BIOACCUMULATION OF HEXACHLOROBENZENE IN THE TERRESTRIAL ISOPOD
PORCELLIO SCABER

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Abstract: A test system to investigate the biomagnification of organic chemicals in the terrestrial isopod Porcellio scaber was developed and validated. Adult isopods were fed on alder leaf powder (Alnus glutinosa) spiked with [14C]hexachlorobenzene (HCB). Test animals, sampled regularly during the uptake (16 d) and depuration phases (16 d), were analyzed, and the kinetics of tissue concentrations were determined. Uptake (k1) and depuration rates (k2) were calculated to estimate kinetic biomagnification factors (BMFs). In addition, the effect of coprophagy on the uptake and accumulation of HCB as well as the tissue distribution of HCB in P. scaber was investigated. The test system was shown to be suitable for investigations into the terrestrial bioaccumulation of chemicals. Coprophagy had no effect on the bioaccumulation of HCB in P. scaber. The hepatopancreas was identified as the main target tissue for HCB accumulation. The low BMF of 0.057 resulted from an assimilation efficiency (α) of 31.42%, a low uptake rate k1 (0.009 d⁻¹), and a high depuration rate k2 (0.164 d⁻¹). The results indicate that the terrestrial bioaccumulation of organic chemicals in P. scaber might not represent a worst-case scenario for biomagnification, limiting the value of the test system for the regulatory assessment of organic chemicals. Environ Toxicol Chem 2016;35:2867–2873. © 2016 The Authors. Environmental Toxicology and Chemistry Published by Wiley Periodicals, Inc. on behalf of SETAC.

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INTRODUCTION

Evaluation of bioaccumulation data is essential for the determination of environmental risk of substances as part of the European Commission’s Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH, Annex XIII) program, which aims to identify persistent, bioaccumulative, and toxic substances, as well as substances with very persistent and very bioaccumulative properties. Assessment of the bioaccumulation potential of chemicals is usually based on biocentration factors (BCFs), which are determined in fish studies according to the Organisation for Economic Cooperation and Development (OECD), technical guidance document 305. The bioaccumulation potential of substances with very low water solubility (log Kow > 5, solubility below ~0.01–0.1 mg/L) can be determined with a dietary bioaccumulation test following the same guideline but resulting in a biomagnification factor (BMF) [1]. The only established test for bioaccumulation assessment on terrestrial animals is conducted with invertebrate oligochaetes according to OECD guidance document 317 [2]. In this test, oligochaetes are exposed to contaminated soil via multiple uptake routes including porewater, direct dermal contact, and ingestion of contaminated soil particles. Therefore, the test endpoint is defined as a bioaccumulation factor (BAF).

A terrestrial test system that considers only the dietary pathway would allow the calculation of terrestrial BMF estimates. The terrestrial isopod Porcellio scaber is the most studied terrestrial isopod [3] and seems to be a good candidate in this respect. Isopods play an important role in terrestrial ecosystems. As primary decomposers, they mobilize chemicals bound to soil and organic material, making them available for higher trophic levels. There are several predators that prey on isopods, such as shrews, toads, hedgehogs, slow-worms, and frogs [4]. Porcellio scaber has been used for metal toxicity testing of various elements such as zinc [5–7], cadmium [7,8], and cobalt [9], considering different lethal and sublethal endpoints. In addition, the toxic effects of nanoparticles like titanium dioxide [10–12] and different organic compounds have been investigated with P. scaber [13–15]. However, a standardized test protocol is still lacking. Studies have been commonly carried out with animals collected in the field, a procedure that does not guarantee standardized size and constant quality. The development of a laboratory culture procedure providing a sufficient supply of animals is recommended.

Porcellio scaber has also been used as test organism for metal bioaccumulation studies [16–18]. Metal storage in terrestrial isopods is located in S-type cells of the hepatopancreas [19], which makes them suitable candidates for studies on metal bioaccumulation. In contrast, research into the bioaccumulation of organic compounds in terrestrial isopods is rare [14,20–22]. Van Brummelen and van Straalen investigated the uptake and elimination of benzo[a]pyrene in P. scaber [21]. The toxicokinetics of lindane in field-collected Porcellionides pruinosus exposed via soil and plant diet were compared by Sousa et al. [22]. Lindane exposure via soil led to a much higher body burden in animals compared with exposure via food. This was confirmed by Vink et al. [23], who compared the food and exposure routes of different pesticides. In contrast, for isopods like P. scaber that live mostly on the soil surface, dietary uptake seems to be the dominant bioaccumulation pathway [22,24], making this species a potential candidate for regulatory biomagnification testing. Biomagnification assessment requires

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a dietary exposure test system that allows the determination of a BMF rather than a BAF. Therefore, a soil-free test system for biomagnification studies with laboratory-cultured *P. scaber* was developed and validated with hexachlorobenzene (HCB). Environmental pollution with HCB is a global problem [25]. Despite prohibition of HCB applied as a pesticide, chemical manufacturing, metal industries, and waste combustion still result in the introduction of HCB into the environment [26].

