Soil ecotoxicology: state of the art and future directions

Cornelis A.M. van Gestel

1 Department of Ecological Science, Faculty of Earth and Life Sciences, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

Corresponding author: Cornelis A.M. van Gestel (kees.van.gestel@falw.vu.nl)

Academic editor: J. Štrus | Received 25 November 2011 | Accepted 26 January 2012 | Published 20 March 2012

Citation: van Gestel CAM (2012) Soil ecotoxicology: state of the art and future directions. In: Štrus J, Taiti S, SFenthourakis S (Eds) Advances in Terrestrial Isopod Biology. ZooKeys 176: 275–296. doi: 10.3897/zookeys.176.2275

Abstract

Developments in soil ecotoxicology started with observations on pesticide effects on soil invertebrates in the 1960s. To support the risk assessment of chemicals, in the 1980s and 1990s development of toxicity tests was the main issue, including single species tests and also more realistic test systems like model ecosystems and field tests focusing on structural and functional endpoints. In the mean time, awareness grew about issues like bioavailability and routes of exposure, while biochemical endpoints (biomarkers) were proposed as sensitive and potential early-warning tools. In recent years, interactions between different chemicals (mixture toxicity) and between chemical and other stressors attracted scientific interest. With the development of molecular biology, omics tools are gaining increasing interest, while the ecological relevance of exposure and effects is translating into concepts like (chemical) stress ecology, ecological vulnerability and trait-based approaches. This contribution addresses historical developments and focuses on current issues in soil ecotoxicology. It is concluded that soil ecotoxicological risk assessment would benefit from extending the available battery of toxicity tests by including e.g. isopods, by paying more attention to exposure, bioavailability and toxicokinetics, and by developing more insight into the ecology of soil organisms to support better understanding of exposure and long-term consequences of chemical exposure at the individual, population and community level. Ecotoxicogenomics tools may also be helpful in this, but will require considerable further research before they can be applied in the practice of soil ecotoxicological risk assessment.

Keywords

Toxicity tests, bioavailability, ecological effects, biomarkers, soil organisms, isopods
Introduction

Ecotoxicology studies the effects of chemicals on organisms in the environment, with the final aim to protect the structure and functioning of ecosystems. This aim generally is achieved by assessing effects on single species of selected test organisms and trying to extrapolate the obtained (no) effect concentrations to safe levels for populations and communities. In the ecotoxicological risk assessment of chemicals, such safe levels are then compared with predicted or measured exposure levels to assess the possible risk for exposed ecosystems.

This paper will give an overview of developments in soil ecotoxicology, focusing on soil invertebrates, starting with a historical overview. Based on that, the state-of-the-art of current soil ecotoxicology will be depicted. This is done by first describing the way soil ecotoxicological data are used in the risk assessment of new and existing chemicals or the assessment of the risks of soil contamination. Next, the development of soil toxicity tests is outlined, followed by considerations on the inclusion of bioavailability, and the use of multiple species, model ecosystem and field tests. Then tools of assessing the possible risk of contaminated soils are described. Finally, some thoughts are given on the future of soil ecotoxicology. As this paper was written on the basis of a presentation at an isopod meeting, special attention will be given to the use of isopods in soil ecotoxicological testing.

Historical perspective of soil ecotoxicology

When thinking of ecotoxicological effects, it is often referred to Rachel Carson, publishing her book ‘Silent Spring’ in 1962. This book was among the first describing the negative side-effects of the increasing use of synthetic pesticides that started from the second World war onwards. The book mainly focused on pesticide effects on birds, especially singing birds that apparently became silent due to the effects of chlorinated pesticides accumulating in the food chain. This book however, was not unique in ringing the alarm bell, although other bells did not sound that loud.

The first soil ecotoxicological papers date back to the 1960s, reporting observations on the negative effects of pesticides on soil invertebrates (e.g., Fox 1964, Edwards 1969). Similar to developments in aquatic ecotoxicology, these observations triggered the performance of toxicity tests with selected organisms to enable prediction of such side-effects in the field. First results of such toxicity tests, using Collomola and earthworms, were published by the end of the 1960s, also on pesticides (e.g., Ghabbour and Imman 1967, Scopes and Liechtenstein 1967). In the mean time, the Organization for Economic Co-operation and Development (OECD) started developing Guidelines for the testing of chemicals, to support the chemical risk assessment and pesticide registration procedures developed in most Western countries. It took another 15 years before the first toxicity test with soil invertebrates was internationally standardized by OECD, using earthworms and only focusing on short-term (acute) responses like sur-
vival (OECD 1984). In the 1980s and 1990s, the development of soil ecotoxicological tests received more attention, e.g. in the SECOFASE project funded by the European Union, that explored the possibilities of developing toxicity tests with different soil invertebrates, including earthworms, enchytraeids, nematodes, Collembola, staphylinid beetles, mites, centipedes, millipedes and isopods (Løkke and Van Gestel 1998). Several of the methods developed in SECOFASE never made it to standardization, but the project laid a basis for testing new species, using sub-lethal endpoints and including interactions between species.

During the last ten years, there has been a renewed attention for effects of mixtures of chemicals in soil (Van Gestel et al. 2011), while the interaction of chemicals with other stress factors, like temperature and soil moisture content, also came into focus (Holmstrup et al. 2010). In addition, the available test methods outline below are nowadays applied to new and emerging chemicals, especially to determine the toxicity of nanoparticles using earthworms (e.g. Shoults-Wilson et al. 2011a,b; Heckmann et al. 2011; Hooper et al. 2011), Collembola (Kool et al. 2011), and isopods (e.g. Jemec et al. 2008; Drobne et al. 2009; Pipan-Tkalec et al. 2010).

**Ecotoxicological risk assessment**

In ecotoxicological risk assessment, two approaches can be distinguished. One approach aims at predicting possible effects of (new) chemicals in order to regulate their use or prevent their introduction onto the market. This predictive approach (prognosis) uses laboratory toxicity tests to obtain toxicity data that are used to derive safe levels of chemicals in the environment. The second approach is assessing the actual ecological risk or damage in case of pollution. This diagnostic approach (diagnosis) enables setting priorities for remediation and risk reduction, and may provide triggers for the management of contaminated land.

Prognosis starts from the paradigms also used in human toxicology. It assumes that the risk of a chemical for ecosystems can be estimated from its toxicity to a number of surrogate test or indicator species, exposed in standard laboratory toxicity tests. These tests aim at assessing toxicity, which is expressed in terms of dose-response relationships for effects on selected endpoints like survival, growth and reproduction. Toxicity is quantified by parameters like LC$_{10}$ and LC$_{50}$ (the concentrations killing 10% and 50% of the exposed test organisms, respectively), EC$_{10}$ and EC$_{50}$ (the concentrations causing 10% and 50% reduction, respectively in a measured endpoint, e.g. growth or number of juveniles produced), and NOEC and LOEC (no-observable and lowest-observable effect concentration, respectively). Since there is no ‘most sensitive species’ a battery of toxicity tests is needed to obtain proper insight into the potential hazard of a chemical for the ecosystem. In prognosis, the outcome of toxicity tests is used to establish thresholds or safe levels of chemicals in soil, which can be compared with measured or predicted exposure data (soil concentrations) to assess the (potential) risk.
A critical part of this procedure is the derivation of safe levels of chemicals on the basis of available toxicity data. When only short-term (acute) toxicity data are available (usually focusing on survival) or data for a limited number of species, somewhat arbitrary application factors are applied to derive safe levels that should protect ecosystems. For example, when only one or two LC_{50} values are available, a factor of 1000 is applied to the lowest LC_{50} value; this factor should be sufficient to extrapolate from acute to chronic effects (factor of 10), from one or few species to many species (factor of 10), and from laboratory to field (factor of 10). When sublethal toxicity data (usually NOEC or EC_{10} values for effects on e.g. reproduction) are available for 3 or more species, application of a factor of 10 to the lowest value is considered sufficiently protective. When many toxicity data are available (preferably ≥ 8) for species representative of different taxonomic groups (see below), a statistical method may be applied. Such a statistical method is used to construct a species-sensitivity distribution (SSD), which assumes a log-normal or log-logistic distribution of the sensitivities of species in an ecosystem. From such an SSD the 95% lower limit is selected as the safe level. At this level, at least 95% of the species in the ecosystem are supposed to be safe (Posthuma et al. 2002).

