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Ecotoxicological Laboratory Test for Assessing the Effects of Chemicals on Terrestrial Isopods

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Several laboratory methods for studying sublethal effects of chemicals on organisms have been developed in recent years. One of the aims of such tests is to identify whether use in the field will result in unacceptable ecological damage to non-target species. Most tests have been developed for single species, but at present, very few are required by law before a product can be released onto the market. Indeed for terrestrial ecosystems, the only test that is widely-used is the earthworm toxicity test (Greig-Smith et al. 1992).

The range of species for which test procedures are being developed is expanding. For example, several authors have recommended methodologies for tests with terrestrial isopods (Eijsackers 1991; Van Straalen 1993; Van Straalen and Verweij 1991; Van Straalen and Van Gestel 1993; Van Wensem et al. 1992). Criteria measured in these studies include growth, reproduction and survival in response to toxicants. However, terrestrial isopods have a long reproductive cycle and can survive for up to 180 days without food (Donker 1992). Thus, there is a need to develop a test which examines a parameter that has ecological relevance, but which can be measured in a relatively short time.

In this paper, we describe a rapid, simple and reproducible procedure to assess the toxicity of metals to terrestrial isopods by measuring food consumption determined from faecal production rates. In order to develop the technique, the effects of cobalt at different concentrations on food consumption of two common species of woodlice (*Porcellio scaber* and *Oniscus asellus*) were studied. Woodlice are good candidates for standard test species. They are common, easy to handle, and are strong bioaccumulators of metals (Hopkin 1993). Effects of chemicals on mortality, reproduction, food consumption, and assimilation are well documented (Donker 1992; Donker et al. 1992, 1993; Hopkin 1989; Tomita et al. 1992; Van Capelleveen 1987), although there is currently no agreed standard procedure for an ecotoxicological test using isopods (Van Straalen and Van Gestel 1993).

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MATERIALS AND METHODS

Approximately 100 specimens of *Porcellio scaber* and *Oniscus asellus*, and a large sample of leaves of Field Maple (*Acer campestre*) were collected from the litter layer of uncontaminated woodlands in the Reading area in February 1993. For the experiment, males and non-gravid females were selected at random and were held individually in plastic Petri dishes (diameter 9 cm) at 20^{o}C under a 16 hr light, 8 hr dark period.

The leaves were air-dried at room temperature for 48 hr and weighed individually. Solutions of cobalt nitrate were applied topically to the leaves as small droplets and allowed to dry overnight at room temperature. The amount of solution applied to each leaf was adjusted to give nominal concentrations of cobalt of 50, 500 or 2500 μg g^{-1} dry weight. Distilled water was applied to a further batch of leaves which acted as controls.

The leaves were rehydrated at 100% relative humidity for 48 hr and then placed individually into Petri dishes. A single specimen of either *Porcellio scaber* or *Oniscus asellus* of c.40 mg freshweight was placed with each leaf. There were 12 replicates of each cobalt concentration and 12 controls for each species giving a total of 96 Petri dishes. Humidity in the Petri dishes was maintained by regularly spraying the internal side of the lids with distilled water. The Petri dishes were stacked in large covered plastic tanks which maintained the relative humidity at 100 %.

The isopods ate between 70-90% of the leaves in four weeks. At the end of this period, six of the 12 isopods from each treatment were dissected into three tissue fractions (hepatopancreas, gut, and "rest") using the technique of Hopkin (1990). The remaining six isopods from each treatment were transferred to uncontaminated leaves for two weeks and then dissected in the same manner.

Every day of the experiment, the faecal pellets voided during the previous 24 hours were counted and removed from each Petri dish. In the group of animals transferred to clean leaves, the faecal pellets were collected in separate dishes and analyzed subsequently for cobalt. At the end of the experiment, the faecal pellets and leaf remains from each Petri dish were weighed. There is very little variation in weights of faecal pellets in individual isopods (<5%). Thus, daily faecal production of each isopod can be determined (without having to weigh every sample) from the formula W/T×D (where W = total weigh of faecal pellets produced by an individual during the experiment, T = total number of faecal pellets and D = number of faecal pellets produced during the previous 24 hr). Assimilation efficiency (AE) was calculated for each individual as AE=(C-F)/C, where C = consumption and F = defecation in mg dry weight. Consumption rate was not measured directly. Preliminary experiments have shown that food consumption is directly proportional to faecal production rates. Thus, the latter are the values given in Figs. 1 and 2. Faecal production rate is presented as a percentage of dry

body weight of each individual to allow for individual weight differences and are given on a weekly basis.