Apart from its relevance for the environment, HCB was used as a test substance because of its highly lipophilic characteristics (log *K*<sub>OW</sub> 5.73) [27] and its known potential to bioaccumulate in earthworms (*Eisenia fetida*) [28]. It is suspected to biomagnify through various trophic levels of the terrestrial food web. In higher trophic levels (birds, mammals), HCB seems to have a higher potential to biomagnify [29]. However, except for the earthworm, there is a lack of terrestrial invertebrate HCB accumulation data. Biomagnification factors were estimated for *P. scaber* and compared with biomagnification data obtained for earthworms.

**MATERIALS AND METHODS**

**Standards and reagents**

*Test chemicals.* Radiolabeled HCB with a specific activity of 0.92 MBq/mg and nonlabeled hexachlorobenzene (purity 99%) were purchased from Sigma-Aldrich.

*Solvents.* Acetone (purity ≥ 99.5%) and methanol (purity ≥ 99.9%) were purchased from Sigma-Aldrich; cyclohexane (purity ≥ 99.9%) and isopropanol (purity ≥ 99.5%) were from J.T. Baker. Oxyxolve C-400 (Zinsser Analytic) was used as scintillant for combustion.

**Porcellio scaber rearing**

*Porcellio scaber* were reared in groups of 5 males and 5 females in plastic containers (16 cm × 12 cm, 1 L) covered with perforated and transluscent lids allowing gas exchange and light incidence. The rearing containers were filled with a 0.5-cm layer of plaster cast material (Danogips), which could be moistened as necessary to guarantee adequate high air humidity (~90%). On top of the plaster, a thin layer (5 mm) of standard soil substrate consisting of 70% sand, 20% kaolin, and 10% humus was applied, which was prepared according to OECD guideline 207 [30]. The pH of the substrate was adjusted to pH 7 by addition of CaCO<sub>3</sub>. Thin plasterboards (3 cm × 5 cm) were placed directly on top of the soil to provide shelter for the isopods. The rearing temperature was 23 ± 2 °C, and the light cycle was set to a 16:8-h light: dark cycle (max. 604 lux, Ø 164 lux). The isopods were fed twice a week ad libitum with dried alder leaves (*Alnus glutinosa*), fresh salad (*Lactuca sativa*), and dry carrot powder (*Daucus carota* subsp. *Sativus*) and supplied with water.

**Preparation of experimental diets**

Experimental diets for the bioaccumulation studies were prepared by spiking powdered alder leaves with radiolabeled [14C]HCB. Alder leaves (*A. glutinosa*) were collected in the field near Schmallenberg, Germany, from an uncontaminated site, diced (for 48 h, at 50 °C), and ground in a mortar to powder. The [14C]HCB was dissolved in acetone and mixed with 10 g A. glutinosa powder, to reach a nominal HCB concentration of 100 mg/kg. The acetone was evaporated for 2 h under the fume hood. To confirm the [14C]HCB concentrations, aliquots of the prepared diet were analyzed by liquid scintillation counting after combustion. The kinetics of substance decay were determined over a 24-h period to test the stability of the experimental diet during feeding. The [14C]HCB-spiked alder leaf powder was transferred into test containers and kept for 24 h under conditions comparable with the BMF test but without animals. After 2 h, 4 h, 8 h, 12 h, and 24 h, food samples (*n* = 5) were removed from each container, combusted, and counted by liquid scintillation counting. A toxicity test was carried out prior to the bioaccumulation study with HCB to verify the test concentration to be applied during the main test.

**Test system and study design**

*Porcellio scaber* with a length > 0.5 cm and a weight of 24.9 ± 0.3 mg (mean ± standard error [SE]) were used as test organisms and were taken from the stock culture. To reduce the risk of unwanted reproduction during the test, only male isopods and female animals without marsupium (brood pouch) were selected. Plastic boxes (16 cm × 12 cm, 1 L) grouted with 0.5 cm of plaster cast material (Danogips) were used as test containers. The plaster was moistened daily with 1 mL of water to maintain the humidity in the test boxes during application at approximately 95%. A watch glass was placed on top of the plaster layer and used as a food reservoir during the experiment. An aluminum foil-coated Petri dish cover (Ø 5.8 cm), with an entrance hole on 1 side, was placed in each test box to provide shelter for the experimental animals. All boxes were covered with perforated translucent test box covers to allow gas exchange. Every test box was stocked with 5 isopods each.

