

TEXTE

201/2020

# **Evaluation of the risk for soil organisms under real conditions**

**Development of a national position for amending  
downstream legislations of the new EU Plant Protection  
Products Regulation**

## **Summary**



TEXTE 201/2020

Environmental Research of the  
Federal Ministry for the  
Environment, Nature Conservation  
and Nuclear Safety

Project No. (FKZ) 3710 67 410  
Report No. EF001129-KURZ,ENG

## **Evaluation of the risk for soil organisms under real conditions**

Development of a national position for amending downstream  
legislations of the new EU Plant Protection Products Regulation

Summary

by

Andreas Toschki (Coordination), Monika Hammers-Wirtz  
gaiac -Research Institute for Ecosystem Analysis and Assessment, Aachen

Claudia Poßberg, Martina Roß-Nickoll, Andreas Schäffer, Burkhard Schmidt,  
Björn Scholz Starke  
RWTH Aachen University, Aachen

Jörg Römbke, Adam Scheffzyk  
ECT- Oekotoxikologie GmbH, Flörsheim am Main

Michael Klein, Udo Hommen  
Fraunhofer IME, Schmallenberg

On behalf of the German Environment Agency

## **Imprint**

**Publisher:**

Umweltbundesamt  
Wörlitzer Platz 1  
06844 Dessau-Roßlau  
Tel: +49 340-2103-0  
Fax: +49 340-2103-2285  
[buergerservice@uba.de](mailto:buergerservice@uba.de)  
Internet: [www.umweltbundesamt.de](http://www.umweltbundesamt.de)

 [/umweltbundesamt.de](https://www.facebook.com/umweltbundesamt.de)  
 [/umweltbundesamt](https://twitter.com/umweltbundesamt)

**Study performed by:**

gaiac-Research Institute for Ecosystem Analysis and Assessment e.V.  
Kackertstr. 10  
52072 Aachen

**Study completed in:**

March 2018

**Edited by:**

Section IV 1.3 Plant Protection Products  
Silvia Pieper

**Publication as pdf:**

<http://www.umweltbundesamt.de/publikationen>

ISSN 1862-4804

Dessau-Roßlau, November 2020

The responsibility for the content of this publication lies with the author(s).

## Table of contents

<b>Background .....</b>	<b>1</b>
<b>Aims .....</b>	<b>1</b>
<b>Methodological requirements .....</b>	<b>2</b>
Study design .....	2
Test items (PPP) and chemical analysis .....	2
Investigated soil organisms .....	3
Exposure modelling .....	3
State of the art.....	4
Terrestrial model ecosystems .....	5
Methods .....	6
<b>Results .....</b>	<b>8</b>
Field study 1 TME: Concentrations of the test substances Lindane and Imidacloprid .....	8
Laboratory study 2: Concentrations using $^{14}\text{C}$ -labelled pesticide .....	8
Comparison of experimental findings and modelling of exposure .....	8
Effects of the tested substances on soil organisms .....	9
<b>Conclusions .....</b>	<b>11</b>
<b>Recommendations .....</b>	<b>12</b>
<b>References .....</b>	<b>14</b>

## Background

For the approval of active substances and for the authorization of Plant Protection Products (PPPs) in Europe (EU), it is necessary to test the active substances and products according to the current state of science and technology to demonstrate that no unacceptable effects on the natural environment will occur following their intended use (Regulation (EC) No 1107/2009 (EU 2009), PflschG 2012). The effects of active substances and/or PPPs on the protection target are assessed by applying the criteria given in Regulation (EU 2011) No. 546/2011. The thorough derivation of the environmental concentration (Predicted Environmental Concentration, PEC) of an applied PPP or active substance(s) in the soil or water body is the crucial basis for the evaluation and assessment of possible detrimental effects on the non-target organisms that are exposed to it. The recent process in the development of guidelines and guidance documents (e.g. EFSA Guidance Documents and EFSA PPR Scientific Opinions 2010a and 2010b, 2012 and 2017) indicates that fundamental new strategies will be followed to derive environmental concentrations in soil in the future.

New conceptions and understandings of the ecological/ecotoxicological effects of PPP on soil organisms and of the active substance's fate and behaviour in soils should lead to a more realistic and relevant calculation of the predicted environmental concentrations (PECs). At the same time, it will result in a much more complex evaluation system than the one in place nowadays. Two crucial issues have to be addressed in the future:

- The spatial correlation between the toxic agent and the effects on soil organisms belonging to specific exposure types are not experimentally proven so far. The initial hypothesis that the behaviour and life form type of soil organisms in respect of their habitat preferences in the soil profile determine quality and duration of exposure is not yet scientifically confirmed.
- The resulting protection level for non-target soil organism of an approach differentiating for soil layers in comparison to the current practice is not consequently analysed, nor are the consequences for the risk assessment outcome acknowledged.

