



Assimilation Efficiency and Toxicokinetics of ^{14}C -lindane in the Terrestrial Isopod *Porcellionides pruinosus*: The Role of Isopods in Degradation of Persistent Soil Pollutants

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Abstract. An achievable way to evaluate the bioavailability of a certain toxic in the environment is to measure the concentration inside soil organisms. Non-target saprotrophic organisms like isopods are often exposed to agrochemicals or other kind of persistent chemicals. In this study the isopod *Porcellionides pruinosus* was exposed to a constant concentration of Lindane (γ -HCH) via food. Using toxicokinetic models the bioaccumulation and fate of the pesticide by isopods was assessed and compared with previous studies, where an unexpected decrease in γ -HCH concentration was observed. Animal body burdens showed higher values, and a lower assimilation rate constant, although the elimination rate constant was twice the value previously observed. It was also observed that a significant amount of γ -HCH had an unknown fate. To discover its possible destiny, a factorial experiment was carried out using two types of CO_2 traps and contaminated leaves in the presence and absence of isopods. It was concluded that isopod activity might have been responsible for a more rapid biotransformation of γ -HCH in leaves, since the amount of the pesticide is reduced in their presence.

Keywords: assimilation efficiency; toxicokinetics; soil contamination; isopods; lindane; *Porcellionides pruinosus*

Introduction

The study of the uptake and elimination of chemicals by edaphic species and the concomitant increase of the interior body concentration of the organisms have been recommended as tools to explain the distribution of pollutants in soils (Moriarty and Walker,

1987; Van Straalen, 1996). These approaches are particularly helpful in the case of potentially persistent chemicals like pesticides and heavy metals, which can be retained by organisms in proportion to their environmental concentration. Persistent organic pollutants are characterized by their stability and resistance to degradation processes in the environment, and their tendency to partition in fats and to accumulate in food chains. However they possess a range of physico-chemical properties that will lead to a partitioning between the gas and particle phases in the atmosphere, and between the air

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compartment and the surface compartments of soil, vegetation and pore water. This will provide them environmental mobility (Jones, 1998). Toxicokinetic models have been useful to describe changes in internal body concentrations of chemicals in aquatic and terrestrial organisms. In one-compartment models, animals fed with contaminated food, or exposed to a contaminated substrate, have an internal body concentration of the pollutant that will increase until equilibrium between uptake and elimination is reached; the equilibrium will be directly proportional to the exposure concentration (Van Straalen, 1996).

Terrestrial isopods play an important role in the decomposition processes and nutrient availability cycling in soil, affecting matter and energy flow through ecosystems (Drobne, 1997). They are usually chosen as model organisms because they are common, widespread and often numerous inhabitants of the leaf litter layer. Within organic matter consumption and microbial activity stimulation, they are primary consumers alongside with millipedes and earthworms (Bayley, 1995; Drobne, 1997). Isopods respond to elevated concentrations of chemicals in their food or substrate, but only a few of these responses can be used as toxicological endpoints. The most suitable are changes in reproduction strategies, food consumption, moulting and bioaccumulation processes. These are the reasons why isopods have gained some importance in the evaluation of the effects of pollutants in the edaphic system, being sensitive to external factors such as xenobiotics and their ability to bioaccumulate them to quite high concentrations. The woodlouse species *Porcellionides pruinosus* was chosen for this study because it has already been used in several ecotoxicological tests such as studies of the bioaccumulation of pesticides (Sousa et al., 2000; Vink et al., 1995; Vink and Van Straalen, 1999) and is one of the species that are in more straight contact with soil particles.

The present study has two major goals. The first is to study the uptake and elimination kinetics of hexachlorocyclohexane (γ -HCH) in the terrestrial isopod *Porcellionides pruinosus*, when exposed to a constant concentration of γ -HCH in food. Chemical degradation over time was evaluated and associated to several factors such as sorption, volatilization or an increased microbial activity by isopods. The second aim of this study is to analyse the effect of isopods on the fate of γ -HCH, mainly on the pesticide

degradation. To achieve this, a factorial experiment, using two types of CO₂ traps, was carried out.

