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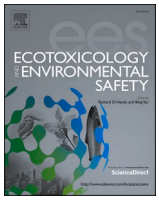

Organic matter dependent toxicokinetic of lindane in soil: Insights from enchytraeid bioaccumulation studies

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ABSTRACT

The bioavailability and toxicity of a given chemical can vary considerably depending on specific soil properties, especially the organic matter (OM) content. These properties determine the partition of organic chemicals between soil particles and porewater, and hence, its bioaccumulation potential in soft bodied soil invertebrates. LUFA 2.2 soil (3 % OM) better represents European arable soils than the standard OECD soil (10 % OM), commonly used in regulatory studies. This discrepancy, may lead to an underestimation of bioaccumulation and toxicity in natural environments in regulation assessments. The current study, therefore, investigates how soil organic matter (OM) content affects the bioaccumulation of lindane in *Enchytraeus crypticus*. A two-week toxicokinetic experiment was conducted using LUFA 2.2 soil (2.85 % OM) and an amended LUFA 2.2 soil (10 % OM). Results showed that kinetic bioaccumulation factors (BAF_k) were twice as high in LUFA 2.2 soil compared to amended LUFA 2.2 soil, mainly due to differences in uptake rate constants. Elimination rate constants remained similar between soil types, suggesting that OM content primarily affects uptake processes. The study also compared findings with literature data on enchytraeids and earthworms, revealing higher uptake rate constants and BAF_k in enchytraeids. The inverse correlation between OM content and BAF_k was consistent across studies, highlighting the importance of soil composition in influencing bioaccumulation. These findings highlight how soil properties influence the bioavailability and potential toxicity of persistent, lipophilic chemicals like lindane, emphasizing the need for risk assessment practices to use soils with representative OM levels for more accurate environmental toxicity predictions.

1. Introduction

Chemical bioavailability in soil is a complex phenomenon influenced by various soil properties, including organic matter (OM) content, pH, and soil texture (Kuperman et al., 2013; van Hall et al., 2023). These characteristics play a crucial role in determining the chemical partition between soil particles and pore water (Lanno et al., 2004). Consequently, the bioavailability and toxicity of a given chemical can vary significantly depending on the specific properties of the test soil, even for the same chemical and test species (van Hall et al., 2023; Šmídová et al., 2021).

Researchers emphasize the importance of incorporating bioaccumulation studies into chemical risk assessments to better understand bioavailability (Lanno et al., 2004; Amorim et al., 2002). Such studies are crucial for identifying persistent, bioaccumulative, and toxic (PBT) chemicals, which can significantly impact ecosystems and human health, and their identification is crucial for regulatory actions (Hoke et al., 2016). Additionally, bioaccumulation studies provide insight into toxicokinetics, as the concentrations of chemicals in bulk soil often differ from their bioavailable concentrations. This discrepancy indicates that chemical uptake and toxicity may not always correlate directly to bulk soil concentrations (Lanno et al., 2004; van Hall et al., 2024). By

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measuring both internal (organism) and external (medium) chemical concentrations, accumulation studies offer valuable insights into uptake and elimination kinetics in different soil types (Amorim et al., 2002; Šmídová and Hofman, 2014).

Enchytraeids have been extensively used in laboratory studies to assess acute and chronic effects of organic chemicals (Roembke et al., 2017). However, few studies have investigated the bioaccumulation potential of lindane by describing the uptake and elimination rate constants (Amorim et al., 2002; Bruns et al., 2001). These studies reported contradictory results regarding the relationship between soil OM content and bioaccumulation, with no consistent evidence supporting a decrease in bioaccumulation potential as soil OM increases. This inconsistency underscores the lack of mechanistic understanding of how soil OM content specifically affects the uptake and elimination rate constants of lipophilic chemicals like lindane in soft-bodied soil invertebrates. Furthermore, lindane (γ-hexachlorocyclohexane; γ-HCH), a broad-spectrum neurotoxic organochlorine insecticide, is classified as a persistent organic pollutant by the Stockholm Convention and despite being banned in Europe in 2001, it can still be detected in European ecosystems (McKnight et al., 2015; Rasmussen et al., 2015). The persistence of lindane in soil (degradation time around 148 days), combined with its ability to volatilize and undergo long-range transport, has led to its ubiquitous presence in various environmental compartments globally (Dorsey et al., 2005). Moreover, lindane's low water solubility (7.3 mg/L at 25°C) and moderate octanol-water partition coefficient (log K_{ow} = 3.72) make it prone to sorption onto soil OM and bioaccumulation in organisms (Dorsey et al., 2005). Nevertheless, our knowledge of how soil OM content influences lindane's kinetic rate constants remain limited, highlighting a significant gap in our understanding of this complex process.

Soil organic matter (OM; approximately related to soil organic carbon (SOC) by OM $\approx 1.724 \times$ SOC) is a crucial factor driving the bioavailability of organic chemicals. In Europe, regulatory toxicity studies typically use OECD artificial soil with a 10 % OM (OECD, 1984). However, this has been criticized for not representing natural conditions (van Hall et al., 2023), because mineral arable topsoils typically contain much less carbon. In a national survey of SOC levels in the UK, 50 % of soil had < 5 % SOC (Emmett et al., 2010). Likewise, across the EU-28, cropland topsoils had a median SOC of 14.3 g kg^{-1} (≈1.43 % SOC; ≈2.47 % OM) and a mean of 17.6 g kg^{-1} (≈1.76 % SOC; ≈3.03 % OM) (Jones et al., 2020). As a more representative alternative, LUFA 2.2 (≈3 % OM) aligns better with typical European arable topsoils, which generally have < 5 % OM (Chelinho et al., 2014; Lehmann and Kleber, 2015). Some studies have reported a decline in soil OM content related to agricultural management practices and climate change across European croplands and grasslands, increasing the potential for bioaccumulation and toxicity of organic chemicals to non-target organisms like enchytraeids (van Hall et al., 2023; Bellamy et al., 2005; Capriel, 2013; Saby et al., 2008). Given this discrepancy between OECD artificial soil and natural European soils, LUFA 2.2 soil with a lower OM content offers a more realistic alternative for evaluating bioavailability and toxicity under laboratory conditions.