To support a national position for risk assessment of soil organisms in line with the new scientific and regulatory developments, it has to be determined whether the assumed connection between spatial distribution of soil organisms, the distribution of PPP in soil and the ecotoxicological effects on soil organisms can be systematically observed. Moreover, it is crucial to check out thoroughly the possibly arising testing-effort and the protection level to develop the new strategy with clear questions and aims.

## Aims

The aim of this project was to develop the technical basis in order to adapt the exposure assessment and risk assessment of PPP in soil and for soil organisms according to newest scientific developments.

In addition to recording the state of knowledge about the relationship between the location of effects on soil organisms within the soil profile and the spatial and temporal distribution of PPPs, in particular, experimental investigations were conducted under controlled conditions, in order to provide a scientific basis for an adapted risk assessment strategy.

The project focussed on the following main questions:

- Can the assumed relationship between spatial distribution of a PPP in the soil profile and the location of ecotoxicological effects be confirmed?
- Is the exposure level and consequently the extent of ecotoxicological effects modulated by the preferred position and the behavior of soil organisms in the soil profile?
- Is the spatial transfer of the maximum concentration of a PPP into different soil layers over time accompanied by a sequence of effects in organism groups with different mode of exposure?
- Do active substances with different properties at a given time interfere with different groups of organisms, each representing a typical mode of exposure?

The results of the experimental studies should help refining the input parameters for current exposure models for soil organisms. The existing simulation models for exposure assessment in environmental risk assessment are able to calculate PECs for discrete soil depths. The aim of this project was to provide evidence on whether the average concentration of a PPP over different soil layers can be used for risk assessment of soil organisms or whether the concentration peak is determinative of the toxicity for soil organisms.

Finally, recommendations for the adaptation of risk assessment strategy for soil organisms were developed. Here a systematic and comprehensive comparison of the results of a risk assessment for soil organisms was performed with the currently established method and according to the specifications of a new adapted strategy. The aim was to document the achieved protection level of different strategies for the protection goal, that no unacceptable impacts on the subject of protection “soil and soil organisms” will occur, and to develop specific recommendations for the adjustment of the risk assessment.

## **Methodological requirements**

To meet the above mentioned challenges within one study, appropriate methods and an adapted experimental design were required.

### **Study design**

The test design provided the possibility to measure toxicological effects on populations of different soil communities and the fate and behaviour of the toxicant at the same time and approximately at the same place. The test design enabled the analyses of different soil layers over time. Additionally, it had to be ensured that the statistical needs were met, i.e. that the sampling design and methods would take the sometimes high variability of soil organisms into consideration in order to be able to detect statistically significant effects. Furthermore, the test system should be stable and mirror realistic conditions as in the field over a relevant period of time (at least one year).

### **Test items (PPP) and chemical analysis**

The study was planned to study PPP with similar mode of action (insecticides), similar persistency but different sorption properties. It was decided to select one agent with a KOC > 500 and another one with a KOC < 500, assuming that one is retained in the upper soil centimetres, while the other is expected to be transported to deeper soil layers. Since the test items were to be measured in different soil layers, it had to be ensured that the analytical methods were standardised and able to detect the assumed small amounts of the agent in deeper soil layers.

Since studies with unlabelled test substances rely on the analysis of extractable fractions only, it was decided to establish studies with radiolabelled compounds in order to quantify the amount of non-extractable residues (NER). More effort was deemed to be necessary to identify the nature of such residues and their binding mode in the soil matrix. Recently, it has been shown that NER comprise three different types, i.e., type I containing xenobiotic residues entrapped in the voids of the inorganic and organic soil matter components, type II xenobiotic residues covalently bound to humic matter, and type III containing completely metabolized residues not distinguishable from natural organic matter, i.e. peptides, proteins, phospholipids etc. (biogenic residues) (Kästner et al., 2014). In the present project, though, we restricted our investigations on the quantitative aspects of NER in order to distinguish between readily and slowly desorbable and not bioavailable residues.

### **Investigated soil organisms**

In the present study we aimed at considering representative groups of soil organisms of the macro- and mesofauna. The selection criteria were based on the respective sensitivity of the organism group to specific modes of actions of active substances in PPPs, the presence of the group in arable land habitats and the knowledge and practicability in dealing with these organisms in the process of risk assessment i.e. determination, classification of life form-type etc. A further advantage would be to select groups of soil organisms that have together a diverse structure - so that effects could be measured on different trophic levels and in different ecological niches, i.e. different exposure scenarios. Therefore, the populations of various animal groups, such as oribatid mites, collembolans, enchytraeids and earthworms were recorded in controlled model terrestrial ecosystems (TMEs) on species level and in different soil layers.

