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TERRESTRIAL ISOPODS—A GOOD CHOICE FOR TOXICITY TESTING OF POLLUTANTS IN THE TERRESTRIAL ENVIRONMENT

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Abstract—Terrestrial isopods are suitable invertebrates for testing the relative toxicities of chemicals present in the terrestrial environment. Terrestrial isopods respond in numerous ways to elevated concentrations of chemicals in their food, but only a few of these responses can be used as toxicological endpoints. The most suitable are changes in reproduction, food consumption, moult cycle duration, and structure of the digestive glands. These responses are able to provide accurate indications of sublethal toxicity. Toxicity tests with terrestrial isopods could be much more reliable through the use of positive controls. A positive control with a reference toxicant could also be supplemented by a reference endpoint. The most suitable reference endpoint is change of food consumption rate. Toxicity testing with terrestrial isopods is a very promising method for fast, routine, and inexpensive laboratory determination of the relative toxicities of chemicals in the terrestrial environment.

Keywords—Toxicity testing Measurement endpoints *Porcellio scaber* Invertebrates Terrestrial environment

INTRODUCTION

Various toxicological endpoints are used to measure relative toxicities of pollutants at different levels of biological organization ranging from molecules to populations [1–3]. Assessment endpoints such as intrinsic population growth rate, decomposition processes, or biomass turnover rate are explicit expressions of the actual environmental values that are to be protected or at least controlled. Measurement endpoints such as survival, growth, or reproduction are measurable responses to a pollutant [1,4,5]. Because the assessment endpoints are often not observable or have fixed values, measurement endpoints are used in their place [1].

Despite their many shortcomings, laboratory-conducted single-species toxicity tests still have a central position in chemical risk assessment [6]. Recently, toxicity tests based on biochemical, physiological, behavioral, or histological endpoints in place of traditional survival, growth, and reproduction responses have been developed [2,3,7]. Advantages of suborganismic toxicological measurements are that they provide data on bioavailability measured by amounts of chemicals present in an organism and data on toxicity of contaminants detected by sublethal effects [2].

In the future, single-species toxicity tests will probably maintain their importance for characterization of chemical impact. However, they have to be conducted with more sensitive endpoints (biochemical, histological, and physiological biomarkers). On the basis of results of these tests, decisions on more complex, more time-consuming, and more expensive tests at higher levels of biological organization could be made.

In this article, the terrestrial isopod *Porcellio scaber* (Lat.) is presented as a suitable organism for toxicity testing of pollutants in the terrestrial environment. The literature on the effects of chemicals on *P. scaber* is reviewed, and findings are compared with results of our experimental work. The responses that could be used as endpoints in toxicity tests are detailed. The interrelationships between different endpoints are discussed. The importance of the use of a reference toxicant and a reference endpoint with a positive control is stressed.

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TOXICITY TESTS WITH TERRESTRIAL ISOPODS

Only a few animal species fulfill the criteria for being used as test organisms for testing the effects of pollutants in the terrestrial environment. Some of these criteria are size, abundance, simple identification, and simple aging [8]. In addition, there must be sufficient background knowledge on the biology of the species, its sublethal responses to chemicals, and information that enables differentiation of measured effects from natural background variability.

A large body of evidence on the different effects of chemicals on terrestrial invertebrates exists; however, only a few of these effects can be used as toxicological endpoints: (1) mortality (lumbricids [9,10], enchytraeids [11], molluscs [12], Collembola [13], beetles [14], predatory mites [14], and spiders [14]); (2) growth (lumbricids [15–17] and Collembola [12]); (3) abundance and biomass (lumbricids and enchytraeids [11]); (4) cocoon production (lumbricids [15,17]); (5) total number of offspring produced (enchytraeids [11] and lumbricids [15,16]); (6) reproduction (lumbricids [18], oribatid mites [19], isopods [20], Collembola [13], beetles [14], and predatory mites [14]); (7) behavior (spiders [14] and beetles [21]); (8) food consumption (isopods [22,23] and molluscs [12]); and (9) survival, reproductive behavior, dormant state, and new shell growth (molluscs [12]).