During the first part of the experiment (uptake phase, 16 d) the animals were fed daily with spiked alder leaf powder. In the following elimination phase (16 d) the isopods were placed in new, uncontaminated test boxes and were fed with nonspiked alder leaf powder. Feed residues were removed and quantified daily to evaluate the feeding behavior in each group. A test group (replicate) had to consume more than 70% of the applied food during the uptake phase as well as during the depuration phase to be accepted for the evaluation. To minimize effects resulting from coprophagy, all feces were removed daily from the test boxes. To prevent saprophagy, dead animals were removed daily. The daily feed portion applied per replicate corresponded to 3% of the average isopod mass at the onset of the uptake phase. The feed rations were recalculated every 8 d. In case of mortality, the diet portions were adjusted accordingly. During the uptake and elimination phase, animals were collected to allow the measurement of tissue concentrations. The sampling schedule included 6 and 7 samplings for both phases. During the uptake phase the sampling of 4 replicates was carried out on days 1, 2, 4, 8, 12, and 16. Depuration phase samplings of 4 replicates took place after 6 h, 12 h, 24 h, and 48 h and on days 5, 10, and 16. For measurement of the animals’ lipid content, 16 isopods were sampled at the beginning of the test. For a second lipid content measurement, 4 additional samples with 4 animals each were collected at the end of the uptake phase. The weight of the collected animals was measured, and the samples were stored at −20 °C prior to analysis. The physical parameters, temperature, air humidity, and light intensity were measured at the beginning and end of the uptake phase and also at the end of the depuration phase. Experimental animals were kept under stable conditions. The average temperature during the HCB experiment was 23.3 °C, light intensity was between 20 lux and 41 lux, and air humidity was constant at approximately 96%.

In addition to the test animals that were fed the contaminated diets, 4 control groups were set up with 5 isopods each; the controls were fed pure alder leaf meal over the full experimental
period. In preliminary studies the toxic effects of solvent residues in the diets proved to be negative, and consequently the use of an independent solvent control was not necessary in the present study. The biomagnification study on HCB involved a total of 60 test boxes.

**Coprophagy test**

To determine the effect of coprophagy on the bioaccumulation of HCB, an additional feeding experiment was carried out. The test design and parameters were analogues to the biomagnification test with [14C]HCB. In total, 16 test groups were set up, including 5 isopods each having an average weight of 52.3 ± 1.2 mg (mean ± SE) and an age of approximately 6 mo. In 8 replicates, coprophagy was stopped by daily removal of feces from the test containers. In the other 8 replicates, feces remained in the test system until the end of the experiment. After 8 d and 12 d of exposure, 4 groups from each treatment were sampled. Feces removed during the test were collected daily. Feces in the other groups were collected at the end of the test. All collected animals and feces were weighed and frozen at −20 °C until analysis by liquid scintillation counting after combustion.

**Mass balance and tissue distribution of HCB**

A further experiment was carried out to explore the distribution of HCB in the test system as well as in the tissues of *P. scaber*. The test system and experimental conditions were in accordance with the BMF test described previously in the Test system and study design section. Two replicated test groups were stocked with 4 adult isopods each with an age of 11 mo and an average mean weight of 92 ± 0.7 mg (mean ± SE). The animals were fed *A. glutinosa* diet (daily feed portion corresponded to 3% of the average isopod mass) spiked with [14C]HCB. During the test feces were removed daily and frozen at −20 °C as separate samples. At the end of the exposure period lasting 7 d, the animals were removed from the tests containers, and their exoskeletons were cut with a scalpel between the head and first pereion segment. The head connected with the hepatopancreas was then removed by means of tweezers, and both parts were separated with a scalpel. A second cut through the exoskeleton was carried out behind the second pleonite. The pleon connected with the hindgut was dissected and separated. The 3 tissue fractions hepatopancreas, hindgut, and body (head, pleon, thorax, hemolymph) obtained from the replicates were directly combusted and analyzed by liquid scintillation counting as described below. For mass balance the layers of plaster cast in the test system were homogenized and analyzed by liquid scintillation counting after combustion.

**[14C]HCB analysis**

Animal, feces, plaster, and food samples were analyzed for [14C]HCB content by combustion followed by liquid scintillation counting. Frozen samples were combusted in a biological oxidizer (OX500, Zinsser) at 900 °C for 4 min in the presence of 335 cc/min O2 and 335 cc/min N2. Radiolabeled CO2 was trapped in scintillation liquid (Oxysolve C-400, Zinsser Analytic) and quantified by liquid scintillation counting (Tricarb TR/LL 2550, Packard Instruments).