Diagnosis may use the same tools as applied for prognoses, but it more heavily relies on ecological methods and environmental chemistry. Basically, toxicity tests or bioassays are used as diagnosis tools to assess toxicity of soil samples from a contaminated site. Results of the bioassays, together with those of chemical measurements and ecological field observations, are used to assess the potential risk of soil contamination. The three tools together form the TRIAD approach (Jensen and Mesman 2006).

Toxicity tests

Both prognosis and diagnosis use toxicity tests, and in both cases a battery of tests is recommended. Criteria to select tests for such a battery have been formulated e.g. by Van Gestel et al. (1997). These criteria among others include:

1. Practicability, referring to the feasibility and cost-effectiveness of a test;
2. Acceptability, including aspects like standardization, reproducibility and statistical validity of a test method as well as its broad chemical responsiveness;
3. Ecological meaning, including sensitivity and ecological realism of the test method.

To obtain a balanced battery of tests, in addition the following criteria need to be taken into account (Van Gestel et al. 1997):

1. Representativeness for the ecosystem to protect: this includes e.g. the representation of organisms having different life-histories, representing different functional groups, different taxonomic groups and different routes of exposure;
2. Representativeness of responses, to make sure responses measured really are relevant for the protection of populations and communities;
3. Uniformity, which refers to the possibility to apply all tests in a battery to the same test media.

By the end of the 1990s and early 2000 toxicity tests, using sub-lethal endpoints like reproduction, were standardized for enchytraeids, earthworms and Collembola by both the OECD and the International Standardization Organization (ISO). But also Environment Canada, the United States Environmental Protection Agency (EPA) and ASTM International (formerly known as the American Society for Testing and Materials) have described similar methods. Recently, for the same organisms, avoidance behaviour tests have been described, while for earthworms and enchytraeids a bioaccumulation test is available. Table 1 provides an overview of the toxicity tests with soil invertebrates available at the moment.

The oldest standardized toxicity test guideline with soil invertebrates, OECD 207 (OECD 1984), describes two short-term toxicity tests, one using 14 days exposure in soil and the other one exposing the worms for 2 days to treated filter paper. Both methods use survival as the only endpoint. The test on filter paper is only rarely applied nowadays, as it does not have any relevance for exposure in soil. It may however, be a

| Test organism | Species                          | Duration (days) | Endpoint          | Guideline                | Reference                  |
|---------------|----------------------------------|-----------------|-------------------|--------------------------|----------------------------|
| Earthworms    | *Eisenia fetida*/*Eisenia andrei*| 14              | Survival          | OECD 207, ISO 11268-1    | OECD (1984), ISO (1993)    |
|               |                                  | 28 (+28)        | Reproduction      | ISO 11268-2, OECD 222    | ISO (1998), OECD (2004b)   |
|               |                                  | 2               | Avoidance         | ISO 17512-1              | ISO (2008a)                |
|               | Field test, different species    | Up to 1 year    | Species diversity; abundance | ISO 11268-3              | ISO (1999b)                |
| Enchytraeids  | *Enchytraeus albidus*, other      | 21 (+21)        | Survival, Reproduction | ISO 16387, OECD 220      | ISO (2004), OECD (2004a)   |
|               | *Enchytraeus* species            | 2               | Avoidance         | No standard guidelines   | Amorim et al. (2008a,b)    |
| Mollusca      | *Helix aspersa*                  | 28              | Survival, Growth  | ISO 15952                | ISO (2006)                 |
| Mites         | *Hypoaspis aculeifer*            | 14              | Survival, Reproduction | OECD 226                | OECD (2008)                |
|               | *Platynothrus peltifer*          | 14, 70          | Survival, Reproduction | No standard guideline    | Van Gestel and Doornekamp (1998) |
|               | *Oppia nitens*                   | 28              | Reproduction      | No standard guideline    | Princz et al. (2010)       |
|               |                                  | 2               | Avoidance         | No standard guideline    | Owojori et al. (2011)      |
useful exposure method for the rapid screening of chemicals, assessing the uptake and/or biotransformation of chemicals or other types of mechanistic research.

Compared to survival, reproduction is a more relevant endpoint when translating effects to the population level. For that reason, an earthworm toxicity test focusing on reproduction has been developed (ISO 1998, OECD 2004b). Although focus is on reproduction, it is essential in this test to also include weight change of the earthworms, since there is evidence for a trade off between reproduction and growth, which may affect their response to toxicants (Van Gestel et al. 1992, Van Gestel et al. 1995). Like for earthworms, the tests with enchytraeids (ISO 2004, OECD 2004a) also focus on reproduction and survival as the endpoint, but for these organisms no separate short-term (acute) and sub-lethal tests were developed. All the reproduction toxicity tests available with oligochaetes typically have test durations of 21-28 days. In case of the earthworm test with *Eisenia fetida* or *Eisenia andrei* and the enchytraeid test with *Enchytraeus albidos*, this means exposing adult worms for 28 and 21 days, respectively, after which they are collected from the soil; the cocoons are incubated for another 28 or 21 days, respectively to enable determining the number of juveniles produced. Nowadays, *Enchytraeus crypticus* seems to be more commonly used for toxicity testing than *Enchytraeus albidos*. Since that species is smaller, adults are not removed from the soil and reproduction is determined after 28 days of exposure. Considering the shorter life cycle of *Enchytraeus crypticus* and its high reproductive output, limiting test duration to 21 days has recently been advocated (Van Gestel et al. 2011).

A standardized test with snails has been developed by ISO (2006), focusing on survival and growth of juveniles snails (*Helix aspersa aspersa*) after 28 days exposure.

Standardized toxicity tests with soil arthropods include the collembolan species *Folsomia candida* (ISO 1999a) and *Folsomia fimetaria* (OECD 2009), the predatory mite *Hypoaspis (Geolaelaps) aculeifer* (OECD 2008), and larvae of the insect *Oxythyrea funesta* (ISO 2005). The tests with the collembolans and the predatory mite typically

| Test organism | Species | Duration (days) | Endpoint | Guideline | Reference |
|---------------|---------|----------------|----------|-----------|-----------|
| Isopods       | *Porcellio scaber* | 28 | Survival, growth | No standard guideline | Hornung et al. (1998a,b) |
| Windows | *Porcellionides pruineosis* | 14 | Survival, reproduction | No standard guidelines | Jansch et al. (2005) |
|              |         | 2 | Avoidance | No standard guidelines | Loureiro et al. (2005) |
| Collembola    | *Folsomia candida* | 28 | Survival, reproduction | ISO 11267 OECD 232 | ISO (1999a); OECD (2009) |
|              | *Folsomia fimetaria* | 2 | Avoidance | ISO 17512-2 | ISO (2011) |
| Insects       | *Oxythyrea funesta* | 14 | Survival | ISO 20963 | ISO (2005) |
| Carabid beetles | *Pterostichus oblongopunctatus; Poecilus cupreus* | Different durations | Adult or larval survival; adult behaviour, respiration | No standard guidelines | Schrader et al. (1998); Bednarska et al. (2010) |
focus on survival and reproduction after 28, 21 and 14 days exposure, respectively for the parthenogenetic *Folsomia candida*, the sexually reproducing *Folsomia fimetaria* and the predatory mite *Hypoaspis aculeifer*. The test with insect larvae only focuses on survival.