Each sample of woodlice tissue was digested in 2 mL of boiling concentrated Analar grade nitric acid (BDH Chemicals) and diluted to 10 mL with double-distilled water. The leaves were digested in 20 mL of boiling Analar nitric acid and diluted to 100 mL. The digests were analyzed for cobalt by flame atomic absorption spectrometry (Varian Spectra 30). Concentrations of cobalt in control leaves were always < 1 $\mu g g^{-1}$. The levels of cobalt in contaminated leaves were within 5 % of the expected values.

RESULTS AND DISCUSSION

Food consumption, as measured by faecal production, was reduced in both species exposed to 2500 µg Co g^{-1} (Fig. 1d, 2d). However, at 500 µg Co g^{-1}, only *Oniscus asellus* showed evidence of a decrease in consumption (Fig. 2c). Transfer of the isopods from the wild to the laboratory stimulated moulting in some animals. Most *Porcellio scaber* shed their exoskeleton during the first 14 days of the experiment, and this is the reason for the decreased faecal production in the second week. However, food consumption recovered in week 3 in all but the 2500 µg Co g^{-1} treatment (Fig.1). In future experiments, it would be advisable to maintain the isopods in the laboratory for a few weeks before exposing them to the test substance to allow them to perform this initial moult.

The decreased consumption rates observed on the most contaminated diets were probably due to feeding deterrence rather than poisoning. None of the isopods died during the experiment, although the long-term effects of cobalt assimilation (Fig.3, Table 1) cannot be determined from this study.

The faeces of the isopods fed on clean leaves for two weeks after consuming treated food was analysed for cobalt. The concentrations were extremely low (<1 µg Co g^{-1}) indicating that the isopods excreted very little of the cobalt they had assimilated during feeding on contaminated leaves. The cobalt is stored in granules in the S cells of the hepatopancreas where it can still be detected by X-ray microanalysis for at least 6 months after transfer to a clean diet (Hopkin 1989).

Food was assimilated with an efficiency of between 25-33% in most treatments (Fig. 4). This is in agreement with other similar studies (Van Capelleveen 1987, Van Straalen and Verweij 1991). An exception was *O. asellus* exposed to the highest concentration of cobalt, where the food assimilation rate was significantly higher (44%). However, consumption in this group of *O. asellus* was slow and after two weeks they stopped eating. Thus the higher food assimilation rate could be explained by longer residence time of food in the gut.