### **Exposure modelling**

The experimental results with regard to the concentration gradient of the applied active substances in the soil profile and the analysis of soil water budget should serve as a basis for a model-based evaluation of the leaching behaviour of the active substances and of temporal and spatial distribution of the applied chemicals in the soil profile.

The present project was divided into four work packages, which are drawn up in Figure 1.

AP II - Experimental Studies - *Fate and Effect* (Chapter 3-6)

TME Study [1] - Outdoor (Chapter 4)

**Test-Substance:**  
 - Lindane  
 - Imidacloprid



**Effects on:**  
 - Collembola  
 - Oribatida  
 - Enchytraeids  
 - Earthworms

TME Study [2] - Indoor (Chapter 5)

**Test-Substance:**  
 - Lindane  
 - Imidacloprid



**Radiolabelled Substances:**  
 - Bounded Residues

TME Study [3] - Outdoor (Chapter 6)

**Test-Substance:**  
 - Carbendazim

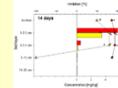


**Effects on:**  
 Earthworms

AP III - Exposure Modelling (Chapter 7)



Evaluation of Exposure and Effects (Chapter 8-9)



AP IV - Recommendations - *Risk Assessment Strategy* (Chapter 10)

Figure 1: Schematic description of the project and report structure

## State of the art

Summarising the available information on the vertical distribution of soil invertebrates at German crop sites it can be stated that:

- Different soil invertebrate species prefer different soil layers, but are usually found either in the litter layer (if present) or in the uppermost 5 - 10 cm of the mineral soil. The most notable exceptions are anecic earthworms which can burrow several meters deep.
- Species living in the same soil layer often have common physiological or morphological properties, i.e. they can be classified into ecological groups. The best known example is the classification of earthworms into three groups (epigeic, endogeic and anecic species (Bouché 1977), but similar groups have also been defined for Enchytraeidae and Collembola (EFSA 2010b).
- Depending on the site properties (soil, climate, land use etc.) typical invertebrate communities consisting of species or ecological groups can be identified.
- Since species and, accordingly, ecological groups differ in their vertical distribution, they might also be differently exposed towards PPPs. The possible movement of the animals in the soil profile should however not be disregarded. PPPs are usually sprayed on the soil surface or on crop plants, meaning that a vertical concentration gradient of these chemicals is the normal exposure scenario.

Soil invertebrates can be exposed towards PPPs via four pathways:

- Pore water
- Contact soil
- Ingestion of food (living or dead matter) and soil particles
- Inhalation of air present in the soil pores

In addition, direct contact with the spray is possible for organisms living on the soil surface, in the litter layer or in vertical burrows (e.g. anecic earthworms).

The relative importance of each of these uptake routes is determined by morphological (e.g. structure of the epidermis), physiological (e.g. mode of uptake of water [drinking versus uptake via the skin], mode of uptake of oxygen, feeding habits) and behavioural properties. A general sub-division may be made between so-called ‘soft-bodied’ organisms (like nematodes, earthworms, enchytraeids and some insect larvae) and ‘hard-bodied’ invertebrates (arthropods like spiders, some mites, insects, some collembolans, millipedes, centipedes, harvestman, isopods, and some other terrestrial crustaceans like some crab species). ‘Hard-bodied’ organisms have evolved special organs for assimilation of oxygen and water, while for ‘soft-bodied’ biota uptake via the skin is the most important route of uptake of water and oxygen. Contaminants and nutrients may also be taken up via these distinct exposure routes while uptake of contaminants via food is possible for all biota. In this context also the uptake via “secondary poisoning”, i.e. predators feeding on contaminated prey (e.g. predatory mites on worms). Consequently, soil dwelling organisms are exposed to chemicals by a variety of pathways. Most organisms share the feature that the relative contribution of each pathway varies. On top of ecological impacts, these contributions depend on factors like the hydrophobicity of the chemical and variations in environmental conditions like soil type, climate, etc.

### **Terrestrial model ecosystems**

The present study was designed to assess the effects of pesticides on soil organisms under realistic conditions and exposure in soil. The study was conducted by means of Terrestrial Model Ecosystems (TMEs, Figure 2). These systems provide the possibility to study natural soil communities under standard conditions over a period up to one year (Schäffer et al., 2008; Scholz-Starke, 2013; Scholz-Starke et al., 2013). The advantage of these systems is that there are replicable and it is generally possible to measure and investigate different taxa at the same time (Sheppard, 1997). They provide the possibility to analyse the behaviour of pesticides over time for different soil layers by using adequate soil sampling approaches which are also suitable to link between laboratory (see study [2] with radiolabelled substances or ecotoxicological single species testing in lower tier risk assessment) and the real conditions in field (Odum, 1984, Scholz-Starke, 2013). In the present study, we used open TMEs that were cored in grassland and contained an undisturbed soil community typical for grassland habitats. For study [1] and [3] they were placed outside (outdoor) for study [2] they were placed in the laboratory (indoor).

