant solutions (e.g., sodium taurocholate), which could not fully mimic the organisms’ gut fluids. Compared to *in vitro* simulations, *in vivo* feeding experiments with aquatic or terrestrial faunas may be better for representing realistic exposure scenarios. Thus far, there is still a debate over whether or not microplastics can serve as a measurable vector for HOC bioaccumulation.<sup>17–21</sup> For example, Besseling et al. studied the effects of polyethylene (PE) microplastics in sediment on PCB uptake by lugworm *Arenicola marina* under environmentally realistic conditions and reported that PE did not act as a measurable vector of PCBs.<sup>17</sup> Conversely, Wang et al. estimated the accumulation of PCBs and PAHs via pre-contaminated

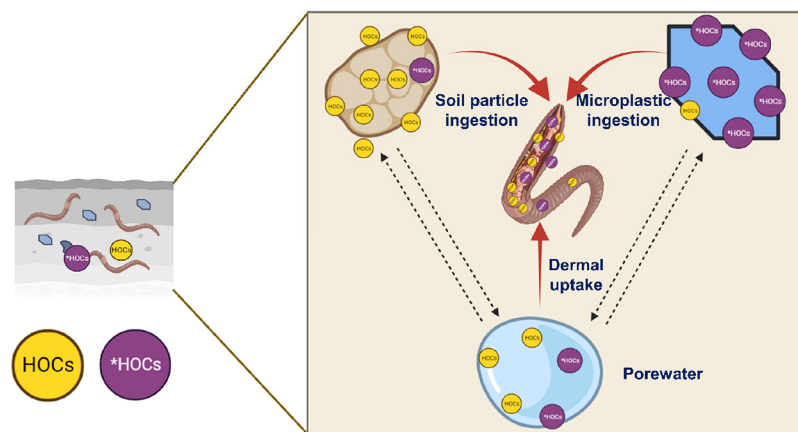
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**Figure 1.** Schematic drawing of HOC and \*HOC accumulation in earthworms.

microplastics in earthworm *Eisenia fetida* and found that microplastic ingestion could be a significant pathway for chemical uptake, likely due to HOCs on the microplastics being in an “over-equilibrium” state.<sup>18</sup> The intense debate surrounding vector effects may be because in vivo feeding studies only partially simulated exposure scenarios by employing only contaminated foods with clean microplastics or clean substances with pre-contaminated microplastics.<sup>19–21</sup> Additionally, the abovementioned studies found it difficult to experimentally determine the relative importance of food versus microplastics for HOC bioaccumulation. Furthermore, these studies may not have represented an environmentally realistic scenario because the food and microplastics are in the same environment and would contain the same contaminants. An organism can simultaneously assimilate certain HOCs from contaminated food and microplastics. Therefore, identifying the same HOC that originates from food or microplastics is pivotal for directly estimating the vector effects of microplastics.

Since stable isotope-labeled analogues and their unlabeled counterparts have the same environmental characteristics, including sorption, desorption, and bioaccumulation,<sup>22</sup> directly estimating that distinguishing between microplastics and food should be easy if the two contain labeled and unlabeled analogues, respectively. Thus, a dual tracer design was used in this study, wherein the microplastics were dosed with isotope-labeled HOCs (\*HOC) and the soil was dosed with unlabeled HOCs. The accumulation of the \*HOCs and unlabeled HOCs (hereinafter referred to as HOCs) by *E. fetida* earthworms in soil was characterized after introducing microplastics composed of different polymer types. This dual tracer design would enable the simultaneous determination of HOC uptake from soil and food as well as a direct comparison of the vector effects of different polymer microplastics.

## MATERIALS AND METHODS

### Plastics, Chemicals, and Other Materials

In this study, high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) particles were used because of their widespread usage and frequent detection.<sup>23–25</sup> Six HOCs, namely, three polychlorinated biphenyls (PCB52, PCB70, and PCB101) and three polyaromatic hydrocarbons, (phenanthrene (PHE), pyrene (PYR), and benzo[ $\alpha$ ]pyrene (BaP)), were used as the model HOCs. Their respective deuterated analogues, PCB52- $d_3$ , PCB70- $d_3$ , PCB101- $d_3$ , PHE- $d_{10}$ , PYR- $d_{10}$ , and BaP- $d_{12}$ , were used for the dual dosing as the \*HOCs. Details about the sources of

microplastics and chemicals are provided in the [Supporting Information \(SI\)](#). Agricultural soil (organic matter content: 1.61%) was collected from the Shangzhuang Agricultural Experimental Field at China Agricultural University. The soil was air-dried at room temperature for 3 weeks, sieved through a 2 mm mesh, and homogenized. The sand, silt, and clay contents were 25.6, 56.1, and 18.3%, respectively. The concentrations of the six HOCs in the soil were measured (1.5–2.3  $\mu\text{g}/\text{kg}$ ), which were negligible in comparison with the spiked concentrations. Adult *E. fetida* earthworms (0.3–0.5 g) were purchased from the TIANJIN JIALIMING Earthworm Farm (Tianjin, China). The earthworms were acclimated under laboratory conditions for 2 weeks.

