Conference Paper

# BEHAVIOURAL RESPONSE IN PAIRED FOOD CHOICE EXPERIMENTS WITH *ONISCUS ASELLUS* (CRUSTACEA, ISOPODA) AS AN INDICATOR OF DIFFERENT FOOD QUALITY*

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This paper describes a study of behavioural response in terrestrial isopod *Oniscus asellus* when offered two food pellets of different quality. One group had a choice of sterilised food and food pellets covered with mould. The other group had a choice of uncontaminated and cadmium-dosed food. During the behavioural test, the animals were monitored by a video camera and each visit to food pellets and time spent around it was counted. The results show that animals spent significantly less time near sterilised and cadmium-dosed food than with uncontaminated or mould-covered food. Discrimination between offered food pellets showed that avoidance behaviour can be used as an indicator of different food quality.

**KEY WORDS:** *avoidance behaviour, cadmium, terrestrial isopods, video monitoring*

Terrestrial isopods are among the most investigated invertebrate groups in terrestrial ecotoxicology. Two most common isopod species *Porcellio scaber* and *Oniscus asellus* were recognised as organisms that fulfil the criteria for use in toxicity testing. The sublethal toxicological endpoints used in toxicity test protocols with terrestrial isopods are food consumption, growth and reproduction (1-3). The main disadvantage of growth and reproduction as endpoints is the long duration of the test due to low growth rate and long reproduction cycle of the isopod species.

Terrestrial isopods have also been proposed as terrestrial biomonitoring organisms for assessing the bioavailability of metals (4, 5) due to their strong affinity for zinc, cadmium, lead and copper. The accumulated tissue concentrations of these metals are the highest among invertebrates (6). The environment in which terrestrial isopods live and feed contains from 0.15 mg Cd/kg dry weight in uncontaminated environments (7) to over 560 mg Cd/kg dry weight in industrially polluted areas (8).

In recent years, much information has been gathered about the food preference of terrestrial isopods. *P. scaber* is strongly attracted by the odour of metabolites released by microorganisms that colonise food (9) and show preference for the food with higher levels of fungal permeation (10). In addition, some results of food choice experiments have suggested that terrestrial isopods may be able to discriminate against food contaminated with metals (11-16).

The aim of this study was to see if avoidance behaviour indicated a certain quality of food. The paper further discusses behavioural response as an endpoint in toxicity testing with terrestrial isopods.

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## MATERIALS AND METHODS

### *Selection of test organisms*

Specimens of woodlouse (*Oniscus asellus*) were collected from the litter layer of a wood near a former smeltery of Nussloch near Heidelberg, Germany. After acclimation to laboratory conditions male animals were selected for behavioural testing. During the test, the animals were receiving complex food pellets made of uncontaminated hazel leaves (*Corylus avellana*), commercial Dr Oetker`s gelatine, and aquarium fish food (JBL Novobel; JBL GmbH & Co. KG).

### *Food preparation*

Dry leaves were pulverised with a coffee mill and sieved through a 0.25 mm mesh. Leaf powder, gelatine, and fish food were mixed in a 63:34:3 ratio and turned into a paste with demineralised water (15 ml per g of gelatine). For the preparation of Cd-dosed food, cadmium nitrate [$Cd(NO_3)_2$, >99 purity, Merck, Germany] solution was added to the paste to give nominal concentrations of 200 mg Cd/kg dry weight of food. The cadmium concentration was selected on the basis of previous experiments with the same population of animals (15) and represents a medium concentration that led to a decrease in food consumption rate. In order to exclude food choice due to nitrate content (6), corresponding amounts of potassium nitrate ($KNO_3$, >99 purity, Merck, Germany) were added to respective control food. 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 again at 70 °C for the next 24 h.

### *Cadmium analysis*

Before the food choice experiments began, the animals were given uncontaminated food pellets for one week to get acquainted with their form and taste. After acclimatisation, the animals were starved for 24 h to empty their guts. Ten woodlice were randomly selected for cadmium content analysis. They were freeze-dried for 48 hours, 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 % $HNO_3$. Copper 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. An average cadmium concentration in animals was 71 mg Cd/kg dry body weight (SE=8.9 mg/kg).

