The benefit of using Terrestrial Model Ecosystems (TME) in Environmental Risk Assessment

Andreas Toschki | gaiac - Research Institute
SETAC Europe 14th Special Symposium, Brussels
19-20th November 2019
Outline

1. Introduction
2. Terrestrial Model Ecosystems (TME) – Conceptual approach
3. Case Study „Linking fate & effects in soil“ – Methods and selected results
4. Conclusion
Soil as living space and basic resource

Maintenance & Support of Ecosystem Services and Functions

Spiders, Coleoptera, Woodlice, Snails, Earthworms

Collembola, Mites, Enchytraeids, Nematoda, Diptera-, Hymenoptera-Coleoptera-Larvae & Eggs

Protozoa, Rotatoria, unicellular Organisms
Regulatory demands


Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms 2017

**Keywords:**

Consideration of:  » non-target species  » ecosystem  
» behaviour  » indirect effects  
» biodiversity  » food web
TME as semi-field test

Variability is the challenge
methods has to be adapted in order to get
• precise results
and not to
• overstress practicability

modified after Schaeffer et al. (2010)
Conceptual TME approach

Exposure: under natural/representative environmental conditions
Outdoor /semi-field

Realism: natural soil community (off crop)
Species–composition and distribution in the soil column is similar to the field

Focus: most abundant representatives of the soil mesofauna
Collembola | Oribatida | Enchytraeidae | Nematoda | Lumbricidae
Methods - TME coring

- Undisturbed grassland
- Regularly mown
- No pesticide application
- Strictly vertical coring (technical device)
- Intact soil cores with minimum compaction (‘undisturbed’)
- Outdoor: Ø: 47 cm, h: 40 cm, m: ≈100 kg
- Coring strategy minimise spatial heterogeneity by sampling as nearby as possible
TME facility and maintenance

- Each TME kept 'isolated' to prevent emigration from or immigration to the soil core
- Drainage to avoid stagnant water
- Irrigation or shelter is possible
- Data logger for moisture and temperature
Standardised sampling procedures

Oribatida | Collembola | Enchytraeidae | Nematoda | Analytics

Sequential sampling for mesofauna & analytics
- max. 19 soil cores (5 cm Ø) / TME (maximal loss of 22 % of the TME surface)
- pitfalltraps for surface activity
- MacFadyen Extraction (Collembola & Oribatida)
- Wet extraction (Enchytraeidae, Nematoda)

Destructive sampling for earthworms
- 100 % of the TME
- hand sorting of the whole column
- cutting of measured slices
Case study

Evaluation of the risk for soil organisms under real conditions

Research & Development Project
Federal Environment Agency


Partner:
Focal issues

Are soil organisms differently affected by pesticides with different persistence and mobility in soil?

Does the preference of different soil layers by soil organisms result in differences of exposure and thus different vulnerabilities?

tracking the test-substances

different soil layers of the soil column
 reaction of soil organism species
### Experimental setup – study overview

<table>
<thead>
<tr>
<th>Biologie</th>
<th>Analytics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field samples</td>
<td>Analytics cold</td>
</tr>
</tbody>
</table>

**TME Study [1] – Outdoor**
- Collembola
- Oribatida
- Enchytraeids
- Earthworms
- Analytics cold

**TME Study [2] – Indoor**
- Radioactive analytics

**TME Study [3] – Outdoor**
- Earthworms
- Analytics cold

### Outdoor Treatments

- **Lindane**
  - 7.5 kg/ha
  - 20 kg/ha

- **Imidacloprid**
  - 0.75 kg/ha
  - 2 kg/ha

- **Carbendazim**
  - 7.5 kg/ha
  - 15 kg/ha

- Different physico-chemical properties (fate in soil)
- Different toxicity on soil organisms
Screening pre-test

28 d laboratory test can be used to find the optimal concentration range

Coring of ø 5 cm soil cores in the field

Application on the single soil cores in the laboratory
control n=8, treatment n=3
Dose response approach

Incubation for 14 -28 days under controlled laboratory conditions

Extraction

Evaluation

<table>
<thead>
<tr>
<th>Test</th>
<th>14 d after application</th>
<th>28 d after application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC₅₀ [mg/kg]</td>
<td>NOEC [mg/kg]</td>
</tr>
<tr>
<td>PPP1</td>
<td>0,96</td>
<td>3,3</td>
</tr>
<tr>
<td>PPP2</td>
<td>4,26</td>
<td>33</td>
</tr>
</tbody>
</table>