Tests with carabid beetles have been performed using adult *Pterostichus oblongopunctatus* or larvae of *Poecilus cupreus*, but these tests have not been standardized and use different life stages (larvae, adults), endpoints (survival, mobility, respiration) and test durations (from few weeks to several months) depending on the aims of the study (e.g. Schrader et al. 1998, Bednarska and Laskowski 2008, Bednarska et al. 2010).

Also the toxicity tests with the oribatid mites *Oppia nitens* and *Platynothrus peltifer* described in the literature (Van Gestel and Doornekamp 1998, Princz et al. 2010) are not yet standardized. These tests focus on survival and reproduction.

For assessing chemical toxicity to isopods, also no standard test guidelines are available. Nevertheless, isopods are used as test organisms, using different test durations, different routes of exposure (food, soil) and different endpoints. Drobne and Hopkin (1995) described a test exposing *Porcellio scaber* via food and determining effects of zinc on feeding rates. Hornung et al. (1998a) developed a draft test guideline, allowing for exposure both via food and in soil, also using *Porcellio scaber* as the test species. Several different endpoints have been proposed, but considering the difficulty of culturing *Porcellio scaber* in the laboratory survival, growth and food consumption rate so far have been used most frequently (see e.g. Odendaal and Reinecke 2004, Nolde et al. 2006, Kolar et al. 2010). Another interesting endpoint may be related to the composition of the gut microflora of isopods (Drobne et al. 2002). Nevertheless, isopod (*Porcellio scaber*) reproduction also has been proposed as a test endpoint (Hornung et al. 1998b). Successful reproduction isopod experiments have been performed, e.g. by Van Brummelen et al. (1996a), in a 48-week test with *Oniscus asellus*, applying dietary exposure to Polycyclic Aromatic Hydrocarbons (PAHs), and by Lemos et al. (2010) assessing the reproduction toxicity to *Porcellio scaber* of some chemicals with suspected endocrine-disrupting effects. Other isopod species, like *Porcellionides pruniosis*, are more easily cultured in the laboratory, and therefore may be more suitable for performing reproduction toxicity tests (e.g., Jänsch et al. 2005).

Recently, avoidance response was introduced as an easy, fast and sensitive endpoint. For some chemicals avoidance response may be as sensitive as reproduction, while for others it is at least as sensitive as survival. Great advantage of avoidance tests is that they are fast, with test durations of no more than 2 days. Standard test guidelines for avoidance tests have been developed for earthworms (ISO 2008a) and Collembola (ISO 2011), but similar tests have also been performed with enchytraeids (Amorim et al. 2008a,b, Novais et al. 2010), oribatid mites (Owojori et al. 2011) and isopods (Loureiro et al. 2005, Zidar et al. 2005).

In addition to these tests with soil invertebrates, ISO and OECD have also developed a number of toxicity tests with plants, which are important in soil ecosystems as primary producers. Also, several tests are available focusing on the effects of microbial communities or processes performed by microorganisms, like nitrification.
Considering the fact that for a proper risk assessment a battery of tests is desirable, it is important to consider the currently available test methods. The current set of available tests (Table 1) shows an underrepresentation of arthropods in comparison with their abundance in the field when compared with other species like Oligochaetes. And of the available or suggested tests with arthropods, only the one with Collembola has been standardized. Development and international standardization of more toxicity tests with representative arthropod species therefore is highly needed (see criteria for the selection of test species outlined above). The ecological relevance of isopods, their typical routes of exposure (soil, food) and life history characteristics, the possibility to determine different endpoint, and the fact that they have already been used for testing for more than 30 years, make them highly suitable test organisms. Standardization of toxicity tests with isopods therefore is highly recommended.

**Bioavailability**

For reasons of standardization and to facilitate comparison of results, all standardized tests use a standard soil type: the so-called OECD artificial soil, first introduced in the earthworm acute toxicity test developed by OECD (1984). This artificial soil is composed by mixing readily available materials like sphagnum peat (10%), kaolin clay (20%) and quartz sand (70%); by adding some CaCO$_3$ pH is adjusted to approx. 6.0. The properties of this soil resemble those of a sandy loam soil. Recently, within OECD the use of 5% peat has been advocated when testing pesticides, in order to increase the ‘worst case’ realism of the artificial test soil for low organic (agricultural) field soils. Also with the aim to increase realism, the SECOFASE project started using the natural LUFA 2.2 soil (Løkke and Van Gestel 1998). The LUFA soils are commercially available from the Landwirtschaftliche Untersuchungs und Forschungsanstalt (LUFA) in Speyer, Germany. Since that time, LUFA 2.2 standard soil seems to become more commonly used for toxicity tests with soil invertebrates, also because several test species like the collembolan *Folsomia candida* and the enchytraeid *Enchytraeus crypticus* seem to perform as good in this natural soil as they do in artificial soil.

The notion that soil type was important when determining the toxicity of chemicals went along with the increasing insight into the concept of bioavailability: only a fraction of the total amount of chemical in the soil is available for uptake by organisms and therefore of relevance for risk assessment. This was, for instance, demonstrated by Bradham et al. (2006), exposing the earthworm *Eisenia andrei* for 28 days to different soil types spiked with one concentration (2000 mg/kg) of lead (Pb). While in some soils all worms died, in other soils no mortality was seen and in the remainder of the soils only part of the earthworms died. This finding could be explained from the differences in soil properties, especially pH, clay and organic matter content that affected the availability of lead for the earthworms. The pore-water hypothesis or equilibrium partitioning theory was developed to enable linking toxicity of organic chemicals to the concentration available in the pore water (Van Gestel and Ma 1988, 1990). For metals,
application of this approach turned out to be less straightforward because metal speciation in soils is much more complex (Van Gestel 1997) and many factors may affect metal bioavailability in soils (e.g., Allen 2002, Lanno et al. 2004). It nowadays is realized that bioavailability is not a static but rather a dynamic concept (Luoma and Rainbow 2005, Van Gestel 2008). This was demonstrated by Van Straalen et al. (2005), exposing isopods (Porcellio scaber) from a metal-contaminated and a non-polluted site to zinc via the food. Both groups showed the same EC$_{50}$ expressed on the basis of zinc concentrations in the diet, but contrary to the expectation effects could not be explained from zinc concentrations in the animals. So, it seems that flux of zinc through the animals rather than total zinc concentration was determining their sensitivity.

These findings also suggest that when considering bioavailability, not only chemical partitioning of chemicals in the exposure medium (soil, food) and pore-water concentrations have to be considered. The biology of bioavailability also needs attention. One aspect of this is the way organisms deal with chemicals. For metals, internal compartmentalization has been shown to be an important aspect (Rainbow 2002), as it determines what fraction of the metal is present in the body in a metabolically available form. Most soil invertebrates have the capacity to sequester at least part of their metal burden in such a way that it does no longer pose a risk. Isopods use the hepatopancreas for a very efficient storage of excess metals (Hopkin and Martin 1982), while earthworms have their chloragogenous tissue that serves the same purpose (Morgan and Morgan 1998, Morgan et al. 1999). As a consequence, both isopods and earthworms show a huge capacity of storing metals like cadmium, which after uptake are hardly eliminated. Other soil invertebrates, like Collembola and beetles, use the midgut epithelium for metal storage. Upon moulting, also the midgut epithelium is renewed enabling these organisms to excrete excess metal (Hopkin 1989). Internal sequestration determines what fraction of the total metal burden in an organism may contribute to its toxicity or is available for trophic transfer to its predators (Vijver et al. 2004). This was for instance demonstrated by Crommentuijn et al. (1994), who found very high Lethal Body Concentrations for cadmium in isopods compared to other arthropods that could be attributed to the highly efficient storage of the metal in an inert form.