The behavior of lindane in different soil types is particularly relevant to this study. In sandy soils with low organic matter, lindane may be more mobile and bioavailable. In contrast, in clay-rich organic soils, it may be more tightly bound and less bioavailable, but potentially more persistent (Singh et al., 2013; World Health and International Programme on Chemical, 1991). The use of standardized soils allows for comparison across different laboratories, reducing uncertainty in toxicity assessments. However, the higher OM content in the recommended OECD artificial soil could lead to an underestimation of bioaccumulation and toxic effects compared to those which occur in many natural soils, especially those in arable habitats that have lower OM content. This discrepancy is particularly significant for lipophilic chemicals like lindane, which tend to strongly sorb to OM due to their low water solubility and consequent greater potential to interact with lipid components in OM (Dorsey et al., 2005; EFSA-PPRPanel, 2017). Understanding these dynamics is crucial for accurately assessing environmental risks and makes lindane an excellent model compound for studying the effects of soil properties on bioaccumulation.

The current study aims to investigate the differential impact of soil OM content on uptake and elimination rate constants of lindane in enchytraeids. We hypothesize that soil OM content affects the uptake rate constant rather than the elimination rate constant. This approach seeks to provide a more mechanistic understanding of how OM influences bioaccumulation processes, extending beyond previous work that has primarily focused on overall bioaccumulation factors. The methodology involves a two-week toxicokinetic in which enchytraeids were exposed to lindane in two different soils: LUFA 2.2 soil with a 2.85 % OM content and an amended LUFA 2.2 soil with 10 % OM content (hereafter refer to as 'amended LUFA soil'). The study calculates uptake and elimination rate constants, as well as kinetic bioaccumulation factors (BAF_k) for both soil types. By enhancing our understanding of these processes, we can improve risk assessment strategies and better predict the environmental impact of such chemicals across various soil conditions and the relevance of different soil types for bioaccumulation assessment.

2. Materials and methods

2.1. Test chemical

The analytical standard Lindane (gamma-hexachlorocyclohexane) (PESTANAL™; CAS: 58–89–9, purity > 98.0 %) was obtained from Sigma Aldrich (Saint Louis, MO, USA) and dissolved in acetone prior to the application in the test soil. The final solution was prepared to reach a nominal concentration of 5 $mg.kg^{-1}$ of soil dry weight.

2.2. Test organisms

Enchytraeus crypticus (Enchytraeidae; Oligochaeta; Annelida) was selected as test organisms given its relevant status in ecotoxicological studies (OECD, 2010, 2016). These enchytraeids are key decomposers in the soil food web, contributing to nutrient cycling and soil structure improvement (Jänsch et al., 2005). They are particularly abundant in the upper layers of soil, where most contaminants tend to accumulate, and are prey for many soil-dwelling predators, making them a potential route for biomagnification of contaminants (Roembke et al., 2017).

The enchytraeids originated from a laboratory culture of the Department of Ecological Science, Vrije Universiteit, Amsterdam (Netherlands) and they have been kept in culture at the UK Centre for Ecology and Hydrology, Wallingford (UK) since 2005. The average weight of an individual enchytraeid was 3.28 mg, calculated from the pooled mass of ten individuals. The experiments were conducted at a constant temperature (20 ± 1 ℃) in constant dark. The organisms were fed 2 mg oatmeal per g dry weight equivalent (dw) of soil at the start of the experiment and every week thereafter.

2.3. Soils

A natural LUFA 2.2 soil (LUFA Speyer, Germany) and an amended version of the LUFA 2.2 soil were used in this study to compare the toxicokinetics of lindane in enchytraeids in two types of soil. The first soil was a natural LUFA 2.2 soil with the following main characteristics: pH (0.01 M $CaCl_2$) of 5.93 ± 0.04 SD, organic carbon (%OC) content of 1.66 ± 0.6, organic matter (OM%) content 2.86 %, maximum water-holding capacity (WHC max) of 44.2 %, cation exchange capacity (meq/100 g) 8.5 ± 1.8, and a particle size distribution of 10.8 % clay, 15.7 % silt and 73.5 % sand. We used the van Bemmelen Conversion Factor of 0.58 (or the reverse value of 1.72) to convert organic carbon to organic matter content, assuming OM contains 58 % carbon (Pribyl, 2010).

For the amended LUFA soil, we added a fine composted dry bark from sustainable coniferous trees to increase the organic matter content of the LUFA 2.2 to 10 % of the total soil dw. The composted bark was obtained from Melcourt Industries Limited® and desiccated overnight in the oven at 90℃. The bark was ground to a fine powder, sieved mixed with LUFA 2.2 soil. The pH of the amended LUFA soil (6.38 ± 0.06 SD), an increase of 0.39 pH units compared to the unameded soil, was measured in a solution of 0.01 M $CaCl_2$ as described by Kalra and Maynard (1991).

The spiking solution was prepared in acetone to reach a nominal concentration of 5 mg lindane/kg dw soil. The solution was spiked into both soils four days before the experiment began, and the acetone was left to evaporate overnight. The soil was then checked to confirm it was completely dry, ensuring that all the solvent had evaporated. After solvent evaporation, each soil was thoroughly mixed and stored at room temperature for four days. Distilled water was added to the soil 48 h before the start of the experiment to reach 50 % of the maximum WHC. The control group was treated in the same way but without lindane to assess the potential impacts of the solvent on the fitness, and therefore, the toxicokinetic of enchytraeids.

To assess the stability of lindane in soil, a 14-day dissipation study was conducted separately in LUFA 2.2 and amended LUFA soils. Lindane concentrations after 14 days were expressed as a percentage of the initial (day 0) concentrations, representing the fraction of lindane remaining in the soil.

2.4. Experimental design

A total of 33 pots were prepared per soil (three replicates per time-point) with 60 g of dw soil per pot. An extra three replicates were also set as a control group for each soil type. A cohort of 120 individuals were introduced in each test pot with pierced lids to allow aeration, and were then weighed. The weight loss of the pots was used as a proxy for water evaporation which was replenished every 4 days. The experiment lasted 14 days, 7 days for the uptake phase and 7 days for the elimination phase. Enchytraeids were sampled at 6 h, 12 h, and 1, 3, and 7 days after the start of exposure in the uptake phase. The remaining treatment groups, along with the control group, were then rinsed with deionized water, blotted dry with filter paper, and transferred to clean (uncontaminated) soil for the elimination phase of the study. The elimination phase resembled the uptake phase, and the enchytraeids were sampled at 6 h, 12 h, 1, 3 and 7 days during the elimination phase. At each time-point, 80 enchytraeids were collected from the soil for chemical analysis, gently rinsed with distilled water, blotted dry in filter paper and transferred to 3 mL vials suitable for sample homogenization. Likewise, enchytraeids from the control group were collected at the end of the elimination phase for chemical analysis. Lastly, these vials were weighed and frozen at −20 ℃ for chemical analysis.

