Temperature-Dependent Toxicokinetics of Phenanthrene in *Enchytraeus albidus* (Oligochaeta)

Wencai Dai,* Stine Slotsbo, Cornelis A.M. van Gestel, and Martin Holmstrup*

**ABSTRACT:** Although the toxicokinetics of organic pollutants in soil invertebrates under optimal and constant temperature has been widely reported, their uptake, elimination, and bioaccumulation under suboptimal temperatures, and especially daily fluctuating temperature (FT) regimes have received only little research attention. In this study, the uptake, elimination, and bioaccumulation of phenanthrene (PHE) in *Enchytraeus albidus* (Oligochaeta) under different constant temperatures, and an FT regime were investigated in a natural soil. In general, the PHE concentrations in worm tissues reached steady state within 14 days at different temperatures. The uptake \( k_u \) and elimination \( k_e \) rate constants and the bioaccumulation increased with increasing temperature likely because of an increased diffusivity of PHE into the worms and an increased metabolic rate. Interestingly, the bioaccumulation factor of PHE in *E. albidus* showed a positive relationship with temperature because the slope of the \( k_u \)-temperature relationship was larger than that of the \( k_e \)-temperature relationship. Further, the uptake and elimination rate constants were larger under the FT regime than at the constant average of the fluctuating temperature. These findings suggest that, climatic conditions, especially daily fluctuating temperatures, should be considered for the assessment of the toxicokinetics of organic pollutants in terrestrial organisms.

**INTRODUCTION**

Polycyclic aromatic hydrocarbons (PAHs) are some of the most widespread hydrophobic organic compounds (HOCs) in the environment and have attracted raising concerns because of their toxic, carcinogenic, and mutagenic properties. Soils are increasingly becoming sinks for PAHs as a result of sewage sludge deposition, incomplete combustion of organic matter and fossil fuels, and other high-temperature industrial processes. Once in the soil, a fraction of the PAHs becomes preferentially adsorbed to organic matter and can be bound there for a long time due to their strong sorption to carbonaceous particles. Due to their lipophilic nature, PAHs may accumulate in organisms. The cytochrome P450 oxygenases and transferases of the phase I and phase II biotransformation systems of organisms can transfer PAHs into more water-soluble metabolites, which are eliminated from the body by transporters. PAHs and their metabolites are directly harmful to soil communities by accumulating in soil biota. The toxicokinetics of PAHs has been investigated in both aquatic and terrestrial organisms, but with most focus on aquatic invertebrates and fish. Much less is known on the uptake and elimination of PAHs in terrestrial invertebrates, such as the enchytraeid, *Enchytraeus albidus*.

Enchytraeids, which belong to the Oligochaeta and are closely related to earthworms, are abundant and play an essential ecological role in the soil. They maintain soil functions, such as soil organic matter decomposition and stimulate microbial activity. Furthermore, they are an important food resource in terrestrial food webs and are eaten by a wide variety of predators. Thus, accumulation of PAHs in enchytraeids not only poses a risk to the enchytraeids but potentially also to top predators through food chain transfer.

Using toxicokinetics analysis, it is possible to integrate uptake, accumulation, and elimination processes of chemicals in organisms. The uptake of lipophilic organic pollutants by soil invertebrates is primarily driven by passive diffusion across the cuticle and then partitioning into their storage lipids and membrane phospholipids. Diffusive uptake is a spontaneous process driven by the partial of molar free energy, which can be expressed by its chemical activity. The organism can take up PAHs through air and direct contact with soil particles. However, the dominant route of diffusive uptake of organic chemicals from moist soil is probably through soil pore water.
Before the organic pollutants are partitioning into organisms, they need to be desorbed from soil organic matter and dissolved in soil pore water as described in the equilibrium partitioning theory.\textsuperscript{17,18} This is a partitioning process, which is influenced by temperature and by the physicochemical properties of the pollutant in question.

