Laboratory and higher-tier effect tests in soil ecotoxicology: state-of-the-art and new developments

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Aims and content

1. Current status of soil effect testing
   - Legal requirements (mainly in the EU);
   - Overview of currently used (standard) tests

2. Gaps and ideas in regulatory soil effect testing

Framework of the content:
Compartment Soil: Upper soil (ca. 20 cm) + litter layer
Organisms: Microbes, invertebrates, plants.
Methods: Laboratory tests and field studies

Briefly: Bioaccumulation tests
Soil ecology

Soils differ enormously in terms of their biological diversity, but also regarding their abiotic properties (e.g. texture, pH).
Legal requirements: Introduction

1. **Prospective risk assessment (i.e. single chemicals)**
   - Pesticides (PPP), veterinary drugs (VMP), industrial chemicals (REACH), biocides
   - Requirements are mainly defined by the EU
   - Tests (to be) standardized OECD

2. **Retrospective risk assessment (contaminated sites)**
   - Not addressed, but a source of ideas and experiences
   - Diverse requirements, defined on the national level.
   - Guidance from ISO available (TRIAD)
   - General soil quality monitoring and assessment
Legal requirements: Approach I

**Single species tests**
- Plant and invertebrate tests
- Bioaccumulation tests

**Multispecies tests**
- Microbial tests, *intermediate tier tests*

**Soil microcosms / soil mesocosms**
- e.g., *TME, MS-3*

**Field studies, monitoring and modelling**
- e.g., Earthworm field test, litter bag test, *bait lamina test*
- *Modelling approaches*
Legal requirements: Approach II

Increasing complexity

Cell
Tissue
Organism
Population
Community
Ecosystem
Biosphere

Higher-Tier

Decreasing precision

Lab Tests
Semi-field Tests
Field Study
Monitoring

Biomarker

Increasing complexity

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## EU legal requirements: Tests I

Potential test requirements with soil organisms

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Note: Rarely required tests are not listed (especially for biocides).
EU legal requirements: Tests II

Potential test requirements with soil organisms
An Earthworm Acute Test is listed under REACH and for biocides.

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<td>Earthworm field ISO 11268-3</td>
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Legal requirements: Lab. Tests III

All invertebrates tests are performed in OECD Artificial Soil, which is defined by a high content of organic matter. The ecological relevance would be higher in field soils.

1 OECD artificial soil: Well standardized

Many natural field soils: Very diverse but "realistic"
Standardized test methods 1:

**Microbial tests:**
- Usually, the natural microbial community of field soils is tested, not single species.
- They focus primarily on microbial functions.
- **OECD 216:** Nitrogen transformation test
- **OECD 217:** Carbon transformation test

**Plant tests:**
- Main focus on testing of crop species
- Residue analysis required (stock solution)
- **OECD 208:** Test with exposure via soil
- **OECD 227:** Vegetative vigour test
Standardized test methods 2: Invertebrate tests:

- Acute tests not required any more
- Cover of different physiological, taxonomic, size and ecological groups
- Important: Different exposure pathways: soft versus hard bodied species

Chronic tests following the same principles

Endpoint: reproduction

- **OECD 222**: Earthworms (Lumbricidae)
- **OECD 232**: Springtails (Collembola)
- **OECD 226**: Predatory mites (Acari)
- **OECD 220**: Pot worms (Enchytraeidae)
Standardized test methods 3: Earthworm field test (ISO 11268-3; OECD Draft)

Substrate: Field sites / soils (especially Europe)
Organisms: Earthworm community (Lumbricidae)
Duration: Usually 12 months (ca. 4 samplings)
Parameter: Diversity, abundance, biomass
Design: Treatment versus control
Note: Ecologically relevant; useful for other groups
Standardized test methods 4:
Litter bag test (OECD Guidance Document 56)

Substrate: Field sites / soils (world-wide)
Duration: Usually 6 - 12 months
Parameter: Mass loss of (e.g.) wheat straw
Design: Treatment versus control
Note: Not very sensitive ==> not required anymore.
Standardized test methods 5.1:

Oligochaete bioaccumulation test

Guideline: OECD (2010)
Species: *Eisenia fetida, E. andrei, Lumbricus rubellus, E. albidus* or *E. luxuriosus*
Substrate: Artificial Soil or field soils, e.g. LUFA
Duration: At least 28 - 42 days
Parameter: Bioaccumulation factor: BAF or BSAF (lipid-normalised)
Design: Uptake and elimination phase
Experience: Limited experience; ring-test performed;

Not an effect issue but relevant for secondary poisoning.
Standardized test methods 5.2:

Test soils

Test chamber

Test species
Summary (thought starters) I.

