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

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Aims and content

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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

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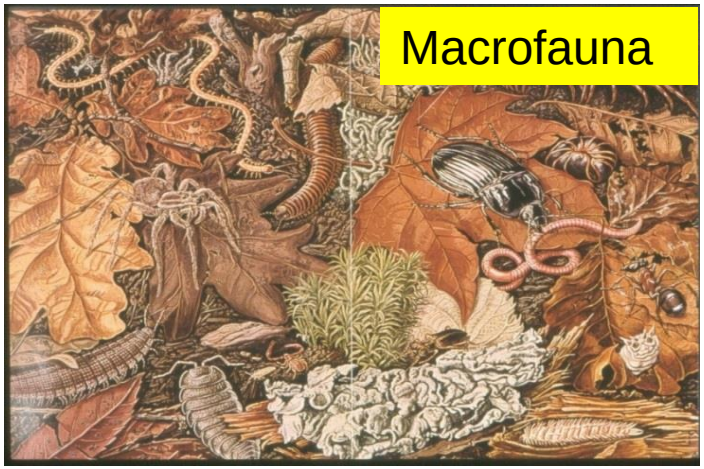
Soils differ enormously in terms of their biological diversity, but also regarding their abiotic properties (e.g. texture, pH).



Legal requirements: Introduction

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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

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Laboratory Tests

Single species tests
- Plant and invertebrate tests
- Bioaccumulation tests

Multispecies tests
- Microbial tests, *intermediate tier tests*

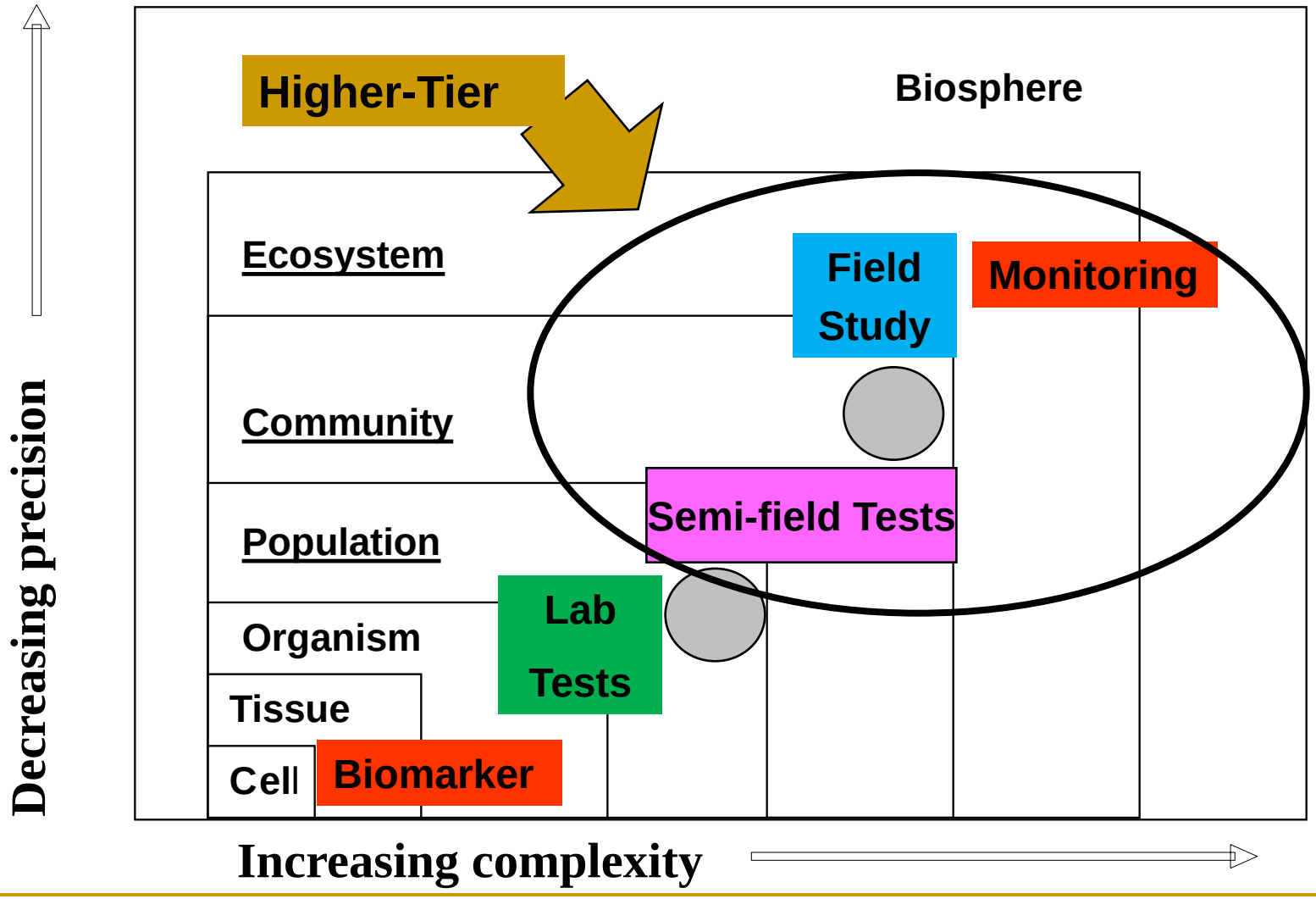
Semi-field Tests

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

Field Tests etc.