The uptake and elimination of pollutants may vary with temperature because diffusivity (or diffusion coefficient governing uptake), as well as metabolic rates of organisms (governing biotransformation and excretion of water-soluble degradation products), are influenced by temperature.\textsuperscript{19–21} The diffusion coefficient is related to Fick’s law and increases only moderately with increasing, but permissible, physiological temperatures because it is proportional to absolute temperature. However, the metabolic rate of ectotherms increases exponentially with temperature within their thermal range, which can be expressed as \( Q_{10} \) defined as the fold change of a metabolic rate with a 10 °C increase in temperature.\textsuperscript{22–24} It may therefore be expected that the uptake rate of pollutants is less influenced by increasing temperature than elimination rates.

Most investigations on the toxicokinetics in terrestrial oligochaetes (earthworms and enchytraeids) have been performed at the organism’s optimal temperature (often 20 °C).\textsuperscript{25} Therefore, laboratory results cannot always be applied to conditions in the field, where temperature changes both on a diurnal and a seasonal scale and along climatic gradients.\textsuperscript{26} Previous studies have shown that organisms’ performance, such as metabolic rate, at daily fluctuating temperature within permissive temperature ranges, is larger than at the constant mean of the fluctuating temperature.\textsuperscript{27} Furthermore, temperature is one of the important factors affecting the partitioning of PAHs between soil organic matter and soil pore water\textsuperscript{28} and its uptake by organisms.

There is a gap in knowledge when it comes to understanding how temperature influences the toxicokinetics of organic pollutants in soil organisms. Some studies have shown that the partitioning process is predominantly enthalpy-driven and bioaccumulation factors (BAFs) are negatively related to temperature.\textsuperscript{19,20,29} However, other studies reported that this process is mainly entropy-driven and that BAFs are positively influenced by temperature.\textsuperscript{20,21} Furthermore, most studies on the uptake and elimination of PAHs and temperature effects on metabolic rates focused on aquatic biota.\textsuperscript{9,19,24,32,33} The temperature effects on uptake, elimination, and bioaccumulation of PAHs in soil biota remain unknown, and no studies have been performed with fluctuating temperature (FT).

To improve the risk assessment of chemicals, it is necessary to predict how ectotherms respond to natural temperature settings. The relationships between the performance of ectotherms (e.g., rates of development or physiological processes) and temperature are slightly nonlinear. Therefore, using physiological rates of ectotherms obtained in constant temperature (CT) experiments to predict the rates in FT regimes will usually result in underestimations because of the Jensen’s inequality phenomenon.\textsuperscript{21} This phenomenon says that the response of the metabolic activity of ectotherms follows an accelerating temperature–performance relationship below the optimal temperature (\( T_{\text{opt}} \)) and a decelerating relationship above \( T_{\text{opt}} \). Diffusion uptake rates of chemicals and organism metabolic rates may be enhanced under FT compared to the constant mean of FT when temperature is below the \( T_{\text{opt}} \) leading to a difference in uptake, bioaccumulation, and elimination in organisms.\textsuperscript{23,27} These considerations encouraged us to study the effects of different CTs and FTs on the toxicokinetics of a representative PAH (phenanthrene, PHE; \( \log K_{\text{ow}} 4.46 \)) in a model soil invertebrate species (Enchytraeus albidus).

The present study was designed to assess the effect of temperature (CT: 5, 10, 15, 20, and 25 °C or FT: 10–20 °C) on the uptake, elimination, and bioaccumulation of PHE in E. albidus. Since both the diffusion of PHE and the metabolic rate of the worms increase at higher temperature, we hypothesized that (1) the uptake and elimination rate of PHE in the worms would increase with increasing temperature. (2) The uptake rate would be more affected by temperature than the elimination rate, and thus, the bioaccumulation factor would show a positive temperature effect. (3) The uptake and elimination rate of PHE in the worms would be higher at FT treatment than that at the constant average of the varying temperatures.

