

Behavioral Response in the Terrestrial Isopod *Porcellio scaber* (Crustacea) Offered a Choice of Uncontaminated and Cadmium-Contaminated Food

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Abstract. The objective of this study was to find out whether *Porcellio scaber* discriminates against Cdcontaminated food. The foraging behavior in animals offered uncontaminated and Cd-contaminated food simultaneously was quantified for 48-h employing computer-aided video tracking. To see whether the isopods' selection of less contaminated food could diminish the influence Cd on food consumption, growth, metal assimilation, moulting and mortality, Cd-dosed food (20, 45, 200 and 450 mg kg⁻¹ dry weight) was offered together with untreated food for 3 weeks. Data from the video tracking experiments revealed that animals visited Cd-dosed food as often as untreated food, but spent much less time near Cd-dosed food Discrimination against Cd-contaminated food increased with previous experience with contaminated food and/or with increased Cd body burden. In 3 weeks exposure uncontaminated food preference rose with time of exposure and cadmium concentration in food and reached a maximal preference ratio of 65% (untreated food): 35% (Cd-dosed food). The decreased consumption of Cd-dosed food was compensated by the increased consumption of control food. Cadmium body burden increased with time of exposure and cadmium concentration in food and reached a maximal preference ratio of 65% (untreated food): 35% (Cd-dosed food). The decreased consumption of Cd-dosed food was compensated by the increased consumption of control food. Cadmium body burden increased with time of exposure and cadmium concentration in food consumed, while the influence of Cd on food consumption, growth and moulting was diminished.

Keywords: food-choice; consumption rate; accumulation; avoidance response; video tracking

Introduction

The terrestrial isopod *Porcellio scaber* Latr. is one of the most investigated arthropods in terrestrial ecotoxicology. The species was successfully used in field studies and monitoring programmes for assessing the bioavailability of metals due to its strong affinity for zinc, cadmium, lead and copper (Wieser et al., 1976; Coughtrey et al., 1977; Hopkin et al., 1986; Dallinger et al., 1992; Hopkin, 1993; Paoletti and Hassall, 1999). The

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accumulated tissue concentrations of these metals are the highest found among invertebrates (Hopkin, 1989; Heikens et al., 2001).

P. scaber was also recognized as an organism that fulfils the criteria for characterization of the relative toxicity of metals (Drobne, 1997). Food consumption, moult frequency, growth and reproduction were proposed as sub-lethal toxicological endpoints in toxicity test protocols with terrestrial isopods (Drobne and Hopkin, 1994, 1995; Drobne and Štrus, 1996; Hornung et al., 1998).

In laboratory conducted toxicity tests, isopods are usually exposed constantly to contaminated food and have no opportunity to select alternative food. In recent years, much information has been gathered about terrestrial isopods' food preference. Some results of food choice experiments have suggested that they can discriminate against food contaminated with metals (Dallinger, 1977; van Capelleveen et al., 1986; Odendaal and Reinecke, 1998, 1999; Zidar, 2000; Kaschl et al., 2002; Zidar et al., 2003a, 2004).

By selecting less contaminated food or moving to a less polluted location terrestrial isopods may mitigate exposure, as metals are not distributed uniformly. In that case, their body loads reflect not only the concentrations of available metals in their food, but also an integrated response of the organism to the polluted environment. This means that the extrapolation of the results from laboratory tests to the field situation becomes even more difficult.

In the present work, we investigated the feeding behavior of *Porcellio scaber* offered uncontaminated and Cd-contaminated food simultaneously. The aim was to find out whether *P. scaber* discriminates against Cd-contaminated food. We also aimed to see whether isopods' selection of less contaminated food could influence food consumption, growth, metal assimilation, moulting and mortality. Behavioral response has been considered as a mechanism, which helps isopods to survive in metal-polluted environments and as an endpoint in toxicity assessments with terrestrial isopods.

Methods

Origin of the animals

Specimens of *Porcellio scaber*, fourth generation laboratory raised, were used in the experiments. The original population was collected in an unpolluted environment in the vicinity of Ljubljana, Slovenia. Animals were kept in a climate chamber, caged in glass containers with moist sand and peat on the bottom. They were fed with leaves from various trees, with periodical addition of commercial food designed for experimental animals (M-K 02, Homec-SI).