The most widely used toxicological endpoints are mortality, growth, and reproduction. The problem with mortality as a toxicological endpoint is its low sensitivity. The use of other responses such as growth and reproduction is sometimes not convenient because of the long duration of the required test

Table 1. Sublethal effects of chemicals (except Zn) on the terrestrial isopod *Porcellio scaber* following exposure to artificially contaminated food and food from metal-polluted locations

Response	Chemicals	LOEC	NOEC	Reference
Survival	Litter from Zn smelter side	8 μg Cd/g 50 μg Cu/g 750 μg Pb/g		[28]
Survival	Added Cd or Cu	500 μg Cd/g	200 μg Cu/g	[28]
Survival	Added Pb_3O_2	12,800 μg Pb/g	6,400 μg Pb/g	[36]
Survival and growth	$CdCl_2 \cdot 2(1/2H_2O)$	LC50, 1,000 μg Cd/g EC10, 95 μg Cd/g		[37]
Growth	Added $Cd(NO_3)_2$	10 μg Cd/g		[38]
Growth	Cd- and Zn-contaminated food from polluted environment		65 μg Cd/g	[39]
Growth and food assimilation	Added B*a*P	125 μg B*a*P/g (growth)	125 μg B*a*P/g (food assimilation)	[40]
Fragmentation activity	Cd- and Zn-contaminated litter from polluted environment	18 μg Cd/g		[39]
Food consumption	Added $CoCl_2 \cdot 6H_2O$	500 μg Co/g	50 μg Co/g	[22]
Food consumption	Cd added to food		11 μg Cd/g	[26]
Food consumption	Herbicides (Anetos, TopKH, Roundup, Krenitezol)	Application rate, 6–12 L/ha		[41]
Brood development time	Added Cd		10 μg Cd/g	[26]
70 kD heat shock protein	Cd, Cu, and Pb in litter from Pb/Zn smelter side	41.9 μg Cd/g 628.4 μg Cu/g 1,658 μg Pb/g	1.6 μg Cd/g 55.5 μg Pb/g	[42]
70 kD heat shock protein	Added $Pb(NO_3)_2$	100 μg Pb/g	10 μg Pb/g	[42]
Respiration	Fe and Mn from polluted environment	2,906 μg Fe/g 324 μg Mn/g		[43]
Ultrastructural changes of gland cells	Added $CdCl_2$ or $PbCl_2$	57 μg Cd/g 517 μg Pb/g		[44]

B*a*P, benzo[*a*]pyrene; EC10, 10% effective concentration; LC50, 50% lethal concentration; LOEC, lowest-observed-effect concentration; NOEC, no-observed-effect concentration.

or the high individual variability of the response. The most sensitive measures are biochemical, histological, and physiological endpoints. Few data on these endpoints in terrestrial invertebrates exist [7].

The usefulness and relevance of a test is also compromised by the nature of the substrate used [24]. When artificial substrates are used in toxicity tests, extrapolation of test results to the field situation is limited. In the future development of test methods, standardization of the natural or regional test substrate is essential.

Besides earthworms, terrestrial isopods are perhaps one of the most frequently used terrestrial invertebrates for testing the effects of chemicals (Table 1). Isopods inhabit the upper layer of the soil and surface leaf litter in a variety of urban and natural habitats. Regarding their body length, they belong to both meso- and macrofauna. They feed mainly on dead organic material, are distributed throughout the world, and are abundant in many different terrestrial environments [25]. They also play an important role in decomposition processes, mainly as fragmentors of dead plant material. Any changes in feeding rates of terrestrial isopods affect the decomposition process and subsequently matter and energy flux through ecosystems. One of the reasons for considering terrestrial isopods as suitable crustaceans for toxicity testing is that the toxicity tests presently available for routine use cover only insects and annelids. Accepted soil ecotoxicity tests using *Eisenia fetida* and *Folsomia candida* are used to assess the quality of soil, while isopods could be used to assess the relative toxicity of the litter layer.