### Spiking of the \*HOCs and HOCs

The microplastic particles were spiked with \*HOCs via a long-term sorption experiment in deionized water. Moreover, the soil was spiked with HOCs according to the US Environmental Protection Agency guidelines.<sup>28</sup> Briefly, 10 g of microplastic particles was placed in a 500 mL glass jar, and 200 mL of sterilized deionized water and 200  $\mu\text{L}$  of the \*HOC stock solution were added. The jar was horizontally shaken at 120 rpm under laboratory conditions. After 30 days of mixing, the microplastic particles were collected by filtration, rinsed with deionized water, freeze-dried, and stored at 4  $^{\circ}\text{C}$  before use. The unlabeled HOCs were spiked into the soil. Briefly, 20 g of silica sand was added into a 2.0 L glass jar, and the stock solution containing a mixture of non-labeled HOCs was then added. After the solvent drying, 1.0 kg of dry soil was added. The glass jar was homogenized on a rotator mixer for 2 weeks under laboratory conditions. The total concentrations of the \*HOCs and HOCs were measured after exhaustive solvent extraction and are given in [Tables S1 and S2](#). The experimental soil was prepared by homogenizing the dosed microplastics and soil for 4 h. Six polymer types (HDPE, LDPE, PP, PS, PVC, and PET) were used in two concentrations (0.1 and 1% w/w). Previous studies have indicated that these concentrations could be considered environmentally relevant for soils subject to intense human activities and could facilitate the quantitative evaluation of HOC transfer.<sup>26,27</sup>

### Earthworm Accumulation Experiments

The guts of adult *E. fetida* earthworms of similar sizes were emptied using previously reported methods.<sup>28,29</sup> Deionized water was added to the experimental soil to maintain its moisture at approximately 20%. Five earthworms were exposed to 200 g of each experimental soil (dry weight) under laboratory conditions for 28 days. Each treatment was performed three times. Earthworms incubated to uncontaminated soil without microplastics were used as the control. After exposure, the earthworms were retrieved, rinsed with deionized water, and placed on damp filter paper for 48 h to void their gut contents. These worms were then freeze-dried and pulverized with liquid nitrogen. The \*HOC and HOC concentrations in the earthworms were measured using GCMS (QP2020 NX, Shimadzu, Japan), and the details can be found in the [SI](#).

## Measurement of the Freely Dissolved Concentration in Soil Porewater

The diffusive mass transfer process, i.e., uptake into organisms, is controlled by the freely dissolved concentration ( $C_{\text{free}}$ ) of the contaminants. The  $C_{\text{free}}$  of the \*HOCs and HOCs in the soil was measured using a previously developed passive sampling method involving POM films,<sup>30</sup> and the details are provided in the SI.

## Biodynamic Modeling of Chemical Accumulation

In the experimental soil, HOCs can be desorbed from soil particles into soil porewater, and a fraction of the desorbed HOCs may be subsequently sorbed by microplastics. Likewise, the \*HOCs can be leached from microplastic particles, and a proportion would be sorbed onto soil particles. Therefore, both HOCs and \*HOCs are assimilated by earthworms via three general routes: dermal uptake via soil porewater, soil-particle ingestion, and microplastic particle ingestion (Figure 1). Thus, a modified biodynamic model was used to evaluate the relative contribution of the three exposure routes to the overall HOC and \*HOC accumulation in earthworms.<sup>17,18</sup>

For HOC bioaccumulation:

$$\frac{dC_{B,t}}{dt} = k_{\text{derm}} \times C_{\text{free}} + IR \times (S_{\text{soil}} \times \alpha_{\text{soil}} \times C_{\text{soil}} + S_{\text{PL}} \times C_{\text{PLR,t}}) - k_{\text{loss}} \times C_{B,t} \quad (1)$$

For \*HOC bioaccumulation:

$$\frac{d^*C_{B,t}}{dt} = k_{\text{derm}} \times ^*C_{\text{free}} + IR \times (S_{\text{soil}} \times \alpha_{\text{soil}} \times ^*C_{\text{soil}} + S_{\text{PL}} \times ^*C_{\text{PLR,t}}) - k_{\text{loss}} \times ^*C_{B,t} \quad (2)$$