Preparation of food pellets

Food pellets used during experiments were made by the recipe of Kaschl et al. (2002). Powdered hazel leaves, gelatine, and fish food in a 63:34:3 ratio were mixed with demineralised water. All ingredients were powdered and homogenized to prevent food selection due to food heterogeneity (Hassall et al., 2002). Food pellets were formed out of the paste by depositing equal amounts in plastic blisters (V = 0.3 ml). The food pellets were left to solidify for 24 h at 5 °C, then they were dried at room temperature for 24 h and then at 70 °C for the next 24 h. For the preparation of Cd-dosed food, cadmium nitrate $(Cd(NO_3)_2, >99)$ purity, Merck, Germany) solution was mixed with the food ingredients. In order to exclude food choice due to nitrate content (Hopkin, 1989), corresponding amounts of nitrate ions (KNO₃, >99 purity, Merck, Germany) were added to the uncontaminated control food. The increased concentration of K⁺ ions in control food has no effect on food consumption rate in P. scaber (Donker and Bogert, 1991; Donker et al., 1996) and has no known effects on food taste. The average dry weight of a single pellet was 75 mg. Prior to use food pellets were kept in a desiccator.

Video tracking of foraging behavior

Foraging activity of animals offered a choice of Cd-contaminated and uncontaminated food was monitored for 48 h by a video camera. Food discrimination in relation to Cd concentration in food and pre-exposure to Cd-contaminated food was studied.

For the experiments 80 males of around 50 mg fresh weight were selected and divided into four groups. Animals from all groups were fed first with uncontaminated food for 1 week to accustom with the form and taste of the food. Prior to video tracking experiments the second and the fourth group was pre-exposed for two more weeks to the food dosed with 45 mg Cd kg⁻¹ dry weight and 200 mg Cd kg^{-1} dry weight, respectively. After pre-treatment animals were starved for 24 h to empty their guts. Then, 10 animals from each group were analyzed for Cd content and 10 used for video tracking. During the video tracking experiment animals from the first and the second group were offered control food and food dosed with 45 mg Cd kg⁻¹ dry weight. Animals from the third and the fourth group were offered a choice of control food and food dosed with 200 mg Cd kg⁻¹ dry weight. All pre-treatments were scheduled in such a way that video tracking of foraging behavior was performed consecutively in intervals of three days.

For the video tracking, 10 animals from same group were separated into 10 arenas (10 cm long, 6 cm wide and 4 cm high) made of glass, with moistened plaster of Paris at the bottom. Each animal was offered two food pellets ($\emptyset = 0.4$ cm). Pellets were covered with white paper discs of the same size as the pellets (to assure contrast between the animals and the background) and fixed to the floor by thin headless needles. The pellets of the control and Cd-dosed food were positioned alternatively to exclude food selection due to food position. Animals were acclimated to the arenas for 2-h prior to monitoring. For a new group of animals newly prepared plaster of Paris and a new set of food pellets, needles and paper discs were used.

Isopods in arenas were followed by a video camera (Iskra, B/W) under weak lighting (0.72 W m^{-2}) and at a room temperature of 23 °C. Video images of the test arenas were digitized into 256×192 pixel frames (30 per second) using a VIDEOMEX-V frame grabber (Columbus Instruments, USA, 1992). In each test arena three zones were assigned; two food zones, one for control and the other for Cd-dosed food, and the third zone without food. The number of visits and the time spent in food zones were quantified. Animals that did not approach to any of food were excluded from calculation.

Cadmium toxicity test with Cd-contaminated and uncontaminated food

During the test animals were exposed to Cd-contaminated food (20, 45, 200, or 450 mg kg⁻¹ dry food weight) combined with uncontaminated food for 3 weeks. Food consumption rate, Cd assimilation, growth, moulting and mortality were quantified in relation to Cd doses and time of exposure.

Animals were separated, sexed and weighed, and kept individually on moist filter paper in plastic Petri dishes ($\emptyset = 9$ cm). Only males and non-gravid females, 30–50 mg fresh weight, were selected for the experiment. Each animal was assigned a serial number. Prior to the experiment they were fed on uncontaminated food pellets for 1 week to accustom them to the form and taste of the food. After a week of acclimatisation animals were starved for 24 h to empty their guts. They were divided into five groups of 40 individuals each, giving a total number of 200 animals. Three animals from each group with the highest serial number were used for cadmium content analyses. The rest of the animals were offered a choice between a control and a Cd-dosed food pellet. Each food pellet was separated in a small plastic dish $(\emptyset = 2 \text{ cm}, \text{ height of rim 3 mm})$ to prevent contact with the other food pellet and moist filter paper. Five combinations of nominal Cd concentrations (all in mg kg⁻¹ dry food weight) were used: "0–0", "0–20", "0–45", "0–200" and "0– 450".