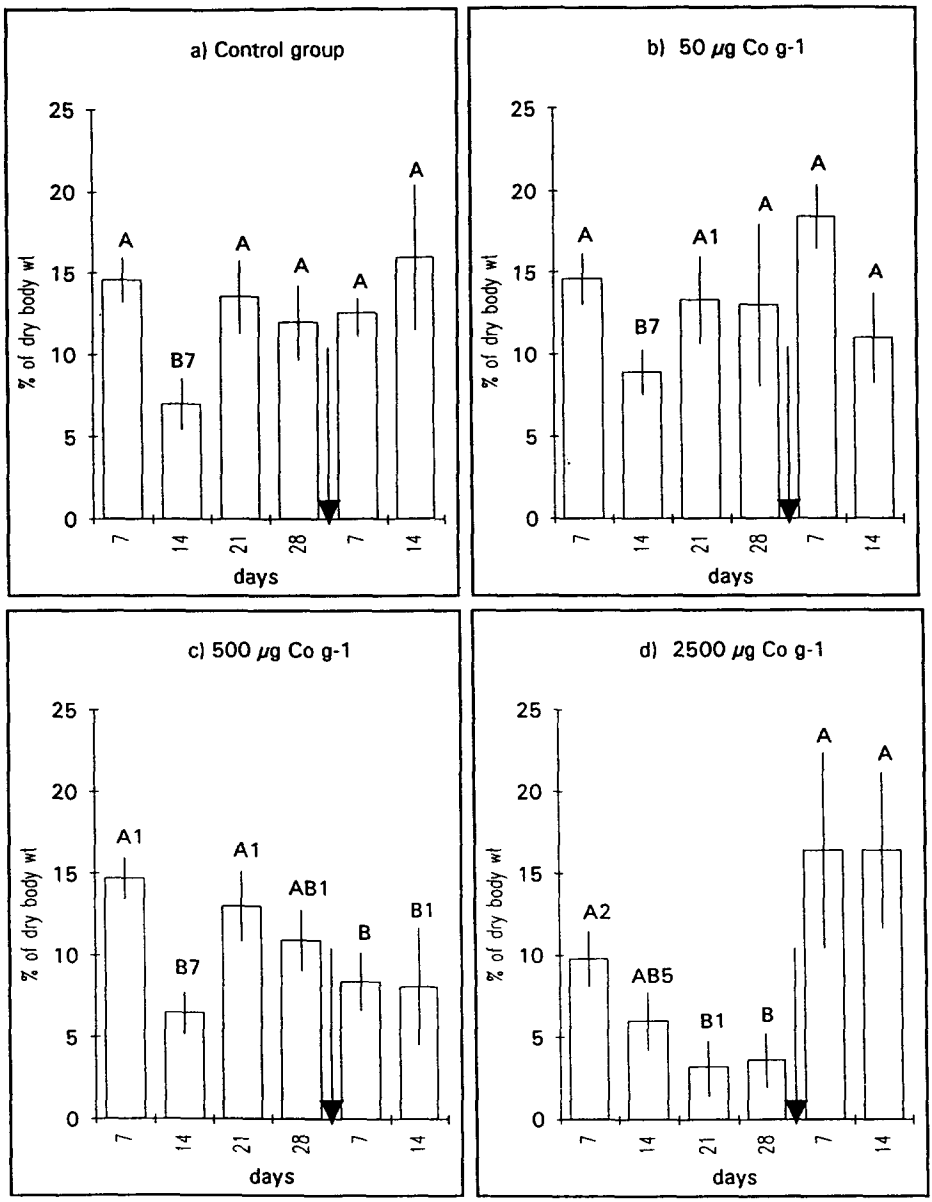

Figure 1. *Porcellio scaber*. Weekly faecal production rates of isopods (expressed as % dry body wt.) feeding on a) uncontaminated leaves and leaves of Field Maple contaminated with b) 50 c) 500 or d) 2500 µg Co g^{-1} dry wt. for four weeks, followed by two weeks (arrow) on uncontaminated leaves. Means and standard error bars (N=12 until day 28, N=6 thereafter) are not significantly different at the 5% level (t test) if they have the same letter (A, B). Numbers indicate the number of animals that were moulting during the 7 day period.