where  $dC_{B,t}/dt$  and  $d^*C_{B,t}/dt$  ( $\mu\text{g} \times \text{g}^{-1} \times \text{day}^{-1}$ ) are the bioaccumulation rate of HOCs and \*HOCs in the earthworm, respectively. The term  $k_{\text{derm}} \times C_{\text{free}}$  or  $k_{\text{derm}} \times ^*C_{\text{free}}$  is the uptake via dermal sorption, where  $k_{\text{derm}}$  ( $\text{L} \times \mu\text{g} \times \text{d}^{-1}$ ) is the dermal uptake rate constant, and  $C_{\text{free}}$  or  $^*C_{\text{free}}$  is the chemical concentration in soil porewater ( $\mu\text{g}/\text{L}$ ). The same  $k_{\text{derm}}$  was used for the certain HOC and its deuterated analogue.  $IR \times (S_{\text{soil}} \times \alpha_{\text{soil}} \times C_{\text{soil}} + S_{\text{PL}} \times C_{\text{PLR,t}})$  is the HOC accumulation through ingested soil and microplastics, where  $C_{\text{soil}}$  is the HOC concentration sorbed to the soil particles and can be approximated with the dosed HOC concentration in soil, and  $C_{\text{PLR,t}}$  is the transferred HOC concentration from microplastics during gut passage at time  $t$ . Similarly,  $IR \times (S_{\text{soil}} \times \alpha_{\text{soil}} \times ^*C_{\text{soil}} + S_{\text{PL}} \times ^*C_{\text{PLR,t}})$  represents the \*HOC accumulation via particle ingestion, where  $^*C_{\text{soil}}$  is estimated based on the partitioning coefficients between the porewater and soil, and  $^*C_{\text{PLR,t}}$  is approximated with the spiked \*HOC concentration on microplastics.  $IR$  is the total diet ingestion rate ( $\text{g} \times \text{g}^{-1} \times \text{day}^{-1}$ ),  $\alpha_{\text{soil}}$  is the absorption efficiency from the soil,  $S_{\text{soil}}$  is the mass fractions of soil in the food, and  $S_{\text{PL}}$  is the mass fractions of microplastic in the diet. The sum of the mass fractions of soil and microplastics in the diet is assumed to be 1. The term  $k_{\text{loss}} \times C_{B,t}$  and  $k_{\text{loss}} \times ^*C_{B,t}$  represent the overall loss of HOCs and \*HOCs due to elimination and egestion, and  $k_{\text{loss}}$  ( $\text{day}^{-1}$ ) is the loss rate constant. More details of this biodynamic model are given in SI.

## Quality Control and Data Analysis

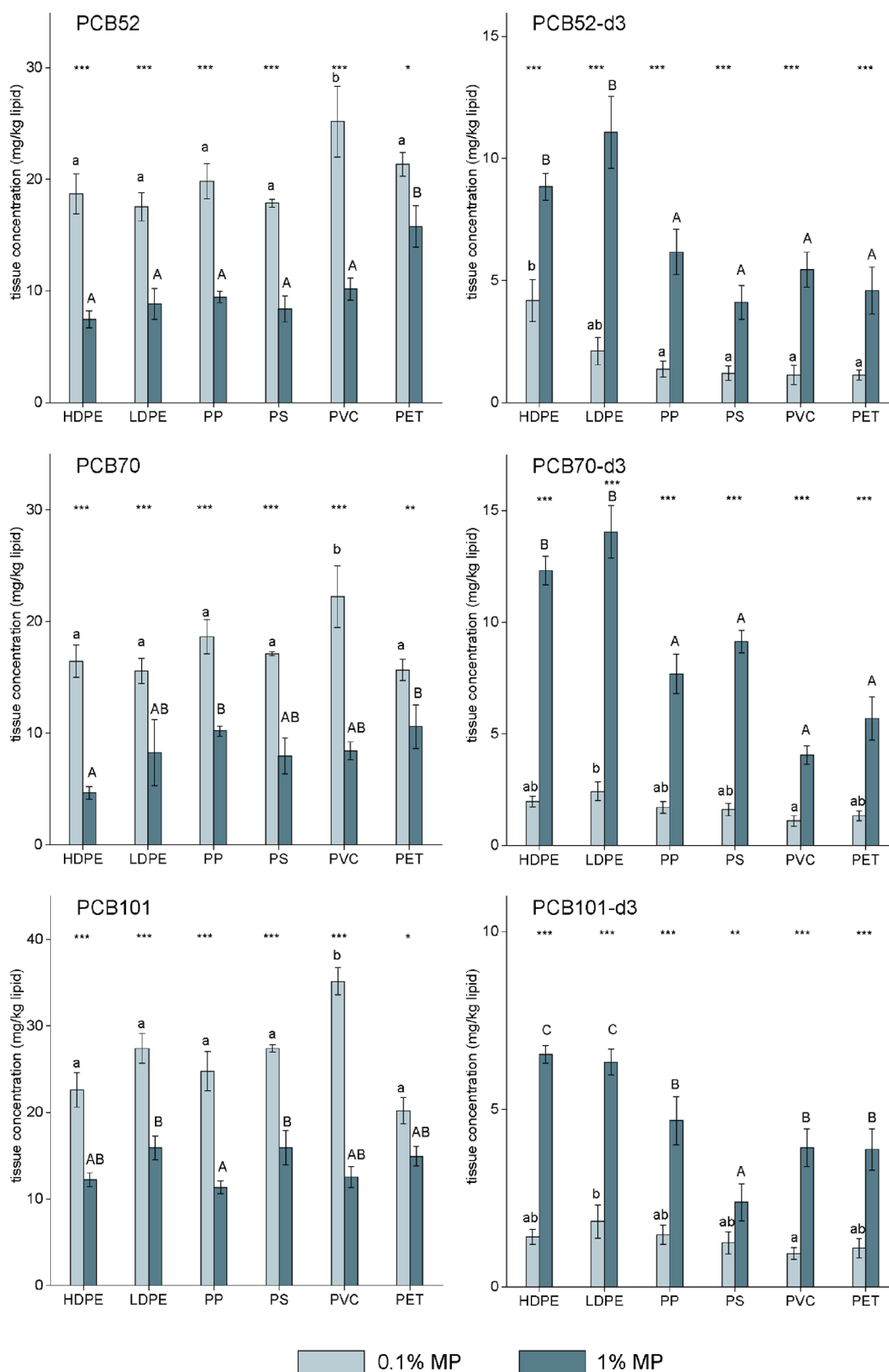
Several practices were carried out to ensure the accuracy of sample analysis. Three replicates were conducted for each measurement, and the results were expressed as means  $\pm$  standard deviation. External surrogates were used to evaluate the recovery of extraction, and internal standards were added to eliminate the potential instrument drift. Clean soil, plastics, and worms were spiked with a known amount of HOCs to measure the method recovery, ranging from  $93.5 \pm 5.7$  to  $106.1 \pm 7.6\%$ . The limits of instrumental detection of the experimental chemicals were 0.5–1 ng/mL. A nine-point calibration curve was prepared from 1 to 1000 ng/mL with a regression coefficient of  $\geq 0.99$ . Statistical differences were evaluated with one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test within SPSS 19.0 (SPSS, Chicago, IL, US). The general linear models were applied to evaluate the relationship between the microplastic vector effects and