Terrestrial isopods fulfill most of the required criteria for characterization of the relative toxicities of chemicals [8] (Appendix 1). Usually, growth, reproduction, and life-cycle investigations are not the most suitable when terrestrial isopods are selected as the test organism. Rates of growth in terrestrial isopods over several weeks are rather variable even for one individual [26]. Reproduction is also difficult to assess because after mating, females may retain the sperm for a long period before reproducing [20]. The life cycle of most terrestrial isopods is relatively long, often more than 6 to 8 months [24].

Many investigations have been performed to discover the effects of metals on organisms (Tables 1 and 2). Usually, the laboratory test is considered to be the worst-case situation because test animals are exposed constantly to contaminated food and have no opportunity to select alternative food or shelter. Under field conditions, exposure may be lower because the chemical is not distributed uniformly in the habitat and bioavailability will often (not always) be lower because of various sorption processes [24]. In addition, in the field, animals *could* select differently contaminated food [26,27] or move to a different location.

Metals in artificially contaminated leaves or leaves from polluted environments can produce different responses at similar concentrations (Table 1). The concentrations of Pb or Cd that reduced survival of *P. scaber* in the field were 20 or 60

Table 2. Sublethal effects of Zn on the terrestrial isopod *Porcellio scaber* following exposure to artificially contaminated food and food from metal-contaminated locations

Response of *P. scaber*	Chemicals	LOEC	NOEC	Reference
Moult frequency	Added $ZnCl_2$	500 μg Zn/g	250 μg Zn/g	[31]
Digestive glands	Added $ZnCl_2$	500 μg Zn/g	250 μg Zn/g	[32]
Respiration, weight increase, and reproduction	Added ZnO	1,650 μg Zn/g	900 μg Zn/g	[45]
Growth	Cd and Zn from polluted environment		1,055 μg Zn/g	[39]
Respiration	Zn, Fe, and Mn from polluted environment	411 μg Zn/g		[43]
Ultrastructural alterations of hepatopancreatic cells	Added $ZnCl_2$	1,975 μg Zn/g		[44]
Food consumption	Added $Zn(NO_3)_2 \cdot 6H_2O$	2,000 μg Zn/g	1,000 μg Zn/g	[23]
Food consumption	Added ZnO	2,990 μg Zn/g		[46]
Food consumption	Added $Zn(NO_3)_2 \cdot 6H_2O$	3,240 μg Zn/g		[20]
Fragmentation activity	Zn from polluted environment	2,200 μg Zn/g		[39]
Survival	Added Zn sulfur	5,000 μg Zn/g		[28]
Mortality	Litter from Zn smelter side	800 μg Zn/g		[28]
Adult survival	Added ZnO	6,400 μg Zn/g	3,200 μg Zn/g	[36]
Survival	Litter from Zn smelter side		4,150 μg Zn/g	[29]
Survival	Added Zn	1,000 μg Zn/g		[29]
Survival	Litter from primary smelting works side	1,430 μg Zn/g		[30]

LOEC, lowest-observed-effect concentration; NOEC, no-observed-effect concentration.

times lower than those in tests with added Pb or Cd [28]. In the case of Zn (Table 2), the concentration of added Zn that affected survival was reported to be five times *lower* than that in food taken from a Zn-polluted location [29]. On the other hand, it was reported that concentrations of Zn added to food that affected survival were approximately five times *higher* than those in food from polluted environments [28]. However, these differences can be explained by the different sensitivities of adults and juveniles, the length of time for which the experiments were conducted, and the chemical forms of the metals in the foods supplied [28–30].