Gesamtanzahl Collembolen nach 28d

<table>
<thead>
<tr>
<th>Konzentration [mg/kg]</th>
<th>Gesamtanzahl Collembolen nach 28d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

PPP1

PPP2

Gesamtanzahl Collembolen nach 28d

Konzentration [mg/kg]

Kontrolle

Anzahl pro Bodenkern

0
50
100
150
200
AgPURE
AgNO₃

Evaluation
Experimental design

A: Analytics  F: Moisture, Temperature  LW: Leached water

May | June | July | August | September | October | November | December | January | February | March | April | May
---|---|---|---|---|---|---|---|---|---|---|---|---
2011

May | June | July | August | September | October | November | December | January | February | March | April | May
---|---|---|---|---|---|---|---|---|---|---|---|---
2012

May | June | July | August | September | October | November | December
---|---|---|---|---|---|---|---
2013
Vertical distribution of pesticides

Application of 7.5 kg/ha of lindane

Log Kow = 3.2 - 3.9

- Highest concentration always in layer A (0-2.5 cm)
  (laboratory experiment 0-1 cm)
- Decrease in concentration over time
- Only low concentrations (<10%) in deeper soil layers
- Concentration in leachate < 0.05%
Vertical distribution of Pesticides

Conclusions for all considered pesticides

- Highest concentration always in layer A (0-2,5 cm) / (laboratory experiment 0-1 cm)
- Decrease in concentration over time
- Only low concentrations (<10%) in deeper soil layers
- Concentration in leachate < 0.05 %
Vertical distribution of soil organisms

**Collembola**
- 0-2.5 cm: 68 % Ind.
- 0-5 cm:  92 % Ind.

**Oribatida**
- 0-2.5 cm: 91 % Ind.
- 0-5 cm:  98 % Ind.

**Enchytraeidae**
- 0-2.5 cm: 60 % Ind.
- 0-5 cm:  88 % Ind.

**Lumbricidae**
- 0-2.5 cm: 36 % Ind.
- 0-5 cm:  58 % Ind.
Ecological life-form types

Behaviour of soil organisms in TME is similar to that in the field

Ecological life form types of the recorded collembolans.
Control abundances of all sampling dates were classified after Stierhoff (2003) and Theißen (2010).
Ecological life-form types

**Lumbriculus castaneus - Control**

<table>
<thead>
<tr>
<th>Soil Layer</th>
<th>0-2,5 cm</th>
<th>2,5-5 cm</th>
<th>5-10 cm</th>
<th>10-20 cm</th>
<th>20-40 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of individuals [%]</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

- epigeic ✓

**Aporrectodea caliginosa - Control**

<table>
<thead>
<tr>
<th>Soil Layer</th>
<th>0-2,5 cm</th>
<th>2,5-5 cm</th>
<th>5-10 cm</th>
<th>10-20 cm</th>
<th>20-40 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of individuals [%]</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

- endogeic ✓

**Lumbriculus terrestris - Control**

<table>
<thead>
<tr>
<th>Soil Layer</th>
<th>0-2,5 cm</th>
<th>2,5-5 cm</th>
<th>5-10 cm</th>
<th>10-20 cm</th>
<th>20-40 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of individuals [%]</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

- anecic ✓

**Aporrectodea rosea - Control**

<table>
<thead>
<tr>
<th>Soil Layer</th>
<th>0-2,5 cm</th>
<th>2,5-5 cm</th>
<th>5-10 cm</th>
<th>10-20 cm</th>
<th>20-40 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of individuals [%]</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

- endogeic ✓

Behaviour of soil organisms in TME is similar to that in the field ✓
Effects on Collembola

**Lindane 7.5 kg a.s./ ha**

<table>
<thead>
<tr>
<th>layer</th>
<th>14</th>
<th>42</th>
<th>140</th>
<th>189</th>
<th>364</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>73</td>
<td>83</td>
<td>-28</td>
<td>X</td>
<td>52</td>
</tr>
<tr>
<td>A</td>
<td>90</td>
<td>100</td>
<td>3</td>
<td>84</td>
<td>51</td>
</tr>
<tr>
<td>B</td>
<td>89</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>C</td>
<td>86</td>
<td>83</td>
<td>88</td>
<td>X</td>
<td>73</td>
</tr>
<tr>
<td>all layers</td>
<td>90</td>
<td>98</td>
<td>37</td>
<td>87</td>
<td>59</td>
</tr>
</tbody>
</table>