\section*{MATERIALS AND METHODS}

\textbf{Test Animals.} Enchytraeus albidus (Henle, 1837) were collected at a beach near Grettislaug, Northwest Iceland (65°52′53″ N; 19°44′11″ W) and cultivated in our laboratory in a mixture of agricultural soil and seaweed (4:1) at 18 ± 1 °C. The worms were fed with oats weekly, and deionized water was added weekly to maintain the moisture content. We used a mixture of randomly selected subadult and adult worms in this study.

\textbf{Test Soil.} An uncontaminated agricultural soil was collected at Askov, Denmark. The soil was dried at 80 °C for 24 h and 2 mm sieved prior to use. The organic carbon content was 1.6% (determined by loss-on-ignition), soil pH\textsubscript{water} was 6.2, water holding capacity was 36% (w/w), bulk density and the total cation exchange capacity (CEC) were 1.135 g cm\textsuperscript{-2} and 8.14 cmolc kg\textsuperscript{-1}, respectively. The coarse sand (200–200 \( μ \)m), fine sand (63–200 \( μ \)m), coarse silt (20–63 \( μ \)m), fine silt (2–20 \( μ \)m), and clay (<2 \( μ \)m) contents of Askov soil were 38.4, 23.6, 10.0, 12.3, and 13.0%, respectively.\textsuperscript{34} PHE Spiked Soil. To facilitate easy and precise detection of PHE levels in the enchytraeid worms, but also secure normal behavior of the animals, we used the lowest observed effect concentration of PHE (LOEC\textsubscript{reproduction}), 40 mg PHE kg\textsuperscript{-1} dry soil,\textsuperscript{8} as the exposure concentration in the present study. The soil was spiked with PHE (Sigma Aldrich, CAS #85-01-8, 98% purity) using acetone (J.T. Baker, HPLC quality) (180 mL kg\textsuperscript{-1} dry soil) as a carrier solvent to obtain the desired concentration. The spiked soil was thoroughly mixed and left overnight under a fume hood to allow evaporation of acetone. The soil was stirred once again to ensure complete evaporation of acetone. Subsequently, the water content of the soil was adjusted to approximately 50% of the water holding capacity (180 mL kg\textsuperscript{-1} dry soil) using 15% NaCl solution. The moist soil was thoroughly mixed.

\textbf{Uptake and Elimination of PHE.} The tests were performed at five constant target temperatures (5, 10, 15, 20, and 25 °C) and one diurnally fluctuating (10–20 °C; mean: 15 °C) target temperature regime for 28 days including 14 day uptake phase followed by 14 day elimination phase. At the beginning of the test, 480 brown glass vials (200 mL) were prepared, each vial containing five randomly selected worms and 25 g of moist spiked soil. The vials were closed with a perforated plastic lid allowing gas exchange and placed in polystyrene boxes to ensure stable temperatures. The boxes...
were placed in different walk-in climate rooms, which were precise to ±1 °C. The actual temperature of representative vials was logged at 5 min intervals using TinyTag temperature data loggers accurate to ±0.1 °C (Gemini Data loggers, Chichester, UK). We used the logged data to calculate the actual average temperatures (constant temperatures: 5.3, 10.1, 14.3, 20.3, and 24.7 °C; fluctuating temperature: 10.1–20.3 °C, mean: 14.9 °C) and used these in the further analysis. The box exposed to the daily fluctuating temperature treatment (FT; 10.1 to 20.3 °C) was shifted every 12 h from 20.3 to 10.1 °C and from 10.1 to 20.3 °C (Figure S1). The animals were fed with oats, and water loss of the moist soil was determined by weighing and replenished weekly if necessary. We destructively sampled the worms at different time points during the test. During the uptake phase, worms were sampled at day 0 (control), 6 h, 12 h, 1, 2, 3, 4, 7, 10, and 14 days. After the 14 day uptake phase, worms of the remaining vials were collected and transferred to another glass vial with 25 g of moist uncontaminated soil for the elimination phase. Worms were then sampled after 12 h, 1, 2, 4, 7, 10, and 14 days during the elimination phase. Five replicates were used for each sampling time at each temperature treatment. At each sampling time, worms were collected from the soil using featherweight forceps and washed three times in deionized water. After checking their survival under a stereomicroscope, the worms were gently dried on filter paper before they were transferred into an Eppendorf tube. The tubes were snap-frozen in liquid nitrogen and stored at −80 °C until further analysis. Soil samples from the uptake phase were stored at −20 °C for the later PHE analysis.