2.5. Quantification of lindane in soil and enchytraeids

The chemical analysis of the bulk soil samples was based on the OECD 317 guideline test (OECD, 2010). In our case, we performed three consecutive extractions followed by a washing step to ensure the maximum recovery of lindane from the soil matrix. A single washing step consisted of 10 mL of cyclohexane that was added to soil in falcon tubes and placed in a rotatory shaker for 1 h. Following this, the samples were centrifuged at 3000 rpm for 5 min, and the supernatant filtered through a 0.2 μm pore size filter into a new Falcon tube for subsequent evaporation to dryness under a constant N_2 flow. This process was repeated three times. After the third extraction, the samples were reconstituted in 1 mL of cyclohexane for subsequent analysis via GC-MS.

The chemical analysis of enchytraeids was conducted following an adapted protocol from Römer et al (Römer et al., 2024). This involved a series of two consecutive extractions. Upon thawing, one ceramic maceration ball and 1.2 mL of cyclohexane was added to each vial. Homogenization of the samples was achieved using a high-speed benchtop (MP Biomedicals™ FastPrep-24™ 5 G) at 6 m/s speed for a duration of 40 s. Subsequently, the samples were macerated for 1 h in a shaker and then centrifuged at 3000 rpm for 5 min. Following centrifugation, the samples were filtered through 0.20 μm pore size filter (CHROMAFILXtra PET-20/13, Macherey- Nagel GmbH & Co.KG, Düren, Germany) into a 4 mL glass vial for solvent evaporation. The overall process was conducted twice, and at the conclusion of each extraction step, the solvents were combined in the 4 mL glass vial for subsequent evaporation to dryness under a continuous N_2 flow. After the final extraction, the samples were reconstituted in 1 mL of cyclohexane for GC-MS analysis.

2.6. GC-MS analysis

Lindane was quantified by gas chromatography coupled to a mass spectrometer (Trace 1310 Gas Chromatograph and ISQ Mass spectrometer, both Thermo Scientific; Autosampler: PAL GC-xt, PAL System). A single ion method (SIM) was used to detect lindane in extracts of enchytraeids and soil samples. The GC was equipped with a Zebron ZB-5MS GC capillary column (15 m x 0.25 mm×0.25 μm, Phenomenex). After equilibrating the GC oven at 70°C for 0.5 min, a sample volume of 5 μl was injected. Injector temperature was 250°C and a split flow of 5 mL/min Helium with a split ratio of 1:4 was used. After injection a temperature gradient was started with an initial temperature of 70°C, which increased with 15 °C/min up till 320 °C. The ISQ Mass spectrometer was operated in Chemical Ionization mode and methane was used with a flow of 1 mL/min as reagent gas. Lindane and the internal standard butylcyclohexane were detected with a scheduled SIM method capturing *m/z* 83 and 140 Da (butylcyclohexane) from minute 2–5 and *m/z* 181 and 219 Da (lindane) after minute 5. Lindane quantification was based on peak area responses fitted to a seven-point linear calibration curve. The calibration range for soil samples was 0.15–10 mg/L, while for enchytraeids samples, it extended from 0.15 mg/L to 600 mg/L. The limit of detection (LoD) and limit of quantitation (LoQ) were both determined to be 0.15 mg/L.

2.7. TK modeling and parameter estimation

The model was calibrated by simultaneously considering both the uptake and elimination phases of the lindane concentration within the enchytraeids. For each time-point, three replicates were used with no weights. The exposure concentration was an averaged value of three replicates of lindane bulk soil concentration. Data was fitted to a one-compartment first-order TK model to simulate the internal chemical concentration in enchytraeids over time. A time-resolved numerical model was used following Eq. (1):

$$\frac{dC_{\text{int}}(t)}{dt} = k_{in} \times C_{soil}(t) - k_{out} \times C_{\text{int}}(t) \quad (1)$$

Where C_{int} is the body burden concentration of lindane ($\mu g_{\text{a.i.}}\, g_{\text{wwt}}^{-1}$), k_{in} the uptake rate constant ($g_{\text{soil}}\, g_{\text{wwt}}^{-1}\, d^{-1}$), C_{soil} is the lindane concentration in bulk soil ($mg_{\text{a.i.}}\, kg_{\text{soil\_wwt}}^{-1}$), and k_{out} the elimination rate constant (d^{-1}), assuming the concentration in enchytraeids equals 0 at the start of the experiment.

The TK model was implemented in MATLAB (version R2022b) within the Bring Your Own Model (BYOM, v6.0_beta5) platform (https://www.debtox.info/byom.html). Model calibration employed maximum likelihood estimation with a normal likelihood function to identify the optimal parameter set yielding the lowest minus log-likelihood (MLL). To determine the prediction intervals for the model fit and confidence internals (CIs) for kinetic parameters, both uptake (k_{in}) and elimination (k_{out}) rate constants were simultaneously fitted through likelihood profiling using the parameter space explorer algorithm (Jager, 2021). TK plots were generated on a logarithmic scale,

with kinetic rate constants constrained between 0.01 and 100 for the CI search grid. The parameter values within the 95 % critical value for the χ^2 distribution (one degree of freedom) were accepted in the parameter-space plot, while rejecting those exceeding the critical value for the minus log-likelihood ratio (MLLR). Parameter sets falling between the dotted horizontal lines around the critical value were utilized for uncertainty propagation to model predictions, resulting in representative prediction intervals (Jager, 2021).

The kinetic bioaccumulation factor (BAF_k) was calculated as the ratio between the uptake rate constant and the elimination rate constant, as shown in Eq. (2).

$$BAF_k = \frac{k_{in}}{k_{out}} \quad (2)$$

Where BAF_k ($g_{soil\_wwt}\ g^{-1}_{wwt}$).