### *Behaviour experiments*

During behavioural testing, the woodlice were monitored by a video camera (Iskra, B/W) under a pale light (0.72 W/m$^2$) at a room temperature of 23 °C. The woodlice were separated in arenas (10 cm long, 6 cm wide, 4 cm high) with moistened plaster of Paris at the bottom. The first group (N=8) was offered sterilised uncontaminated pellets and pellets overgrown with mould to feed on for 48 hours. The pellets were sterilised in autoclave at 121 °C and 1.3 bar for 15 minutes. Mould growth was stimulated by keeping the pellets on a moistened filter paper for two days. The second group (N=10) was offered uncontaminated and Cd-dosed (200 mg /kg dry weight) food for 72 hours. In the arenas, the food pellets (Ø=0.5 cm) were covered by white paper discs of the same size as pellets (to assure contrast between animals and the background) and fixed to the floor by a thin headless needle. Animals were acclimatised for 2 h before video monitoring. Video images of the test arenas were digitised into a 256x192 pixels frame (30 per second) using a VIDEOMEX-V frame grabber (Columbus Instruments, USA, 1992). Each test arena was divided in three zones; two food zones, one for each type of food, and the third zone without food. We counted the number of visits and time spent in either of the food zones. Sterilised food and food dosed with cadmium were considered as low quality food (17).

### *Statistical analyses*

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 %. Time per visit in zones with food covered with mould and untreated food was compared to that in zones with sterilised and Cd-dosed food, respectively, using the non-parametric Mann-Whitney test.

## RESULTS

Figure 1 shows the ratios between time spent in the sterilised food zone and moulded food zone. Save for a single woodlouse, all animals spent up to 35-fold more time in the zone with pellets overgrown by mould than in the sterilised food zone. During the 48 hours

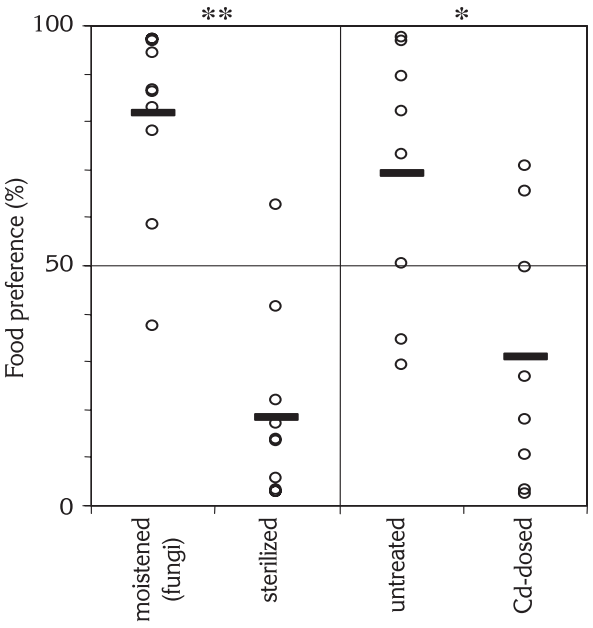


**Figure 1** *Food preference ( %) in woodlouse expressed as percentage of time spent near single food offered in computer-aided video monitoring experiments (circles - individual data, horizontal lines – average values; t-test: *p<0.05, **p<0.01 (tested to a null-hypothesis of 50 %).*

of monitoring, woodlice in average visited sterilised food 51 times, and food covered in fungal hyphae 67 times. This difference is not significant. However, the average time per visit of food with mould was significantly higher (Mann-Whitney test $p<0.05$) than that spent in the sterilised food zone (Figure 2).