Various scientific and international regulatory organizations usually demonstrate interest on various chemicals such as lindane (γ -HCH). This insecticide was one of the pesticides studied by Klepper and Meent (Lock et al., 2002) that caused the largest potentially affected fraction (PAF), the fraction of species exposed above their no observed effects concentration (NOEC), besides its persistence and bioaccumulation character. The toxicity of γ -HCH, and specially its bioaccumulation, to soil invertebrates has rarely been assessed, although its effects on aquatic organisms and terrestrial vertebrates are quite well documented. Although its use is officially forbidden in several countries, the γ isomer of hexachlorocyclohexane is still in use in some Mediterranean countries, such as Portugal and Spain.

Materials and methods

Two experiments were conducted to study the bioaccumulation and fate of γ -HCH in the terrestrial isopod *Porcellionides pruinosus* Brandt, 1833. Animals were exposed to contaminated food in a plastic box filled with a thin layer of plaster of Paris and activated charcoal (8:1). In the first experiment (Exp. 1) alder leaves were contaminated every four days with γ -HCH (0.2 μ g/g soil) to maintain a constant concentration of the insecticide and then administered to isopods. In the second experiment, isopods were also exposed to contaminated food but only fed in the beginning of the experiment (Exp. 2). Two different types of CO₂ traps were also added in each box and were constituted of a solution of sodium hydroxide (NaOH 30%) or soaked filter paper with the same solution and used to evaluate the loss of pesticide by volatilization and microbial activity. Some boxes containing only contaminated leaves were kept throughout the experiment with both CO₂ traps.

Test organisms and test chemical

Isopods were hand collected from an unpolluted horse manure heap at the Agriculture School of Coimbra (Escola Superior Agrária de Coimbra, Portugal). Collected animals were transported to the laboratory and maintained in culture boxes

(rectangular plastic boxes 350 mm × 240 mm) with natural soil and manure also collected from the Agricultural School neighbourhood. They were kept in a climatic chamber at $21 \pm 2^\circ\text{C}$ and 30–40% humidity, with a 16:8 h (light:dark) photoperiod. One day before starting the experiments, animals were selected by weight (15–25 mg) and kept singly in a plastic box, covered with a thin layer of plaster of Paris, to empty their guts. Sexes were not distinguished.

In the two experiments [^{14}C]- γ -HCH (Internationale Isotope Munich, Germany) was used as test chemical. The radio-chemical purity (>99.5%) was examined by Thin Layer Chromatography. In all the experiments only labelled compound was used (specific activity of 3.82 MBq * mg γ -HCH).

Chemical application in alder leaves

Alder leaves were selected as food for all experiments. Green leaves were collected from trees in the Baixo Mondego region and were air dried and stored at $21 \pm 2^\circ\text{C}$ till lindane application. Leaf portions (± 25 mg dry weight) were rewetted with distilled water before contamination. γ -HCH was applied directly, in a form of acetone solution, to each portion of alder leaves; after solvent evaporation they were fed to the isopods. In the first experiment, leaves were contaminated every four days, just before being used; in the second experiment, contamination was made only in the beginning of the exposure. A non-lethal concentration of 0.2 μg lindane/g leaf dry weight was chosen. The homogeneity of leaf inoculation was assessed with the contamination of extra portions of leaves, which were contaminated at the same time as those used in the experiment, and then stored at -20°C before analysis.

Experimental design

Exp. 1—A total of 50 animals were used in this experiment. Each animal was exposed to contaminated food individually in a plastic box (8 cm diameter and 4.5 cm high) filled with a thin layer of plaster of Paris as substrate. The first phase of the experiment (uptake phase) lasted 21 days; animals were exposed to a new set of contaminated leaves every four days. At the end of each four-day period, a random sample of five replicates was removed from

the experiment; the remaining animals were then fed with a fresh portion of contaminated leaves to maintain a relatively constant concentration of the test chemical. To prevent coprophagy, faeces from all animals were also removed every four days to an individual vial. All the times observations comprised of animals, leaves and faeces samples. Leaves and faeces were dried for 48 h at 60°C , weighed and frozen at -20°C until further analysis. Isopods were also preserved at -20°C before analysis. After the first 21 days all animals left in the experiment were transferred to clean boxes without food, to empty their gut, for one day and then they were fed with uncontaminated leaves for a further period of 21 days (elimination phase). During this period the previous sampling procedure was used (Table 1).

