Assessment of Ecotoxicological Effects of Pesticides on the Soil Fauna and Soil Processes under Tropical Conditions

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Abstract

The work reported here is planned for the SHIFT project "Management of plant residues and its effect on decomposition and soil fauna in central Amazonian agro-ecosystems". The results of SHIFT ENV52-I project show that the macrofauna, especially ants, earthworms, diplopods, isopods and termites, is mainly responsible for the decomposition of litter. Assuming that a natural decomposition rate is necessary for the sustainable use of agroforestry polyculture plantations, negative effects on these organisms must be avoided. In order to transfer the results of ENV52-I to practical agroforestry activities in the Manaus region, fungicides and insecticides have been taken into consideration. No data are available to assess the risk of these compounds to macrofauna species active in litter decomposition. Even worse, no standardized methods are available to measure side effects of pesticides on macrofauna species under tropical conditions. This statement is true for single species tests in the laboratory as well as for monitoring (bioindicator) studies in the field.

In Brazil, EMBRAPA is responsible for the improvement of agroforestry measures. Therefore, the agency must be able to recommend (or ban) the use of certain pesticides in order to avoid harmful effects of these substances on macrofauna species since otherwise important ecosystem functions like litter decomposition and therefore, in the long-run, the economically sound and sustainable use of such cultures could be seriously affected.

The work is planned to be divided in the following steps:

1. Identification of suitable test species, test conditions and test endpoints;
2. Performance of successful (i.e. reproducible) laboratory tests using two pesticides relevant for EMBRAPA;
3. In close interaction with other parts of the planned project: Extrapolation of these experiences to the microcosm and field level (agroforestry plantations);
4. Inclusion of functional endpoints on the latter two levels (e.g. the bait-lamina test (measuring the feeding rate of the macrofauna) and the litter-bag test (measuring the decomposition rate of organic material);
5. Formulation of test guidelines (according to OECD standards).

Keywords

Ecotoxicology, Test methods, Pesticides, Agroforestry, Earthworms, Isopods, Amazonas

1 Introduction

The work reported here is planned for the SHIFT project "Management of plant residues and its effect on decomposition and soil fauna in central Amazonian agro-ecosystems", forming the fourth module of the whole work plan (HANAGARTH et al. 2000). The results of SHIFT ENV52-I project show that the macrofauna, especially ants, earthworms, diplopods, isopods and termites, is mainly responsible for the litter decomposition of various forest sites in the EMBRAPA area (HÖFER et al. 2000). Assuming that a natural decomposition rate is necessary for the sustainable use of agroforestry polyculture plantations, negative effects on these organisms must be avoided. In order to transfer the results of this project to the agroforestry activities on farms in the Manaus region, fungicides and insecticides have been taken into consideration (PREISINGER & GASPAROTTO 1998).

2 Pesticide use in Amazonia: Some background data

Unfortunately, only few data are available about the use of pesticides in the region of Manaus based on data from Manacapuru, 14 – 85% of the farmers use pesticides regularly (FABRE et al. 1999). The percentage is especially high for vegetable crops, but it is also increasing in plantations (in particular in those located close to cities). As an indication of the importance of pesticides the value of these compounds imported to the state of Amazonas is given as follows:

A wide variety of pesticides is commercially available in the region of Manaus (Tab. 2). Practically all of them are marketed for many years and usually have a broad efficacy

Year	1996	1997	1998	1999
Value (R$)	1.300.000	3.400.000	2.500.000	≈ 2.900.000

Tab. 1: Amount of money (value given as R$) used for the import of pesticides to the State of Amazonas between 1996 and 1999 (last year estimated based on the data of the first six months) according to FABRE et al. (1999)