chemical properties as well as polymer characteristics. The relative contribution of these properties on the observed variation in microplastic vector effects was further estimated by using R package "relaimpo" with the function "calc.relimp".

## RESULTS AND DISCUSSION

### $C_{\text{free}}$ of the HOCs and \*HOCs in Soil Porewater

The  $C_{\text{free}}$  values of the HOCs and \*HOCs were first measured to compare their potential contribution through the soil and microplastics. For most chemicals, the soil treated with 1% microplastics had lower  $C_{\text{free}}$  values than that treated with 0.1% microplastics (Table S3). For example, the  $C_{\text{free}}$  values of PCB52, PCB70, and PCB101 in the soil treated with 0.1% LDPE microplastics were 22.2, 8.9, and 5.4 ng/L, respectively, which were significantly ( $p < 0.05$ ) higher than those in the soil treated with 1% LDPE (13.8, 7.1, and 2.8 ng/L for PCB52, PCB70, and PCB101, respectively). The  $^*C_{\text{free}}$  values of the \*HOCs displayed an opposite trend, whereby the microplastics with a high content in the soil strongly desorbed the \*HOCs into the soil porewater (Table S4). These observations directly indicate that the microplastics not only served as sorbents to reduce the bioavailability of the HOCs in the surrounding environment but also served as contaminant sources for organisms to assimilate. The  $C_{\text{free}}$  values of the HOCs and \*HOCs were also varied in the soil with different polymer microplastics. The LDPE-treated soil was frequently observed to have the lowest values of  $C_{\text{free}}$  values, followed by HDPE- and PP-treated soils, potentially suggesting that polyethylene (PE) has a higher affinity for HOCs than the other polymers. Similar results were observed in previous studies. For instance, Rochman et al. investigated the long-term field sorption of organic contaminants to five types of plastic pellets and found that the sorption of PAHs and PCBs to HDPE, LDPE, and PP were consistently faster and greater than those to PET and PVC.<sup>48</sup> The static contact angles of the microplastics were measured to describe their external surface hydrophobicity and to confirm their different sorption properties.<sup>31</sup> The PE and PP polymers had high static contact angles, indicating that they both have high surface hydrophobicity (Figure S1). Additionally, at ambient temperature (approximately 25 °C), the amorphous domains in PE and PP were in a rubbery state and had relatively flexible structures, whereas those in PS, PVC, and PET were glassier and had rigid and condensed structures.<sup>32–34</sup> Previous studies have reported that the rubbery domains exhibit higher HOC sorption than the glassy domains.<sup>35–37</sup> Hence, we can conclude that the PE and PP microplastics sorbed more HOCs from the soil porewater than the other microplastics, causing the six HOCs to have relatively low  $C_{\text{free}}$  values. The free fractions of the \*HOCs were calculated by dividing the  $^*C_{\text{free}}$  over the total spiked concentration ( $C_{\text{free}}/C_{\text{plastic}}$ ) to compare the desorption capacity of the different microplastics (Figure S2). The PE microplastics consistently had higher free fractions of most \*HOCs than PS, PVC, and PET, indicating greater desorption capacity. The PS, PVC, and PET microplastics are glassy polymers with abundant condensed domains, which may restrict the \*HOC mobility within their polymer chains.<sup>37</sup> Furthermore, the three microplastics have lower molecular flexibility than PE and PP because of the heteroatom in their polymer chains.<sup>38</sup> Consequently, these properties may have contributed to the sequestration or very slow desorption of the \*HOCs, resulting in low  $C_{\text{free}}$  in the soil porewater.