Another way organisms may deal with potentially toxic chemicals is by biotransformation. The process of biotransformation aims at making chemicals more hydrophilic and in this way facilitating their excretion. Isopods and Collembola have been shown to be extremely efficient in biotransforming organic chemicals like Polycyclic Aromatic Hydrocarbons (PAHs), which are excreted by these organisms with half lives of approximately 1 day (Van Brummelen and Van Straalen 1996, Howsam and Van Straalen 2003, Stroomberg et al. 2004). Earthworms seem less efficient in doing so. Possible consequence of this rapid biotransformation is that potentially toxic metabolites may be produced. This has been shown in isopods, with even DNA adducts being formed upon exposure to PAHs (Van Brummelen et al. 1996a). This may also lead to long-term damage and possible effects on subsequent generations as has been shown for phenanthrene in the collembolan Folsomia candida (Leon Paumen et al. 2008). As
very little is known about such multi-generation effects, further research on the long-term effects of chemicals is urgently needed.

An important biological aspect that may affect the exposure of soil invertebrates to chemicals is their behaviour. Soil by definition is a heterogeneous environment. As a consequence, also the distribution of chemicals in soil is heterogeneous. Chemicals reaching soil by areal deposition for instance accumulate in the topsoil layer, leading to a depth-related concentration gradient as was shown for PAHs in forest soils (Van Brummelen et al. 1996b). Depending on the habitat and mobility, organisms may be more or less exposed to chemicals present in the topsoil layer. Similarly, the effect of pesticides on earthworms was shown to be highly related to their mobility with epigeic and anecic species being much more vulnerable compared to endogeic species. Especially anecic species, like *Lumbricus terrestris*, which come to the soil surface to forage and mate at night, may experience a very high exposure shortly after pesticide spraying (Edwards and Brown 1982). Also in case of spatially heterogeneous soil pollution, behaviour may affect exposure, as was shown for earthworm exposure to copper in a heterogeneously polluted soil by Marinussen and Van der Zee (1996) In the latter study, knowledge of the uptake and elimination kinetics showed to be very helpful in predicting metal concentrations in the earthworms living in a heterogeneously polluted environment. Also in case of isopods, behaviour is an important factor determining exposure. Unfortunately, no research has been done on the chemical exposure of isopods and consequent effects in relation to their behaviour in the field.

**Multiple species, model ecosystem (microcosm) and field tests**

All standardized toxicity tests with soil invertebrates focus on assessing the effects of chemicals on single species of organisms. To enable assessment of toxic effects in a more realistic setting, micro- or model ecosystems have been developed, ranging from artificially composed set-ups with a number of selected different species introduced in a well homogenized soil (e.g., Burrows and Edwards 2004) to intact soil columns extracted from the field and incubated in the laboratory (e.g., Knacker et al. 2004). Such model ecosystems or microcosms allow assessing effects at the community level, taking into account the interactions between species. Although basically considered single-species tests, earthworm and isopod tests focusing on decomposition or feeding activity in fact also are multispecies tests as in these tests also the interaction with microorganisms in the gut and in the soil or food are important. In addition, the endpoint (decomposition) has high ecological relevance for assessing potential effects on the functioning of the soil ecosystem (e.g. Hobbelen et al. 2006). The only field test available aims at assessing pesticide effects on earthworms (ISO 1999b), but can be combined with a decomposition or litter bag test (Römbke et al. 2003, OECD 2006, Dinter et al. 2008).

Since the introduction of the term Ecotoxicology, the question for “putting more eco into ecotoxicology” has been raised. Some authors even argued that ecotoxicology
Soil ecotoxicology: state of the art and future directions

should not be seen as a sub-discipline of toxicology but rather as a case of stress ecology (Van Straalen 2003). This notion has triggered the focus on more ecologically relevant test designs, integrated approaches including responses at different levels of biological organization, and taking into account the normal operating range of parameters describing the structure and functioning of soil ecosystems.

Since early 2000, with the notion of stress ecology, more complex issues have been receiving attention, with ecological vulnerability, trait-based analysis and effects on functional endpoints (so-called ecosystem services) being key items (e.g., De Lange et al. 2009, Saad et al. 2011). The application of these trait-based approaches in soil ecotoxicology on one hand offers promising perspectives, on the other hand it also demonstrates an enormous lack of knowledge on the traits represented by different species and groups of soil invertebrates.

Diagnosis

Many of the tests initially developed for assessing the toxicity of single chemicals are also used for assessing the toxicity of field samples. In addition to the tests mentioned above, a bioassay using the nematode Caenorhabditis elegans has been developed by ISO (2010) Such bioassays may be applied together with chemical analysis and field observations. The resulting TRIAD approach is a useful tool for the actual risk assessment of contaminated sites (Jensen and Mesman 2006). ISO (2008b) gives guidance on the choice of different bioassays, depending on the purpose of the risk assessment and taking into account aspects like land use.

Other diagnostic tools include effects at the biochemical level. Such biomarkers may act as a sensitive, early warning indicator of possible effects at higher levels of biological organization (Spurgeon et al. 2005), and also may provide information on the mode of action of a chemical (Kammenga et al. 2000). Biomarkers may be applied both to organisms captured from the field and to test organisms exposed to field samples under controlled laboratory conditions (see e.g. Van Gestel et al. 2009). Isopods may be used for such biomarker studies (Köhler et al. 1999, Stroomberg et al. 1999, Stanek et al. 2006, Drobné et al. 2008, Lemos et al. 2009), while also their potential of accumulating metals has been proposed as a suitable monitoring tool (‘woodlouse watch’ scheme) especially in metal-contaminated areas (Hopkin et al. 1993).

Spurgeon et al. (2005), comparing different biochemical endpoints, demonstrated that responses at the gene level were most sensitive. This notion also plays a role in the recent developments of genetic tools (genomics, proteomics and transcriptomics etc.), which has resulted in a vast extension of the ecotoxicological tool box. Ecotoxicogenomics nowadays is seen as a tool to enable better understanding of molecular mechanisms of action of chemicals (Snape et al. 2004), while it may provide insight into the way soil invertebrates are able to develop resistance to pollution, e.g. metal or pesticide tolerance (Van Straalen et al. 2011). Ecotoxicogenomics may also help unravelling the mechanisms by which (metal-based) nanoparticles affect organisms, as e.g. was
determined for the nematode *Caenorhabditis elegans* (Roh et al. 2009, 2010). Ecotoxicogenomics may also open the way for developing new diagnostic tools for assessing possible effects of soil pollution (Van Straalen and Roelofs 2008, Nota et al. 2010). Some authors have advocated that ecotoxicogenomics may enable bridging the gap between genes and populations (Fedorenkova et al. 2010). It remains however, uncertain whether time is ready for such ‘from gene to population extrapolations’ (Van Straalen et al. 2010). In a recent review, Van Straalen and Feder (2012) discuss the possible use of environmental genomics in the ecotoxicological risk assessment of chemicals. Community and population genomics may provide insight into the species composition at different sites and the possible relationship with pollution. Genome scans may also provide information on genetic changes in specific species that have been exposed to contaminated soils over many generations. Gene expression profiling may provide information on toxicant-induced changes in gene expression. The meaning of these changes however, remains unclear as the linkage between gene expression (transcriptomics) and the functioning of the genes (proteomics) often is not straightforward. At the moment, gene expression analysis is applied to only few species for which the genome has more or less completely been described, like the nematode *Caenorhabditis elegans*, the springtail *Folsomia candida* and the earthworms *Lumbricus rubellus* and *Eisenia fetida*, thus limiting wider application. Information on background gene expression is lacking, hampering a proper interpretation of responses under stressed condition. Van Straalen and Feder (2012) therefore conclude that more research is needed before genomics tools can make a sound contribution to the risk assessment of chemicals.