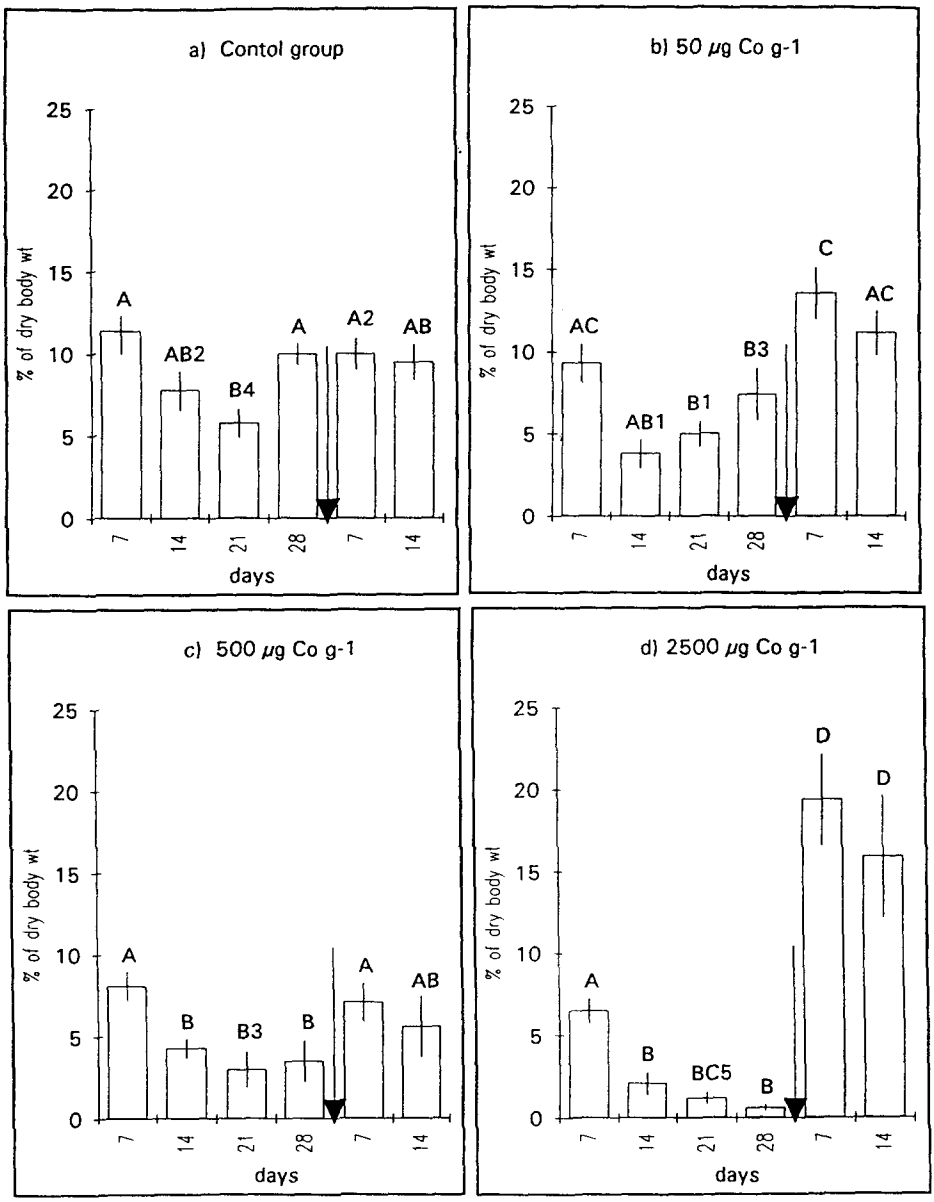

Figure 2. *Oniscus asellus*. Weekly faecal production rates of isopods (expressed as % dry body wt.) feeding on a) uncontaminated leaves and leaves of Field Maple contaminated with b) 50 c) 500 or d) 2500 µg Co g^{-1} dry wt. for four weeks, followed by two weeks (arrow) on uncontaminated leaves. Means and standard error bars (N=12 until day 28, N=6 thereafter) are not significantly different at the 5% level (t test) if they have the same letter (A, B, C, D). Numbers indicate the number of animals that were moulting during the 7 day period.