**Lipid content measurement**

A slightly modified method for lipid extraction originally described by Smedes [31] was applied. Collected isopods were dried for 6 h at 40 °C. Pooled samples of dry isopods were transferred into test tubes and homogenized with 4.5 mL cyclohexan/isopropanol mix (5:4) by an Ultra-Turrax. Then the samples were vortexed and centrifuged for 12 min at 1650 rpm with 2.75 mL ultrapure water. The organic phase was transferred into preweighed glass vials. Then a solution of 87% cyclohexane and 13% isopropanol (2.5 mL) was added to the remaining aqueous phase. The test tubes were vortexed and centrifuged again. The organic phase was removed and pooled with the previously obtained fraction. The organic phases were evaporated to dryness under a flow of nitrogen. The samples were then further dried over night at 75 °C. The lipid content of the fresh isopods was calculated.

**Data analysis and statistics**

The biomagnification potential of HCB in *P. scaber* was determined in 3 different ways. A kinetic BMF_{kin1} (Equation 1) was determined according to OECD guideline 305 [1] based on ingestion rate (I), assimilation efficiency (α) (Equation 2), and the depuration rate constant k2. For the calculation of k2 a linear regression of ln (C_{isopod}) versus time was performed assuming 1-compartment kinetics of the test item. The slope of this regression was used as an estimate of the depuration rate constant k2.

\[
BMF_{kin1} = \frac{I \times \alpha}{k2}
\]

\[
\alpha = \frac{C_{0, isopod} \times k2}{I \times C_{food}} \times \frac{1}{1 - e^{-k2t}}
\]

where k2 represents the depuration rate constant (d⁻¹), I is the uptake rate constant (d⁻¹), α is the assimilation efficiency, I is the ingestion rate (g food/g isopod/d), C_{food} represents the concentration in food (µg/g), C_{isopod} is the concentration in isopod (µg/g), C_{isopod} is the concentration in isopod at time 0 of the depuration phase (µg/g), and t represents the duration of the uptake period (d).

The BMF_{kin1} was confirmed by an alternative kinetic approach BMF_{kin2} (Equation 3) using k1 and k2 and assuming that steady-state conditions have been reached at the end of the uptake period. The uptake rate constant (k1) was calculated by nonlinear regression analysis of the ratios C_{isopod}/C_{food} against time during the uptake phase using the depuration rate k2 fitted before.

\[
BMF_{kin2} = \frac{k1}{k2}
\]

Finally, tissue and feed concentrations were used to derive a steady-state biomagnification factor (BMF_{SS}; Equation 4) considering the constant isopod tissue concentrations measured at the last 3 sampling points of the uptake phase.

\[
BMF_{SS} = \frac{C_{isopod}}{C_{food}}
\]

Data were analyzed by t test and analysis of variance followed by Tukey’s post test using GraphPad Prism software v5.01. The kinetics of HCB in the experimental diet (decay) and animals collected during the bioaccumulation study were fitted by nonlinear regressions.

**RESULTS**

**Biomagnification study with HCB**

The measured HCB concentration in the spiked *Alnus glutinosa* powder (122.8 mg/kg) was slightly higher than the target concentration (100 mg/kg). The decay rate of [14C]HCB
concentration in food under experimental conditions was 0.88 d⁻¹ (Figure 1), equivalent to an HCB loss of 15.68% and 28.72% after 12 h and 24 h, respectively. A previously performed toxicity test showed no effects of the applied HCB concentration on _P. scaber_ (no-observed-effect concentration [NOEC] ≥ 100 mg/kg [nom.]). Food consumption was stable over the entire test period of 32 d. During the uptake and depuration phase, on average 95.6 ± 1.0% (mean ± SE) and 94.1 ± 2.1% of the applied food was consumed (Table 1). Two replicates did not reach the 70% threshold during the experimental period and were thus not considered in the statistical evaluation. The exclusion of the 2 groups reduced the number of replicates collected after 5 d and 16 d of the uptake period was 5.3 ± 0.8% and 3.1 ± 0.3% (based on wet weight), respectively. Two isopods in the test groups developed a marsupium during the present study. However, the single animals that had access to feces showed a slight trend toward higher HCB concentrations. The HCB concentrations in feces after 8 d were significantly lower (Figure 3B) when collected from groups kept with feces (6.70 ± 0.80 μg/g fresh wt) than without feces (10.08 ± 0.11 μg/g fresh wt). This was confirmed after 12 d, at which point HCB concentrations in feces of 9.91 ± 0.70 μg/g fresh weight (feces removed) and 6.33 ± 0.32 μg/g fresh weight (with feces) were determined.

### Coprophagy test

No mortality was observed in the coprophagy test in any replicate. The test animals consumed approximately 97% of the applied food. After 8 d of exposure there was no significant difference in HCB concentration in _P. scaber_ between replicates with (7.24 ± 0.68 μg/g fresh wt; mean ± SE) and without feces (7.33 ± 0.63 μg/g fresh wt; Figure 3A). Also after 12 d no significant differences between the groups with (10.12 ± 0.54 μg/g fresh wt) and without feces (8.51 ± 0.84 μg/g fresh wt) could be observed. However, the isopods that had access to feces showed a slight trend toward higher HCB concentrations.