The Petri dishes were kept in a climate chamber at a relative humidity of 90% and under a 16 h light and 8 h dark regime. Temperature was kept constant at 21 °C (\pm 1 °C). Animals were checked every two days, and the filter paper was moistened with commercial bottled water if necessary. Any dead animals were removed immediately and their data excluded from the results. Moulting animals or animals that did not produce faecal pellets were registered.

After 7 days of the food choice experiment, the animals were transferred to new Petri dishes and starved for 24 h again. The last eight animals of each group were analyzed for cadmium content. The food pellets were collected, dried at room temperature, cleaned from faeces and dried at 70 °C for the next 48 h. Consumption of food was calculated according to the differences in weight before and after the seven-day period of exposure to the animals. Faecal production was not measured.

The remaining animals were offered a new pair of food pellets with the same Cd concentrations as before. The whole cycle of feeding, removal of food, 24-h starvation, and selection of animals for analysis of Cd content was repeated three times, resulting in a total exposure period of 24 days for the last cohort of animals. The mass gained or lost during the experiment was calculated by the difference in the animals' fresh weight after 1, 2 or 3 weeks of feeding. Animals were starved for 24 h before being weighed.

Analysis of data

In the video tracking experiment the number of visits and time spent in either of the food zones were recorded for each animal. The percentage of time spent in each food zone was compared to a null-hypothesis of 50% with a two-tailed Student's *t*-test, as the total time spent in either of the two food zones was 100%. Number of visits and time per visit in zones with control food was compared to that in zones with Cd-dosed food, using the non-parametric Mann–Whitney test.

In the toxicity test consumption rate, food selection, Cd assimilation and mass gain were determined for each animal. These data were analyzed using one-way or two-way ANOVA with the cadmium concentration (five levels) and time of exposure (three levels) as fixed factors. Food consumption rates (CR) were calculated as the absolute consumption of food per week divided by the dry weight of the animals. The percentage of uncontaminated food consumed was calculated and compared to a null-hypothesis of 50% with a two-tailed Student's *t*-test. Standard errors (SE) and 95% confidence intervals were calculated where appropriate.

All calculations were executed using SPSS 11.0 for Windows statistics software.

Cadmium analyses

To determine the total body concentration of Cd, animals were freeze-dried for 48 h, weighed and digested in glass tubes using a mixture of nitric and perchloric acid (7:1 v/v; Ultrex quality) at increasing temperatures (85, 160 and 185 °C). After evaporation of the acid, the residue was dissolved in 0.2% HNO₃ (V = 1.5 ml). Cadmium body burdens were determined on a flame atomic absorption spectrometer (Perkin Elmer AAAnalyst 100) in an air-acetylene flame with deuterium correction of non-specific absorption. As certified material, reference lobster hepatopancreas (TORT-2, National Research Council of Canada) was used.

The food pellets were digested and analyzed for the total concentration of Cd in the same way. The actual concentrations of Cd-dosed food did not differ from the nominal concentration by more than 5%. The cadmium concentration in control pellets was 0.06 mg kg⁻¹ dry weight (± 0.02 SE) (for details see Kaschl et al., 2002).

The amount of water-soluble cadmium in the food pellets was measured by soaking pellets in demineralised water (V = 4 ml) for 24 h at increasing temperatures up to 40 °C, centrifuging the solution and analysing the supernatant for Cd content by flame AAS. The water-soluble concentration of cadmium in food pellets was around 8% of the actual cadmium concentration (for details see Kaschl et al., 2002).

Results

Video tracking of foraging behavior

Visiting time in zones around food in relation to dose and Cd pre-exposure

The data showed that animals spent less time near Cd-dosed food compared to control food (Table 1). Preference for control food was significant in the animals pre-exposed to Cd-dosed food prior to video tracking.

During the 48 h of monitoring, animals of all groups visited Cd-dosed food as often as control food. In all groups, the average time per visit to control food was higher than of Cd- dosed food (Table 1). The difference was significant only in animals pre-exposed to Cd-dosed food.