**Outlook**

Final aim of (soil) ecotoxicology is the understanding of the long-term effects of chemicals on ecosystems. As such, focus on long-term sub-lethal effects is essential, but it also requires detailed understanding of the processes of exposure, uptake, internal processing (metabolism, sequestration) and intoxication in individual organisms as well as the translation of effects to higher levels of biological organization. From the overview presented in this paper, it may have become clear that soil ecotoxicology has shown a tremendous development in the past 40 years. From the initial realization that chemicals may affect soil organisms, through the development of standardized toxicity tests and the use of soil chemistry to develop the concept of bioavailability, soil ecotoxicology has grown to a mature field of science. The incorporation of biochemical and omics tools on one hand and the link with ecology on the other hand, does guarantee that soil ecotoxicology remains an important player in the field of stress ecology. In spite of the promising developments outlined above, the following aspects need further attention in the near future:
Toxicity tests

Although several toxicity tests are available for soil organisms (Table 1), it is obvious that the current battery is not complete and also not well balanced. As mentioned above, it seems there is an under-representation of arthropods. Isopod toxicity testing seems most advanced, while these organisms also represent an ecologically important and relevant group of soil arthropods. In addition, they offer the possibility of exposure via soil and food, while effects may be determined at different levels including biochemical and genomics, individual (growth, behaviour) as well as ecological (feeding activity). It therefore is recommended to put more effort on standardizing isopod toxicity tests for sublethal endpoints. Finally, it has to be noted that the currently available toxicity tests may need adjustment to make them applicable for determining the toxicity of new and emerging chemicals, like nanoparticles.

Bioavailability

For better enabling extrapolation from laboratory tests to the field and among soil types, it is essential to get better understanding of the routes of uptake of chemicals in organisms. This not only requires attention for the chemical aspects, but also needs a greater emphasis of the ‘biological’ aspects of bioavailability. This may also require paying closer attention to the way organisms are exposed in the field, and attention for the dynamics of exposure and bioavailability.

Kinetics

For a better understanding of bioavailability but also of the toxicity of single chemicals and mixtures, it is essential to increase our understanding of toxicokinetics and toxicodynamics. Such understanding will also enhance the possibilities to extrapolate effects in time and to higher levels of biological organization, like the population level. Kinetics also is of great importance when considering the toxicity of new chemicals, like nanoparticles, that may show changing properties with time as a consequence of aggregation, agglomeration and dissolution processes. Finally, kinetics should not only address whole organisms but should also include the way organisms deal with chemicals internally (biotransformation, sequestration, internal distribution and translocation).

Ecology

For better understanding exposure in the field and predicting ecosystem effects, our knowledge on the ecology of soil invertebrates needs much better development. Such knowledge also is crucial for the description of the normal operating range of structural
and functional endpoints and for the application of trait-based approaches to understand and predict effects of chemicals on soil invertebrate communities and ecosystem services provided by these communities.

**Ecotoxicogenomics**

For the application of genomics tools in the diagnosis of soil pollution it is essential to better understand the link between gene expression level responses and ecologically relevant endpoints. A better understanding of gene expression responses may also help unraveling the mechanisms of action of chemicals, single and in mixtures, and as such be helpful in predicting toxicity. In the long run, a better understanding of responses at the genomics level may even provide tools for cross-species extrapolation and the development of completely new models for mixture toxicity, especially when combined with toxicokinetics and toxicodynamics data. Genomics tools may also help unraveling the causes of long-term effects of chemicals, e.g. multi-generation effects as a consequence of accumulation of damage in earlier generations. But all these applications will require an enormous amount of information on the meaning of gene expression profiles in relation to background conditions, in relation to chemical exposure both outside and inside the body and related to ecologically relevant endpoints like growth and reproduction.

**References**

Allen HE (Ed) (2002) Bioavailability of Metals in Terrestrial Ecosystems: Importance of Partitioning for Bioavailability to Invertebrates, Microbes, and Plants. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, Florida, USA.

Amorim MJB, Novais S, Römbke J, Soares AMVM (2008a) Avoidance test with *Enchytraeus albidus* (Enchytraeidae): Effects of different exposure time and soil properties. Environmental Pollution 155: 112–116. doi: 10.1016/j.envpol.2007.10.028

Amorim MJB, Novais S, Römbke J, Soares AMVM (2008b) *Enchytraeus albidus* (Enchytraeidae): A test organism in a standard avoidance test? Effects of different chemical substances. Environment International 34: 363–371. doi: 10.1016/j.envint.2007.08.010

Bednarska AJ, Gerhardt A, Laskowski R (2010) Locomotor activity and respiration rate of the ground beetle, *Pterostichus oblongopunctatus* (Coleoptera: Carabidae), exposed to elevated nickel concentration at different temperatures: novel application of Multispecies Freshwater Biomonitor®. Ecotoxicology 19: 864–871. doi: 10.1007/s10646-010-0467-2

Bednarska AJ, Laskowski R (2008) Effects of nickel and temperature on the ground beetle *Pterostichus oblongopunctatus* (Coleoptera : Carabidae). Ecotoxicology 17: 189–198. doi: 10.1007/s10646-007-0183-8

Bradham K, Dayton EA, Basta NT, Schroder J, Payton M, Lanno RP (2006) Effect of soil properties on lead bioavailability and toxicity to earthworms. Environmental Toxicology and Chemistry 25: 769–775. doi: 10.1897/04-552R.1
Soil ecotoxicology: state of the art and future directions

Burrows LA, Edwards CA (2004) The use of soil microcosms to assess the impact of carbendazim on soil ecosystems. Ecotoxicology 13: 143-161. doi: 10.1016/S1164-5563(02)01153-6

Carson R (1962) Silent Spring. Houghton Mifflin Company, New York.

Crommentuijn T, Doornkamp A, Van der Pol JJC, Bedaux JJM, Van Gestel CAM (1994) Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. Environmental Toxicology and Chemistry 13: 1781–1789.

De Lange HJ, Lahr J, Van der Pol JJC, Wessels Y, Faber JH (2009) Ecological vulnerability in wildlife: an expert judgment and multicriteria analysis tool using ecological traits to assess relative impact of pollutants. Environmental Toxicology and Chemistry 28: 2233–2240. doi: 10.1897/08-626.S1

Dinter A, Coulson M, Heimbach F, Keppler J, Krieg W, Kölzer U (2008) Technical experiences made with the litter bag test as required for the risk assessment of plant protection products in soil. Journal of Soils and Sediments 8: 333–339. doi 10.1007/s11368-008-0028-y

Drobne D, Hopkin SP (1995) The toxicity of zinc to terrestrial isopods in a “standard” laboratory test. Ecotoxicology and Environmental Safety 31: 1–6. doi: 10.1006/cesa.1995.1037

Drobne D, Jemec A, Pipan Tkalec Z (2009) In vivo screening to determine hazards of nanoparticles: Nanosized TiO2. Environmental Pollution 157: 1157–1164. doi: 10.1016/j.envpol.2008.10.018

Drobne D, Blazic M, Van Gestel CAM, Leser V, Zidar P, Jemec A, Trebse P (2008) Toxicity of imidacloprid to the terrestrial isopod Porcellio scaber (Isopoda, Crustacea). Chemosphere 71:1326–1334. doi: 10.1016/j.chemosphere.2007.11.042

Drobne D, Rupnik M, Lapanje A, Štrus J, Janc M (2002). Isopod gut microflora parameters as endpoints in toxicity studies. Environmental Toxicology and Chemistry 21: 604-609. doi: 10.1897/1551-5028(2002)021<0604:IGMPAE>2.0.CO;2

Edwards CA (1969) Soil pollutants and soil animals. Scientific American 220: 88-99. doi: 10.1038/scientificamerican0469-88

Edwards PJ, Brown SM (1982) Use of grassland plots to study the effect of pesticides on earthworms. Pedobiologia 24: 145–150.