### Mass balance and tissue distribution of HCB

The mass balance of [14C]HCB in the BMF test system is presented in Figure 4A. The distribution of [14C]HCB in the test system was 16 ± 3% (mean ± SE) animal, 19 ± 3% feces, and 10 ± 2% plaster. The remaining 55% represents the fraction of the test item that was obviously lost via the gas phase. No samplings after 2 d and 4 d showed a further increase of the tissue concentrations up to 2.08 ± 0.58 μg/g fresh weight and 3.26 ± 0.79 μg/g fresh weight, respectively (Figure 2A). The 3 remaining samplings at days 8, 12, and 16 of the uptake period showed no further significant increase in concentration (4.93 ± 1.86 μg/g fresh wt, 4.78 ± 0.43 μg/g fresh wt, and 4.97 ± 1.33 μg/g fresh wt), showing that a steady-state concentration had been reached. For calculation of BMF, an average concentration of HCB in the food of 103.55 mg/kg was assumed considering a loss of 15.68% HCB after 12 h (Figure 1). A BMFss of 0.047 was calculated (Table 2). Animals collected 6 h after the beginning of the depuration phase showed an average tissue concentration of 6.05 ± 2.25 μg/g fresh weight (Figure 2B). At the further samplings after 12 h, 24 h, and 48 h of depuration, a significant decrease in tissue concentrations to approximately 4 μg/g fresh weight was observed. After 5 d and 10 d of depuration, the tissue concentrations further decreased to 2.46 ± 1.36 μg/g fresh weight and 1.64 ± 1.10 μg/g fresh weight and finally reached a level of 0.29 ± 0.16 μg/g fresh weight at the last sampling after 16 d of depuration. Uptake and depuration rate constants of 0.009 d⁻¹ (k₁) and 0.164 d⁻¹ (k₂), respectively, were determined, which resulted in a BMFkin2 of 0.057. With the calculated ingestion rate I (2.7%) and assimilation efficiency α (31.42%), a BMFkin1 of 0.052 was calculated (Table 2).

### Plant food consumption

The isopods consumed 2.1% of the applied food was consumed (Table 1). Two isopods in the test groups developed a marsupium during the present study. However, the single animals that had access to feces showed a slight trend toward higher HCB concentrations. The HCB concentrations in feces after 8 d were significantly lower (Figure 3B) when collected from groups kept with feces (6.70 ± 0.80 μg/g fresh wt) than without feces (10.08 ± 0.11 μg/g fresh wt). This was confirmed after 12 d, at which point HCB concentrations in feces of 9.91 ± 0.70 μg/g fresh weight (feces removed) and 6.33 ± 0.32 μg/g fresh weight (with feces) were determined.

### Table 1. Number of isopods, mean weight at start of uptake phase, food consumed during uptake and depuration phases, and overall survival after hexachlorobenzene (HCB) exposure of _Porcellio scaber_

| Treatment | No. of isopods | Mean weight start uptake phase (mg/isopods) | Consumed food during uptake phase (%) | Consumed food during depuration phase (%) | Survival (%) |
|-----------|---------------|--------------------------------------------|--------------------------------------|-------------------------------------------|--------------|
| HCB       | 250           | 24.9 (±0.3)                                 | 95.6 (±1.0)                          | 94.1 (±2.1)                               | 96.4 (±1.2)  |
| Control   | 20            | 23.9 (±1.2)                                 | 99.0 (±0.6)                          | 92.0 (±4.9)                               | 95.0 (±5.0)  |

Values are presented as means (± standard error). The HCB and control values were compared using 1-tailed unpaired _t_ tests. There were no significant differences.
mortality was observed in the experiment. The experimental food was ingested almost completely (98%) by the test animals. The HCB content in *P. scaber* measured at the end of the study was distributed in the body (58%), hepatopancreas (32%), and hindgut (10%; Figure 4B). With respect to the specific organ wet weights, the average HCB concentrations in the different tissue fractions were calculated as: body 1.79 ± 0.41 mg/g fresh weight, hepatopancreas 16.62 ± 7.45 mg/g fresh weight, and hindgut 3.76 ± 0.67 mg/g fresh weight (Figure 4C).