2.8. Statistical analysis

The differences in mean for the internal concentration of lindane in enchytraeids from LUFA 2.2 soil and amended LUFA soil were statistically assessed according to Halsey et al. (2015). The 95 % CI of the difference in means was calculated using an alpha value of 0.05. The statistical analysis was performed using Rstudio version 4.4.0 (R Core Team, 2024).

3. Results

3.1. Soil exposure concentration

The chemical analysis revealed that the soils were homogeneously mixed (Table S1 and Table S2). The average exposure concentration in the LUFA 2.2 soil was 4.89 $mg/kg_{wwt} \pm 0.74$ SD lindane, whereas in the amended LUFA soil was 4.47 $mg/kg_{wwt} \pm 0.54$ SD (Table 1). The limited degradation of lindane during the uptake period confirms that the first-order uptake kinetics model can be applied without modifications to account for the degradation of the test chemical

The average exposure concentration in the LUFA 2.2 soil was 4.89 $mg/kg_{wwt} \pm 0.74$ SD lindane, whereas in the amended LUFA soil was 4.47 $mg/kg_{wwt} \pm 0.54$ SD (Table 1). Lindane concentrations after 14 days remained at 86 % of the initial concentration in LUFA 2.2 soil and 91 % in amended LUFA soil. The dissipation of lindane was the result of volatilization or mineralization in soil. The analytical results for samples collected on day 10 were unreliable due to technical issues during the analytical process. Consequently, these data points were excluded from further analysis to prevent potential bias in the interpretation of results.

3.2. Kinetic rate constants

Despite the substantial weight variability among enchytraeid replicates in both soils, the average sample weights were similar for LUFA 2.2 and amended LUFA soil. The average weight for the enchytraeids in LUFA 2.2 samples was 99.7 mg ± 31.9 SD, while the enchytraeids in the amended LUFA soil weighted 139 mg ± 43.1 SD.

The toxicokinetic profiles of lindane in LUFA 2.2 and amended LUFA soil are presented in Fig. 1. Both soil types exhibited a similar pattern of rapid uptake followed by elimination, but with notable differences in the magnitude of body burdens. In LUFA 2.2 soil, the internal concentration of lindane in enchytraeids increased sharply during the first 7 days, reaching a peak of 287 $\mu g_{lindane}\ g^{-1}_{worm\_wwt}$. Subsequently, elimination was observed with concentrations declining to about 125 $\mu g_{lindane}\ g^{-1}_{worm\_wwt}$ by day 14 (Table S3). The k_{in} value was 37.8 $g_{soil}\ g^{-1}_{worm\_wwt}\ d^{-1}$ (95 % CI: 17.9; 52.6) and the k_{out} was 0.57 d^{-1} (95 % CI: 0.26; 0.94) (Table 2). After 7 days of elimination, the enchytraeids were unable to eliminate lindane completely during the experimental period and about half of lindane body residues remained.

Consistent with our prior hypothesis, the maximum internal concentrations in the enchytraeids were substantially lower in the amended LUFA soil, with a maximum average peak of 117 $\mu g_{lindane}\ g^{-1}_{worm\_wwt}$ after 7 days of exposure (Table 1). The elimination phase in amended LUFA soil showed a similar trend to LUFA 2.2 soil, but with consistently lower concentrations throughout the elimination period given the lower maximum concentration achieved at the end of the uptake phase. The overall uptake in amended LUFA soil was almost four-fold that of LUFA 2.2 soil, as evidenced by the lower k_{in} of 10.9 $g_{soil}\ g^{-1}_{worm\_wwt}\ d^{-1}$ (95 % CI: 4.69; 27.8) (Table 2). Likewise, the elimination rate constant was half that of LUFA 2.2. soil (0.30 d^{-1}, 95 % CI: 0.09; 1.07). The enchytraeids were unable to eliminate lindane completely during the experimental period and about half of the peak concentration of lindane body residues remained after 7 days (Table S4).

3.3. BAF_k

The rapid uptake of lindane demonstrated a high potential for bioaccumulation in enchytraeids. The BAF_k in LUFA 2.2 soil was double (66.3 $g_{soil}\ g^{-1}_{wwt}$, 95 % CI: 58.7; 71.9), compared to the amended LUFA soil (36.4 $g_{soil}\ g^{-1}_{wwt}$, 95 % CI: 30.9; 40) (Table 2). As illustrated in Fig. 1, the BAF_k values supported the bioaccumulation pattern observed in both soils and were consistent with k_{in} values. The absence of steady state during the uptake phase indicates an ongoing bioaccumulation potential over extended period.

4. Discussion

4.1. BAF_k in LUFA 2.2 and amended LUFA soil

The present study supports the hypothesis that bioaccumulation of organic chemicals is inversely correlated with soil OM content. This relationship was clearly demonstrated by the observation that bioaccumulation was more than doubled in enchytraeids from LUFA 2.2 soil (BAF_k: 66.3 $g_{soil}\ g^{-1}_{wwt}$) compared to those from amended LUFA soil with higher OM content (BAF_k: 36.4 $g_{soil}\ g^{-1}_{wwt}$). The primary factor driving the observed difference was the uptake rate constant, which was approximately four-fold higher for enchytraeids from LUFA 2.2 soil compared to the amended LUFA soil. The significant difference in enchytraeid internal concentrations between LUFA 2.2 soil and amended LUFA soil was confirmed by comparing the difference between the mean values (TableS5). The mean internal concentration for enchytraeids from LUFA 2.2 soil was 287 $\mu g_{lindane}\ g^{-1}_{worm\_wwt}$ (± 39.7 SD), considerably higher than the 117 $\mu g_{lindane}\ g^{-1}_{worm\_wwt}$ (± 2.56 SD) observed in enchytraeids from amended LUFA soil. The statistical analysis further supported this finding, with a mean difference of 170

Table 1
Soil characteristics from a two-week bioaccumulation study of lindane using *Enchytraeus crypticus* in LUFA 2.2 soil (2.85 %) and amended LUFA 2.2 soil with dry bark (10 % OM). Soil pH was measured by $CaCl_2$ method. C_{soil} indicates lindane concentrations in the exposure soil, and C_{worm} represents internal lindane concentrations in enchytraeids after one week of exposure.