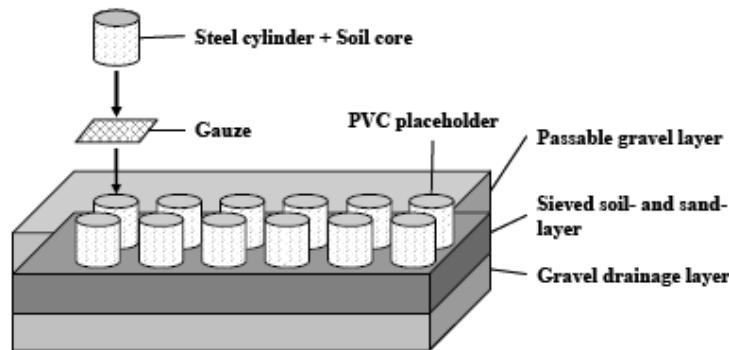


Figure 2: left: Terrestrial Model Ecosystem (TME) Ø 467 mm, height 400 mm; right: schematic picture of the study facility in Aachen

## Methods

In this project, three separate studies were conducted in order to assess the exposure and effects of pesticides on soil organisms. We conducted two different outdoor studies in Terrestrial Model Ecosystems (TMEs) to monitor (a) the movement of pesticides in soil over time and (b) the exposure and effects on soil organisms during the same time. Additionally it was conducted an indoor TME study to measure the fate of the radiolabelled pesticides Lindane and Imidacloprid and the formation of non-extractable residues in soil.

Table 1 : Application rates of the two pesticides involved in the first outdoor TME study [1] and sampling of different soil organisms

	(Conc. g a.s./ha)	Orib., Coll., Enchy.	Earthw.	Leach.
<b>Imidacloprid</b>				
Conc. low	750	x	x	
Conc. high	2000	x		x
<b>Lindan</b>				
Conc. low	7500	x	x	
Conc. high	20000	x		x

Conc. Earthw. = Concentration used for earthworm TME;

Leach. = Concentration used for Leached Water TME

Study [1] The first outdoor study was conducted with two different pesticides with different physico-chemical properties, Lindane ( $\log K_{ow} > 3$ ) and Imidacloprid ( $\log K_{ow} < 1$ ), to assess the effects on soil organisms under realistic conditions. The application rates were 0.75 kg/ha and 2 kg/ha for Imidacloprid and 7.5 kg/ha and 20 kg/ha for Lindane (Table 1).

Study [2] The second study was conducted in the laboratory with the same two but radiolabelled pesticides and with the same applied concentrations to assess the fate of the

active substances but also the formation of non-extractable residues as part of the total exposition to soil organisms.

Study [3] The third study was additionally conducted as an outdoor experiment to assess the toxic effects on earthworms because acute effects could not be measured in the first study. In this third study it was used the agent Carbendazim which is known as earthworm toxic. The application rates were 7.5 kg/ha and 15 kg/ha. The application rates were chosen with the aim to produce significant effects on earthworms - while total erasure of the population should be avoided.



		Outdoor		Indoor
Terrestrial Model Ecosystems				
		Biology	Chemical Analysis	
		Field Samples	unlabelled	radiolabelled
Layer	A: 0-2.5 cm	Collembola		
	B: 2.5-5 cm	Orbitalida		
	C: 5-10 cm	Enchytraeidae		
	D: 10- 20 cm	Earthworms	Analytics Parent and Metabolites	Analytics Parent and Metabolites Non extractable Residues
	E: 20- 40 cm			

Figure 3: Link of analytical and biological data out of the field (outdoor study [1] and indoor laboratory study [2] by sampling in Terrestrial Model Ecosystems (TMEs).

All three have in common that during the study the pesticide concentration and the toxic effect on different soil animal taxa were measured in different soil layers over time (Figure 3) as well as the water inputs (precipitation) and outputs (leachate water, Figure 4) for the whole TME. This approach should provide the possibility to merge the specific data with one another and with the approaches and calculations that were made in the registration process for pesticides at present time.