Exp. 2—In this experiment two types of CO_2 traps were used: one containing a sodium hydroxide solution (NaOH 30%) and the other a filter paper soaked in the same solution. An Eppendorff vial attached to the inner side of the plastic box was used as trap container. Each set composed of one trap type was divided in two more sets: one containing only contaminated leaves and the other containing isopods in the presence of contaminated leaves (Table 1).

All groups started with 12 replicates and the experiment lasted for 12 days, a period of time previously found to be enough to achieve an equilibrium between assimilation and elimination of γ -HCH by the isopods (Sousa et al., 2000). Every four days four samples were collected; each sample was composed of one animal (if present), the faeces, the leaves portion, and the CO_2 trap. Every sampling time faeces from nonselected isopods were collected to an individual vial, preventing coprophagy. Simultaneously, CO_2 traps were replaced for new ones. The faeces and contaminated leaves were dried for 48 h at 60°C , weighed and frozen at -20°C till analysis was made. The CO_2 traps and isopods were also frozen prior to analysis.

[^{14}C]- γ -HCH analysis

Animals, leaves, faeces and filter paper from the CO_2 traps were all analysed for [^{14}C]- γ -HCH contents using a combustion method prior to scintillation counting. Samples were burned in an oxidizer (OX 500 Harvey Instrument, Harvey Instruments, Buffalo, NY, USA) at 900°C in the presence of a

Table 1. Experimental setup of the two experiments.

	Exp. 1	Exp. 2			
		Pt	Pt Isop	Lt	Lt Isop
Exposure route	Food (alder leaves) (every 4 days new contaminated food supplied)	Food (alder leaves)		Food (alder leaves)	
Nominal concentration	0.2 µg/g leaf	0.2 µg/g leaf (starting concentration)		0.2 µg/g leaf	
Real concentration	Highest value—0.1257 µg/g leaf Lowest value—0.0421 µg/g leaf (during the all 4 day periods)	Highest value—0.150 µg/g leaf Lowest value—0.103 µg/g leaf			
Test duration	42 days (21 uptake + 21 elimination)	12 days (12 uptake)		12 days (12 uptake)	
Isopods CO ₂ trapping	50 —	0 Filter paper soaked with NaOH (30%)	12	0 Liquid solution of NaOH (30%)	12

Pt: Filter paper trap, absence of isopods; Lt: liquid solution trap, absence of isopods; Pt Isop: Filter paper trap, presence of isopods; Lt Isop: Liquid solution trap, presence of isopods).

mixture of O₂ and N₂. The labelled CO₂ formed was trapped in a scintillation vial containing scintillation liquid (Zinsser Oxysolve 400, Wheaton Science Products, Millville, NJ, USA). A cocktail of scintillation liquids (Ethylenglycolmonomethylether, High flash point LSC cocktail for aqueous/non-aqueous samples and Lumagel Safe-Advanced Safety LCS Cocktail) was added to the NaOH solution from traps of Exp. 2 to prevent crystallization. The amount of ¹⁴C present was measured using a scintillation counter (Bechman LS 6500, Bechman Instruments, Fullerton, CA, USA) for a maximum period of 20 min. The results were obtained in Bq and then converted to γ-HCH (pg) using the specific activity values (3.82 MBq/mg γ-HCH). Blanks, with scintillation liquid only, received the same treatment as the samples and the obtained radioactivity was subtracted from each sample value.

Kinetic models

In Exp. 1, the uptake and elimination kinetics of the toxicant in the animals was described using a one-compartment model. The estimation of the assimilation rate (*a*) and elimination rate constant (*k*) in the uptake and elimination phases was made separately resulting in a better fit to the experimental data.

The uptake phase was modelled using the following equation (Widianarko and Van Straalen, 1996):

$$Q_t = a/k * (1 - e^{-k*t}) \quad (1)$$

where *Q_t* is the internal body burden at time *t* (pg), *a* the assimilation rate (pg/animal/day), *k* the elimination constant (/day) and *t* the time (days).

In the elimination phase the model used the following equation:

$$Q_t = C * e^{-k_2*t} \quad (2)$$

where *Q_t* is the internal body burden at time *t* (pg), *C* the initial internal body burden (pg), *k₂* the elimination constant (/day) and *t* the time (days).