Soil type	OM [%]	pH [SD]	C_{soil} ($mg_{lindane}\ kg_{soil\_wwt}$) [SD]	C_{worm} at day 7 ($\mu g_{lindane}\ g_{worm\_wwt}$) [SD]
LUFA 2.2	2.86	5.93 [0.04]	4.89 [0.74]	287 [39.7]
Amended	10	6.38 [0.06]	4.47 [0.54]	117 [2.56]

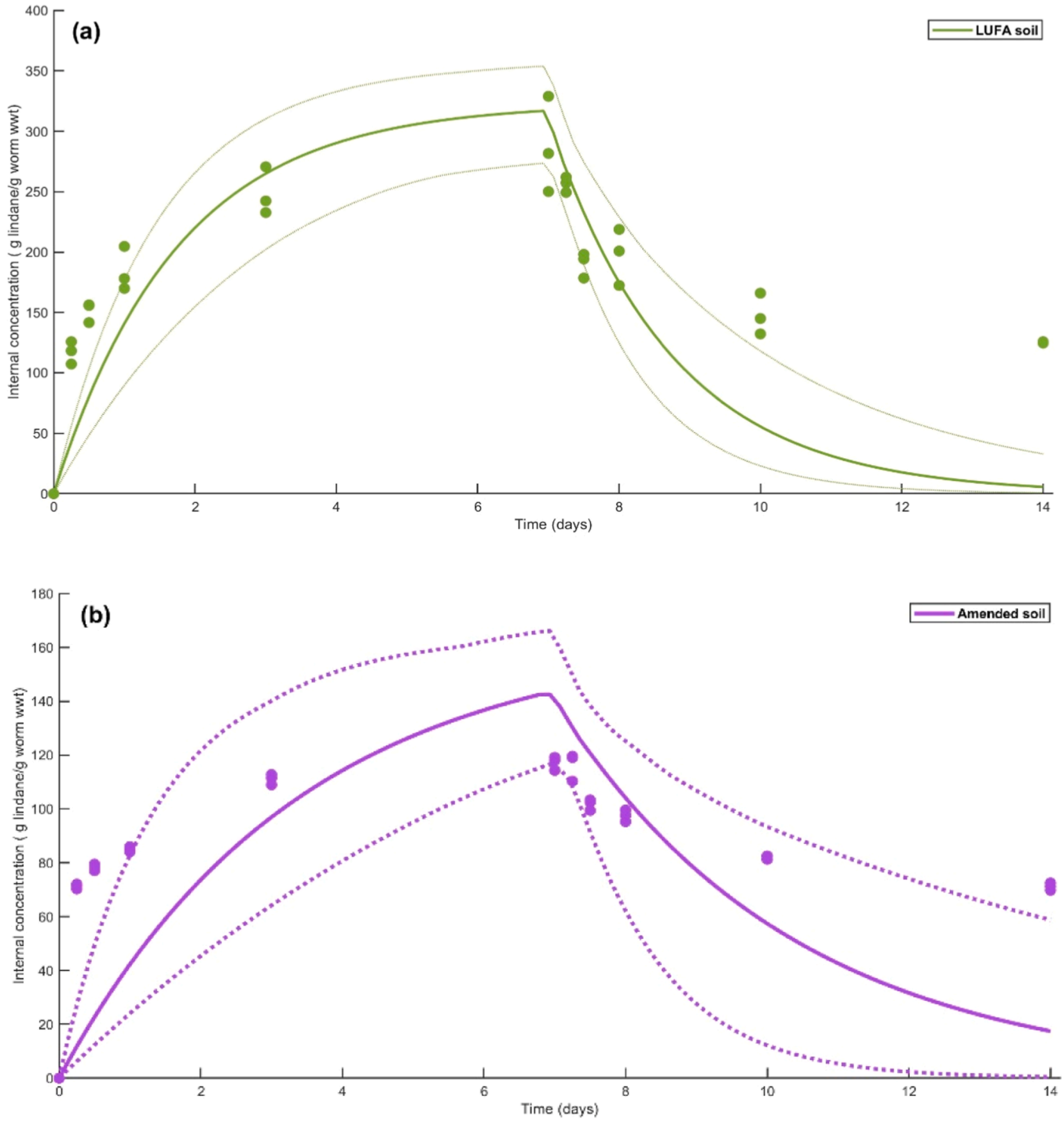

Fig. 1. Toxicokinetic of lindane in Enchytraeus crypticus exposed to: a) LUFA 2.2 soil (2.86 % OM), and b) amended LUFA 2.2 soil with added organic matter (10 % OM). Enchytraeids were exposed to 4.89 and 4.47 mg lindane/kg wet soil, respectively, for 7 days, followed by transfer to clean soil for the elimination phase. The figure shows the toxicokinetic best model fit (solid line), prediction interval (dashed lines), and the experimental data points (dots).

$\mu g_{lindane}\ g^{-1}_{worm\_wwt}$ and a 95 % CI of 106–234 $\mu g_{lindane}\ g^{-1}_{worm\_wwt}$ (TableS5). The fact that this CI does not include zero reinforces the conclusion that there is a significant difference between the two soil types.

Despite differences in the uptake rate between the two soils the elimination rate constants were similar between the two soil types. This suggests that the OM content primarily affects the uptake process rather than the process linked to elimination. These findings highlight the importance of soil composition in influencing the bioaccumulation of lindane in enchytraeids, with a more pronounced effect of OM on uptake than on elimination processes.

4.2. Bioaccumulation potential

Our results show that organisms exposed to soil with lower organic matter content (2.85 %) exhibited substantially higher uptake rate constants and bioaccumulation factors compared to those in high organic matter soil (10 %). Moreover, BAF_k values reported in the literature for lindane in enchytraeids span a wide range, from as low as 3.46 $g_{soil}\ g^{-1}_{wwt}$ reported by Bruns et al. (2001) to over 165 $g_{soil}\ g^{-1}_{wwt}$ in

Table 2
Overview of toxicokinetic studies on lindane bioaccumulation in enchytraeids (Enchytraeus crypticus, Enchytraeus albidus, and Enchytraeus luxurious) and earthworms (Eisenia andrei and Eisenia fetida) in natural and artificial soils with varying organic matter content. Reported parameter include the uptake rate constant (k_{in}), elimination rate constant (k_{out}), kinetic bioaccumulation factor (BAF_k), and the time to reach 95 % of steady state. Values within square brackets refer to 95 % CI.