Figure 4: Schematic profile (left) and picture of the facility for measurement of leachate water (right)

## Results

### Field study 1 TME: Concentrations of the test substances Lindane and Imidacloprid

Concentrations of Lindane (20 kg/ha) in the top soil layer (0-2.5 cm) were 61.5 mg/kg at day 1 and decreased to 13.0 mg/kg after one year; more than about 90 % of the extracted substance remained in this layer for half a year, and after one year still 71 % were present in this layer. The next layer (2.5-5 cm) contained 23 % of the extracted amount after one year (3.2 mg/kg), whereas the lower layers had concentrations way below 1% of the extracted amount. Imidacloprid (2 kg/ha) was only slightly more mobile with 9.1 mg/kg at day 1 and 0.75 mg/kg after one year in the top layer (97 % and 56 % of the extracted amount, respectively) and 0.28 mg/kg (day 1) and 0.49 mg/kg (day 365), equivalent to 3 % and 37 % of the extracted amount in the layer 2.5-5 cm. Concentrations in lower layers were below about 0.1 mg/kg.

### Laboratory study 2: Concentrations using <sup>14</sup>C-labelled pesticide

Lindane equivalent concentrations in the first cm layer were 168.2 mg/kg at day 1 and 74.9 mg/kg at day 180, and in the second layer (1-2.5 cm) 0.62 mg/kg and 39.52 mg/kg at day 1 and day 180, respectively. Concentrations in the next two layers (2.5-5 cm and 5-10 cm) were each 2.87 mg/kg after 180 days; the bottom layer (10-20 cm) contained below 0.1 mg/kg Lindane equivalents at day 180. Also Imidacloprid equivalents had the highest concentrations in the first cm of soil with 17.35 mg/kg (day 1) and 4.48 mg/kg (day 180), respectively. Corresponding values in the second layer (1-2.5 cm) were 0.11 mg/kg (day 1) and 1.86 mg/kg (day 180). The layer below contained less than 1 mg/kg (2.5-5 cm) and less than about 0.1 mg/kg in the bottom layer (10-20 cm) after 180 days.

### Comparison of experimental findings and modelling of exposure

#### Lindane, application rate 20 kg/ha (Example 1)

Initial concentrations (day 1) in the top 2.5 cm layer were 61.5 mg/kg (field study) and 55.1 mg/kg calculated by inverse modelling. In the lab study Lindane initial concentration in the first cm layer (0-1 cm) was 168.2 mg/kg and the modelled concentration in this layer at that time was 133.3 mg/kg.

After one year in the field experiment the concentration in the 0-2.5 cm decreased to 13.0 mg/kg (= 71 % of the applied amount); modelling resulted in a concentration of 14.8 mg/kg after that time. In the first cm layer after one year, experimental concentrations (obtained in the lab study) was 74.9 mg/kg, the modelled concentration was 50.7 mg/kg.

The next soil layer (2.5-5 cm) after one year contained 3.2 mg/kg in the field TMEs, whereas inverse modelling resulted in a concentration of 0.3 mg/kg. After one year 0.34 mg/kg were detected in the field TMEs (5-10 cm layer), but only 0.001 mg/kg were modelled. Only minor (experimental) or zero amounts (modelled) were detected and expected in the lower soil layers below 10 cm.

Thus, experimental findings and modelled concentrations were quite similar showing that Lindane remained mainly in the top soil layers even one year after application. However, modelling underestimated the concentration of Lindane in lower soil layers.

#### ***Imidacloprid, application rate 2 kg/ha (Example 2)***

Initial concentrations (day 1) in the top 2.5 cm layer were 9.1 mg/kg (field study) and 8.5 mg/kg calculated by inverse modelling. In the lab study Imidacloprid initial concentration in the first cm layer (0-1 cm) was 17.4 mg/kg and the modelled concentration in this layer at that time was 21 mg/kg.

After one year in the field experiment the concentration in the 0-2.5 cm decreased to 0.75 mg/kg (= 56% of the applied amount); modelling resulted in a concentration of 0.1 mg/kg after that time. In the first cm layer after one year, experimental concentrations (obtained in the lab study) was 4.5 mg/kg, the modelled concentration was 1.2 mg/kg.