**DISCUSSION**

The experimental diet spiked with HCB was readily accepted by the test animals and induced an immediate increase of tissue concentrations of the test chemical. Because it is a highly lipophilic compound (log $K_{OW}$ 5.31) [27], HCB leads to lipid-based bioaccumulation. The HCB concentrations in *P. scaber* reached steady-state conditions after approximately 7 d. However, the feeding study showed that HCB does not biomagnify in the test animals. The BMF estimates calculated in different ways are all well below the biomagnification threshold of 1. Basically, to reach the BMF threshold of 1, an animal had to consume food in an amount corresponding to 100% of their body weight while accumulating 100% of the applied substance. However, a low assimilation efficiency and rapid elimination of the test item obviously mitigated against the formation of significant tissue concentrations. The assimilation efficiency measured for HCB was 31.42%. Comparable assimilation efficiencies have been observed in dietary experiments with *P. scaber* fed benzo[a]pyrene-spiked poplar leaves, in which assimilation efficiencies of 33.0% [21] and 25.6% [15] were calculated. The similar assimilation efficiencies suggest that assimilation through the gut of *P. scaber* might be comparable even for substances with different chemical properties. However, this is in contradiction to the results of the feeding experiments with *Porcellionides pruinosus* presented by Sousa [22], in which animals exposed to

| Table 2. Kinetic parameter estimates in the experiment with hexachlorobenzene (HCB)* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| BMF$_{SS}$ | BMF$_{km1}$ | BMF$_{km2}$ | $k_1$ | $k_2$ | $\alpha$ | $I$ (%) |
| 0.0472 | 0.0574 | 0.0517 | 0.0094 | 0.1640 | 0.3142 | 2.7 |

*Parameters are the biomagnification factors (BMF$_{SS}$; BMF$_{km1}$; BMF$_{km2}$), depuration rate constant ($k_2$), uptake rate constant ($k_1$), assimilation efficiency ($\alpha$), and ingestion rate ($I$).
applied HCB and the HCB contents in isopods, feces, and plaster. (\(**\), \(\times\) variance followed by a Tukey test (**, \(\times\)) of HCB in the individual tissue fractions (\(\times\)). Data points are presented as means (± standard error). Tissue concentrations were compared by analysis of variance followed by a Tukey test (\(**\), \(p < 0.01\); \(***\), \(p < 0.001\)).

\([^{14}C]\)Y-hexachlorocyclohexane-spiked alder leaves showed an assimilation efficiency of only 17.7%.

Tissue concentrations measured in \(P. scaber\) might simply reflect the intestinal content of the animals. However, the tissue distribution test with \([^{14}C]\)HCB showed that the main part of HCB found in animals collected after 7 d of exposure was stored in the body (58%), in the hepatopancreas (32%), and only a lower proportion (10%) in the hindgut, clearly showing bioaccumulation of the test item. Comparable results were shown by van Brummelen et al. [21] for the distribution of benzo[a]pyrene in \(P. scaber\); 53% of the accumulated test item was found in the body (14% head + 39% remainder), 22% in the hepatopancreas, and 25% in the gut.

The elimination rate (\(k_2\)) for HCB was 0.164 d\(^{-1}\), which is similar to the \(k_2\) value of 0.10 d\(^{-1}\) estimated for \([^{14}C]\)Y-hexachlorocyclohexane in \(P. pruinosus\) [22]. However, a study on the bioaccumulation of benzo[a]pyrene in \(P. scaber\) showed a much higher elimination rate of 1.1 d\(^{-1}\) [21]. This high elimination rate might be explained by a high activity of detoxifying enzymes leading to an increased excretion of benzo[a]pyrene metabolites [21]. To rule out metabolic effects in the HCB test, thin-layer chromatography was carried out with extracts from animals exposed to \([^{14}C]\)HCB, to identify potential metabolites. As expected, the results showed that HCB was not metabolized by \(P. scaber\), which is in accordance with studies with other invertebrates like \(Tubifex tubifex\) [32]. Generally, there is only limited knowledge about the metabolism of xenobiotics in terrestrial isopods, and further studies elucidating the specific biodegradation pathways are required.

\(P. scaber\) has no urinary excretion mechanism [4].

The routes of substance elimination are limited to excretion of feces and exhalation of water vapor. The mass balance test with \(P. scaber\) showed that 16% of the radiolabeled test substance was found in the animals, 19% in the feces, and 10% in the plaster. As metabolism of HCB in \(P. scaber\) can be ruled out, the remaining part (55%) was obviously lost by evaporation from food and feces, or exhaled by the test organism. Whereas HCB has a relatively low vapor pressure, it is extremely volatile in the presence of high air humidity [33]. \(P. scaber\) breath through pseudotracheae, which are characterized by an extremely thin respiratory epithelium consisting of flat cells. The cells form a thin cytoplasmatic layer (0.5 µm) that separates the hemolymph from air [34,35]. In a study on the bioaccumulation of benzo[a]pyrene in terrestrial isopods by van Brummelen [21], it was shown that part of the test chemicals was transferred into the hemolymph. In the same way HCB may have reached the air tubes of the pseudotracheae in the present study. It cannot be ruled out that part of the assimilated substance was finally released into the atmosphere by diffusion through the respiratory epithelium.