Are we using relevant species for testing?
► Some important groups are already covered.
► For new ones transparent criteria are needed:

Ecological relevance  ==>  Functional role clear?
Keeping and breeding  ==>  Easy, short generation?
Exposure situation:  ==>  Soft-bodied or hard-bodied?
Endpoints:            ==>  Chronic / behavioural endpoints?
Sensitivity:         ==>  Moderately but with a wide range?

Species fulfilling these criteria are (partly) available

Examples: Nematods, Isopods, Gastropoda….
Summary (thought starters) II.

Are we using appropriate test methods?

► Impressive list of OECD (ISO?) methods available.
► Again, for new ones transparent criteria are needed:

Exposure pathway:  ==>  Pore water, soil ingestion, air (?)  
Standardization  ==>  Acceptance by OECD preferred  
Practicability:  ==>  Simple, quick and cheap  
Usefulness:  ==>  Fit into existing batteries (e.g. SSD)  
Cover of test levels:  ==>  Suitable for semi-field and field  

Examples: Earthworm or Collembola avoidance tests  
Tests with functional endpoints are lacking.
Summary (thought starters) III.

**Microbial (PFLA) test on structural diversity**: ISO 29843 (2010)

**Nematod Reproduction Test** (*Caenorhabditis elegans*): ISO 10872 (2011)

**Earthworm Avoidance Test** (*Eisenia fetida/andrei*): ISO 17512-1 (2008)

**Snail Growth Toxicity Test** (*Helix aspersa*): ISO 15952 (2003)

**Isopod Chronic Test** (*Porcellio scaber*)

Lökke & Van Gestel (1998)
Microbial functional diversity: BIOLOG assay (metabolic fingerprinting; based on rate of substrate use; not standardized)

Functional genes involved in nitrogen cycling (amoA, nirK, nirS, nosZ1) used as molecular markers (Bru et al., 2011)

Water infiltration rate: DIN 19882-7. Very demanding method

Bait-Lamina test: Feeding rate of soil invertebrates: ISO 18311
Terrestrial Model Ecosystems (TME) (PERAS 2007)

Substrate: Field soils (world-wide)
Duration: Usually 4 - 12 months
Parameter: Various structural / functional endpoints
Design: Treatments versus control
Experience: High, mainly with pesticides
Further issues (thought starters) A.

Can functional diversity be considered as providing a sufficient level of protection, incl. structural diversity?

► *In theory, yes. In practice we do not know.*

► *Higher tier (i.e. community) and long-lasting functional tests are necessary to address this question.*

**Further needs in this context:**

► *Regional ecological differences have to be considered.*

► *Simplification of diversity evaluation using barcoding*

► *Both time and space have to be included in ERA.*

► *Interactions with other stressors have to be studied.*
Further issues (thought starters) B.

How to improve applicability and test design of higher-tier testing in the regulatory context?

- Inclusion of "intermediary" (= complex) lab studies, e.g. Collembola two-generation tests
- Set-up of a tiered battery of standardized tests (e.g. TMEs) and evaluation methods (e.g. SSDs)
- Development and validation of modelling approaches

Further needs in this context:

- Improvement of basic ecological and biogeographical data sets, ideally by EU-wide connected databases
Further issues (thought starters) C.

How to account for bioavailability in toxicity tests?

► General agreement that the bioavailable fraction of a chemical should be used in ERA – but which one?
► Experiences with metals in prospective (REACH) and retrospective (site-specific) ERA should be checked.

Further needs in this context:

► Validation of any surrogate chemical methods needed.
► Influence of environmental (soil) factors to be clarified.
► Complexity of the issue not to be mirrored 1 : 1 in ERA
► Analytical verification of exposure already in lab tests?
Thank you for your attention!