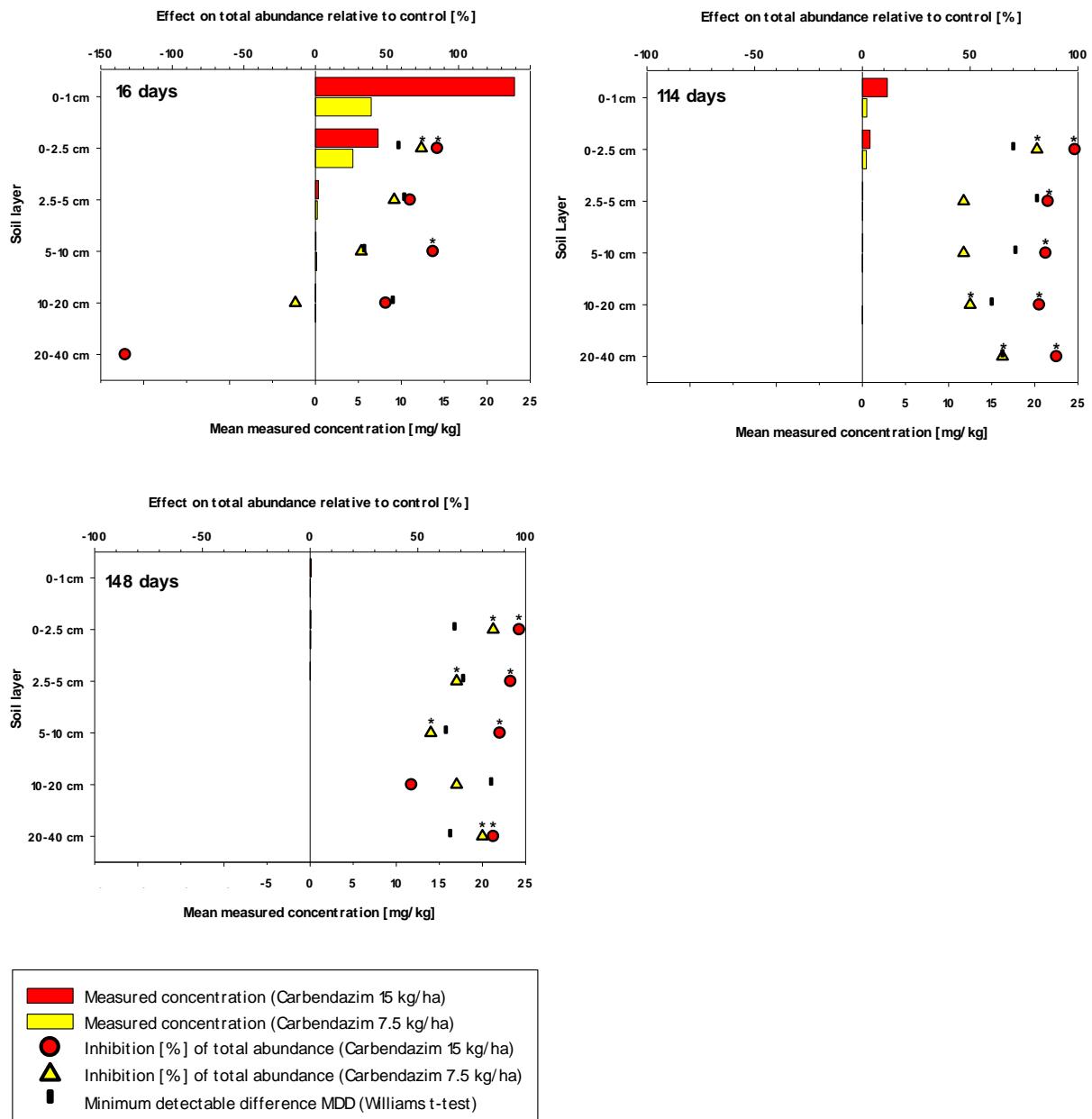
The next soil layer (2.5-5 cm) after one year contained 0.49 mg/kg in the field TMEs, whereas inverse modelling resulted in a concentration of 0.14 mg/kg. Only minor (experimental and modelled, < 0.1 mg/kg) amounts were detected and expected in the lower soil layers below 5 cm.

Thus, experimental findings and modelled concentrations were quite similar showing that Imidacloprid - despite a much lower Kow - remained mainly in the top soil layers even one year after application.

#### **Effects of the tested substances on soil organisms**

All four observed organism groups (Collembola, Oribatida, Enchytraeidae, and Lumbricidae) showed group and species specific vertical distribution patterns in the soil. These distribution patterns were found to be also species-specific, but in each group the individual species could be classified accordingly into three groups (e.g. the well-known epigeic, endogeic and anecic earthworm groups). Consequently, different exposure patterns of species/ecological groups to the respective pesticide could be in principle assumed. The highest numbers of individuals were found in the uppermost soil layer (0-2.5 cm) for all organism groups (Collembola, 68 %; Oribatida, 91 %; Enchytraeidae, 60 %; Lumbricidae, 36 %). Except of lumbricids, more than 80 % of all individuals were observed in the uppermost 5 cm (Collembola, 92 %; Oribatida, 91 %; Enchytraeidae, 88 %). Effects of the three pesticides on soil organisms could be detected in every soil layer (0-10 cm soil depth for Collembola, Oribatida, and Enchytraeidae and 0-40 cm soil depth for Lumbricidae). They were found to be species and substance specific, (e.g. collembolans were affected especially by the insecticides). Both, acute effects (14 days after application) as well as long lasting

effects (up to one year) were observed in the uppermost soil layer, as expected, due to the high application rates chosen, to affect all groups. In the case of Lindane, the deliberately chosen high concentrations remained high during the experiment in the upper soil layers; the measured concentrations in the upper soil layers remained above the lethal values derived from laboratory tests with the corresponding standard test species (i.e. *Folsomia candida* for collembolans) over the course of the study.