Study	Exposure duration (days)	Oligochaeta species	Soil type (OM%)	k_{in} ($g_{soil}\ g_{wwt}^{-1}\ d^{-1}$)	k_{out} (d^{-1})	BAF_k ($g_{soil}\ g_{wwt}^{-1}$)[a]	T95 % SS (days)[b]
This study	7	*Enchytraeus crypticus*	LUFA 2.2 (2.85 %)	37.8 [17.9; 52.6]	0.57 [0.26; 0.94]	66.3 [58.7; 71.9]	5.26
			Amended (10 %)	10.9 [4.69; 27.8]	0.30 [0.09; 1.07]	36.4 [30.9; 40]	9.99
Amorim et al. (2002)	10	*Enchytraeus albidus*	Natural (2.96 %)	151	0.91[e]	165	3.29
			OECD (7.6 %)	88.9	1.58[e]	56.2	1.90
Bruns et al. (2001)	21	*Enchytraeus luxurious*	LUFA 2.2 (3.78 %)	32.4	0.90 ± 0.2 SE[e]	36.0	3.33
	21		OECD (9.63 %)	36.9	0.80 ± 0.1 SE[e]	46.1	3.74
	21	*Enchytraeus albidus*	LUFA 2.2 (3.78 %)	9.70	2.80 ± 1.2 SE[e]	3.46	1.07
	28		OECD (9.63 %)	8.60	1.40 ± 0.9 SE[e]	6.14	2.14
Svobodova et al. (2020)	21	*Eisenia andrei*	Natural S1 (2.75 %)[d]	2.64[c]	0.19 ± 0.22 SE	13.9	15.8
			Natural S2 (16 %)[d]	1.08[c]	0.65 ± 0.22 SE	1.66	4.61
Smidova and Hofman (2014)	21	*Eisenia fetida*	Natural S1 (0.81 %)[d]	5.47[c]	0.52 ± 0.09 SE	10.5	5.76
			Natural S2 (2.67 %)[d]	4.22[c]	0.56 ± 0.11 SE	7.54	5.35
			Natural S3 (3.23 %)[d]	1.84[c]	0.21 ± 0.03 SE	8.76	14.3
			Natural S4 (4.45 %)[d]	0.90[c]	0.31 ± 0.03 SE	2.90	9.66
			Natural S5 (13.55 %)[d]	1.92[c]	1.55 ± 0.58 SE	1.24	1.93
			Natural S6 (34.73 %)[d]	0.67[c]	0.91 ± 0.21 SE	0.74	3.29

[a] BAF_k calculated as the ratio k_{in}/k_{out}
[b] Calculated as T95 % ss (d) = $-\ln(1-0.95)\ /\ k_{out}$
[c] Calculated assuming 81 % water content for earthworms (Dalby et al., 1996)
[d] TOC (%) × 1.72 (conversion factor) (Pribyl, 2010)
[e] Values calculated from a two-compartment model or one-compartment model with a double exponential equation.

enchytraeids as described by Amorim et al. (2002) (Table 2). The results of this study showed that enchytraeids exposed to soil with lower OM content (2.85 %) exhibited substantially higher k_{in} and BAF_k compared to those in high OM soil (10 %). The same pattern was observed in the study of Amorim et al. (2002), who also observed higher BAF_k of lindane in *Enchytraeus albidus* from natural soils with lower OM content (2.96 %) compared to the OECD soil (7.6 % OM), despite the BAF_k values being approximately twice as high as those reported in the present study. The higher accumulation observed in the low OM soil (LUFA 2.2) is likely due to increased chemical bioavailability, since organic compounds tend to bind to soil OM, thereby limiting their uptake by organisms. The difference in BAF_k values between this study and the one from Amorim et al. (2002) could be attributed to differences in the size (surface-to--volume ratio) of the enchytraeid species that were studied, experimental conditions, or soil properties (Roembke et al., 2017). Except in the case of Bruns et al. (2001), where a higher BAF_k ($g_{soil}\ g_{wwt}^{-1}$) of lindane was observed in *Enchytraeus luxurious* and *Enchytraeus albidus* from high OM soil (OECD soil with 9.63 % OM) compared to LUFA 2.2 soil (3.78 % OM), a clear trend showed higher BAF_k values in soils with lower OM content. This is consistently observed across different studies and species. For instance, in earthworm studies, Šmídová and Hofman (2014) and Svobodová et al. (2020) also reported higher lindane BAF_k values ($g_{soil}\ g_{wwt}^{-1}$) in soils with lower OM content for *Eisenia fetida* and *Eisenia andrei*, respectively (Table 2). Apart from BAF_k for lindane, in general, some authors observed higher BAF_k of organic chemicals in earthworms in soils with low OM content, with examples being present for a range of POPs (persistent organic pollutants) including lindane (Vlčková and Hofman, 2012) and the PAH phenanthrene in enchytraeids (Hofman et al., 2008).

These corroborating findings support a trend of higher BAF_k values with lower soil OM content that appears to be consistent across various oligochaete species including enchytraeids as identified here. This relationship can be attributed to the strong sorption capacity of OM for organic compounds. In soils with higher OM content, a larger fraction of organic chemicals tends to be sorbed to soil particles, effectively reducing the fraction of freely dissolved organic compound in the porewater (Belfroid and Sijm, 1998). This sorption process is crucial as the main uptake route for soft-body species is via passive diffusion across their skin (Jager et al., 2003; Peijnenburg et al., 2012). Consequently, higher OM content limits the uptake and bioaccumulation of organic chemicals in soils for enchytraeids and earthworms.

4.3. Uptake rate constants

In studies in the literature reporting on toxicokinetic studies, k_{in} data shows considerable variation across studies and species examined when exposed to lindane, values ranging from 8.60 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$ to as high as 151 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$ in enchytraeids. A notable trend is that k_{in} tends to be higher in soils with lower OM content. For instance, in the study by Amorim et al. (2002), k_{in} for *Enchytraeus albidus* was 151 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$ in soil with 2.96 % OM, compared to 88.9 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$ in soil with 7.6 % OM. However, the experiment in the natural soil (low OM content) started immediately after spiking, whereas in the OECD soil (high OM content), the experiment started one week later. Nevertheless, since the kinetic rate constants in a first-order kinetic model are independent of exposure concentration, the differences in k_{in} values are unlikely to result from variations in the initial exposure concentration. Instead, they likely reflect differences in soil properties and their interactions with organic compounds.