Individual body burdens were used to fit all kinetic models. Parameters were estimated using a Non-Linear Regression method (StatSoft, 1997).

Pesticide decay and feeding parameters

The percentage of the pesticide decay on the leaves was calculated by dividing the difference between the initial and final concentrations by the initial concentration of the leaves, times 100. This value was calculated for every four-day period.

Consumption and assimilation rates and food assimilation efficiency (all calculated on a dry weight basis) were chosen as feeding parameters in the first experiment (Exp. 1). The γ-HCH consumption was calculated by multiplying the mean concentration in the food (based on measured values at the end of each of the four-day period) by the amount of food consumed; assimilation rate of γ-HCH was obtained by fitting individual body burdens to the kinetic model as previously stated (Equation 1). The γ-HCH

assimilation efficiency was calculated by dividing the assimilation rate obtained from Equation 2 by the consumption rate of γ -HCH.

Results

Experiment 1

Some variability associated with the inoculation procedure was observed in all the inoculation periods (coefficient of variation values between 48.8% and 16.9%). Moreover, measured concentrations of lindane were about half the nominal concentration used vary significantly during most of the four-day period.

The γ -HCH decay was also highly variable within each four-day period, since three periods exhibit decays lower than 5% and the other two periods (day 1 to 4 and day 8 to 12) showed a decay in the amount of γ -HCH of 53% and 45%, respectively.

Throughout the 21 days of the uptake phase, the food consumption rate remained constant, with the exception of the period between the 12th and the 16th day where a significant increase was observed (One-way ANOVA; $F_{4,20} = 4.573$; $p = 0.009$). During the first four-day period, isopods started ingesting the plaster that was used as a substrate. Faecal pellets from this ingestion were separated from the other faecal pellets and the amount of ^{14}C was also measured. The plaster faeces production showed a tendency to increase reaching a peak from day 12 till day 16 with a slight decline thereafter.

During exposure, animal body burdens showed a minimum value of 21.6 pg of γ -HCH observed in the fourth day and a maximum value of 97.1 pg on day 16 during this period (Fig. 1). Internal body burdens reached a peak after 16 days of exposure, with a mean value of 79.8 ± 14.3 pg/animal but declined to a mean value of 49.1 ± 10.5 pg/animal at the end of the period. According to the one-compartment model, when animals are in contact with contaminated food or substrate the concentration of the chemical in the body will increase until equilibrium is reached between the uptake and elimination (Van Straalen, 1996), as it was found in this experiment between day 12 and 16. The value obtained from this equilibrium is equal to 63.4 pg/animal, calculated as a/k (Table 2). The γ -HCH consumption rate tends to decrease over

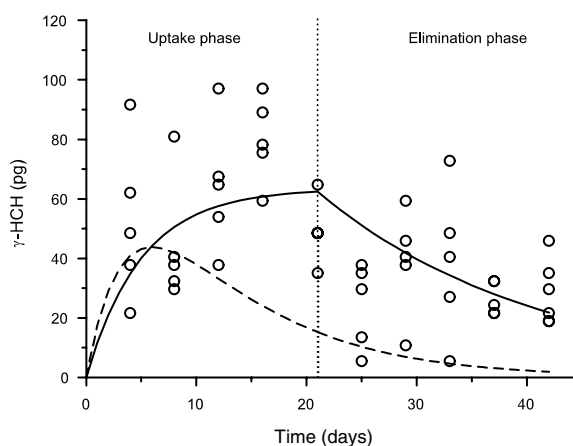


Figure 1. Uptake and elimination of [^{14}C] γ -HCH in the terrestrial isopod *Porcellionides pruinosus* exposed to contaminated food. The line (—) was obtained in Experiment 1 (Equations 1 and 2), using individual body burdens (pg). The dashed line (---) is the curve obtained from the study of Sousa et al. (2000) with a nonconstant concentration of γ -HCH.

time. During the first four days it had a mean value of 138.8 pg/animal/day and between day 12 and 16 reached 89.3 pg/animal/day. In the last four-day period (day 16 till day 21) γ -HCH consumption rate decreased to 42.1 pg/animal/day. The mean value of the γ -HCH assimilation efficiency was $17.5 \pm 9.6\%$ (mean \pm SD). The amount of γ -HCH present in the faeces increased significantly from day 12 to 21 (Kruskal–Wallis One Way Analysis of Variance on Ranks; $H = 14.073$ with four degrees of freedom; $p = 0.007$). The plaster faeces followed the same trend, increasing significantly after the eighth day (One Way ANOVA; $F_{4,20} = 9.653$; $p < 0.001$). Nevertheless, a significant amount of ^{14}C - γ -HCH (in a range between 10% and 20% of the total) still had an unknown fate.