**Figure 5: Decrease of total abundance of lumbricid species in the Carbendazim-treatments 7.5 kg a.s./ha and 15 kg a.s. /ha (5 replicates each) for the different soil layers in comparison to the control (5 replicates). Columns showing the measured concentration for the two application rates at the respective sampling date. \*: significant difference according to Williams t-test; bars showing the minimum detectable difference (MDD) as value for the statistical power. MDD values higher than 100 % are not shown.**

For all substances investigated, effects were observed in deeper soil layers even if the measured concentrations were below the no observed effect concentrations known from literature. This was found for collembolans exposed to Imidacloprid and Lindane as well as for earthworms exposed to Imidacloprid and Carbendazim. An example is given for the effects on earthworms in different soil layers of TMEs treated with Carbendazim (Figure 5). These effects, e.g. on deep-digging earthworms, can be explained by the vertical movement of these organisms within the soil column reaching upper layers with higher pesticide concentrations.

## Conclusions

General conclusions are given by answering the questions asked in the beginning of the project:

- **Can the assumed functional relationships between spatial distribution of a PPP in the soil profile and the location of ecotoxicological effects be confirmed?**

In the most cases the applied amount of pesticides led to high concentrations in the different soil layers, as foreseen at the beginning of the experiment. Thus, the allover occurrence of measured effects was expected according to the applied amounts of the different pesticides. The main research questions of the experiment, though, regarded the distribution in the soil profile of the applied chemicals and the respective ecotoxicological effects on different soil organisms.

The results showed that effects of the applied chemicals were measured in the uppermost soil layers as to be expected, but that especially in deeper soil layers effects were detected that could not be explained or could not have been assumed by the related measured low concentration of the pesticide.

- **Is the exposure level and consequently the extent of ecotoxicological effects modulated by the preferred position and the behaviour of soil organisms in the soil profile?**

According to the high amounts of pesticides applied in our experiment, that was chosen in order to elicit effects on soil organisms deliberately, only in a few cases the concentration in the uppermost soil layer was lower than the assumed NOECs derived from standard laboratory tests. Thus, only in these cases a decoupling of exposure and effect was observed already in the first centimetres of the TMEs. Regarding lower soil layers in the TMEs, often the concentrations were at all times lower than the NOEC reported in the literature, but effects could be nevertheless be observed already at the first sampling dates. This can be most plausibly explained by vertical movement of the soil organisms. The vertical movement is known for different soil organisms, e.g., earthworms that burrow deep in the soil but regularly move to the soil surface for feeding. Consequently it can be stated that soil organisms were affected, sometimes decoupled from the exposure derived from their preferred position in soil, especially in the case of species preferring deeper soil layers. In addition to the exposure measured in the preferred position in soil, the “real exposure” is triggered by the behaviour i.e. vertical movement of the soil organisms, hence both factors

will influence the extent of ecotoxicological effects. This means, that the assessment approach has to be extended by inclusion of the migration behaviour of organisms. This is particularly relevant for soils in which pesticides are distributed heterogeneously displaying a concentration gradient.

- **Is the spatial transfer of the maximum concentration of a PPP into different soil layers over time accompanied by a sequence of effects in organism groups with different mode of exposure?**

For none of the three PPP a time-dependent shift of relevant proportions of the applied amounts in deeper soil layers was observed. Only small shares of the applied amounts were measured in deeper soil layers for both the lipophilic Lindane and the much less lipophilic Imidacloprid. These amounts did probably not cause delayed effects in deeper soil layers, since these concentrations were below no observed effect concentrations reported in the literature. At the same time, in some cases a recovery could be observed later in the experiments in the uppermost soil layers.

- **Do active substances with different properties at a given time interfere with different groups of organisms, each representing a typical mode of exposure?**

In the soil type chosen for our experiments, the used pesticides did not behave very differently with regards to their movement in soil. Consequently, the active substances affected more or less the same organism groups at each individual point in time. This means that effects were detected in deeper soil layers - in organisms also preferring those layers - even if concentrations of pesticides with different properties were not high enough there to elicit the observed effects. Derived from the present study, it seems that the behaviour/mobility of the soil organisms plays a more important role in the overall effect pattern than the distribution of the substance in the different soil layers.