The same trend was observed in earthworms, where Svobodová et al. (2020) and Šmídová and Hofman (2014) reported decreasing k_{in} values with increasing soil OM content. In the latter, a substantial eight-fold difference was observed between the soil with the lowest OM content (0.81 %) and the highest OM content (34.73 %). These results further support our hypothesis that toxicokinetic depend on the OM content and quality/composition of the soil and their characteristics, as they impact

the rate at which organisms absorb the compound.

4.4. Elimination rate constants

Elimination rate constants reported in literature studies also showed wide variability across studies, ranging from 0.80 d^{-1} to 2.80 d^{-1} in different enchytraeids species (Table 2). Unlike k_{in}, there is no clear trend relating k_{out} to soil OM content. Our results align with Bruns et al. (2001), nevertheless, Amorim et al. (2002) reported an opposite trend, with higher k_{out} values in high OM soils. In the latter case, enchytraeids could reach steady-state faster, but at a lower overall tissue concentration. However, this finding was not observed in our study or by Bruns et al. (2001). Similarly, no consistent trend was observed in earthworm studies (Šmídová and Hofman, 2014; Svobodová et al., 2020). For instance, Šmídová and Hofman (2014) found that k_{out} values for *Eisenia fetida* did not consistently increase or decrease with varying OM content. These results suggest that elimination is more related to species-specific physiological traits rather than to soil properties.

Interestingly, some studies observed a biphasic elimination pattern for lindane (Amorim et al., 2002; Šmídová and Hofman, 2014; Bruns et al., 2001; Miao et al., 2018), characterized by an initial fast elimination phase followed by a slower elimination phase. A biphasic pattern can occur when gut content is not removed from test species, leading to rapid initial chemical elimination through egestion followed by slower tissue-based elimination. This pattern might be more common in larger earthworms and might explain why earthworms are typically allowed to purge their gut content. However, unlike in (Amorim et al., 2002) and (Bruns et al., 2001), this pattern was not observed in the present study, possibly due to differences in experimental design, duration of the elimination phase, or species-specific responses. Nevertheless, in Table 2, we included elimination rate constants from a first-order one-compartment model where the elimination was calculated using a double exponential equation (Amorim et al., 2002), and from a two-compartment model, with one compartment for fast elimination and the second for slow elimination processes (Bruns et al., 2001). Hence, we acknowledge the limitation of making a direct comparison of elimination rate constants.

4.5. Interspecies differences of BAF_k

The data collected in Table 2 clearly demonstrate a significant difference in kinetic rate constants and BAF_k between enchytraeids and earthworms. Enchytraeids generally exhibit higher uptake rate constant and BAF_k compared to earthworms. This becomes evident when comparing the k_{in} values for enchytraeids (ranging from 8.60 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$ to 151 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$) to those for earthworms (ranging from 0.67 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$ to 5.47 $g_{soil}\ g_{wwt}^{-1}\ d^{-1}$). The data for lindane suggests that species-specific traits influence significantly TK parameters. Despite both being soft-bodied terrestrial invertebrates, earthworms and enchytraeids have distinct physiological, behavioral, and ecological characteristics, which significantly influence their exposure pathways and susceptibility to soil contaminants (Peijnenburg et al., 2012). In particular, the lipid content and species surface-to-volume ratio have been reported to impact in the bioaccumulation of organic chemicals (Dalhoff et al., 2020; Goto and Sudo, 2018; Li et al., 2024a). Recently, Li et al. (2024b) developed a bioaccumulation model that performed better than previously known bioaccumulation models (Jager et al., 2003; Belfroid et al., 1995). In their model they used several chemical, soil, and species trait characteristics, highlighting that a combination of lipid content and earthworm Specific Surface Area (SSA) played a significant role in the bioaccumulation potential. However, the model needs to be applied and validated in enchytraeids before it can be concluded it performs equally well for enchytraeids, and hence, acknowledge that lipid content and surface-to-volume ratio indeed are the main species traits causing species-specific bioaccumulation patterns.

4.6. Modeling discrepancies

The internal concentration data showed minimal variability between replicates, and the narrower model prediction intervals for the amended LUFA soil data, represented by dashed lines, indicate a strong alignment between the model predictions and experimental observations (Fig. 1). Nevertheless, the uptake and elimination kinetics were not perfectly explained by the simple one-compartment TK model. By visual inspection of the data and model fits, our TK model seemed to underestimate the uptake kinetics, especially for the LUFA 2.2 soil, whereas the model slightly overestimated the elimination kinetics This is not an anomalous observation, as several researchers also observed that one-compartment TK model overestimated elimination kinetics (Amorim et al., 2002; Sousa et al., 2000). Authors have advocated for a biphasic elimination modelling, with two distinct k_{out} values to better represent the elimination kinetics (Amorim et al., 2002; Bruns et al., 2001). Nevertheless, none provided an insightful mechanistic explanation on the possible causes of such a kinetic behavior.

4.7. Additional soil factors potentially impacting BAF_k values

The influence of soil OM on bioaccumulation varies across organic chemicals and test species. Bioavailability depends on the physicochemical properties of the compound (e.g., Log K_{ow}, water solubility), the specific composition of the soil (e.g., OM content, humic acids, pH), and the biological traits of earthworm species (e.g., size, lipid content, feeding behaviour) (Li et al., 2024a; Ehlers and Loibner, 2006; Zhang et al., 2018).

Soil texture, defined by the proportions of sand, silt, and clay, plays a crucial role in contaminant behavior. The clay fraction, with its high surface area and cation exchange capacity (CEC), provides numerous reactive sites for molecular interactions (Adamu and Aliyu, 2012). Similarly, organic matter, clay minerals, and other soil components influence pH, which typically ranges between 4.0 and 8.0 (Reuter et al., 2008; Wamelink et al., 2019). Soils rich in organic matter exhibit greater buffering capacity, resisting pH changes, whereas sandy soils are more prone to acidification (Reuter et al., 2008). Likewise, findings from Libohova et al. (2018) suggest that enhancing soil organic matter can significantly improve the water retention capabilities of soils, which is crucial for agricultural practices and soil conservation efforts.