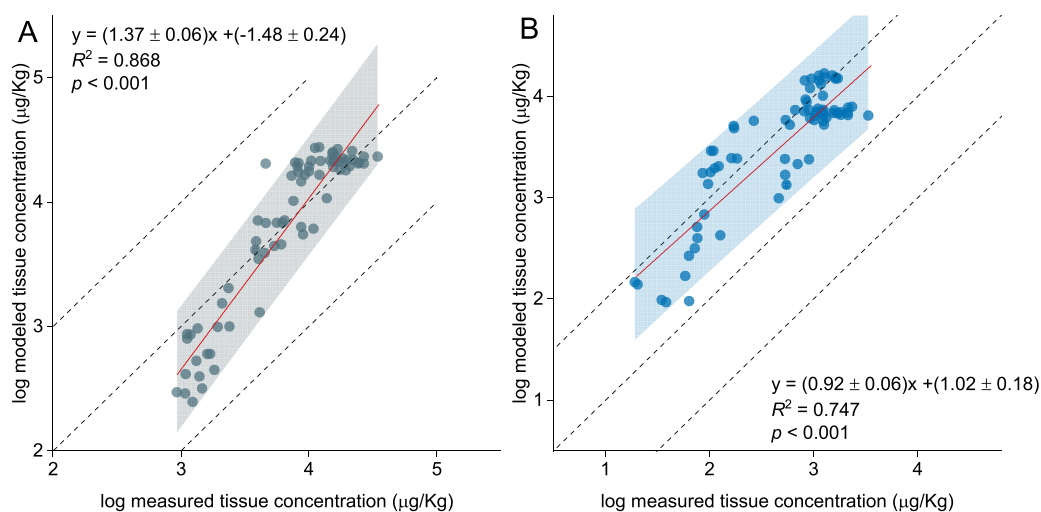
**Figure 2.** Accumulation of PCBs and \*PCBs in earthworms in different microplastic treatments ( $n = 3$ ). Capital letters and lowercase letters respectively designate significant differences at the concentrations of 1 and 0.1% between different microplastic treatments. Asterisks indicate significant differences between the same microplastic treatments with different concentrations ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ).

#### Accumulation of HOCs and \*HOCs in the Earthworms

The accumulation of the HOCs and \*HOCs was evaluated by deriving lipid-normalized chemical concentrations in the

earthworms. The bioaccumulation of the PCBs was significantly ( $p < 0.05$ ) higher in the soil treated with 0.1% microplastics than in the soil treated with 1% microplastics (Figure 2). For example, the PCB concentration in the earthworms ( $C_{\text{worm}}$ ) ranged from