Animals pre-exposed to control food only contained 2.0 mg Cd kg⁻¹ dry body weight $(\pm 0.04 \text{ SE})$. The Cd concentration in animals additionally fed with 45 mg Cd kg⁻¹ for 2 weeks prior to video tracking was 18.6 mg Cd kg⁻¹ dry weight $(\pm 2.63 \text{ SE})$. In animals pre-exposed to food of 200 mg Cd kg⁻¹ dry weight for 2 weeks the Cd concentration increased to 96.8 mg Cd kg⁻¹ dry body weight $(\pm 7.61 \text{ SE})$.

Cadmium toxicity test

Food selection

Control animals that were offered two control food pellets ("0–0") consumed both pellets equally over the whole three-week period (Fig. 1). Save for the group "0–45" in the first week, all animals preferred the control food if offered together with Cd-dosed pellets. Control food

Table 1. Video tracking of *Porcellio scaber* foraging activity offered a choice of Cd-contaminated and uncontaminated food, monitored for 48 h. The number of visits and time spent in either of the food zones were recorded for each animal from which average values are presented (standard error values in parentheses)

	Food zone		Visiting time in 48 l	1		Test	
Group	(conc. of Cd in food)	No. of visits	Seconds	%	Time/visit (s)		
1 (n = 7)	0 45 <i>p</i> -Values	$\begin{array}{c} 68 \ (\pm 20.7) \\ 55 \ (\pm 20.0) \\ 0.655 \end{array}$	21658 (\pm 7278.0) 7952 (\pm 1635.6) 0.225	66 (±12.5) 34 (±12.5)	$\begin{array}{c} 300 \ (\pm 68.4) \\ 195 \ (\pm 66.9) \\ 0.338 \end{array}$	M–W test	
2 (n = 8) Pre-exposure 45 mg Cd kg ⁻¹	0 45	44 (±14.3) 41 (±15.3)	$30302 (\pm 6727.7)$ $15836 (\pm 5862.7)$	$70 (\pm 8.7)$ $30 (\pm 8.7)$	913 (± 186.9) 336 (± 80.0)	<i>i</i> -test	
	<i>p</i> -Values	0.674	0.115	0.047	0.016	M–W test <i>t</i> -test	
3(n = 7)	0 200	47 (±15.3) 41 (±16.7)	$\begin{array}{c} 10152 \ (\pm 2760.2) \\ 4379 \ (\pm 1195.1) \end{array}$	65 (±11.1) 35 (±11.1)	349 (±140.9) 182.80 (±80.4)		
	<i>p</i> -Values	0.565	0.110	0.205	0.277	M–W test <i>t</i> -test	
4 ($n = 10$) Pre-exposure 200 mg Cd kg ⁻¹	0 200	$\begin{array}{c} 45 \ (\pm 5.0) \\ 36 \ (\pm 5.3) \end{array}$	$\begin{array}{l} 40854 \ (\pm 10292.4) \\ 12240 \ (\pm 5690.5) \end{array}$	83 (±4.5) 17 (±4.5)	$\begin{array}{l} 894 \ (\pm 196.4) \\ 293 \ (\pm 105.0) \end{array}$		
	<i>p</i> -Values	0.353	0.008	0.000	0.013	M–W test <i>t</i> -test	

M-W = Mann and Whitney non-parametric test.

preference slightly rose with Cd concentration in food and time of exposure. The maximal preference ratio between control food and food dosed with Cd was 65% (control food) and 35% (Cd-dosed food).

Food consumption rate

Food consumption rate was not Cd-dependent but varied with time of exposure (Table 3). In the first 2 weeks combined consumption rates (CR) of control and Cd-dosed food ranged from 1.8 to



Figure 1. Uncontaminated food preference in *Porcellio scaber* followed over 3 weeks. Consumption rate (CR) of uncontaminated food shown as percentage values (\bullet) of combined CR of uncontaminated and Cd-contaminated food (means and 95% conf. int.): (a) 1st week, (b) 2nd week, (c) 3rd week of food choice experiments. Five combinations of nominal Cd concentrations in food (all in mg kg⁻¹ dry food weight) were used: 0–0, 0–20, 0–45, 0–200 and 0–450. Stars above bars represent significant differences between CR of uncontaminated food and hypothetical value of 50% (horizontal line) (*t*-test: * = p < 0.05; *** = p < 0.001).