Terrestrial bioaccumulation studies with earthworms resulted in a BAF for HCB of 2.33 [28]. However, the multiple potential uptake pathways in oligochaetes make the evaluation and comparison of dietary bioaccumulation impossible. Looking at the elimination of previously accumulated HCB, a depuration rate constant \(k_2\) of 0.16 d\(^{-1}\) was observed in earthworms, which is similar to that of \(P. scaber\) (0.164 d\(^{-1}\)). However, the uptake rate was much higher in earthworms (1.79 d\(^{-1}\)) [28] compared with isopods (0.0094 d\(^{-1}\)). This might be explained by the much greater food consumption of earthworms, with a daily ration equivalent of up to 125% of their own weight [36], compared with 3% consumed by isopods per day. Further exposure routes like skin uptake may also influence the assimilation of substances by earthworms [37].

Coprophagy may be an essential factor influencing the uptake of trace elements like copper in terrestrial isopods [38,39]. Preventing coprophagy, as in the present study by constant removal of feces, might potentially lead to a decrease in growth of \(P. scaber\). However, Kautz et al. [40] showed that there is no consistent evidence that \(P. scaber\) benefits from coprophagy. Their study showed that coprophagy will only be beneficial when the available diet is of low nutritional value. In the present study (coprophagy test with \([^{14}C]\)HCB), no significant effects on food consumption, growth, or survival were observed when coprophagy was stopped. The HCB concentration in the feces of the group in which feces were not removed was lower compared with that of the group whose feces were removed, indicating that ingestion of feces at least in part improved absorption of HCB from the feed. However, only a slight tendency to higher HCB concentrations in animals was observed following coprophagy. Because of the limited effect of coprophagy on the bioaccumulation of HCB in \(P. scaber\), it is obviously not necessary to remove feces during biomagnification tests.

The test system described in the present study requires a large set of test containers and test animals when a full kinetic approach including the estimation of uptake and elimination rates (BMF\(_{\text{kin1}}\)) or the calculation of a steady-state BMF (BMF\(_{\text{SS}}\)) are taken into consideration. However, similar BMF estimates were obtained in the present study independent of the calculation process applied. Aiming only for BMF\(_{\text{kin1}}\), which is based on ingestion rate (\(I\)), assimilation efficiency (\(\alpha\)), and the
degradation rate constant $k_2$, could reduce the amount of test animals by 50% because no samplings during the uptake phase are required. This could significantly facilitate the performance of a study without impairing the result.

CONCLUSIONS

The test system described in the present study allows investigation of the bioaccumulation of chemicals in terrestrial isopods considering the dietary pathway only. In general, assimilation efficiency in P. scaber seems to be too low and substance elimination too fast to result in a significant substance accumulation. The biomagnification test with P. scaber might thus not represent a worst-case scenario for assessing terrestrial bioaccumulation of organic chemicals, which would limit its value for regulatory use. However, with respect to the high potential of P. scaber as test organism for research into metal bioaccumulation, the test system described in the present study might have potential to be used for investigations on the bioaccumulation of metals and nanomaterials in the lower terrestrial food chain.

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Data availability—Essential data are presented in the manuscript. Raw data are available on request from the corresponding author (christian.schlechtriem@ime.fraunhofer.de).

REFERENCES

1. Organisation for Economic Co-operation and Development. 2012. Test 305: Bioaccumulation in fish: Aqueous and dietary exposure. OECD Guidelines for the Testing of Chemicals. Paris, France.

2. Organisation for Economic Co-operation and Development. 2010. Test 317: Bioaccumulation in terrestrial oligochaetes. OECD Guidelines for the Testing of Chemicals. Paris, France.

3. Drobne D. 1997. Terrestrial isopods—A good choice for toxicity testing of pollutants in the terrestrial environment. Environ Toxicol Chem 16:1159–1164.

4. Sutton S, Harding P, Burn D. 1972. The bioaccumulation of organochlorine contaminants in a terrestrial foodweb on the Niagara Peninsula, Ont. Arch Environ Contam Toxicol 1:95–105.

5. Donker MH, Abdel-Lateif HM, Khalil MA, Bayoumi BM, Van Gestel CA, Dewi I, Bedaux J. 1997. The importance of the exposure route when testing the toxicity of pesticides to saprotrrophic isopods. Environ Toxicol Chem 14:1225–1232.

6. Bibi A, Drobne D, Strus J, Byrne AR. 1997. Assimilation of zinc by Porcellio scaber (Isopoda, Crustacea) exposed to zinc. Bull Environ Contam Toxicol 58:814–821.

7. Christen O, Drobne D, Donnemman C-J, Doornekamp A, Van Der Pol JJ, Van Gestel CA, Bedaux J. 1994. Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. Environ Toxicol Chem 13:1781–1789.

8. Godet J-P, Demuynck S, Waterlot C, Lemiere S, Souty-Grosset C, Scheiffer R, Douay F, Leprêtre A, Prouvo C. 2011. Growth and metal accumulation in Porcellio scaber exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicol Environ S 74:451–458.