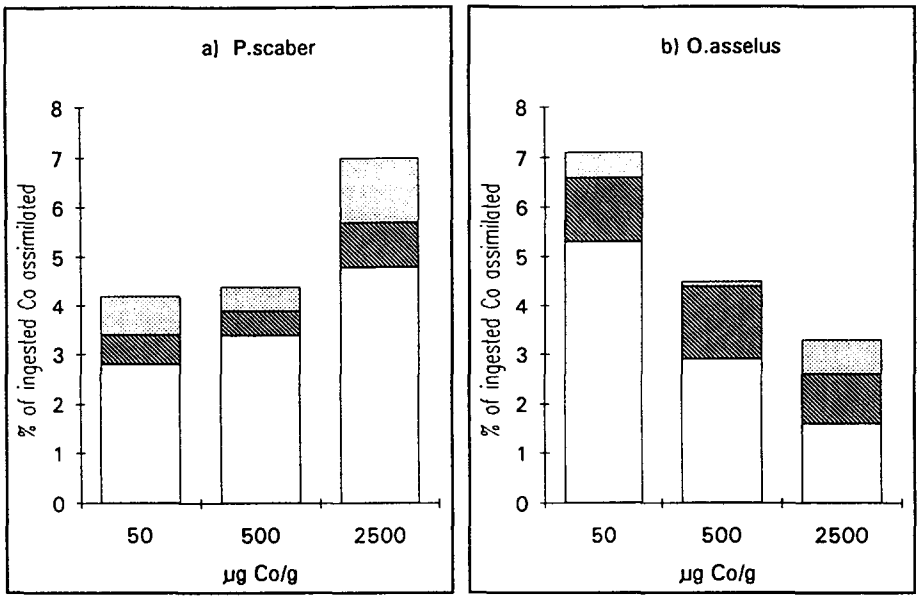

Figure 3. Mean percentage of total amount of cobalt ingested with the food (100%) that were present in the three tissue fractions of a) *Porcellio scaber* and b) *Oniscus asellus* after the first four weeks of the experiment (dot shading="rest", line shading=gut and unshaded=hepatopancreas). N=12 for each treatment. Note that most of the cobalt is stored in the hepatopancreas and that the overall assimilation rate is low (always < 7 %).

Table 1. Mean concentrations of cobalt in the hepatopancreas (hep.), gut and rest body fractions of *Porcellio scaber* and *Oniscus asellus* exposed to three different Co concentrations for four weeks (N=12, SE=standard error). Concentrations of cobalt in tissue fractions of isopods fed on control leaves were always < 5µgg^{-1}.

P.scaber	µg Co g-1 dry wt		
µg Co g-1	hep.	gut	rest
50	135	40.0	<1
SE	35.0	25.0	<1
500	1820	255	10.0
SE	210	85.0	3.0
2500	3600	955	35.0
SE	665	255	10.0

O.asellus	µg Co g-1 dry wt		
µg Co g-1	hep.	gut	rest
50	130	30.0	<1
SE	30.0	20.0	<1
500	915	210	<1
SE	165	70.0	<1
2500	1110	540	20.0
SE	145	110	5.0

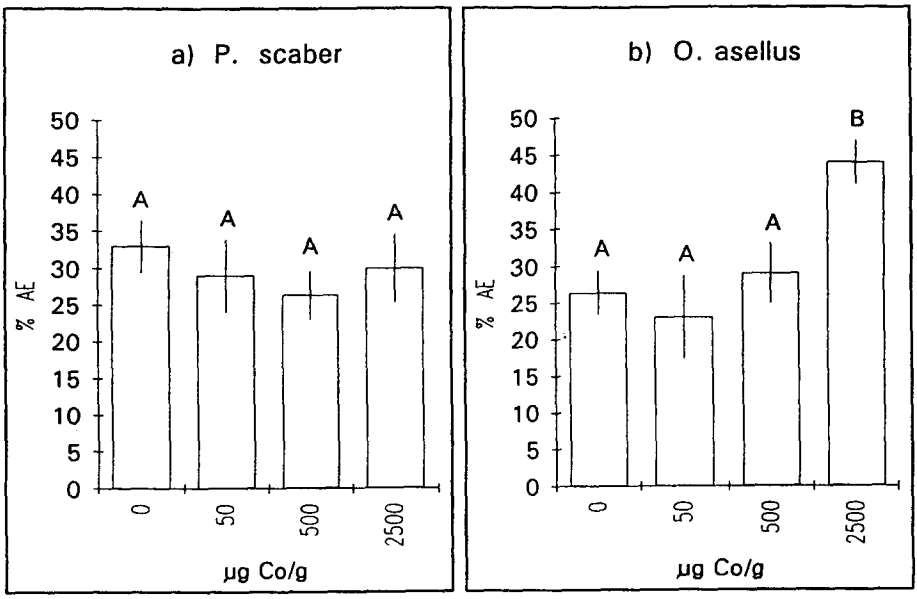

Figure 4. Mean food assimilation efficiencies (AE) in a) *Porcellio scaber* and b) *Oniscus asellus* feeding for four weeks on leaves of Field Maple contaminated with cobalt. Means and standard error bars are not significantly different at the 5% level (t-test) if they have the same letter (A, B). N=12 for each treatment.

The ecotoxicological test described in this paper is inexpensive, easy to perform and provides information on food consumption rate in terrestrial isopods. Assimilation is measured which allows potential food chain transfer of chemicals to predators of isopods to be assessed. However, further work is needed on other species and chemicals to standardise the methodology for international use. In addition, the "critical concentrations" that reduce food consumption in the laboratory need to be related to field populations. Work is currently underway to test the effects of other metals on food consumption and assimilation in *Porcellio scaber* and *Oniscus asellus*.

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