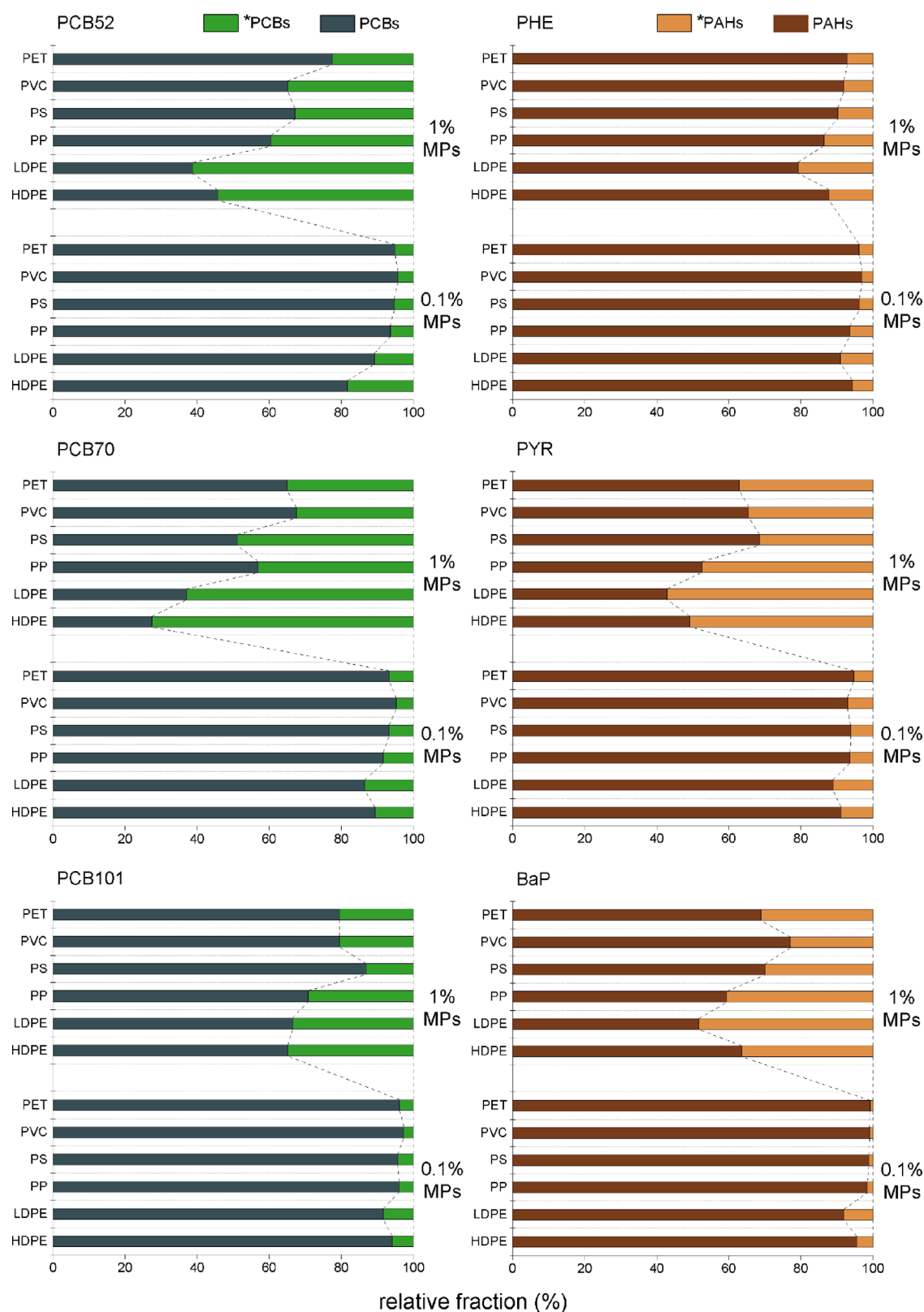


**Figure 3.** Relationship between biodynamic modeled tissue concentration and the experimentally measured tissue concentration in earthworms. (A) PCBs and \*PCBs. (B) PAHs and \*PAHs. The solid red lines represent the linear correlations, and the black dashed lines represent the 1:1 relationship and the one-order of magnitude deviation intervals.

15.6 mg/kg (PCB70 in 0.1% LDPE treatment) to 35.2 mg/kg (PCB101 in 0.1% PVC treatment) lipid in the soil with 0.1% microplastics, whereas it ranged from 4.6 (PCB70 in 1% HDPE treatment) to 15.9 (PCB52 in 1% PET treatment) mg/kg in the soil with 1% microplastics. However, the 1% microplastics induced relatively high \*PCB accumulation (Figure 2). The  $C_{\text{worm}}$  values of PCB52- $d_3$ , PCB70- $d_3$ , and PCB101- $d_3$  in the soil with 1% microplastics were 1.9–6.2 times higher than those in the soil treated with 0.1% microplastics. Distinct PCB and \*PCB accumulation were observed in the treatments with different microplastics. For instance, the soil treated with LDPE and HDPE had lower ( $p < 0.05$ ) tissue concentrations of PCBs than that treated with PVC microplastics at the concentration of 1%. However, the soil treated with PE microplastics had relatively higher \*PCB concentrations than those treated with other microplastic types. A similar pattern was also observed for the tissue concentrations of the three PAHs and \*PAHs, but a statistically significant difference was not always found (Figure S3). For example, there was no significant difference in the PHE tissue concentrations between the soils treated with 1% and 0.1% microplastics, probably because of the uncertainties introduced by the potential metabolic transformation in the organisms.<sup>39,40</sup>

A biodynamic model was subsequently run to better illustrate the chemical uptake and loss during the accumulation. The performance of the model was further evaluated by plotting the modeled tissue concentration on day 28 against the experimentally measured  $C_{\text{worm}}$  values after log transformation. There was a highly significant linear correlation ( $R^2 = 0.868$ ,  $p < 0.001$ ) between the modeled and measured tissue concentrations of the three PCBs (Figure 3). Additionally, all the scatter plots were around the 1:1 line, indicating that this biodynamic model can accurately predict PCB accumulation in earthworms. Although the modeled tissue concentrations of the three PAHs agreed very well ( $R^2 = 0.868$ ,  $p < 0.001$ ) with the measured  $C_{\text{worm}}$  values, all the relationships were above the 1:1 line (Figure 3). This observation implies that this model overestimated the bioaccumulation of PAHs in the earthworms, which may be attributed to the metabolic transformation of PAHs in earthworms.<sup>39,40</sup> The last term in the biodynamic model,  $k_{\text{loss}} \times C_{\text{B,t}}$  or  $k_{\text{loss}} \times *C_{\text{B,t}}$  represents the elimination of the chemicals

