MICROSOIL – Investigation of Alternative Test Methods to Correctly Assess the Impact of Plant Protection Products, Biocides and Pharmaceuticals on Soil Microorganisms

Marie Winter[1,2], Gabrielle Broll[2], Frank Zielinski[3], Jens Schoenfeld[3], Pia Kotschik[3], Silvia Pieper[3], and Karsten Schlich[1]

1 Fraunhofer Institute for Molecular Biology and Applied Ecology, Auf dem Aberg 1, 57392 Schmallenberg, Germany
2 Institute of Geography, Osnabrück University, Seminarstraße 19ab, 49074 Osnabrück, Germany
3 German Environment Agency, Wörlitzer Platz 1, 06844 Dessau-Roßlau, Germany
Contact: karsten.schlich@ime.fraunhofer.de

Introduction and aims

Currently, the **risk assessment of agrochemicals** considers the effects on nitrogen transformation (OECD 216 [1]). However, the determination of one central function may not reflect the complex soil functions of soil microbial communities [2]. Within the project MICROSOIL five alternative test methods in three differing test soils, treated with six substances each, are compared to data based on the current standard test (OECD 216) to **determine systems with a higher sensitivity of respective endpoints**. Here, recent results of effects on nutrient cycles (**MicroResp™** [3]), enzymatic activities (**DIN EN ISO 20130** [4]) and specialists (potential ammonium oxidation (PAO), **DIN EN ISO 15685** [5]) are presented.

Materials & Methods

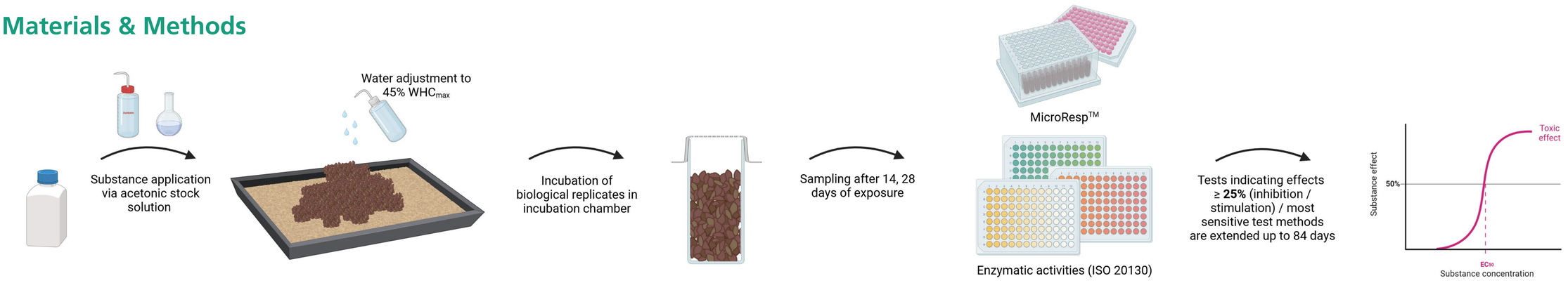

Figure 1: Application scheme of the test substances, implementation of three different test systems and evaluation of results.

The study design (Figure 1) followed the requirements of OECD 216 concerning storage, soil handling, substance application and conditions of exposure for comparability of results. OECD 216 tests were conducted for each substance and test soil in terms of data validation.

Information on test soils
Lufa 2.1: 86% sand; 10% silt; pH 4.7; C_{org} <0.1%
RefeSol 04A: 79% sand; 15% silt; pH 5.2; C_{org} 2.9%
RefeSol 02A: 2% sand; 83% silt; pH 6.5; C_{org} 1.1%

Results

Table 1a: Lufa 2.1. - Results of the impact [%] of three test concentrations [mg a.s./kg dw soil] for six different test substances on the microbial activitiy at day 28.

Ethofumesate	**2**	**10**	**20**
PAO	-1	-12	11
Enyzme activity	11 (Arylsulfatase)	**39 (Arylsulfatase)**	**87 (Arylsulfatase)**
MicroResp™	9 (y-Amino butyric acid)	19 (y-Amino butyric acid)	15 (y-Amino butyric acid)
OECD 216	n.d.	-6	n.d.
Propamocarb	**3**	**15**	**30**
PAO	-16	18	**94**
Enyzme activity	**26 (Phosphatase)**	14 (Phosphatase)	5 (Phosphatase)
MicroResp™	**-28 (Citric acid)**	**-29 (Citric acid)**	-14 (Citric acid)
OECD 216	n.d.	n.d.	pending
Pyraclostrobin	**3**	**15**	**30**
PAO	1	-20	-12
Enyzme activity	19 (Arylamidase)	16 (Arylamidase)	21 (Arylamidase)
MicroResp™	-15 (Citric acid)	-11 (Citric acid)	-10 (Citric acid)
OECD 216	6	n.d.	n.d.
Tebuconazole	**1**	**5**	**10**
PAO	-3	**-45**	-19
Enyzme activity	9 (Arylamidase)	-21 (Arylamidase)	1 (Arylamidase)
MicroResp™	-13 (Citric acid)	**-29 (Citric acid)**	-4 (Citric acid)
OECD 216	n.d.	n.d.	-3
DDAC	**3**	**30**	**300**
PAO	1	**33**	**100**
Enyzme activity	-11 (Arylsulfatase)	-6 (Arylsulfatase)	**-39 (Arylsulfatase)**
MicroResp™	7 (N-Acetyl glucosamine)	7 (N-Acetyl glucosamine)	6 (N-Acetyl glucosamine)
OECD 216	n.d.	n.d.	pending
Tiamulin	**0.36**	**3.6**	**7.2**
PAO	-1	15	**46**
Enyzme activity	**26 (Arylsulfatase)**	**58 (Arylsulfatase)**	7 (Arylsulfatase)
MicroResp™	-12 (Citric acid)	-7 (Citric acid)	-7 (Citric acid)
OECD 216	n.d.	1	n.d.