Fedorenkova A, Vonk JA, Lenders HJR, Ouborg NJ, Breure AM, Hendriks AJ (2010) Ecotoxicogenomics: Bridging the gap between genes and populations. Environmental Science and Technology 44: 4328–4333. doi: 10.1021/es9037287

Fox CJS (1964) The effect of five herbicides on the number of certain invertebrate animals in grassland soil. Canadian Journal of Plant Sciences 44: 405–409. doi: 10.4141/cjps64-080

Ghabbour SI, Imam M (1967) The effect of five herbicides on three Oligochaete species. Revue d’Ecologie et du Biologie du Sol 4: 119–122.

Heckmann L-H, Hovgaard M, Sutherland D, Autrup H, Besenbacher F, Scott-Fordsmand J (2011) Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm Eisenia fetida. Ecotoxicology 20: 226–233. doi: 10.1007/s10646-010-0574-0

Hobbelen PHF, Koolhaas JE, van Gestel CAM (2006) Effects of heavy metals on the litter consumption by the earthworm Lumbricus rubellus in field soils. Pedobiologia 50:51–60. doi: 10.1016/j.pedobi.2005.10.004
Holmstrup M, Bindesbøel AM, Oostingh GJ, Duschl A, Scheil V, Kohler HR, Loureiro S, Soares A, Ferreira ALG, Kienle C, Gerhardt A, Laskowski R, Kramarz PE, Bayley M, Svendsen C, Spurgeon DJ (2010) Interactions between effects of environmental chemicals and natural stressors: A review. Science of the Total Environment 408: 3746–3762. doi: 10.1016/j.scitotenv.2009.10.067

Hooper HL, Jurkschat K, Morgan AJ, Bailey J, Lawlor AJ, Spurgeon DJ, Svendsen C (2011) Comparative chronic toxicity of nanoparticulate and ionic zinc to the earthworm Eisenia veneta in a soil matrix. Environment International 37: 1111–1117. doi: 10.1016/j.envint.2011.02.019

Hopkin SP, Martin MH (1982) The distribution of zinc, cadmium, lead and copper within the woodlouse Oniscus asellus (Crustacea, Isopoda). Oecologia 54: 227–232. doi: 10.1007/BF00378396

Hopkin SP (1989) Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Science, Barking, UK.

Hopkin SP, Jones DT, Dietrich D (1993) The isopod Porcellio scaber as a monitor of the bioavailability of metals in terrestrial ecosystems: towards a global ‘woodlouse watch’ scheme. The Science of the Total Environment, Supplement: 357–365. doi: 10.1016/S0048-9697(05)80036-1

Hornung E, Farkas S, Fischer E (1998a) Tests on the isopod Porcellio scaber. In: Løkke H, Van Gestel CAM (Eds) Handbook of Soil Invertebrate Toxicity Tests. John Wiley & Sons, Chichester, 207–226.

Hornung E, Fischer E, Farkas S (1998b) Isopod reproduction as a tool for sublethal-toxicity tests. Israel Journal of Zoology 44: 445–450.

Howsam M, van Straalen NM (2003) Pyrene metabolism in the springtail Orchesella cincta L. (Collembola, Entomobryidae). Environmental Toxicology and Chemistry 22: 1481–1486. doi: 10.1897/1551-5028(2003)22<1481:PMITSO>2.0.CO;2

ISO (1993) Soil quality – Effects of pollutants on earthworms (Eisenia fetida) – Part 1: Determination of acute toxicity using artificial soil substrate ISO 11268-1. International Standardization Organization, Geneva.

ISO (1998) Soil quality – Effects of pollutants on earthworms. Part 3: Guidance on the determination of effects in field situations. ISO 11268-3. International Standardization Organization, Geneva.

ISO (1999a) Soil Quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants. ISO 11267. International Standardization Organization, Geneva.

ISO (1999b) Soil Quality – effects of pollutants on earthworms. Part 3: Guidance on the determination of effects in field situations. ISO 11268-3. International Standardization Organization, Geneva.

ISO (2004) Soil quality – Effects of pollutants on Enchytraeidae (Enchytraeus sp.) – Determination of effects on reproduction and survival. ISO 16387. International Standardization Organization, Geneva.

ISO (2005) Soil quality – Effects of pollutants on insect larvae (Oxythyrea funesta) – Determination of acute toxicity. ISO 20963. International Standardization Organization, Geneva.
ISO (2006) Soil quality – Effects of pollutants on juvenile land snails (Helicidae) – Determination of the effects on growth by soil contamination. ISO 15952. International Standardization Organization, Geneva.

ISO (2008a) Soil quality – Avoidance test for determining the quality of soils and effects of chemicals on behaviour – Part 1: Test with earthworms (Eisenia fetida and Eisenia nndrei). ISO 17512-1. International Standardization Organization, Geneva.

ISO (2008b) Soil quality – Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials. ISO 17616. International Standardization Organization, Geneva.

ISO (2010) Water quality – Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda). ISO 10872. International Standardization Organization, Geneva.

ISO (2011) Soil quality – Avoidance test for determining the quality of soils and effects of chemicals on behaviour – Part 2: Test with collembolans (Folsomia candida). ISO 17512-2. International Standardization Organization, Geneva.

Jänsch S, Garcia M, Römbke J (2005) Acute and chronic isopod testing using tropical Porcellio-nides pruinosus and three model pesticides. European Journal of Soil Biology 41: 143–152. doi: 10.1016/j.ejsobi.2005.09.010

Jemec A, Drobne D, Remškar M, Sepčić K, Tišler T (2008) Effects of ingested nano-sized titanium dioxide on terrestrial isopods (Porcellio scaber). Environmental Toxicology and Chemistry 27: 1904–1914. doi: 10.1897/08-036.1

Jensen J, Mesman M (Eds) (2006) Ecological Risk Assessment of Contaminated Land. Decision Support for Site Specific Investigations. National Institute for Public Health and the Environment, RIVM, Bilthoven, The Netherlands.

Kammenga JE, Dallinger R, Donker MH, Köhler H-R, Simonsen V, Triebskorn R, Weeks JM (2000) Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment. Reviews of Environmental Contamination and Toxicology 164: 93–147.