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

Legal requirements: Approach II



EU legal requirements: Tests I

Potential test requirements with soil organisms

Test and Guideline	REACH	Biocides	PPP	Hum. Ph.	Vet. Ph.
Microbes: N-cycle OECD 216	X	X	X	X	X
Microbes: C-cycle OECD 217	X	X			
Plants acute, 3-10 species OECD 208	X	X	X	X	X
Plants chronic, 2 species ISO 22030	X	X	(X)		

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.

Test and Guideline	REACH	Biocides	PPP	Hum. Ph.	Vet. Ph.
Earthworm chronic OECD 222	X	X	X		X
Enchytraeid chronic OECD 220	X	X			
Collembola chronic OECD 232	X	X	X		(X)
Predatory mite chronic OECD 226		X	X		
Earthworm field ISO 11268-3			X		

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:

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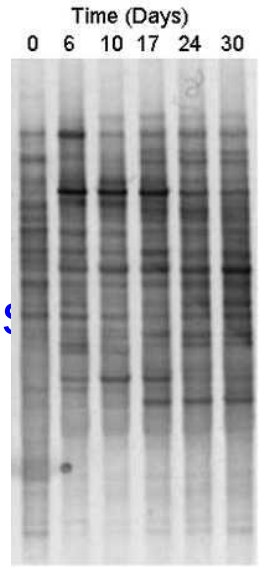
Microbial tests:

- 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:

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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:

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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:

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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:

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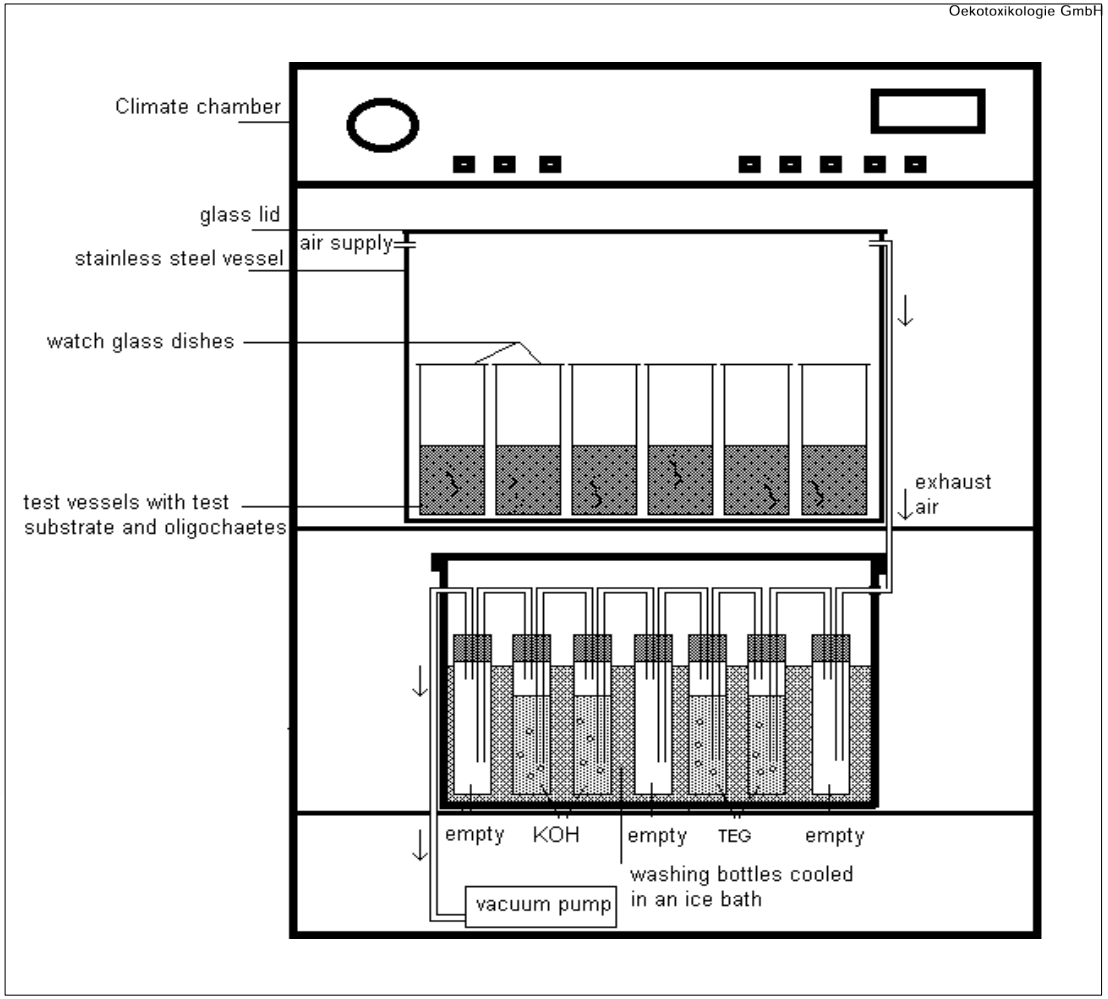
Test soils