Table 1b: RefeSol 04A. - Results of the impact [%] of three test concentrations [mg a.s./kg dw soil] for six different test substances on the microbial activitiy at day 28.

Ethofumesate	**2**	**10**	**20**
PAO	14	-7	9
Enyzme activity	20 (ß-glucosidase)	**48 (ß-glucosidase)**	3 (ß-glucosidase)
MicroResp™	-19 (D-(+)-Glucose)	-19 (D-(+)-Glucose)	-7 (D-(+)-Glucose)
OECD 216	n.d.	21	n.d.
Propamocarb	**3**	**15**	**30**
PAO	-22	3	**36**
Enyzme activity	-23 (Arylsulfatase)	**-33 (Arylsulfatase)**	**-37 (Arylsulfatase)**
MicroResp™	-12 (L-malic acid)	-10 (L-malic acid)	-23 (L-malic acid)
OECD 216	n.d.	n.d.	1
Pyraclostrobin	**3**	**15**	**30**
PAO	7	9	20
Enyzme activity	**-59 (Arylsulfatase)**	10 (Arylsulfatase)	7 (Arylsulfatase)
MicroResp™	**-49 (Citric acid)**	**-27 (Citric acid)**	2 (Citric acid)
OECD 216	n.d.	n.d.	**30**
Tebuconazole	**1**	**5**	**10**
PAO	-4	0	9
Enyzme activity	**31 (Phosphatase)**	15 (Phosphatase)	10 (Phosphatase)
MicroResp™	-6 (L-malic acid)	-18 (L-malic acid)	**-28 (L-malic acid)**
OECD 216	n.d.	n.d.	4
DDAC	**3**	**30**	**300**
PAO	3	17	**84**
Enyzme activity	-2 (ß-glucosidase)	-3 (ß-glucosidase)	**-29 (ß-glucosidase)**
MicroResp™	**-25 (D-(+)-Glucose)**	**-27 (D-(+)-Glucose)**	-13 (D-(+)-Glucose)
OECD 216	n.d.	n.d.	16
Tiamulin	**0.36**	**3.6**	**7.2**
PAO	8	21	**38**
Enyzme activity	**52 (ß-glucosidase)**	**-32 (ß-glucosidase)**	9 (ß-glucosidase)
MicroResp™	**-39 (Citric acid)**	**-26 (Citric acid)**	**-32 (Citric acid)**
OECD 216	n.d.	n.d	7

Note: Enzyme activity was observed for phosphatase, ß-glucosidase, arylsulfatase, arylamidase and urease. For MicroResp™ 8 substrates (deionized water, D-(+) glucose, L-cystein, L-malic acid, y-amino butric acid, N-acetyl-gulcosamine, citric acid and L-alanine) were used. Only the enzyme/substrate most sensitive regarding to the test substance is presented in the table. **Blue values:** Stimulation above 25%; **Red values:** Inhibition above 25% - values compared to control treatment.

- **MicroResp™:** Low sensitivity; neither for basal nor substrate-induced respiration statistically significant dose-response relationship after 28 days or 84 days, respectively.
- **Enzyme activity:** Medium sensitivity; effects >25% but mainly no continuous dose-response (except Ethofumesate in LUFA 2.1) at specific time points followed by recovery on following measurement points
- **PAO:** Most sensitive; Propamocarb, DDAC and Tiamulin (LUFA 2.1 and RefeSol 04A test results), concentration related effects > 25% were determined after 28 days of exposure

Conclusion & Outlook

- PAO is the most sensitive test method for the chosen test substances and test soils (only tow soils are tested so far).
- Investigations of arbuscular mycorrhiza might give first indications of fungi sensitivity under substance exposure compared to other test systems.
- Structural analysis of the microbial community (bacteria and fungi) by applying the ARISA method will complete the data set.

Acknowledgement: The authors thank the German Environment Agency for granting the project (FKZ: 3720 64 411 0) and Leonie Homes and Ruben Schlinkert for their help in the lab.

References: [1] OECD 216 (2000): OECD Guideline for the Testing of Chemicals. Soil microorganisms: Nitrogen Transformation Test. **[2]** Ockleford, C et al. (2017): Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017 (2): 4690 –4915.**[3]** MicroResp™. Technical Manual. **[4]** DIN EN ISO 20130 (2020): Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates **[5]** DIN EN ISO 15685 (2020): Soil quality – Determination of potential nitrification.