The uptake phase and the elimination phase were treated separately, so body burden values of the elimination phase were fitted using Equation 2 (Fig. 1). Upon elimination, the depuration rate constant (k_2), also called elimination rate constant, had a lower value when compared to the one obtained in the uptake period: 0.05/day. The amount of γ -HCH in isopods presented a higher decrease in the first seven days in the presence of uncontaminated food, reaching a mean value of 24.8 pg/animal at the end of the experiment.

Table 2. Parameters measured in this study and in Sousa et al., 2000

Experiment	Food			Lindane (γ -HCH)								
	Uptake phase			Elimination phase			Kinetics		Uptake phase		Ending body-burdens	
	Consumption rate (mg/day)	Assimilation rate (mg/day)	Assimilation efficiency (%)	Consumption rate (mg/day)	Assimilation rate (mg/day)	Assimilation efficiency (%)	a (pg/animal/day)	k /day	Consumption rate (pg/animal/day)	Assimilation efficiency (%)	Uptake phase (pg/animal)	Elimination phase (pg/animal)
Exp. 1	1.33 \pm 0.5	1.14 \pm 0.3	86.49 \pm 9.4	0.83 \pm 0.3	0.69 \pm 0.3	80.9 \pm 19.25	12.67	0.2	141.99 \pm 43.7	17.46 \pm 9.6	49.07	24.81
Sousa et al.	1.42 \pm 0.5	0.84 \pm 0.4	59.4 \pm 18.0	2.71 \pm 1.4	2.17 \pm 1.5	75.6 \pm 21.2	20.66	0.1	116.80	17.7	15.40	0.22

Experiments 2—paper trap and liquid trap

With both types of CO₂ traps, when animals were present the concentration of the pesticide on the leaves showed a higher decay rate when compared with the experiment where animals were absent (Fig. 2). This was also reflected in the percentage of pesticide that decayed in both situations, with the presence of animals inducing a higher decay of the chemical when compared to those trials where animals were absent (Table 3).

Food consumption rate decreased during the experiment, as well as the assimilation rate and assimilation efficiency. Faeces production presented a significant increase after the fourth day of the experiment (Kruskal–Wallis one way analysis of variance on ranks; $H = 11.796$ with two degrees of

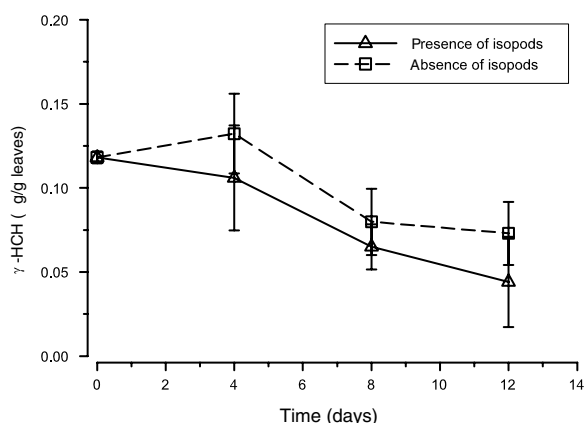


Figure 2. Decrease in the concentration of γ -HCH present on the leaves during the 12 days of Experiment 2 (pg/mg leaves), in the presence and absence of isopods (mean \pm 95% confidence limits).

Table 3. Decay parameters used to study the pesticide unknown fate

Experiment 2 (12 days)	% ¹⁴ C decay in leaves ^a	% ¹⁴ C trapped (cumulative) ^b	k_0 (/day)
Filter paper trap			
Pt	44.37	15.53	0.05
Pt Isop	64.57	14.38	0.11
Liquid solution trap			
Lt	49.66	21.14	0.056
Lt Isop	69.29	17.39	0.1

^a(initial concentration – final concentration)/initial concentration; ^b(¹⁴C-CO₂ in trap/¹⁴C in total leaves) *100; (–) not determined; k_0 —decay rate (/day).

freedom; $p = 0.003$). No plaster faeces were produced by the isopods in this experiment.