Knacker T, Van Gestel CAM, Jones SE, Soares AMVM, Schallnass H-J, Förster B, Edwards CA (2004) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – An Instrument for testing potentially harmful substances: conceptual approach and study design. Ecotoxicology 13: 9–27. doi: 10.1023/B:ECTX.0000012402.38786.01

Köhler H-R, Knödler C, Zanger M (1999) Divergent kinetics of hsp70 induction in Oniscus asellus (Isopoda) in response to four environmentally relevant organic chemicals (B[a]P, PCB52, γ-HCH, PCP): suitability and limits of a biomarker. Archives of Environmental Contamination and Toxicology 36: 179–185. doi: 10.1007/s002449900458

Kolar L, Jemec A, Van Gestel CAM, Valant J, Hrzenjak R, Kozuh Erzen N, Zidar P (2010) Toxicity of abamectin to the terrestrial isopod Porcellio scaber (Isopoda, Crustacea). Ecotoxicology 19: 917–927. doi: 10.1007/s10646-010-0473-4

Kool PL, Diez Ortiz M, van Gestel CAM (2011) Chronic toxicity of ZnO nanoparticles, non-nano ZnO and ZnCl2 to Folsomia candida (Collembola) in relation to bioavailability in soil. Environmental Pollution 159: 2713–2719. doi: 10.1016/j.envpol.2011.05.021
Lanno R, Wells J, Conder J, Bradham K, Basta N (2004) The bioavailability of chemicals in soil for earthworms. Ecotoxicology and Environmental Safety 57: 39–47. doi: 10.1016/j.ecoenv.2003.08.014

Lemos MFL, Van Gestel CAM, Soares AMVM (2009) Endocrine disruption in a terrestrial isopod under exposure to bisphenol A and vinclozolin. Journal of Soils and Sediments 9: 492–500. doi: 10.1007/s11368-009-0104-y

Lemos MFL, Van Gestel CAM, Soares AMVM (2010) Reproductive toxicity of the endocrine disrupters vinclozolin and bisphenol A in the terrestrial isopod Porcellio scaber (Latreille, 1804). Chemosphere 78: 907–913. doi: 10.1016/j.chemosphere.2009.10.063

Leon Paumen M, Steenbergen E, Kraak MHS, Van Straalen NM, Van Gestel CAM (2008) Multigeneration exposure of the springtail Folsomia candida to phenanthrene: from dose-response relationships to threshold concentrations. Environmental Science and Technology 42: 6985–6990. doi: 10.1021/es8007744

Løkke H, Van Gestel CAM (Eds) (1998) Handbook of Soil Invertebrate Toxicity Tests. John Wiley & Sons, Chichester.

Loureiro S, Soares AMVM, Nogueira AJA (2005) Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. Environmental Pollution 138: 121–131. doi: 10.1016/j.envpol.2005.02.013

Luoma SN, Rainbow PS (2005) Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science and Technology 39: 1921–1931. doi: 10.1021/es048947c

Marinussen MPJC, Van der Zee SEATM (1996) Conceptual approach to estimating the effect of home-range size on the exposure of organisms to spatially variable soil contamination. Ecological Modelling 87: 83–89. doi: 10.1016/0304-3800(94)00207-X

Morgan AJ, Stürzenbaum SR, Winters C, Kille P (1999) Cellular and molecular aspects of metal sequestration and toxicity in earthworms. Invertebrate Reproduction and Development 36: 17–24. doi: 10.1080/07924259.1999.9652673

Morgan JE, Morgan AJ (1998) The distribution and intracellular compartmentation of metals in the endogeic earthworm Aporrectodea caliginosa sampled from an unpolluted and a metal-contaminated. Environmental Pollution 99: 167–175. doi: 10.1016/S0269-7491(97)00193-0

Nolde N, Drobne D, Valant J, Padovan I, Horvat M (2006) Lysosomal membrane stability in laboratory- and field-exposed terrestrial isopods Porcellio scaber (Isopoda, Crustacea). Environmental Toxicology and Chemistry 25: 2114–2122. doi: 10.1897/05-593R1.1

Nota B, Verweij RA, Molenaar D, Ylstra B, van Straalen NM, Roelofs D (2010) Gene expression analysis reveals a gene set discriminatory to different metals in soil. Toxicological Sciences 115: 34–40. doi: 10.1093/toxsci/kfq043

Novais SC, Soares A, Amorim MJB (2010) Can avoidance in Enchytraeus albidus be used as a screening parameter for pesticides testing? Chemosphere 79: 233–237. doi: 10.1016/j.chemosphere.2010.01.011

Odendaal JP, Reinecke AJ (2004) Effect of metal mixtures (Cd and Zn) on body weight in terrestrial isopods. Archives of Environmental Contamination and Toxicology 46: 377–384. doi: 10.1007/s00244-003-2303-7
OECD (1984) OECD Guidelines for the Testing of Chemicals No. 207. Earthworm, Acute toxicity tests. Organisation for Economic Co-operation and Development, Paris.

OECD (2004a) OECD Guidelines for the Testing of Chemicals No. 220. Enchytraeid reproduction test. Organisation for Economic Co-operation and Development, Paris.

OECD (2004b) OECD Guidelines for the Testing of Chemicals No. 222. Earthworm reproduction test (*Eisenia fetida*/*Eisenia andrei*). Organisation for Economic Co-operation and Development, Paris.

OECD (2006) Guidance document on the breakdown of organic matter in litter bags. OECD series on testing and assessment. No 56. Organisation for Economic Co-operation and Development, Paris.

OECD (2008) OECD Guidelines for the testing of Chemicals 226. Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. Organisation for Economic Co-operation and Development, Paris.

OECD (2009) OECD Guidelines for the testing of Chemicals 232. Collembolan reproduction test in soil. Organisation for Economic Co-operation and Development, Paris.

Owojori OJ, Healey J, Princz J, Siciliano SD (2011) Can avoidance behavior of the mite *Oppia nitens* be used as a rapid toxicity test for soils contaminated with metals or organic chemicals? Environmental Toxicology and Chemistry 30: 2594–2601. doi: 10.1002/etc.658

Pipan-Tkalec Z, Drobne D, Jemec A, Romih T, Zidar P, Bele M (2010) Zinc bioaccumulation in a terrestrial invertebrate fed a diet treated with particulate ZnO or ZnCl2 solution. Toxicology 269: 198–203. doi: 10.1016/j.tox.2009.08.004

Posthuma L, Suter II GW, Traas T (Eds) (2002) Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, USA.

Princz JI, Behan-Pelletier VM, Scroggins RP, Siciliano SD (2010) Oribatid mites in soil toxicity testing – the use of *Oppia nitens* (C.L. Koch) as a new test species. Environmental Toxicology and Chemistry 29: 971–979. doi: 10.1002/etc.98

Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what? Environmental Pollution 120: 497–507. doi: 10.1016/S0269-7491(02)00238-5

Roh J-Y, Park Y-K, Park K, Choi J (2010) Ecotoxicological investigation of CeO2 and TiO2 nanoparticles on the soil nematode *Caenorhabditis elegans* using gene expression, growth, fertility, and survival as endpoints. Environmental Toxicology and Pharmacology 29: 167–172. doi: 10.1016/j.etap.2009.12.003

Roh J-Y, Sim SJ, Yi J, Park K, Chung KH, Ryu D-y, Choi J (2009) Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. Environmental Science and Technology 43: 3933–3940. doi: 10.1021/es803477u

Römbke J, Heimbach F, Hoy S, Kula C, Scott-Fordsmand J, Sousa P, Stephenson G, Weeks J (Eds) (2003) Effects of Plant Protection Products on Functional Endpoints in Soil (EPFES). SETAC, Lisbon, 24–26 April 2002. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, Florida, USA.