2.1 mg mg⁻¹ dry body weight per week (Fig. 2). In the third week, the CR increased in all groups up to 2.7 mg mg⁻¹ dry body weight per week.

Assimilation of Cd

The total Cd body burden of the animals selected prior to the experiment was 3.31 (± 0.28 SE) mg Cd kg⁻¹ dry weight. The Cd concentration in control animals after the experiment did not exceed that value. In Cd-exposed animals, concentration significantly (Table 3) increased with Cd concentration in food and time of feeding (Fig. 3).

Mass gain

Animals' mass significantly increased with time of feeding in all the experimental groups (Tables 2 and 3), irrespective of the Cd concentration in food.

Moulting

In the first 2 weeks of the experiment two to eight animals moulted per week (Table 4) with no significant differences between groups. None of the animals moulted in the third week.



Figure 2. Food consumption rate (CR, in mg mg⁻¹ dry body weight) in *Porcellio scaber* followed over 3 weeks of the food choice experiment, presented as mean values of combined consumption rates of uncontaminated and contaminated food (\pm SE; $n \ge 8$). Five combinations of nominal Cd concentrations in food (all in mg kg⁻¹ dry food weight) were used: 0–0, 0–20, 0–45, 0–200 and 0–450.



Figure 3. Concentration of cadmium in *Porcellio scaber* ($n \ge 8$) measured by flame AAS after 1, 2, and 3 weeks of the food choice experiment. Five combinations of nominal Cd concentrations in food (all in mg kg⁻¹ dry food weight) were used: 0–0, 0–20, 0–45, 0–200 and 0–450. Concentration of cadmium in animals from the laboratory population is represented on the far left (mean \pm SE). Values presented with asterisks are significantly higher than values from the control (0–0') group (one-way ANOVA, Tukey test, p < 0.01).

Table 2. Average mass gain with the corresponding error value (SE) in *P. scaber* individuals offered a choice of control food and food dosed with five different concentrations of cadmium at the same time for 1, 2 or 3 weeks. All mass values are in milligrams

0–0			0–20			0–45			0–200			0-450			
Time	mass	SE	n	mass	SE	n	mass	SE	n	mass	SE	n	mass	SE	п
Food choice	(mg Cd k	g ⁻¹ dry	weight)											
1 week	1.1	0.7	8	1.8	0.8	8	2.3	0.5	8	0.9	1.3	8	2.0	0.8	8
2 weeks	3.9	0.9	8	3.4	0.5	8	1.6	0.8	8	4.8	0.7	8	3.4	0.5	8
3 weeks	6.2	0.7	13	5.3	0.5	12	7.0	0.5	10	5.8	0.5	12	4.0	0.3	8

Letter n indicates number of animals measured in selected time period.

Table 3. Two-way ANOVA (p-values) for combined consumption rate (CR) of control and differently Cd-dosed food, Cd accumulation and mass gain in *Porcellio scaber* over 3 weeks of paired food choice experiment

Dependent variable:	CR	Cd accumulation	Mass gain		
Fixed factors:					
Cd	0.974	0.000	0.788		
Time	0.000	0.000	0.000		
$Cd \times time$	0.981	0.000	0.014		

Table 4. Number of moulted (m) and dead animals (d) in food choice experiment with Porcellio scaber over 3 weeks of observation

	0–0		0–20		0–45			0–200			0–450				
Time	m	d	n	m	d	n	m	d	n	m	d	n	m	d	n
Food choice (n	ng Cd k	g ⁻¹ dry	weight)												
1st week	8	0	37	7	0	37	5	2	37	7	0	37	6	2	37
2nd week	5	0	29	5	0	29	2	1	27	5	0	29	4	2	27
3rd week	0	8*	21	0	9*	21	0	8*	18	0	9*	21	0	9*	17

Letter *n* indicates number of animals in each food choice group. Asterisk indicates significantly higher mortality (ANOVA, p < 0.05) in the third week.

Mortality

Mortality of experimental animals increased in the third week (Table 4). Mortality was not Cddependent; the number of animals that died in the control group was almost the same as in the Cdexposed groups.

Discussion

Our results show that *Porcellio scaber* is quite obviously capable of selecting food according to Cd concentration, as was previously reported for *Porcellio laevis* (Odendaal and Reinecke, 1999) and *Oniscus asellus* (Kaschl et al., 2002; Zidar et al., 2003a). Video tracking of food choice experiments displayed avoidance behavior as a rapid response to Cd-dosed food and could be easily assessed by the experiments described above.