Determination of PHE in Animal Tissue and Soil. Phenanthrene concentrations in the animal tissues were determined according to a method previously described in detail by Holmstrup et al.,35 but with some modifications. Briefly, the fresh weights of single worms of each replicate were determined by placing them on a pre-weighed small piece of filter paper using a Sartorius Micro SC 2 balance accurate to ±1 mg (Sartorius AG, Goettingen, Germany). The samples were transferred into 1.5 mL brown glass vials together with the filter paper and 500 μL of acetonitrile was added. The samples were placed on ice and sonicated for 90 min and then kept at room temperature for 24 h, frozen at −18 °C for 24 h, and finally kept at room temperature for 24 h. The samples were again sonicated for 90 min, and the supernatant was transferred to autosampler vials and stored at −80 °C until analysis by gas chromatograph-mass spectrometry (GC-MS). PHE standards, including blanks, were run in parallel and subjected to the same extraction procedure. A Shimadzu GCMS-QP2010 with an autosampler was used to perform the analysis. We did not detect phenanthrene in the nonexposed worms. The limit of detection (LoD) and the limit of quantification (LoQ) of worms were 1.54–3.50 and 5.54–12.6 mg kg−1 fresh wt worms, respectively. The LoD and LoQ of soil were 0.108 and 0.360 mg kg−1 dry soil, respectively. Recovery was tested by spiking an uncontaminated animal material with known amounts of PHE. The recoveries of PHE ranged between 93.2 and 108.4%, with an average (±standard deviation) of 101 ± 6%.

Phenanthrene concentration in soil samples was determined according to the method of Dai et al.36 Briefly, 1 g of moist soil samples were extracted with 4 mL of acetonitrile by shaking at 200 rpm for 24 h at room temperature followed by centrifugation at 3000 rpm for 5 min. The supernatant was transferred to autosampler vials and analyzed as described above.

Modeling. The rate of PHE degradation in the soil during the uptake phase was calculated assuming a first-order kinetic model

\[ C(t) = C_0 e^{-kt} \]  

where \( C(t) \) is the PHE concentration in soil at time \( t \) (mg kg−1 dry soil), \( C_0 \) is the initial PHE concentration in the soil (mg kg−1 dry soil), and \( k \) is the rate constant for a decrease in the PHE concentration in the soil (day−1). The kinetics of the PHE concentration in the enchytraeid body was described by a first-order one compartment model,37 written as

\[ \frac{dQ}{dt} = k_u C(t) - k_e Q(t) \]  

This equation can be integrated with eq 1 yielding \( Q(t) \). For the uptake phase (\( t \leq t_e \)), the model reads

\[ Q(t) = \frac{k_u}{k_e - k_0} \times C_{exp} \times (e^{-k_u t} - e^{-k_e t}) \]  

and for the elimination phase (\( t > t_e \))

\[ Q(t) = \frac{k_u}{k_e - k_0} \times C_{exp} \times (e^{-k_u t} - e^{-k_e t}) \times e^{-k_0 (t-t_e)} \]  

where \( Q(t) \) is the PHE concentration in the enchytraeid at time \( t \) (mg kg−1 fresh wt worm), \( k_u \) is the uptake rate constant (kg dry soil kg−1 fresh wt worm day−1), \( k_e \) is the elimination rate constant (day−1), \( k_0 \) is the PHE degradation rate constant in soil (day−1), \( C_{exp} \) is the PHE concentration in the test soil at the start of the uptake phase (mg kg−1 dry soil), and \( t_e \) is the time at which the animals were transferred to clean soil (days). The bioaccumulation factor (BAF) is defined as the ratio of the mean concentration in the enchytraeids (mg PHE kg−1 fresh body wt worm) at a steady state and the concentration in the soil (mg PHE kg−1 dry soil). BAF can also be expressed as the ratio of the uptake (\( k_u \)) and elimination rate constants (\( k_e \)) obtained from the toxicokinetics.