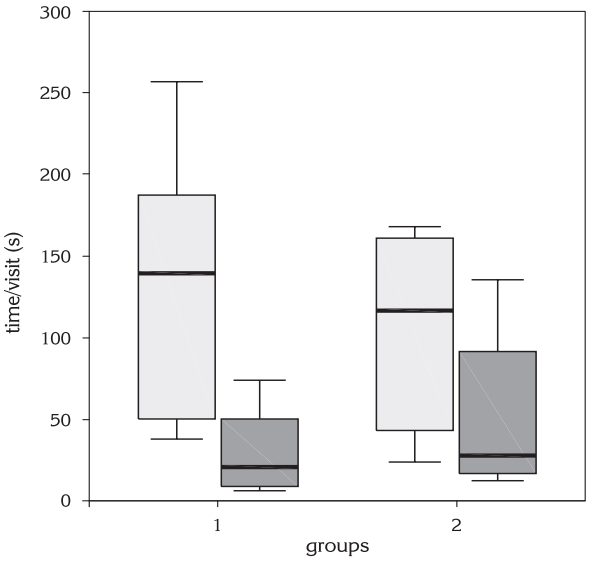


**Figure 2** *Behavioural tests with woodlouse. Time per visit (visiting time divided by the number of visits) in food zones, expressed as median and quartile values (group one: light grey – food covered with mould, dark grey – sterilised food; group two: light grey – uncontaminated food, dark grey – Cd-dosed food; Mann-Whitney test: p<0.05).*

Six woodlice in the group offered uncontaminated and Cd-dosed food spent more time in the uncontaminated food zone while two animals lingered longer in the Cd-dosed food zone (Figure 1). In 72 hours, the average number of visits to zones with uncontaminated and Cd-dosed food was 106 and 136, respectively. The average time per visit of uncontaminated food zone was significantly higher than that of Cd-dosed food (Mann-Whitney test $p<0.05$; Fig. 2).

## DISCUSSION

Our results show that woodlice are quite obviously capable of selecting food not only according to its fungal hyphae content, but also according to Cd concentration. Avoidance response to less palatable food is rapid and can easily be assessed by video monitoring experiments described above.

The mechanism of avoidance behaviour still needs to be investigated. An almost equivalent number of visits to zones with sterilised and mould-covered food pellets, but significantly less time spent with sterilised food suggests that isopods probably can not smell the difference between the offered food pellets. Food with mould was most likely selected only after the woodlice tasted it. This has already been demonstrated by *Gunnarson* (10). Woodlice offered uncontaminated and Cd-dosed food showed similar behaviour. They visited both food zones equally, but spent much more time with uncontaminated food. The question still remains whether isopods can taste cadmium. They have not yet been discovered metal receptors. The avoidance of Cd-dosed food could also be due to adverse metabolic effects of ingested cadmium as suggested by *Kaschl and co-workers* (15). In that case, one could also expect a dose-dependent behavioural response. For example, higher doses of Cd would produce stronger effects and the woodlice would spend even less time with the contaminated food.

As terrestrial isopods are given a selection of differently contaminated food in their natural environment, their body load probably does not reflect the concentration of available chemicals in food they have access to. Besides, avoidance may lead to lower exposure than would be predicted from standard laboratory tests in which animals are usually not given the choice of food.

## CONCLUSION

Avoidance behaviour of terrestrial isopod *Oniscus asellus* indicates lower food quality due to Cd contamination. In comparison to other endpoints in toxicity tests with terrestrial isopods, behavioural response to metal-contaminated food is very fast and still reliable.

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***Sažetak***

IZBJEGAVANJE UZIMANJA HRANE ONISCUS ASSELLUSA KAO POKAZATELJ KAKVOĆE HRANE

Autori su istraživali ponašanje *Oniscus asellusa* (Crustacea, Izopoda) pri uzimanju različitih pripravaka hrane, a sa svrhom da se utvrdi mogućnost primjene ovakvih testova u vrednovanju kakvoće hrane. Ispitivanim pokusnim životinjama, pomno odabranim i razvrstanim u skupine, nuđena je hrana od usitnjenog sušenog lišća lijeske, želatine i hrane za ribe pomiješana s demineraliziranom vodom. Jednoj je skupini životinja nuđen izbor takve hrane kontaminirane plijesnima i jednake, ali sterilizirane hrane, a drugoj skupini jednaka hrana kojoj je dodan samo nitrat (koji je bio i u ispitivanoj hrani skupa s kadmijem) i ona kontaminirana kadmijevim nitratom (200 mg/kg hrane). Pri praćenju ponašanja pokusnih životinja autori su se koristili videokamerom. Utvrdili su da je skupina koja je imala na izbor pljesnivu i steriliziranu hranu preferirala pljesnivu, a druga je skupina očito izbjegavala hranu kojoj je pridodan kadmijev nitrat. Autori smatraju da bi ovakvi pokusi mogli pripomoći utvrđivanju onečišćenja hrane kadmijem.

**KLJUČNE RIJEČI:** *kadmij, ponašanje pri izboru hrane, praćenje videokamerom, zemaljski jednakonošci*

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