Toxicological endpoints differ in sensitivity to chemicals and in the concentrations that produce the same effects on organisms when exposed to artificially contaminated food or food from polluted environments. Some toxicological endpoints (survival and growth) might exhibit integrated responses to all chemicals; others probably do not. Several factors probably influence extrapolation of the effects of chemicals from laboratory tests to the field. Based on the literature, it is evident that this extrapolation factor is related to the measurement endpoint and the chemical tested.

A body of evidence exists on the effects of elevated concentrations of Zn on *P. scaber* (Table 2). Our results have shown that the most sensitive responses to elevated concentrations of Zn are moult cycle and ultrastructure of the digestive glands [31,32]. The Zn concentrations in artificially contaminated substrate that produced the observed effect on food consumption and survival were four and 10 times higher, respectively, than those that affected the moult cycle or digestive glands. Physiological and histological endpoints are more sensitive to the same metal than whole-organism endpoints. Therefore, these endpoints could be used for early warning studies. Whole-organism responses are much more relevant in terms of environmental risk assessment.

Similar data indicating that whole-organism responses such as survival are less sensitive to elevated concentrations of metals in the food than biochemical or histological responses such as a 70 kD heat shock protein or ultrastructural changes of gland cells also exist for other metals like Pb and Cd.

Presently, *P. scaber* is by far the most studied terrestrial isopod and therefore is proposed as the organism of choice for testing the toxicity of metals in the terrestrial environment [22,23]. If terrestrial isopod toxicity tests are standardized for international use, then in some cases, other species of terrestrial isopods might be used instead of *P. scaber*. For example, this substitution may be appropriate in areas where other species of terrestrial isopods are more common and ecologically significant. Future development of toxicity testing with terrestrial isopods will probably also be focused on the use of isopods that have shorter life cycles and that are parthenogenetic (e.g., *Porcellionides pruinosus* or *Trichoniscus pusillus*).

REFERENCE TOXICANTS AND REFERENCE TOXICOLOGICAL ENDPOINTS

All toxicity assays must be conducted using well-established negative (clean) controls. Toxicity assays should also include positive controls conducted with reference toxicants. Reference toxicants are used to provide insight into the changes in sensitivity that might occur as a result of disease or handling stress. Concurrent tests using a reference toxicant should be implemented at regular intervals and for each new batch of test organisms obtained [33,34].

When terrestrial isopods are used as test organisms, positive controls are important because the animals can become tolerant to polluted environments [26,27]. Tolerant populations will respond differently when exposed to metals [26,27]. Therefore, it is necessary to test each new population of test organisms for sensitivity to pollutants before conducting toxicity testing. Although a laboratory culture must be maintained for standard toxicity tests, the sensitivity of this population to pollutants can also change, so it should be regularly tested with reference toxicants.

Our results show that zinc chloride or zinc nitrate could be used as a reference toxicant in toxicity tests with *P. scaber* [23,31,32]. Zinc-positive controls could provide data on the sensitivity of different testing populations of terrestrial isopods. Studies with artificially contaminated substrates were conducted with $ZnCl_2$, $Zn(NO_3)_2$, and ZnO and have proved that all are suitable reference toxicants for positive controls (Table 2). With regard to the salts applied, there are no differences in toxicity of these Zn salts to *P. scaber*.

Besides reference toxicants, reference endpoints could also be used as positive controls. Our results show that feeding rate is the most suitable parameter to be standardized as a toxicological endpoint in a positive control if toxicity of metals is tested [22,23].

DISCUSSION

Toxicity testing systems can be preventive or auditive, either predicting potential toxicities of compounds before release or assessing the relative toxicities of compounds already present in contaminated areas [35]. Accurate assessment of the relative toxicities of chemicals requires proper test organisms with proper toxicological endpoints and proper exposure of chemicals so that the bioavailable concentrations of chemicals are known or can be calculated.