to water through feces and through growth dilution, with the elimination due to metabolism being neglected. This means that the modeled HOC tissue concentrations experienced no metabolic transformation. Hence, the modeled PCB tissue concentrations could match the experimentally measured  $C_{\text{worm}}$  values because the PCB metabolic rates are known to be negligible.<sup>41</sup> However, the experimentally measured PAH tissue concentrations were lower than the modeled  $C_{\text{worm}}$  values because substantial PAH metabolism may occur within organisms. The metabolic transformation rates of PAHs in invertebrates were further included to modify the biodynamic model.<sup>42–44</sup> There was an enhanced correlation between the measured and modeled  $C_{\text{worm}}$  values of the PAHs, with all the relationships being close to the 1:1 line (Figure S4), confirming the accurate estimation of our models. Next, the relative contributions of the three uptake routes, i.e., dermal uptake, microplastic ingestion, and soil particle ingestion, to the total HOC and \*HOC accumulation were determined. Dermal uptake and soil particle ingestion dominantly contributed to HOC and \*HOC accumulation at the 0.1% treatment rate, whereas microplastic ingestion only contributed less than 5% (Figure S5). Moreover, microplastic ingestion did not substantially contribute to the uptake of the low hydrophobic contaminants (i.e., PHE, PYR, and PCB52) at the high treatment rate of 1% (Figure S6). For instance, the relative contributions of microplastic ingestion to the bioaccumulation of unlabeled low hydrophobic chemicals were less than 1%, while the relative contributions to the bioaccumulation of isotope-labeled low hydrophobic analogues ranged from 0.05 to 3.7%. However, microplastic ingestion substantially contributed to the uptake of the high hydrophobic contaminants (i.e., BaP, PCB70, and PCB101). The relative contributions of microplastics to the bioaccumulation of labeled high hydrophobic contaminants can be up to 87.8% (\*PCB101 in PP treatment). Additionally, microplastic ingestion consistently contributed more to \*HOC assimilation than to HOC uptake (Figure S5). As previously suggested,<sup>17</sup> the \*HOCs on the microplastics were in an “over-equilibrium” state (the desorption of chemicals from microplastics into the gut fluid is greater than the sorption from the solution to the particles), and the fugacity gradient between the ingested plastics and the worm gut systems caused the



**Figure 4.** Values of relative fractions of isotope-labeled contaminants under different microplastic treatments.

outstanding contribution of microplastic ingestion to the overall bioaccumulation. However, the HOCs were desorbed from the soil particles into the porewater, and some of the desorbed HOCs were sorbed by the microplastics. These HOCs on the microplastics were in an “under-equilibrium” state (HOCs are in

the process of rate-limited sorption to the microplastics) and made a minor contribution to the total accumulation. Additionally, as a soil-feeding organism, earthworms live in direct contact with soil porewater and particles, and they accumulate HOCs simultaneously through their intestine and skin via

alimentary and dermal uptake. In the microplastic-contaminated soil, earthworms ingest the plastic particles along with the soil particles. Hence, the behaviors of the chemicals from microplastics should be similar with those from soil (Figure 1). The chemical transfer among soil, microplastics, and porewater would be the critical factor influencing the bioaccumulation. If the microplastics stay in the soil for a long time (e.g., close to equilibrium), the assimilation of the chemicals from the microplastics and soil would be different in comparison to the current study.

### Vector Effects of Microplastics

To compare the vector effect of the different microplastics, the  $*C_{\text{worm}}$  fractions in the total tissue concentrations ( $*C_{\text{worm}} / (*C_{\text{worm}} + C_{\text{worm}})$ ) were directly calculated (Figure S7). The  $*C_{\text{worm}}$  fractions ranged from 0.8 to 18.3% in the soil treated with 0.1% microplastics, and the values increased to 13.0–72.5% in the soil treated with 1% microplastics. The  $*\text{HOC}$  accumulation fractions were significantly higher ( $p < 0.05$ ) in the soil treated HDPE and LDPE. For example, the  $*C_{\text{worm}} / (*C_{\text{worm}} + C_{\text{worm}})$  values of PCB52- $d_3$  in the soil treated with 1% LDPE and HDPE microplastics were 61.2 and 54.2%, respectively, whereas the values in the soils treated with the other polymers ranged from 22.5 to 39.5%. Considering that the HOC and  $*\text{HOC}$  dosing concentrations varied in the different treatments, a direct comparison between the  $C_{\text{worm}}$  and  $*C_{\text{worm}}$  may not reveal the actual vector effects of the microplastics. Therefore, the BSAF of the HOCs, which is the ratio of the concentration in the earthworm (normalized over the lipid content) to the concentration in the soil (normalized over organic carbon), and the BSPF of the  $*\text{HOCs}$ , which is the ratio of the lipid concentration to the concentration on the microplastics, were calculated to eliminate the influence of the spiked concentrations. The vector effects of the microplastics can be obtained using the relative fractions of the  $*\text{HOCs}$ :

$$\text{relative fraction (RF)} = \frac{\text{BSPF}}{\text{BSAF} + \text{BSPF}} \times 100\% \quad (3)$$

The microplastics with a relatively high treatment rate (1%) had stronger vector effects on most HOCs (Figure 4). For example, the RF values of the  $*\text{PCBs}$  were 18.1–55.8% in the soils treated with 1% microplastics, which were 2.9–10.4 times higher than those in the soils treated with 0.1% microplastics. The other polymer microplastics had significantly weaker vector effects than the rubbery PE microplastics. The RF values of 1% HDPE and 1% LDPE for PCB52- $d_3$  were 45.9 and 52.5%, respectively, whereas the values of PP, PS, PVC, and PET only ranged from 19.7 to 32.0%. Concurrently, the vector effects varied for the same polymer and were greater for the low hydrophobic contaminants than for the other chemicals.