\[ \text{BAF} = \frac{C_{enchytraeid}}{C_{soil}} = \frac{k_u}{k_e} \]  

The half-life for PHE degradation in soil was calculated as

\[ t_{1/2} = \frac{\ln 2}{k_0} \]  

The temperature coefficient (Q10) is defined as the fold change of biochemical reactions as a consequence of a 10 °C temperature change.22 It was calculated according to eq 7

\[ Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_2-T_1)} \]  

where \( R_1 \) and \( R_2 \) are the rates of biochemical processes, in this case, uptake (\( k_u \)) or elimination (\( k_e \)) rates at the temperatures \( T_1 \) and \( T_2 \) (°C), respectively.

Data Analysis. The effect of temperature on PHE degradation was analyzed using generalized linear models (glm) in the package “lme4” in R (version 3.5.2). All model parameters were estimated by nonlinear regression in R using nls() function based on experimental data. It is well-established that temperature–rate relationships are of nonlinear when a temperature range is extended to higher and lower limits for
permissible temperatures. In our study, we have extended the temperature range to include suboptimal low and high temperatures and we therefore applied a polynomial model to describe the relationships between \( k_u \), \( k_e \), and BAF, and temperature. For the prediction of the effect of the fluctuating temperature on PHE accumulation in the enchytraeid worms, we first obtained the relationships between \( k_u \), \( k_e \), and temperature from the CT treatments. Then, first-order Euler methods (numerical method) following eq 2 were used to calculate the predicted PHE concentration in the worms using the measured temperature in the FT treatment. For the \( k_u \) rate constants, and BAF values comparison, we first fitted the polynomial model using the results from CT treatments, and then calculated the \( k_u \), \( k_e \) rate constants, and BAF at constant 14.9 °C based on the fitted model, and subsequently, we used these calculated values to make the comparisons with the results obtained from the FT treatment. All results are expressed as mean ± standard error (SE).

### RESULTS

**Phenanthrene Degradation in Soil.** During the 14 day uptake phase, the PHE concentration in the soil decreased slowly, but significantly at all CT temperatures (g/l, \( P < 0.05 \)). For the FT treatment, \( k_u \) was estimated to 0.0073 d\(^{-1}\), but the slope was not significantly different from 0 (g/l, \( P > 0.05 \)). In general, the average relative degradation rate at different temperatures ranged from 9 to 19% at the end of the 14 day uptake phase. The degradation rate in soil increased with increasing temperature. The PHE degradation rate constants and the corresponding half-lives at different temperatures are shown in Table 1.

<table>
<thead>
<tr>
<th>temperature (°C)</th>
<th>( k_u ) (d(^{-1}))</th>
<th>( P ) value</th>
<th>( R^2 ) for the model</th>
<th>half-life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>0.0055 (0.0011–0.0100)</td>
<td>0.016</td>
<td>0.127</td>
<td>126</td>
</tr>
<tr>
<td>10.1</td>
<td>0.0058 (0.0007–0.0109)</td>
<td>0.027</td>
<td>0.109</td>
<td>120</td>
</tr>
<tr>
<td>14.3</td>
<td>0.0085 (0.0041–0.0129)</td>
<td>&lt;0.001</td>
<td>0.261</td>
<td>82</td>
</tr>
<tr>
<td>20.3</td>
<td>0.0088 (0.0036–0.0139)</td>
<td>0.001</td>
<td>0.215</td>
<td>79</td>
</tr>
<tr>
<td>24.7</td>
<td>0.0099 (0.0048–0.0150)</td>
<td>&lt;0.001</td>
<td>0.261</td>
<td>70</td>
</tr>
<tr>
<td>FT</td>
<td>0.0073 (0.0014–0.0132)</td>
<td>0.017</td>
<td>0.126</td>
<td>95</td>
</tr>
</tbody>
</table>