Test species

Test chamber



Summary (thought starters) I.

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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.

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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.

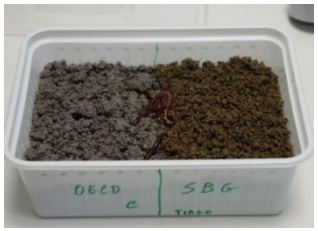
Microbial (PFLA) test on structural diversity: ISO 29843 (2010)

Nematod Reproduction Test (*Caeno-rhabditis elegans*): ISO 10872 (2011)

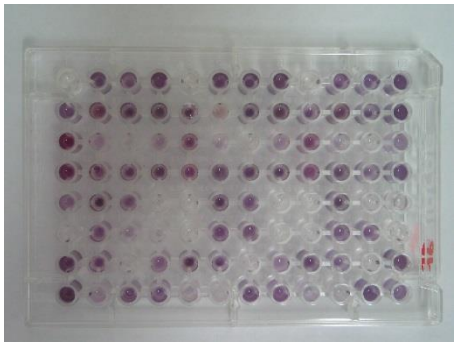
Earthworm Avoidance Test (*Eisenia fetida/andrei*): ISO 17512-1 (2008)

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

Isopod Chronic Test (*Porcellio scaber*) Lökke & Van Gestel (1998)



Summary (thought starters) IV.



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

Summary (thought starters) V.

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.

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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.

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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.

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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!

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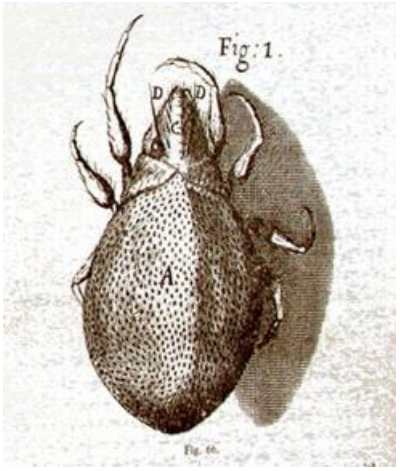

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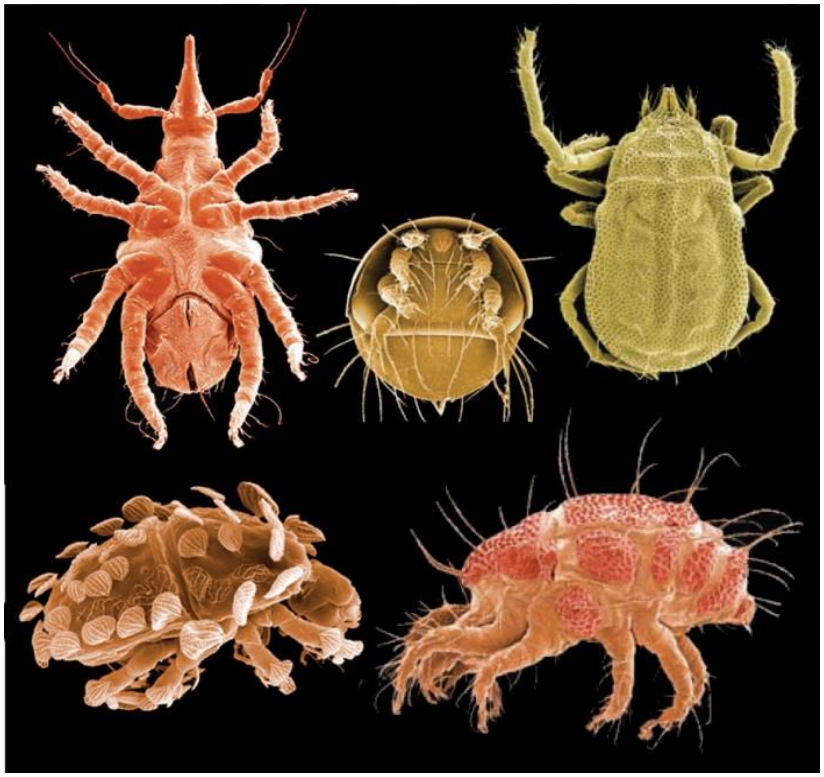
Soil Biodiversity Atlas



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