**Lindane 20 kg a.s./ ha**

<table>
<thead>
<tr>
<th>layer</th>
<th>14</th>
<th>42</th>
<th>140</th>
<th>189</th>
<th>364</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>94</td>
<td>91</td>
<td>94</td>
<td>X</td>
<td>76</td>
</tr>
<tr>
<td>A</td>
<td>97</td>
<td>99</td>
<td>100</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>57</td>
<td>92</td>
<td>88</td>
<td>X</td>
<td>100</td>
</tr>
<tr>
<td>all layers</td>
<td>95</td>
<td>93</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

% decrease in **total abundance**

bold: statistically significant results

: effects > 50 %
Community assessment

Principal Response Curve - Lindane - all layers

- control
- 7.5 kg a.s./ha
- 20 kg a.s./ha

Principal Response Curve - Layer A -

- control
- 7.5 kg /ha
- 20 kg /ha
Fate & Effect Collembola

**Effect on total abundance relative to control [%]**

### 14 days

- **Surface**
- **0-2.5 cm**
- **2.5-5 cm**
- **5-10 cm**
- **10-20 cm**

**LC$_{50}$**: $< 2.21$ mg/kg

### 364 days

- **Surface**
- **0-2.5 cm**
- **2.5-5 cm**
- **5-10 cm**
- **10-20 cm**

**NOEC$_{repro}$**: $< 0.06$ mg/kg

**Legend**

- Red: Measured concentration (Lindane 20 kg/ha)
- Yellow: Measured concentration (Lindane 7.5 kg/ha)
- Red dot: Inhibition [%] of total abundance (Lindane 20 kg/ha)
- Yellow dot: Inhibition [%] of total abundance (Lindane 7.5 kg/ha)
- Triangle: Minimum detectable difference MDD (Williams t-test)

**LC$_{50}$:** *Folsomia candida* | Mortality

**NOEC:** *Folsomia candida* | Reproduction
Fate & Effect

Explanation for effects in deeper layers?

1. ‘Direct effects’: Sensitivity of species in TME has to be much higher then the one of the test species *F. candida*

2. ‘Indirect effects’: Effects are based on animal movement in the soil column

Explanation for increase over time?

1. Additive effects and no recovery

2. Long-term effects on reproduction
Conclusion – case study

• The mobility of all tested pesticides were found to be similar
• Exposure to all tested pesticides mainly in the top soil layer (TME study 0-2.5 cm, 0-1 cm indoor)
• For all organism groups, a relevant proportion was found in the upper 2.5 cm layer
• Effects occur in all layers and also for soil organisms that prefer soil layers that are not exposed (low measured concentration)
• Long-term effects on reproduction can occur even when the test substance is no longer detectable

PEC calculations in RA should be based on the uppermost soil layers (0-2.5 cm, 0-1 cm)
Conclusion – TME approach

With TME studies

• the fate of pesticides and their effects on soil organisms can be investigated in space and time

• the diversity, behaviour, interaction of species under realistic conditions can be investigated

• different scenarios (drought, multiple application, mixture toxicity etc.) can be investigated on community level

TME studies can lead to a better understanding of the fate and effect of substances in soil that are yet unknown!
Conclusion - TME in Risk assessment

It would be advisable to integrate this approach in Risk Assessment!

Consequences to consider

- Testing high numbers of species (86 in case study) leads to high probability to get a false positive result
- The positioning of community studies in RA should be reconsidered to protect biodiversity. Representativeness of single species tests for the community is limited.
Thanks to...

**Project team**
Cornelia Bandow, Dennis Becker, Julia Bless, Anette Fürste, Melanie Grolms-Aal, Monika Hammers-Wirtz, Udo Hommen, Michael Klein, Pascale Lorenz, Susanne Miller, Thomas Moser, Claudia Poßberg, Johanna Oellers, Jörg Römbke, Martina Roß-Nickoll, Andreas Schäffer, Adam Scheffczyk, Björn Scholz-Starke, Rüdiger Schmelz, Burkhard Schmidt, Tido Strauss, Andreas Toschki, Pascal Weitzel

toschki@gaiac-eco.de
www.gaiac-eco.de

**Federal Environment Agency**
Carsta Hufenbach, Wolfram König, Silvia Pieper