The mechanism of avoidance behavior still needs to be investigated. Isopods might detect metal-rich food by chemoreception as (in the case of copper) was stated by Weissenburg and Zimmer (2003). The almost equivalent number of visits to zones with control and Cd-dosed food pellets, but significantly less time spent with Cd-dosed food suggest that isopods probably did not smell the difference between the offered food pellets. Similar behavior was also shown in experiments with *Oniscus asellus* (Zidar et al., 2003a) where animals were offered uncontaminated and Cd-dosed food, as well as sterilized and mould-covered food. Food

with mould was most likely selected by taste, as has already been demonstrated by Gunnarson (1987). The question still remains whether isopods can taste cadmium as receptors for metals have not yet been described. Hassal and Rushton (1982) suggested that isopods might taste the differences in microbial populations on food, caused by different metal treatment. Whether Cd²⁺ ions (and possibly also K^+ ions) indirectly affect the taste of the food need some further investigations. Our experiment showed that discrimination against Cd-dosed food significantly increased in animals pre-exposed to Cd-contaminated food. Therefore, food selection may be related to Cd body loads and/or previous experience with contaminated food. The avoidance of Cd-dosed food could also be due to the adverse metabolic effects of ingested cadmium, as suggested by Kaschl et al. (2002). In that case, a dose-dependent behavioral response could be expected. For example, higher doses of Cd would produce stronger effects and the woodlice would spend even less time with the contaminated food. In this study a trend toward a higher avoidance response at higher doses and body loads of Cd was observable.

In the experiment where food consumption was followed for 3 weeks, food with Cd had no adverse effects on consumption if the animals had been offered Cd-dosed and uncontaminated food at the same time. In accordance with the data obtained by Donker and Bogert (1991) consumption of Cddosed food decreased already at the concentration of 20 mg Cd kg⁻¹ dry food weight. In our case a decreased consumption of Cd-dosed food was compensated by an increased consumption of control food. This is not necessarily a general pattern as was shown in experiments with copper (Zidar et al., 2004), where decreased consumption of Cu-dosed food was not compensated fully by consumption of uncontaminated food.

In our experiment, the growth of isopods was not affected. In previous studies with *P. scaber* and *P. laevis* where animals were exposed only to Cdcontaminated food, inhibition of growth was recorded (Donker and Bogert, 1991; Khalil et al., 1995; Odendaal and Reinecke, 1999). Decreased growth was explained by increased use of energy to resist the contaminant (Donker, 1992; Odendaal and Reinecke, 1999). It seems that animals offered contaminated and uncontaminated food simultaneously may have enough energy for growth and to cope with assimilated cadmium.

As the uncontaminated food preference did not exceed 65% of the total food consumed, cadmium body burden increased with the time of exposure and the concentration of Cd in the food. Animals' body concentrations were comparable to those obtained in the experiments of Donker and Bogert (1991) although the animals were offered only Cddosed food. According to results of Hames and Hopkin (1991) in P. scaber cadmium excretion from the body is very slow and probably cannot occur while animals fed on clean food if available. The discrepancy in findings from our previous experiments with P. scaber (Zidar et al., 2003b) can be ascribed to the type of food used in the experiments. Farcas et al. (1996) demonstrated differences in results when P. scaber was fed with maple leaves only and when was fed with food mixture (maple leaves - 50%, potato powder -10% and commercial rabbit food -40%) contaminated with copper. The bioavailability of metals from dead plant material prepared by spraying is probably higher than that from a food mixture. In the food pellets used in this study only around eight percentage of the total cadmium concentration was water-soluble. Unfortunately, only data about the total concentration of metals are available from previous experiments (Zidar et al., 2003b).

When alternative food is offered the addition of cadmium to food seems to have no effect on moulting processes. For a conclusive statement about that, at least 6 weeks of observations is necessary (Drobne and Štrus, 1996), considering the duration of the moult cycle of 33 days (Zidar et al., 1998). It is well known that a couple of days before axuviation animals stop feeding (Zidar et al., 1998). As no animals moulted in the third week this might explain the increased consumption rate in the last week.

In contrast to our food choice experiments with copper (Zidar et al., 2004) in short-term exposure to cadmium no additional mortality of animals was caused by cadmium. The isopods' physiological mechanisms such as storage of metals in intracellular granules of the hepatopancreas (Wieser and Klima, 1969; Hopkin and Martin, 1982) and complexation of metals by metal-binding proteins (Donker et al., 1990), combined with avoidance behavior, seems to be more efficient in preventing toxic effects from inessential cadmium than from essential copper.

