Sampling methods for soil living organisms
(in regulatory studies)
Assessment of effects of plant protection products on soil organisms (10 years)

Effects on soil and organism needs to be assessed

TIER-ed testing approach is used for soil organisms

International standards or publication are available or are developed

- ISO, 2007: 23611-3 Soil quality — Sampling of soil invertebrates — Part 3: Sampling and soil extraction of enchytraeids
Functional tests – only litterbag test

Macrofauna (ISO)- earthworm (Lumbricina)

Mesofauna (ISO) - springtails (Collembola), mites (Acariformes), enchytraeids (Enchytraeidae) and nematods (Nematoda)

Test run for all fauna types 1 year

Positive and negative control always included

Test design - limit test

Effects are allowed and recovery within 1 year is currently accepted

<table>
<thead>
<tr>
<th>Effect class</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Large effects</td>
<td>pronounced reduction above 65 %</td>
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<tr>
<td>Medium effects</td>
<td>reduction between 35 % and 65 %</td>
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<td>Small effects</td>
<td>reduction above 10 % and below 35 %</td>
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<td>Negligible effects</td>
<td>reduction up to 10 %</td>
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</table>

Scaling of magnitude of effects (Source: EFSA 2015)
Litter bag studies (functional studies)
Decomposition of organic material in soil

- Test guidelines: Römbke et al. 2003 (Draft guideline), OECD (No. 56) 2006
- Timing of applications adapted to realistic worst case scenario
- Litterbags with 4 g of straw dug into soil after application
- Sampling dates 1, 3, 6, 9 and 12 months after litter bag incorporation
- Determination of ash-free dry weight in the lab

Other systems bait lamina or micro container
Field exposure studies can support extensions of existing use patterns, if adverse effects to earthworms have been observed in lower tiered studies.

Earthworm field studies are required according to SANCO/10329/2002 if the calculated TER (toxic exposure ratio) for soil is above 5 or in case bioaccumulation cannot be excluded.

Challenge: for study ≥ 60 (adult) earthworms/m² at first sampling for all treatment groups (ISO 11268-3, 2014). Species of different ecological life forms (i.e. endogeics, such as *Apporrectodea caliginosa* and anecics, such as *Lumbricus terrestris*) have to be present in sufficiently high numbers of at least 10 individuals per m² or at least 10 % of total adult earthworm abundance. Abundance on average 77.89±98.94 individuals per m²; (Phillips et al 2019) 1-7 species in central European agricultural sites (Römbke et al 1997)
Earthworm field studies

- Earthworm studies run in bare soil, grassland and crops
- Start spring or autumn with field search
- Methodology
  - Randomized block design
  - Negative control, x treatments, positive control (carbendazim) with 4 replicates each
  - Irrigation after application
  - Assessment of baseline before application and assessment of recovery after application, e.g. after 1, 6 and 12 months, or several years
- Abundance and biomass are recorded
Earthworm field studies

- Methods works by irritating earthworms other methodology octet method (electric extraction)
- Sample areas of 50 x 50 cm or other sizes using frames
- Efficiency check by hand-sorting after using the selected extraction method
- Extraction by allyl isothiocyanate (AITC) solution
- Problems: -low efficiency when soil is dry / cold
Earthworm field studies

- Sample areas of 50 x 50 cm or other size
- Excavation depth 20-30 cm
- Problems:
  - mainly endo- and epigeic species
  - time consuming

Handsorting
Earthworm field studies

- Sample areas of 50 x 50 cm or other size
- Excavation depth 20 - 30 cm
- Extraction by allyl isothiocyanate (AITC) solution
- Advantages:
  - endogeic, epigeic and anecic species
  - best sampling results in agriculture environment
  - independent of draught, cold temperatures
- Problem: most time consuming
Earthworm field studies

Distribution of earthworms within 2 different field sites (70 m x 70 m)
Earthworm field studies

Layout of study and individual plots with positions of sample areas for earthworm population samplings (the letters A-D indicate the sampling dates SEW1-4, the numbers 1-4 indicate the sub-samples (50x50 cm and 20 depth per sampling date), areas for monitoring (M1, M2), sample areas for soil core and deposition tray sampling.
Mesofauna field studies are required if the calculated TER (toxic exposure ratio) for soil is above 5.

Abundance especially of Collembola is very variable. No numbers are given but there should be at least 10 specimen per soil core on average.

Abundance Collembola 1,500 – 33,000 for central European grassland sites 17 -38 species (Römbke et al 1997)

Abundance mites < 1,000 – 5,000 for central European grassland sites 3 - 10 species (Römbke et al 1997)
No current guideline only publication available (RÖMBKE et al. 2009) bare soil, grassland and crops

Methodology

- Randomized block design
- Negative control, x treatments, positive control with 6 replicates each
- Assessment of baseline before application and assessment of recovery after application, e.g. after 1, 3, 6 and 12 months
- Abundance is recorded

Sampling and Extraction

- Sampling soil cores (5 cm Ø and minimum 5 cm depth)
- Dry extraction of mites and collembolans with Macfayden extractor in 8 days (accord. to ISO Guideline 23611-2) alternative is wet extraction
- Optional activity based pitfall, slide/mine traps and suction sampling
- Optional surface species suction sampling
- Other groups possible to sample nematodes and enchytraeids
Sampling with slide/mine trap or underground pitfall trap (Collembola)

New sampling method for collembola
Take into account vertical movement

Advantage - Sample for longer periods of time and lower depth, different depth as the same time
Disadvantage - only active collembola sampled

Different species composition than soil cores

Bakker et al. 2017
Sampling with suction sampling (springtails and mites)

Sampling method for springtails and mites on soil surface and vegetation

Advantage
- Sample has relation to a surface area
- Large amount of specimen can be samples at the same time than soil cores

Different species composition than soil cores
Identification more complicated

Bakker et al. 2019
## Identification Work:

<table>
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<th>Group</th>
<th>Identification Level</th>
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<td>Earthworms</td>
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<td>Collembola</td>
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<td>Enchytraeidae</td>
<td>Species level (only live animals can be identified)</td>
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<td>Nematodes</td>
<td>Functional groups</td>
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Results Earthworms

Range of MDD’s for different earthworm taxa

MDD – Minimum detectable differences

Results from control samplings of 26 field studies with 4 replicates.
Seasonal abundance of Collembola
## Results Mesofauna

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<th>6 M</th>
<th>12 M</th>
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</table>

- **bold** statistically significant difference to the control at alpha = 0.05
- **bold** statistically significant difference to the control at alpha = 0.1
- **bold** statistically significant difference to the control at alpha = 0.01
Results Mesofauna

Population Response Curve negative and positive control
Further Developments

- Development of a new earthworm OECD guidance document
- New field study design for other groups?
- Positive controls, what to use in the future?
- EFSA Guidance for risk assessment in soil very compartmental for several taxonomic groups
- Inclusion of e-DNA in studies for additional samplings?

Thank you for your attention!