During this experiment it was observed that the liquid trap seemed to be more efficient in trapping CO₂ when compared to the paper trap (three-way ANOVA: $F_{1,36} = 156.87$; $p < 0.001$). Moreover, significant interaction was found between the factor Isopods (presence or absence) and the observation period (4, 8 and 12 days) (three-way ANOVA: $F_{2,36} = 5.83$; $p = 0.006$). *Post hoc* comparisons using the Tukey test between combinations of these factors showed that the amount of CO₂ trapped varied between the different time periods although no significant changes were observed when the isopods were present. The exception was day 12 where the values measured in the presence of isopods were different from those when animals were absent (Fig. 3). During the 12 days, we still found an

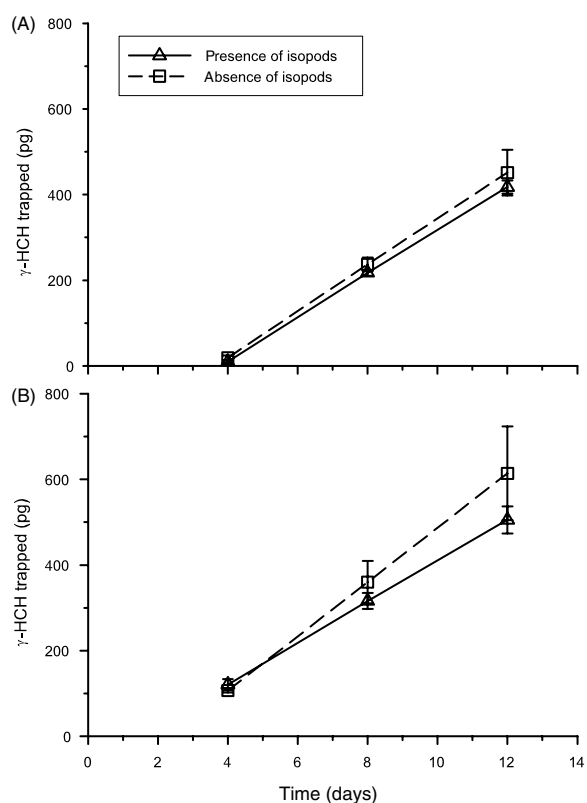


Figure 3. Amount of ¹⁴C-CO₂ trapped (pg) in the filter paper (A) and liquid solution (B) traps used during 12 days in Experiment 2, in the presence and absence of isopods (mean \pm 95% confidence limits).

interval between 10% and 30% of ^{14}C - γ -HCH whose fate was unknown.

Discussion

The amount of γ -HCH present in the leaves is difficult to maintain constant if the concentration is not controlled during the test period. Although we tried to control it, in the first experiment we observed a significant decay in some four-day period (>5%). Previous work (Vink and Van Straalen, 1999) also found that low concentrations of pesticides are easily degraded when compared to higher concentrations.

Furthermore the inoculation procedure could also be improved to decrease contamination variability and to approximate the real concentrations to the nominal concentration.

Comparing constant with nonconstant exposure situation

Previous studies (Sousa et al., 2000) with isopods of the same species (*Porcellionides pruinosus*) were carried out at the same concentration of γ -HCH used in this study. However, no attempt was made to keep the concentration of γ -HCH constant. As a result the concentration of pesticide decreased rapidly during the first four days of exposure and the internal body burdens reached their peak with the mean value of 43.8 pg/animal on the seventh day of exposure decreasing till day 21, following the decrease of HCH in the leaves. In the first experiment of this study, internal body burdens of isopods reached their highest values after 16 days (mean value of 75.5 ± 12.3 pg/animal) (Fig. 1) and the equilibrium between the assimilation and elimination was achieved mainly due to the maintenance of a relatively constant concentration of HCH on the leaves, representing the maximum level that is expected and giving a particular level of exposure. These differences between experiments were likely due to the different external concentration in the two experiments. The contrast found between body burdens at the end of the uptake and elimination phases (Table 2), when comparing Exp. 1 with the study of Sousa et al. (2000), was also expected. The values obtained at the end of the experiment may indicate that 21 days were an insufficient period for a complete elimination of the compound.