*Note that FT is the daily fluctuating temperature regime from 10.1 to 20.3 °C.*

**Bioaccumulation, Uptake, and Elimination.** No mortality was observed during the 14 day uptake phase or the 14 day elimination phase, and all worms were lively moving without any visible damage at all sampling points. Thus, the experiment was in compliance with the quality criteria of the OECD guideline. Although we did not systematically assess weight change of the enchytraeid, masses of the enchytraeid analyzed for the PHE body concentration at each point in time did not show much variation (Table S1). This suggests that the worms did not gain or lose much weight, therefore, no correction for weight change was included in the kinetics model. The uptake and elimination of PHE in *E. albidus* followed a first-order kinetics model (Figure 1). The internal PHE concentration in the worms reached a steady state within the 14 day exposure period at all temperature treatments except for 5.3 °C. At this temperature, the model predicted steady state to be reached after about 24 days. The PHE concentration at the end of the uptake phase in *E. albidus* exposed at 24.7 °C was 2.08, 1.46, 1.35, 1.14, and 1.23-fold higher than at 5.3, 10.1, 14.3, and 20.3 °C and the FT treatment, respectively (Figure 1).

**The Relationship between \( k_u \), \( k_e \), BAF, and Temperature.** The estimated uptake and elimination rate constants of PHE in *E. albidus*, and the BAF values (Table S2) increased significantly and in a polynomial fashion with increasing temperature (Figure 2). Both \( k_u \) and \( k_e \) from 24.7 °C. The \( k_e \) in *E. albidus* at 24.7 °C was 3.30, 1.91, 1.49, 1.12, and 1.24-fold higher than at 5.3, 10.1, 14.3, and 20.3 °C and the FT treatment, respectively. The \( k_u \), \( k_e \), and BAF values in the FT treatment were 17.5, 15.8, and 4.9%, respectively, larger than estimated values at constant 14.9 °C, which was the average temperature for FT. The predicted development of PHE concentration in the worms at FT (based on parameters derived from CT experiments) is shown in Figure 3 and was in good agreement with observed internal concentrations. Body concentrations of PHE were also estimated on a lipid basis using literature values of water and lipid content of worms (Table S4), as well as \( k_u \), \( k_e \), and BAF values (Table S5), and relationships between \( k_u \), \( k_e \), BAF, and temperature (Table S6). The \( Q_{10} \) values calculated based on the uptake and elimination rate constants are shown in Table 2. In general, \( Q_{10} \) values were larger at low temperatures than at high temperatures.

### DISCUSSION

The present study determined the influence of both constant temperatures and diurnally fluctuating temperatures on the uptake, elimination, and bioaccumulation of phenanthrene in *E. albidus*. This is the first time that a study has involved diurnally fluctuating temperatures in toxicokinetics studies. The results showed that the PHE uptake and elimination rate constants and bioaccumulation increased with an increasing temperature. The uncritical use of biological rate constants derived at constant temperatures for predictions of rates at fluctuating temperature will often lead to underestimations. In the following, we will discuss the temperature effects on PHE degradation in soil, and its uptake and elimination in *E. albidus*, and the discrepancies between constant and fluctuating temperature regimes. We will also discuss the mechanisms underlying these effects and put our results in a risk assessment perspective.

The relative degradation of PHE in soil was less than 20% during the 14 day uptake period, which means that the animals in this experiment were continually exposed to a relatively constant concentration (spiked concentration). The degradation rate at 20 °C was lower than observed in previous studies using LUFA 2.2 soil or artificial soil. This discrepancy was probably due to our use of the uncontaminated agricultural soil from Askov with less organic matter compared to the commonly used LUFA 2.2 or artificial soil. Further, we dried the soil at 80 °C for more than 24 h and stored it dry for a long time prior to use. Due to drying and heating, the microbial activity in this soil was likely limited.