9. Prosi F, Storch V, Janssen HH. 1983. Small cells in the midgut glands of the terrestrial isopod Porcellio scaber: Sites of heavy metal accumulation. Zoomorphology 102:53–64.

10. Santos SA, Sousa JP, Frost M, Soares AM. 2003. Time-dependent toxicokinetics of [14C]lindane in the terrestrial isopod Porcellionides pruinosus. Environ Toxicol Chem 22:2221–2227.

11. Van Brummen T, Van Stralen N. 1996. Uptake and elimination of benzo[a]pyrene in the terrestrial isopod Porcellio scaber. Arch Environ Contam Toxicol 31:277–285.

12. Sousa JP, Loureiro S, Pieper S, Frost M, Kratz W, Nogueira AJ, Soares AM. 2000. Soil and plant diet exposure routes and toxicokinetics of lindane in a terrestrial isopod. Environ Toxicol Chem 19:2557–2563.

13. Van Brummen T, Van Gestel CA, Dewi I, Bedaux J. 1997. Assimilation and growth efficiency in the terrestrial isopod Porcellio scaber. Environ Toxicol Chem 16:1159–1164.

14. Novak S, Drobne D, Valant J, Pelicon P. 2006. Stoffbericht tubificid sludgeworms (Oligochaeta) under standardised laboratory conditions. Environ Toxicol Chem 25:1718–1724.

15. van Stralen NM, Verweij RA. 1991. Effects of benzo(a)pyrene on food assimilation and growth efficiency in Porcellio scaber (Isopoda). Bull Environ Contam Toxicol 46:134–140.

16. Drobne D, Strus J, Byrne AR. 1997. Assimilation of zinc by Porcellio scaber (Isopoda, Crustacea) exposed to zinc. Bull Environ Contam Toxicol 58:814–821.

17. Christen O, Drobne D, Donnemman C-J, Doornekamp A, Van Der Pol JJ, Van Gestel CA, Bedaux J. 1994. Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. Environ Toxicol Chem 13:1781–1789.

18. Godet J-P, Demuynck S, Waterlot C, Lemiere S, Souty-Grosset C, Scheiffer R, Douay F, Leprêtre A, Prouvo C. 2011. Growth and metal accumulation in Porcellio scaber exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicol Environ S 74:451–458.

19. Prosi F, Storch V, Janssen HH. 1983. Small cells in the midgut glands of the terrestrial isopod Porcellio scaber: Sites of heavy metal accumulation. Zoomorphology 102:53–64.

20. Santos SA, Sousa JP, Frost M, Soares AM. 2003. Time-dependent toxicokinetics of [14C]lindane in the terrestrial isopod Porcellionides pruinosus. Environ Toxicol Chem 22:2221–2227.

21. Van Brummen T, Van Stralen N. 1996. Uptake and elimination of benzo[a]pyrene in the terrestrial isopod Porcellio scaber. Arch Environ Contam Toxicol 31:277–285.

22. Sousa JP, Loureiro S, Pieper S, Frost M, Kratz W, Nogueira AJ, Soares AM. 2000. Soil and plant diet exposure routes and toxicokinetics of lindane in a terrestrial isopod. Environ Toxicol Chem 19:2557–2563.

23. Van Brummen T, Van Gestel CA, Dewi I, Bedaux J. 1997. Assimilation and growth efficiency in the terrestrial isopod Porcellio scaber. Environ Toxicol Chem 16:1159–1164.

24. Bibi A, Drobne D, Strus J, Byrne AR. 1997. Assimilation of zinc by Porcellio scaber (Isopoda, Crustacea) exposed to zinc. Bull Environ Contam Toxicol 58:814–821.

25. Christen O, Drobne D, Donnemman C-J, Doornekamp A, Van Der Pol JJ, Van Gestel CA, Bedaux J. 1994. Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. Environ Toxicol Chem 13:1781–1789.

26. Godet J-P, Demuynck S, Waterlot C, Lemiere S, Souty-Grosset C, Scheiffer R, Douay F, Leprêtre A, Prouvo C. 2011. Growth and metal accumulation in Porcellio scaber exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicol Environ S 74:451–458.

27. Prosi F, Storch V, Janssen HH. 1983. Small cells in the midgut glands of the terrestrial isopod Porcellio scaber: Sites of heavy metal accumulation. Zoomorphology 102:53–64.

28. Santos SA, Sousa JP, Frost M, Soares AM. 2003. Time-dependent toxicokinetics of [14C]lindane in the terrestrial isopod Porcellionides pruinosus. Environ Toxicol Chem 22:2221–2227.

29. Van Brummen T, Van Stralen N. 1996. Uptake and elimination of benzo[a]pyrene in the terrestrial isopod Porcellio scaber. Arch Environ Contam Toxicol 31:277–285.