Since the sorption properties of microplastics and HOCs influence the vector effects, a generalized linear model was also used to quantify their relationships. The contact angles of the polymers were used to represent the microplastic hydrophobicity, and the log-transformed octanol/water partition coefficient of the HOCs was used to represent the chemical hydrophobicity ( $\log K_{\text{OW}}$ ). Both measured and modeled results were included because the experimental tests may have introduced uncertainties:

$$\begin{aligned} &\text{vector effect (measured)} \\ &= (0.3083 \pm 0.0218) \times \% \text{microplastics} \\ &\quad + (0.0026 \pm 0.0007) \times \text{CA} + (-0.0578 \pm 0.0185) \\ &\quad \times \log K_{\text{OW}} + (0.1193 \pm 0.1303) \end{aligned} \quad (4)$$

$$\begin{aligned} &\text{vector effect (modeled)} \\ &= (0.2521 \pm 0.0226) \times \% \text{microplastics} \\ &\quad + (0.0017 \pm 0.0007) \times \text{CA} + (-0.0362 \pm 0.0141) \\ &\quad \times \log K_{\text{OW}} + (0.0580 \pm 0.1075) \end{aligned} \quad (5)$$

where CA is the contact angle.

Significant fitting was obtained for both models ( $p < 0.001$ ). The regression coefficients were all significant ( $p < 0.05$ ). Both linear models demonstrated that the vector effects of the microplastics had a significantly negative correlation with the HOC hydrophobicity and a positive correlation with the polymer hydrophobicity. On the one hand, microplastic particles with a high surface hydrophobicity could carry large amounts of chemicals into organisms after ingestion, potentially resulting in strong vector effects; on the other hand, the vector effects of microplastics on the hydrophobic HOCs would be low as these chemicals may exhibit high resistance to desorption from microplastic particles. Additionally, it was evident that increasing the microplastic concentration could enhance their vector effects on HOCs, which is consistent with previous findings. The relative contribution of microplastic concentration, surface hydrophobicity, and contaminant hydrophobicity to the observed variation in the vector effect was further explored using the R package “relaimpo” (Figure S8).<sup>45</sup> All three regressors explained 79.9% of the variation in measured vector effects, and plastic concentration was the dominant cause of the variation (71.4%), followed by microplastic surface hydrophobicity (4.9%) and contaminant hydrophobicity (4.2%), confirming the importance of plastic concentration.

A limitation of this study was the environmentally unrealistic microplastic concentration used during the earthworm exposure. Although previous studies have indicated that the microplastic concentrations in soils with intense anthropogenic activities can be up to 6.7%,<sup>26</sup> most studies have revealed that the mass concentrations of microplastics may be only several hundred milligrams per kilogram of soil.<sup>46,47</sup> This condition, coupled with our models, may suggest that microplastics do not have a vector effect in realistic soil environments.

Microplastics are a multifaceted contaminant group with a vast array of polymers, sizes, and morphologies. The ubiquity and broad size range of microplastics make them available to hundreds of species across food webs in aquatic and terrestrial environments. Whether microplastics can transfer HOCs to organisms is central to the perceived hazard and risk of plastic waste in the environment. It is extremely difficult to accurately quantify the vector effects of microplastics under these complex scenarios to assess their risk. In this study, a dual labeled design was used to trace the chemical transfer from microplastics and soil particles, respectively (Figure 1), and an empirical formula was developed for estimating the vector effects using several simple and available parameters. The rubbery PE microplastics had relatively high vector effects, potentially suggesting high risks. This method can be easily incorporated into different microplastic-contaminated scenarios, such as evaluating the

importance of particle size to vector effects. Such evaluations may contribute to a mechanistic understanding of processes and factors regulating the vector effects of microplastics. To determine if microplastics can substantially act as HOC carriers in different environments, the quantitative models must be improved using similar designs under other scenarios (e.g., other terrestrial species, aquatic faunas, and different microplastic sizes).

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsenvironau.3c00024>.

Chemicals and microplastics; contact angles; measurement; measurement of HOCs; instrumental analysis; dynamic model for chemical accumulation; (Table S1) soil HOC concentration; (Table S2) microplastic \*HOC concentration; (Table S3)  $C_{\text{free}}$  values of HOCs; (Table S4)  $C_{\text{free}}$  values of \*HOCs; (Table S5) species-specific parameters in the model; (Table S6) chemical-specific parameters; (Figure S1) contact angles; (Figure S2) free fraction values of \*HOCs; (Figure S3) PAH and \*PAH accumulation; (Figure S4) correlation; (Figure S5) relative contribution of different pathways; (Figure S6) relative contribution of different pathways; (Figure S7) values of  $*C_{\text{worm}}/(*C_{\text{worm}} + C_{\text{worm}})$ ; (Figure S8) contribution of different properties on the variance of vector effects (PDF)

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## Notes

The authors declare no competing financial interest.

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