Although not the focus of this study, soil pH affects the behavior of organic contaminants, particularly ionizable compounds, by influencing cation exchange and sorption (Franco et al., 2009). Maintaining a stable pH is essential for toxicity studies, as it plays a key role in shaping bacterial diversity and community composition (Griffiths et al., 2016). While pH may not be the primary factor controlling bioavailability, consistent soil properties help sustain microbial communities, ensuring comparable degradation and transformation processes.

To minimize the effects of several soil factors, in the current study we added dry compost to the soil and maintained a similar pH in both test soils, thereby isolating the variables of interest in our terrestrial toxicity assessment. These various soil properties form a complex interaction that influence the bioavailability and accumulation of organic compounds in soils. Understanding these intricate relationships is crucial for accurately assessing environmental risks.

4.8. Proposed mechanistic basis for model-data discrepancies

The observed incomplete elimination of lindane in enchytraeids during the depuration phase may be partially explained by the phenomenon of reuptake. Even in clean soil, residual lindane may be present due to excretion by the enchytraeids during the initial depuration period (Peijnenburg et al., 2012). This residual contamination could lead to a continuous, low-level reuptake of lindane by the organisms, eventually slowing down the overall elimination process. This hypothesis is supported by the soil data collected during the experiment, where

a slight increase in soil lindane concentration was recorded during the depuration phase (Table S1 and Table S2). Although this increase was not large enough to fully account for the observed biphasic elimination pattern, it could contribute to the persistence of lindane in the enchytraeids. This effect may have been particularly relevant towards the end of the experimental duration, where a considerable amount of lindane was measured in the soil. However, we accounted for the reuptake of lindane from the soil in our model and compared scenarios with constant exposure of zero value and the actual measured lindane residues in soil. Interestingly, the model did not show significant differences in kinetic rate constants between these scenarios. Furthermore, mass balance calculations showed that if all the lindane was excreted by the enchytraeids in clean soil, and all of it could be re-uptake slowly, it would only account for 25.3 $\mu g_{lindane}\ g_{wwt}^{-1}$ (95 % CI: 22.4–27.4 $\mu g_{lindane}\ g_{wwt}^{-1}$) in enchytraeids from LUFA 2.2 soil, whereas this value would only represent 7.91 $\mu g_{lindane}\ g_{wwt}^{-1}$ (95 % CI: 6.71–8.69 $\mu g_{lindane}\ g_{wwt}^{-1}$) in enchytraeids from amended LUFA soil TableS6). These values clearly could not account for all the residual lindane in enchytraeids by the end of the experiment. Similarly, Belfroid and Sijm (1998) also considered this hypothesis, although they concluded that re-uptake alone could not explain the differences in elimination by earthworms in soils with varying OM content. The presence of residual lindane in the soil and its potential for re-uptake adds complexity to the elimination kinetics. However, other plausible mechanistic explanations may also be relevant.

In addition to reuptake, several other mechanisms may have contributed to the complex elimination kinetics observed in enchytraeids. While biotransformation of lindane has been observed in various organisms, including earthworms (Park et al., 2012; Viswanathan, 1994) and aquatic species (Feroz et al., 1990; Yuan et al., 2021) its occurrence in enchytraeids remains unclear. The residues remaining after seven days in the elimination period indicated that biotransformation was either very slow or not occurring. If biotransformation was more effective, a greater decrease in body residues would have been observed during the elimination period. A rather complementary explanation to that of low biotransformation rate, lies in the lipophilic nature of lindane and its partitioning behavior. The high affinity of lindane for biological membranes and lipid bilayers could lead to accumulation in storage lipids and membrane phospholipids (Antunes-Madeira and Madeira, 1985; Sabra et al., 1996), resulting in different elimination kinetics from various tissues. Additionally, lindane is able of interacting with membrane proteins within the nervous system, in particular with ionotropic receptors like $GABA_A$, which mediates neuronal inhibition by reducing hyperpolarization in postsynaptic neurons (Sallard et al., 2021). The reversible binding of lindane to allosteric sites on these receptors and its accumulation in neuronal membranes may lead to prolonged effects and delayed elimination (Law and Lightstone, 2008; Vale et al., 2003). The combination of these factors likely contributes to the complex elimination kinetics observed in enchytraeids, highlighting the need for further research to fully elucidate these mechanisms.

5. Conclusion

The current study provides insights into the bioaccumulation of lindane in enchytraeids and the influence of OM content, on this process. The inverse correlation between soil OM content and the BAF_k reported in this study further supports the importance of soil OM in driving the bioaccumulation process. In accordance with our hypothesis, the BAF_k in LUFA 2.2 soil (lower OM content) was nearly twice as high as in amended LUFA soil (higher OM content), a difference primarily driven by the uptake rate constant, which was also approximately four-fold higher in LUFA 2.2 soil. This indicates that OM content has a more pronounced influence on uptake rather than elimination processes. A literature-based comparison of accumulation patterns between enchytraeids and earthworms reveals that enchytraeids generally exhibit higher uptake rate constants and associated BAF_ks compared to earthworms. These interspecies differences emphasize the importance of species-specific traits in influencing toxicokinetics. Our findings highlight the necessity for additional research to elucidate specific mechanisms of bioaccumulation of lindane and other organic chemicals in soft-bodied terrestrial invertebrates.

CRediT authorship contribution statement

Alex Robinson: Methodology, Conceptualization. **Dave Spurgeon:** Writing – review & editing, Conceptualization. **Nico van den Brink:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Roman Ashauer:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Oihane Del Puerto:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kevin Noort:** Methodology, Investigation. **Sidney Behringer:** Methodology. **Kristin Höfer:** Methodology.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used AI technology in order to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Del Puerto, Oihane reports financial support was provided by Syngenta Crop Protection AG. Del Puerto, Oihane reports a relationship with Syngenta Crop Protection AG that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

The supplementary material contains the internal body burden residues of enchytraeids, soil exposure concentrations of lindane, the mass balance calculations for the re-uptake of lindane from clean soil, the differences in means calculation between LUFA 2.2 and amended LUFA soil for body burden residues, and the parameter space plots. It also contains a text file with the MATLAB code to reproduce the analyses.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2026.119717.

Data availability

MATLAB codes are provided in the document called "Data availability"

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