Saad R, Margni M, Koellner T, Wittstock B, Deschenes L (2011) Assessment of land use impacts on soil ecological functions: development of spatially differentiated characterization factors within a Canadian context. International Journal of Life Cycle Assessment 16: 198–211. doi: 10.1007/s11367-011-0258-x
Schrader G, Metge K, Bahadir M (1998) Importance of salt ions in ecotoxicological test with soil arthropods. Applied Soil Ecology 7: 189-193. doi: 10.1016/S0929-1393(97)00035-8
Scopes NEA, Lichtenstein EP (1967) The use of Folsomia fimetaria and Drosophila melanogaster as test insects for the detection of insecticide residues. Journal of Economic Entomology 60: 1539–1541.
Sholts-Wilson W, Zhurbich O, McNear D, Tsyusko O, Bertsch P, Unrine J (2011a) Evidence for avoidance of Ag nanoparticles by earthworms (Eisenia fetida). Ecotoxicology 20: 385–396. doi: 10.1007/s10646-010-0590-0
Sholts-Wilson WA, Reinsch BC, Tsyusko OV, Bertsch PM, Lowry GV, Unrine JM (2011b) Effect of silver nanoparticle surface coating on bioaccumulation and reproductive toxicity in earthworms (Eisenia fetida). Nanotoxicology 5: 432–444. doi: 10.3109/17435390.2010.537382
Snape JR, Maund SJ, Pickford DB, Hutchinson TH (2004) Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology. Aquatic Toxicology 67: 143–154. doi: 10.1016/j.aquatox.2003.11.011
Spurgeon DJ, Ricketts H, Svendsen C, Morgan AJ, Kille P (2005) Hierarchical responses of soil invertebrates (earthworms) to toxic metals stress. Environmental Science and Technology 39: 5327–5334. doi: 10.1021/es050033k
Stanek K, Drobne D, Trebse P (2006) Linkage of biomarkers along levels of biological complexity in juvenile and adult diazinon fed terrestrial isopod (Porcellio scaber, Isopoda, Crustacea). Chemosphere 64:1745-1752. doi: 10.1016/j.chemosphere.2005.12.070
Stroomberg GJ, Ariese F, Van Gestel CAM, Van Hattum B, Velthorst NH, Van Straalen NM (2004) Pyrene biotransformation and kinetics in the hepatopancreas of the isopod Porcel-lio scaber. Archives of Environmental Contamination and Toxicology 47: 324–331. doi: 10.1007/s00244-004-3097-y
Stroomberg GJ, De Knecht JA, Ariese F, Van Gestel CAM, Velthorst NH (1999) Pyrene metabolites in the hepatopancreas and gut of the isopod Porcellio scaber, a new biomarker for polycyclic aromatic hydrocarbon exposure in terrestrial ecosystems. Environmental Toxicology and Chemistry 18: 2217–2224. doi: 10.1897/1551-5028(1999)018<2217:PMIT>2.3.CO;2
Van Brummelen TC, Van Gestel CAM, Verweij RA (1996a) Long-term toxicity of five polycyclic aromatic hydrocarbons for the terrestrial isopods Oniscus asellus and Porcellio scaber. Environmental Toxicology and Chemistry 15: 1199–1210. doi: 10.1897/1551-5028(1996)015<1199:SCLTTO>2.3.CO;2
Van Brummelen TC, Van Straalen NM (1996) Uptake and elimination of benzo[a]pyrene in the terrestrial isopod Porcellio scaber. Archives of Environmental Contamination and Toxicology 31: 277–285. doi: 10.1007/BF00212378
Van Brummelen TC, Verweij RA, Wedzinga SA, Van Gestel CAM (1996b) Enrichment of polycyclic aromatic hydrocarbons in forest soils near a blast furnace plant. Chemosphere 32: 293–314. doi: 10.1016/0045-6535(95)00339-8
Van Gestel CAM (1997) Scientific basis for extrapolating results from soil ecotoxicity tests to field conditions and the use of bioassays. In: Van Straalen NM, Løkke H (Eds) Eco-
Van Gestel CAM (2008) Physico-chemical and biological parameters determine metal bioavailability in soils. Science of the Total Environment 406: 385–395. doi: 10.1016/j.scitotenv.2008.05.050

Van Gestel CAM, Borgman E, Verweij RA, Diez Ortiz M (2011) The influence of soil properties on the toxicity of molybdenum to three species of soil invertebrates. Ecotoxicology and Environmental Safety 74: 1–9. doi: 10.1016/j.ecoenv.2010.10.001

Van Gestel CAM, Dirven-Van Breemen EM, Baerselman R (1992) Influence of environmental conditions on the growth and reproduction of the earthworm Eisenia fetida in an artificial soil substrate. Pedobiologia 36: 109–120.

Van Gestel CAM, Doornekamp A (1998) Tests on the oribatid mite Platynothrus peltifer. In: Løkke H, Van Gestel CAM (Eds) Handbook of Soil Invertebrate Toxicity Tests. John Wiley & Sons, Chichester, 113–130.

Van Gestel CAM, Jonker MJ, Kammenga JE, Laskowski R, Svendsen C (Eds) (2011) Mixture Toxicity. Linking Approaches from Ecological and Human Toxicology. SETAC Press, Pensacola, USA.

Van Gestel CAM, Koolhaas JE, Hamers T, Van Hoppe M, Van Rooyt M, Korsman C, Reincke SA (2009) Effects of metal pollution on earthworm communities in a contaminated floodplain area: Linking biomarker, community and functional responses. Environmental Pollution 157:895–903. doi: 10.1016/j.envpol.2008.11.002

Van Gestel CAM, Léon CD, Van Straalen NM (1997) Evaluation of soil fauna ecotoxicity tests regarding their use in risk assessment. In: Tarradellas J, Bitton G, Rossel D (Eds) Soil Ecotoxicology. CRC Press, Inc., Boca Raton, 291–317.

Van Gestel CAM, Ma W (1988) Toxicity and bioaccumulation of chlorophenols in earthworms, in relation to bioavailability in soil. Ecotoxicology and Environmental Safety 15: 289–297. doi: 10.1016/0147-6513(88)90084-X

Van Gestel CAM, Ma W (1990) An approach to quantitative structure-activity relationships (QSARs) in earthworm toxicity studies. Chemosphere 21: 1023–1033. doi: 10.1016/0045-6535(90)90125-D

Van Gestel CAM, Zaal J, Dirven-Van Breemen EM, Baerselman R (1995) Comparison of two test methods for determining the effects of pesticides on earthworm reproduction. Acta Zoologica Fennica 196: 278–283.

Van Straalen NM (2003) Ecotoxicology becomes stress ecology. Environmental Science and Technology 37: 325A–330A.

Van Straalen NM, Donker MH, Vijver MG, Van Gestel CAM (2005) Bioavailability of contaminants estimated from uptake rates into soil invertebrates. Environmental Pollution 136: 409–417. doi: 10.1016/j.envpol.2005.01.019

Van Straalen NM, Feder ME (2012). Ecological and evolutionary functional genomics-how can it contribute to the risk assessment of chemicals? Environmental Science & Technology 46: 3–9. doi: 10.1021/es2034153
Van Straalen NM, Janssens TKS, Roelofs D (2011) Micro-evolution of toxicant tolerance: from single genes to the genome’s tangled bank. Ecotoxicology 20: 574–579. doi: 10.1007/s10646-011-0631-3

Van Straalen NM, Roelofs D (2008) Genomics technology for assessing soil pollution. Journal of Biology 7: 19. doi: 10.1186/jbiol80

Van Straalen NM, Roelofs D, Van Gestel CAM, De Boer TE (2010) Comment on “Ecotoxicogenomics: bridging the gap between genes and populations”. Environmental Science and Technology 44: 9239-9240. doi: 10.1021/es102651z

Vijver MG, Van Gestel CAM, Lanno RP, Van Straalen NM, Peijnenburg WJGM (2004) Internal metal sequestration and its ecotoxicological relevance: a review. Environmental Science and Technology 38: 4705–4712. doi: 10.1021/es040354g

Zidar P, Bozic J, Strus J (2005) Behavioral response in the terrestrial isopod Porcellio scaber (Crustacea) offered a choice of uncontaminated and cadmium-contaminated food. Ecotoxicology 14: 493–502. doi: 10.1007/s10646-005-0005-9