PAHs have a strong binding affinity for soil organic matter due to their low water solubility and high hydrophobicity. Previous studies have shown that the route of uptake in soil invertebrates of PAHs with \( K_{ow} < 5 \) is mainly through soil pore
Uptake and bioaccumulation of PAHs by soil organisms can be considered as the result of a partitioning process between the compounds in the soil pore water and the storage lipids and membrane phospholipids of the organism. In potworms like *E. albidus* and in earthworms, partitioning may also involve uptake across the gut epithelium, as well as across the body wall due to similar partitioning processes. In the present study, the worms were not fed any PHE contaminated food, the uptake comes either from dermal contact with or ingestion of soil. In both cases, uptake will be driven by pore water concentrations, thus, we assumed that the uptake of PHE was mainly from the soil pore water across the body wall and the gut wall by passive diffusion. Exposure of the organism to the contaminated soil will lead to (local) depletion of the chemical from the soil pore water, leading to a new equilibrium between soil pore water and soil organic matter. It is therefore possible that the uptake rate is influenced by the compound’s rate of desorption from organic matter and soil particles into the soil pore water. Because both the soil solid phase and the organism compete for the chemical, the uptake rate may be limited when the amount of compound desorbed from the soil is lower than the depletion by the organism. Under some circumstances, uptake rates of chemicals in earthworms can be limited by too slow desorption rates. For example, *Lumbricus terrestris* bioaccumulated about 50% more dieldrin in stirred soil than in unstirred soils, which was taken as a sign of uptake rate limitation in the unstirred soil. In the present study, the glass containers were shaken gently once a week and the accumulated PHE in worms was, at its highest, less than 1.5% of the total amount of PHE in the soil. Since the total biomass or worms in each vial was low, approx. 28.9 mg fresh wt per 25 g of moist soil (equivalent to about 21 g of dry soil), we suggest that the uptake rate of PHE by the worms was not rate limited by local depletion of the pore water due to slow desorption.

Previous studies have shown that the desorption rate of PHE from soil organic matter and the diffusivity from soil pore water into lipid increase with temperature, thereby influencing the PHE concentration in soil pore water. Increasing temperature can enhance the uptake rate of lipophilic compounds into an organism. However, diffusivity is described by Fick’s law (which uses absolute temperature), and hence, only moderately influenced by the experimental range of temperatures used here. Increasing diffusivity alone

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**Figure 1.** Uptake and elimination kinetics of phenanthrene in *Enchytraeus albidus* exposed to 40 mg kg\(^{-1}\) dry soil at different constant temperatures (a–e) or a fluctuating temperature (FT) regime (f). The black circles represent the internal concentrations. Solid lines present the results from fitting a first-order, one-compartment model to the data, taking into account the rate of degradation of phenanthrene in the soil. The statistical details (*k_u* and *k_e* values with corresponding confidence intervals, *P* values, *R*\(^2\)) and BAF values are presented in Table S2.
across a temperature increase in 20 °C (from 5 to 25 °C) can therefore not explain the observed 5-fold increase in $k_u$. Other yet unknown biological factors might contribute to the significant increase in an uptake rate at elevated temperature. The error bars show SE. The statistical details (confidence intervals, $R^2$ and $P$ values) are presented in Table S3.

The elimination of PAHs is mainly due to passive elimination related to the body concentration reached and active detoxification by biotransformation. Since the metabolic rate of ectotherms increases with temperature within permissive temperature ranges, enzyme activities (e.g., cytochrome P450) are enhanced. This means that the rate of biochemical biotransformation is enhanced, leading to a faster elimination at higher temperature. In line with this, we saw elimination rates of PHE in E. albidus increasing 5-fold from 5 to 25 °C (Figure 2). This has also been observed in other ectotherms such as crustaceans, with the elimination rate of pyrene in Gammarus setosus being higher at a higher temperature. In the present study, the $Q_{10}$ values for both uptake and elimination constants were in the range 1.6–3.0, which agrees with typical $Q_{10}$ values for metabolic rates in ectotherms.