Conclusions

- Porcellio scaber discriminates against Cd-contaminated food.
- Discrimination against Cd-contaminated food depends on previous experience with contaminated food and/or with a high Cd body load.
- Selection of food with less Cd is probably based on taste or adverse metabolic effects of ingested Cd.
- Uncontaminated food selection diminished the influence of Cd on food consumption, growth and moulting.
- Food selection mitigates but probably cannot prevent Cd-accumulation in the field as some contaminated food was consumed as well.
- The avoidance behavior of the terrestrial isopod *P. scaber* indicates lower food quality arising from Cd contamination. In comparison to other endpoints in toxicity tests with terrestrial isopods, behavioral response to metal-contaminated food is very fast and reliable.

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References

- Coughtrey, P.J., Martin, M.H. and Young, E.W. (1977). The woodlouse, *Oniscus asellus*, as a monitor of environmental cadmium levels. *Chemosphere* **12**, 827–32.
- Dallinger, R. (1977). The flow of copper through a terrestrial food chain. III. Selection of an optimum copper diet in isopods. *Oecologia* **30**, 273–76.
- Dallinger, R., Berger, B. and Birkel, S. (1992). Terrestrial isopods: useful biological indicators of urban metal pollution. *Oecologia* 89, 32–41.
- Donker, M.H. (1992). Physiology of metal adaptation in the isopod *Porcellio scaber*. Amsterdam: Vrije Universiteit, The Netherlands, Ph.D. thesis.

- Donker, M.H. and Bogert, C.G. (1991). Adaptation to cadmium in three populations of the isopod *Porcellio scaber*. *Comp. Biochem. Physiol.* 12, 143–6.
- Donker, M.H., Koevoets, P., Verkleij, J.A.C. and Straalen, N.van (1990). Metal binding compounds in hepatopancreas and haemolymph of *Porcellio scaber* (Isopoda) from contaminated and reference areas. *Comp. Biochem. Physiol.* 97C, 119–26.
- Donker, M.H., Raedecker, M.H and Straalen, N.M.van (1996). The role of zinc regulation in the zinc tolerance mechanism of the terrestrial isopod *Porcellio scaber. J. Appl. Ecol.* 33, 955–64.
- Drobne, D. (1997). Terrestrial isopods a good choice for toxicity testing of pollutants in the terrestrial environment. *Environ. Toxicol. Chem.* 16, 1159–64.
- Drobne, D. and Hopkin, S.P. (1994). An ecotoxicological laboratory test for assessing the effects of chemicals on terrestrial isopods. *Bull. Environ. Contam. Toxicol.* 53, 390–7.
- Drobne, D. and Hopkin, S.P. (1995). The toxicity of zinc to terrestrial isopods in a standard laboratory test. *Ecotox. Environ. Saf.* **31**, 1–6.
- Drobne, D. and Štrus, J. (1996). Effect of zinc contaminated food on moulting frequency of terrestrial isopod (*Porcellio* scaber). Environ. Toxicol. Chem. 15(2), 126–30.
- Farcas, S., Hornung, E. and Fischer, E. (1996). Toxicity of copper to *Porcellio scaber* Latr. (Isopoda) under different nutritional status. *Bull. Environ. Contam. Toxicol.* 57, 582– 8.
- Gunnarsson, T. (1987). Selective feeding on a maple leaf by Oniscus asellus (Isopoda). Pedobiologia 30, 161–5.
- Hames, C.A.C. and Hopkin, S.P. (1991). Assimilation and loss of 109Cd and 65Zn by the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. *Bull. Environ. Contam. Toxicol.* 47, 440–7.
- Hassall, N., Truck, J.M., Smith, D.W., Gilroy, J.J. and Addison, R.K. (2002). Effect of spatial heterogeneity on feeding behaviour of *Porcellio scaber* (Isopoda: Oniscidea). *Eur. J. Soil Biol.* 38, 53–7.
- Heikens, A., Peijnenburg, W.J.G.M. and Hendriks, A.J. (2001). Bioacumulation of heavy metals in terrestrial invertebrates. *Environ. Pollut.* **113**, 385–93.
- Hopkin, S.P. (1989). Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Sciences, London, New York.
- Hopkin, S.P. (1993). Deficiency and excess of copper in terrestrial isopods. In: R. Dallinger and P.S. Rainbow (eds). *Ecotoxicology of Metals in Invertebrates*, pp. 359–82. SE-TAC Spec. Publ. Ser., Lewis Publishers.
- Hopkin, S.P. Hardisty, G.N. and Martin, M.H. (1986). The woodlouse *Porcellio scaber* as a biological indicator'of zinc, cadmium, lead and copper pollution. *Environ. Pollut.* 11, 271–90.
- Hopkin, S.P. and Martin, M.H. (1982). The distribution of zinc, cadmium, lead and copper within the hepatopancreas of a woodlouse. *Tissue cell* 14, 703–15.
- Hornung, E., Farkas, S. and Fischer, E. (1998). Tests on the isopod Porcellio scaber. In H. Løkke and C.A.M. Gestelvan (eds*Handbook of Soil Invertebrate Toxicity Tests. Ecological* and Environmental Toxicology Series, pp. 207–26. Chichester: Wiley J & Sons.