## **Recommendations**

Based on these results the following recommendations can be given:

### **Recommendation 1: Protection Goals**

*For the use of Terrestrial Model Ecosystems, field tests or ecological models e.g. as a higher tier options in risk assessment, it is mandatory to develop operational, spatially explicit protection goals.*

### **Recommendation 2: Environmental chemistry**

*Regarding pesticide exposure, further research is needed to address the (partly not expected) behavior of the three PPPs of the present studies in the soil profiles.*

### **Recommendation 3: Exposure Modelling**

*The reliability of mechanistic computer models like PELMO has to be improved for simulating loss processes at the soil surface such as photo- or microbial degradation and volatilisation.*

#### **Recommendation 4: TME Performance**

*A TME or field study to determine the effects of pesticides on the soil community should mirror the (field) conditions of the target system and should be representative for the regional circumstances i.e. climatic conditions or soil properties. The organism groups to be monitored should be selected according to the special mode of action of the pesticide and the results of available laboratory tests.*

#### **Recommendation 5:**

*For the performance of TME or field studies in the context of risk assessment it is not necessary to differentiate between different soil layers. To assess effects of pesticides on soil organisms, a representative capture of abundance of the different species of the respective organism group must be guaranteed (and thus a certain soil depth has to be sampled). The recommended soil depth for sampling of the four soil organism groups studied here is 0-5 cm for Collembola, Oribatida, and Enchytraeidae as well as 0-40 cm for Lumbricidae and corresponds to a number of more than 80 % of their total abundance.*

#### **Recommendation 6:**

*To get a thorough evaluation of the protection level of lower tiers using calculated predicted environmental concentrations (PEC values) for different soil layers, it is recommended to compare the effect concentrations derived from lower tier studies with the effect concentrations derived from higher tiers, e.g. (semi-) field studies. A review on the comparison of the different assessment steps for different pesticides is desirable.*

#### **Recommendation 7:**

*For Environmental Risk Assessment schemes, the predicted environmental concentrations in soils ( $PEC_{soil-initial}$ ) should be derived from the calculation of the concentration of the uppermost centimetre(s) and compared with the determined Effect Concentration.*

#### **Recommendation 8:**

*Basic soil ecological questions have to be answered in order to improve the environmental risk assessment of plant protection products (PPP) for the soil compartment, especially for the evaluation of higher-tier tests such as TMEs or field studies.*

## References

In this chapter only the cited literature of the abstract is listed. Please see the final project Report for the comprehensive list of references.

Bouché, M.B. (1977): Stratégies lombriciennes. In: Lohm, U. & Persson, T. (Hrsg.): Soil organisms as components of ecosystems. Ecological Bulletins NFR 25: 122-132.

EFSA (European Food Safety Authority) (2010a): Selection of scenarios for exposure of soil organisms to plant protection products. EFSA Journal 2010; 8(6): 1642, 82 pp.

EFSA (European Food Safety Authority) (2010b): Scientific Opinion on the development of a soil ecoregions concept using distribution data on invertebrates. EFSA Journal 2010 8(10): 1820, 77 pp

EFSA (European Food Safety Authority) (2017): EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2017; ;15(10):4982 [115 pp.], doi 10.2903/j.efsa.2017.4982

EFSA PPR (EFSA Panel on Plant Protection Products and their Residues) (2012): Scientific Opinion on the science behind the guidance for scenario selection and scenario parameterisation for predicting environmental concentrations of plant protection products in soil. EFSA Journal 2012;10(2):2562 [76 pp.]. doi: 10.2903/j.efsa.2012.2562

European Union EU (2009): European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309/1, 24.11.2009, pp. 1-50

European Union EU (2011): Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products.

PflSchG Pflanzenschutzgesetz (2012): Gesetz zum Schutz der Kulturpflanzen. Pflanzenschutzgesetz vom 6. Februar 2012 (BGBl. I S. 148, 1281), das zuletzt durch Artikel 4 Absatz 84 des Gesetzes vom 18. Juli 2016 (BGBl. I S. 1666) geändert worden ist.

Schäffer, A., van den Brink, P.J., Heimbach, F., Hoy, S.P., de Jong, F.M.W., Römbke, J., Roß-Nickoll, M. & Sousa, J.P. (2008): Guidance from the SETAC Europe Workshop: Semi-field Methods for the Environmental Risk Assessment of Pesticides in Soil (PERAS). CRC Press, Boca Raton, USA. 105 pp.

Scholz-Starke, B. (2013): Assessing the risk of pesticides on soil communities using terrestrial model ecosystems. Dissertation RWTH-Aachen University, Aachen.

Scholz-Starke, B., Beylich, A., Moser, T., Nikolakis, A., Rumpler, N., Schaffer, A., Theissen, B., Toschki, A., Ross-Nickoll, M. (2013): The response of soil organism communities to the application of the insecticide Lindane in terrestrial model ecosystems. Ecotoxicology 22, 339-362.

Sheppard, S. (1997): Toxicity testing using microcosms. In: Tarradellas J, B.G., Rossel D (Ed.), Soil Ecotoxicology. Lewis Publishers, Boca Raton, pp. 345-373.