The relationships between BAF values of PAHs in organisms and temperature are dependent on thermodynamic properties of the partitioning process and species’ lipid composition. Previous studies showed that BAFs of PAHs (including PHE) in aquatic organisms decreased with increasing temperature and that the partitioning process was predominantly enthalpy-driven. In the present study, we observed the opposite because the slope of the $k_e$-temperature relationship was lower than that of the $k_u$-temperature relationship. Thus, the BAF of PHE in E. albidus increased significantly with increasing temperature.

A previous study showed that the partitioning of hydrophobic endocrine disrupting chemicals in unsaturated and saturated liposomes is different and enthalpy gain drives the partitioning into unsaturated liposomes (i.e., biaccumulation decreases with temperature), whereas partitioning into saturated liposomes is predominantly entropy-driven. This

Table 2. Temperature Coefficient $Q_{10}$ Calculated Based on the Uptake ($k_u$) and the Elimination ($k_e$) Rate Constants for the Biaccumulation of Phenanthrene in Enchytraeus albidus Determined at Different Temperatures

<table>
<thead>
<tr>
<th>temperature difference (°C)</th>
<th>designed temperature step (°C−°C)</th>
<th>$Q_{10}$ based on $k_u$</th>
<th>$Q_{10}$ based on $k_e$</th>
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<tbody>
<tr>
<td>5</td>
<td>5–10</td>
<td>2.99</td>
<td>4.89</td>
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<tr>
<td>10</td>
<td>10–15</td>
<td>1.63</td>
<td>1.93</td>
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</tr>
<tr>
<td>20</td>
<td>5–25</td>
<td>1.82</td>
<td>2.50</td>
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suggests that changes in lipid composition can influence the partitioning of lipophilic chemicals in organisms. Furthermore, the proportion of unsaturated fatty acids in the lipids of earthworms increases with decreasing temperature. The proportion of saturated fatty acid in our test species, E. albidus (~18% saturated fatty acids) seems to be lower in the aquatic worm Lumbriculus variegatus (~33% saturated fatty acids), which was used by Muijs and Jonker. Although speculative, the somewhat different fatty acid compositions of these species could contribute to the discrepancy between our study (positive relationship between BAFs of PHE and temperature) and the study by Muijs and Jonker (negative relationship between BAFs of PHE and temperature). However, apart from effects of temperature, the fatty acid composition of an organism also depends on the lipid composition of the food, complicating their relationships. Further research is needed to clarify the significant of lipid composition of an organism for bioaccumulation of lipophilic contaminants.

The optimal temperature for the reproduction and growth in E. albidus is 15−22 °C, and the response of the metabolic activity of ectotherms follows an accelerating temperature− performance relationship below an optimal temperature. We confirmed our hypothesis that the uptake and elimination rate constants and BAFs of PHE in E. albidus were higher when exposed to fluctuating temperatures than when exposed to constant temperature of the same average. Our study demonstrates that the prediction of the effects at fluctuating temperature regimes (natural temperature settings) may be underestimated (by 21.2% on k_u, 18.7% on k_e, and 5.2% on BAF) when using the rates obtained at constant temperature without considering the temperature response curve of ectotherms. Thus, the nonlinearity of temperature effects on toxicokinetic parameters (k_u, k_e, and BAFs) lead to a greater increase during high temperature periods than the reduction occurring during lower temperature periods, resulting on average in higher parameter values.

Here, we have built a relationship between the bioaccumulation in E. albidus and temperature, which can be used to predict the uptake and elimination of PHE in these worms under naturally fluctuating temperature conditions in the field. Our study may be used as a basis for similar evaluations of temperature effects on BAF for other lipophilic compounds, not just PAHs.

In the light of realistic climatic conditions, one should also consider that elevated temperatures may enhance the evaporation of water from soil. These two factors, temperature and drought, may interact in ways that can influence terrestrial organisms. In addition, soil water content will likely influence the bioaccumulation of lipophilic compounds in worms and thus influence the amount possibly entering the food chain. Further studies on the combined effects of soil water content and temperature on the bioaccumulation of chemicals are needed for better understanding of the bioaccumulation under natural climate regimes and improve the current risk assessment of lipophilic compounds in soil invertebrates.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06182.