- Khalil, M.A., Donker, M.H. and Straalen, N.M.van (1995). Long-term and short-term changes in the energy budget of *Porcellio scaber* Latreille (Crustacea) exposed to cadmium polluted food. *Eur. J. Soil Biol.* **31**, 163–72.
- Kaschl, U.I., Zidar, P., Štrus, J. and Storch, V. (2002). Food choice experiments with cadmium nitrate dosed food in terrestrial isopod *Oniscus asellus* (Crustacea). *Acta Biol. Slov.* **45**(2), 3–14.
- Odendaal, J.P. and Reinecke, A.J. (1998). The effect of high lead concentrations on the mortality, mass and behaviour of *Porcellio scaber* Latr. (Crustacea, Isopoda) in laboratory tests. S. Afr. J. Zool. 33, 143–46.
- Odendaal, J.P. and Reinecke, A.J. (1999). The sublethal effects and accumulation of cadmium in the terrestrial Isopod Porcellio laevis Latr. (Crustacea, Isopoda). *Arch. Environ. Contam. Toxicol.* **36**, 64–9.
- Paoletti, M.G. and Hassall, M. (1999). Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agr. Ecosyst. Environ. 74, 157–65.
- Van Capelleveen, H.E., van Straalen, N.M., van den Berg, M. and van Wechem, E. (1986). Avoidance as a mechanism of tolerance for lead in terrestrial arthropods. In: *Proceedings* of the Third European Congress of Entomology, Amsterdam, pp. 251–54.
- Weissenburg, M. and Zimmer, M. (2003). Balancing nutritional requirements for copper in the common woodlouse, *Porcellio* scaber (Isopoda: Oniscidea). Appl. Soil Ecol. 23, 1–11.

- Wieser, W. and Klima, J. (1969). Compartmentalization of copper in the hepatopancreas of isopods. *Microscopie* 24, 1–9.
- Wieser, W., Busch, G. and Büchel, L. (1976). Isopods as indicators of copper content of soil and litter. *Oecologia* 23, 107–14.
- Zidar, P., Drobne, D. and Štrus, J. (1998). Determination of moult stages of *Porcellio scaber* (Isopoda) for routine use. *Crustaceana* 71(6), 646–54.
- Zidar, P. (2000). Effects of metal ions on food consumption of terrestrial isopod *Porcellio scaber* (Crustacea: Isopoda). Ljubljana: Biotechnical faculty, University of Ljubljana, Slovenia; Ph.D. thesis.
- Zidar, P., Drobne, D., Strus, J. and Blejec, A. (2003b). Intake and assimilation of Zn, Cu and Cd in the terrestrial isopod *Porcellio scaber* Latr. (Crustacea, Isopoda). *Bull. Environ. Contam. Toxicol.* **70**, 1028–35.
- Zidar, P., Drobne, D., Strus, J., Gestel, C.A.M.van and Donker, M. (2004). Food selection as a means of Cu intake reduction in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda). *Appl. Soil Ecol.* 25(3), 257–65.
- Zidar, P., Kaschl, I.U., Štrus, J., Drobne, D. and Božič, J. (2003a). Avoidance behaviour in *Oniscus asellus* (Crustacea, Isopoda) as an indicator of food quality. *Arch. Ind. Hyg. Toxicol.* 54, 177–81.