Surprisingly, the assimilation rate (a) had a lower value, and the elimination rate constant was twice the values obtained by Sousa et al. (2000) (Table 2). The exposure to a constant concentration of γ -HCH probably lead to a higher excretion strategy so that the pesticide level inside isopods did not reach the lethal level.

γ -HCH consumption rates and assimilation efficiency showed significant variability, as in Sousa et al. (2000). The mean value for the γ -HCH assimilation efficiency presented a similar value of $\pm 17\%$ in both experiments. During the uptake period, the food consumption and food assimilation rates were similar in both experiments (nonconstant at a constant concentration). However, the food assimilation efficiency showed higher values in the experiment where the pesticide concentration was maintained constant (Table 2).

Although some of these parameters were similar in both experiments during the uptake phase, the same did not happen during the elimination phase. Contrary to what Sousa et al. (2000) found, in this study and during the elimination phase food consumption and assimilation rates showed a significant decrease when compared to the first 21 days of exposure. During both uptake and elimination phase assimilation efficiency showed a similar range of values, although the values observed by Sousa et al. (2000) showed a significant increase during elimination. When animals were transferred to uncontaminated food, an unexpected decrease in food consumption was observed. This could be explained by a possible inhibition of the digestive processes by the constant concentration of the pesticide during the uptake phase (De Knecht et al., 2001) or by the damage of the olfactory receptors (Zimmer et al., 1996) during the exposure period, that should have detected the absence of pollutants in the last 21 days.

Between day 8 and 12 the decrease of the pesticide concentration on the leaves was followed by an increase of the ingestion rate in the following period. This was probably the result of the isopods perceiving a reduction in toxic concentration. Body burdens reached their highest values between day 12 and 16, due to the increase of leaf consumption and an associated decrease in plaster ingestion. This shows that isopods switched to plaster associated fungi and bacteria when the quality of leaf material deteriorates, e.g. in the presence of high levels of toxicants. However, as leaf quality improves they switch back

to leaf ingestion. The existence of ^{14}C in the plaster faecal pellets indicates that the plaster had some contamination from being in direct contact with contaminated leaves. Even though its ingestion could have been due to bacteria and fungi colonization, known to be palatable to isopods. Alternatively, the ingested plaster could have been used as an elimination vehicle.

Experiment 2

The comparison of γ -HCH decay in leaves in the presence and absence of isopods showed that isopods are likely to induce a more rapid degradation of the pesticide. This fact might prove that these edaphic animals can play an important role on soil processes for degrading chemicals, as it was shown for other edaphic organisms like earthworms, enchytraids and mites (Haimi, 2000). By grazing on fungi and releasing in their faecal pellets considerable amounts of bacteria that are present in their guts, isopods can increase the metabolic activity of soil microbes, enhancing the degradation of the chemical compounds that exist in the environment.

When comparing the two kinds of traps used in this experiment (Fig. 3), it was observed that a significantly higher amount of ^{14}C was trapped by the NaOH liquid than by the filter paper soaked with the same solution every four-day period (two-way ANOVA; $F_{1,42} = 97.023$; $p < 0.001$). However a significant percentage of γ -HCH remained with an unknown destiny. This may be explained by the transfer of the pesticide to the plaster used as substrate in the test boxes, pesticide volatilization, or the over saturation of the NaOH solution.

Conclusions

When organic compounds are used in ecotoxicological experiments, chemical inoculation has to be done cautiously because these compounds can be volatilized, rapidly degraded or metabolized.

Isopods play an important role as bioaccumulation/degradation pathways of pesticides such as lindane, through consumption of contaminated organic matter or exposure to polluted soil, and also by other unknown routes. Thus, it is essential to develop a broader understanding about the uptake

routes of pesticides for isopods, when animals are in contact with contaminated soil or/and organic material.

Terrestrial isopods are important ecotoxicological test-species (e.g. bioaccumulation studies, analyzing their internal body concentration) to assess the presence of chemicals in contaminated or bioremediated soils. Furthermore, isopods can be helpful in the bioremediation processes, because they promote microbial and fungal proliferation through faecal production, thus reducing the time associated with the degradation of the pesticides.

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