At our present state of knowledge, terrestrial isopods are one of the most promising organisms in terrestrial ecotoxicology. Toxicity studies on terrestrial isopods performed by different investigators show some common features (Tables 2 and 3). (1) Lower levels of biological organization (biochemical, histological, and physiological) respond to lower concentrations of metals in the food than the whole organism. (2) Safety factors for extrapolation of results from toxicity tests with artificially contaminated food to situations with field-contaminated food depend on many parameters. The most important of these are the properties of tested chemicals and the selected toxicological endpoint. For Zn, field concentrations could be higher or lower than concentrations of Zn added artificially to the food to produce the same effect. (3) Food consumption rate, moult cycle, and structure of digestive glands fulfill most of the criteria for good measurement endpoints (Table 3).

Future development of toxicity tests with terrestrial isopods should be focused on the use of more endpoints in one toxicity test, which could give a more accurate measurement of relative toxicity. For example, if the structure of digestive glands, moult cycle, food consumption rate, and reproduction of *P. scaber* were used as endpoints, the interrelationship between different endpoints could be established. Moult cycle and structure of digestive glands show the earliest indication of changes caused by Zn-contaminated food. Food consumption rate has an impact on ecological functions of isopods, and reproduction changes affect population and community structure. Reproduction and food consumption are affected when animals are exposed to approximately four times higher concentrations of zinc than those that affect moult cycle and digestive glands. The interrelationship between these four endpoints needs to be investigated for other chemicals and compared with the effects of food polluted in the field.

Table 3. Sublethal effects of chemicals on the terrestrial isopod *Porcellio scaber*—How three toxicological endpoints of *P. scaber* fulfill the criteria of a good measurement endpoint [1]

	Toxicological endpoint		
	Food consumption	Moult cycle	Digestive glands
Readily measured?	Yes	Yes	No
Appropriate to the scale of the disturbance/pollution?	Yes	Yes	Yes
Appropriate to the route of exposure?	Yes	Yes	Yes
Appropriate temporal dynamics/persistence of measured response	No	No	No
Low natural variability?	Yes	Yes	No
Broadly applicable?	Yes	Yes	Yes
Correspond to or predictive of an assessment endpoint?	Yes	?	?
Existing data series?	No	No	No

Besides standardization of test animals and endpoints, standardization of test substrate is also important. Drobne and coworkers [22,23,31] proposed the use of field maple (*Acer campestre*) or hazelnut (*Corylus avellana*) leaves treated with chemicals in the laboratory and the same substrate from polluted environments.

The duration of the test procedure is related to the selected toxicological endpoint. Digestive glands and food consumption respond to chemicals within weeks, whereas moult cycle and reproduction respond within months. Food substrate is subjected to changes due to laboratory conditions (e.g., bacterial and fungal growth). Therefore, toxicity tests of shorter duration might provide more accurate data on relative toxicities than those of long duration.

Despite these other predictive methods, laboratory-conducted single- or multispecies toxicity tests cannot be replaced in the future. They will become more sensitive by application of more sensitive endpoints. When terrestrial isopods are used as test animals, traditional endpoints can be replaced with histological, physiological, and whole-organism responses to chemicals. Standardized laboratory testing proposed by Drobne and Hopkin [22,23] can provide background data for selection of more complicated measurements and toxicity tests at higher levels of biological organization.

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APPENDIX

Criteria of species selection for monitoring the impact of pollutants in the field listed by Beeby [8] and how the terrestrial isopod *Porcellio scaber* meets these criteria

Criterion	*P. scaber*
Abundant?	Yes
Wide distribution?	Reasonably yes
Life span at least 1 year?	Yes
Large enough to provide enough tissue for analysis?	Yes
Easy to identify and collect?	Yes
Easy to age?	No[a]
Easy to handle in the laboratory?	Yes
Established body of knowledge on the biology of the species?	Yes
Sensitive indicator of likely impact on the rest of the community?	Few data

[a] Smaller animals are not always younger.