Active Ingredient	Use Type	Toxicology Class	Registered in Germany ?
Benomyl	Fungicide	IV (low toxicity)	Yes until 2003
Copperoxichloride	Fungicide	IV (low toxicity)	Yes until 2000
Deltamethrin	Insecticide	II (high toxicity)	Not marketed
Malathion	Insecticide	III (median toxicity)	Not marketed
Metamidophos	Insecticide	II (high toxicity)	Not marketed
Parathion	Insecticide	I (extremely toxic)	Yes until 2003
Glyphosate	Herbicide	IV (low toxicity)	Yes until 2009
Paraquat	Herbicide	II (high toxicity)	Yes until 2008

Tab. 2: Examples of commonly used pesticides in the area of Manaus. Presented are the name of the active ingredient, the use class, the toxicology class according to the Brazilian law and the current registration status in Germany based on own investigations.

Parameter	Benomyl		Parathion	
	Holarctic	Tropics	Holarctic	Tropics
Soil degradation (DT50)	6 months	≈1 month	1 month	≈ 2 months
Toxicity to earthworms (in mg/kg)	High	?	Medium	?
	LC50: 2 – 10		LC50: 100?	
Toxicity to arthropods	Low - medium	Low ?	High	?
Toxicity to microbes	Low - high	Low ?	Low	?
Litter decomposition	Inhibition	Inhibition ?	No effect	?

Tab. 3: Comparison of important ecotoxicological data for two pesticides (the insecticide parathion and the fungicide benomyl) coming from holarctic and tropical regions.

spectrum (i.e. many side effects). For this reason, especially the use of these insecticides have been restricted or banned in the industrialized countries. This fact is indicated by the registration status in Germany. However, all listed pesticides are registered according to the Brazilian Pesticide Law. Finally, it should be noted that the classification of these substances according to toxicological criteria (class I – IV) is quite different to a classification based on ecological criteria which are not taken into account in Brazil so far. As an example how much is known about the fate and effects of pesticides in terrestrial compartments of different regions of the world, in Tab. 3 such data are compiled for two selected compounds, a fungicide (benomyl) and an insecticide (parathion). It must be remarked that the data from the holarctic region are based on several standardized tests (compiled e.g. in The Agrochemicals Handbook 1994) whereas the data from the tropics are usually coming from just one research project (AGARWAL et al. 1994, VINK 1994). No data are available to assess the risk of these compounds to macrofauna species active in litter decomposition. Even worse, no standardized methods are available to measure side effects of pesticides on macrofauna species under tropical conditions. This statement is true for single species tests in the laboratory as well as for monitoring (bioindicator) studies in the field.

In Brazil, EMBRAPA is responsible for the improvement of agroforestry measures. Therefore, the agency must be able to recommend (or ban) the use of certain pesticides in order to avoid harmful effects of these substances on macrofauna species since otherwise important ecosystem functions like litter decomposition and therefore, in the long-run, the economically sound and sustainable use of such cultures could be seriously affected.

3 Outline of the planned work

Theoretical background will be the Environmental Risk Assessment (ERA) which is the current basis for pesticide registration in the European Union and North America (EU 1991, FURLONG 1995). Methodologically, an adaptation of existing "temperate" test guidelines for tropical conditions has to be done. In order to check the results, data will be collected by an intensive testing of two selected pesticides under laboratory, semi-field and field conditions. The work is planned to be divided into the following steps:

Fig. 1: Potential test organisms in ecotoxicological tests systems for tropical regions: Upper half: *Pontoscolex corethrurus (Glossocolecidae, Annelida);* lower half: *Trigonoiulus corallinus* (Trigoniulidae, Diplopoda)

3.1 Identification of suitable test species, test conditions and test endpoints

Species to be identified as potential test organisms must be kept under standardized conditions in the laboratory in such a way, that they reproduce (fortunately as a mass culture); help from other institutions would greatly enhance the chance of success and at the same time clearly minimize the time needed. The ecological requirements of each species should be known in order to decide which one is useful for testing purposes. Main criteria for test species selection are:

- Ecologically relevant (e.g. high biomass, role as an ecosystem engineer)
- Practicable (e.g. easy to breed, short generation cycle, experience available)
- Sensitive (e.g. affected by various chemicals but less by abiotic conditions)
- Ecotoxicologically relevant (e.g. covering different exposure scenarios)

It is proposed to use the following species (Fig. 1):

1. One earthworm species: e.g. *Eudrilus eugeniae* (Eudrilidae), *Eisenia fetida* (Lumbricidae) or *Pontoscolex corethrurus* (Glossoscolecidae)
2. One macroarthropod species: e.g. *Circoniscus gaigei* (Scleropactidae, Isopoda), or *Trigonoiulus corallinus* (Trigoniulidae, Diplopoda).

The first earthworm species listed here has been chosen since it has been used intensively in scientific studies investigating composting (e.g. KNIERIEMEN 1984). The use of *Eisenia fetida* might be surprising since it is known as ecotoxicological test organism in Europe and the USA (OECD 1984). However, this species is well adapted to the environmental conditions in the region of Manaus; probably because it is preferring relatively high temperatures in the holarctic too: According to the current guidelines it should be tested at 20** 2 °C, but they

Test condition	Existing guideline	Proposed modification
Standardised Test	**Substrate Artificial soil according to OECD (1984)**	**Modified artificial soil (dried fern stem material instead of peat)**
Natural Soil	**e.g. Lufa St. 2.2 (Germany)**	**Tierra preta ?**
Temperature	**20 °C - 2 °C**	**28 °C - 2 °C**
Food source	**e.g. dried cow manure**	**Cow manure, litter ?**
Test duration	**2 – 8 weeks**	**1 – 6 weeks ?**

Tab. 4: Important properties of terrestrial ecotoxicological guidelines, their current status in existing guidelines for holarctic regions and proposed modifications in order to use them under tropical conditions

reproduce well until at least 28 °C. Finally, *Pontoscolex corethrurus* is a peregrine earthworm species, distributed all over the world in tropical ecosystems. The first two species belong to the ecological group of epigeic worms (i.e. living in the litter layer), whereas the third one is a typical inhabitant of the mineral soil (Römbke et al. 1999). Unfortunately, much less is known about isopod species which might be suitable as test organisms under tropical conditions (Vink & van Straalen 1999). Some important test conditions have to be changed in order to mimic tropical conditions (Tab. 4). In total, two control runs per species and substrate according to a factorial design (24) will be performed. These results will also be used to define validity criteria (i.e. those requirements which have to be fulfilled in order to use test results for environmental risk assessment purposes).

3.2 Performance of laboratory tests using two pesticides relevant for EMBRAPA

Probably two test chemicals will be used: one "old", very well-known insecticide (data available on fate and effects including tropical sites) like parathion, endosulfan or deltamethrin and one "field-relevant" fungicide applied in Amazonia regularly will be used. In detail, the following work has to be done as basis for test method modification and standardization:

- Two test runs per species in modified artificial soil per test substance (range-finding and definitive test)
- Different statistical designs (NOEC versus ECx)
- Comparison of results gained in modified artificial soil with effects gained in a field soil according to a factorial design (24)
- Estimation of the concentration of the test chemicals using Rapid Kits.

In general, the results of this work step should be used to check the proposed test method modifications whether they are suitable for a routine use under topical conditions. The reproducibility of test results will be the main criterion.

3.3 Extrapolation of the laboratory experiences to the microcosm and field level

This work will be done in close interaction with other parts of the planned project. For example, the microcosm experiments will contain the following elements (Morgan & Knacker 1994):
Method: Terrestrial Model Ecosystems (TME); i.e. intact soil cores with a diameter of 17 cm and a maximum height of 40 cm
Substrate: The same field soil as used in the laboratory test (preferrably from experimental field sites in order to get well characterized soil samples)
Organisms: Natural composition of soil fauna
Conditions: Partly controlled (e.g. test performance in a greenhouse)
Substance: The same pesticides as in the laboratory tests (incl. Rapid Kits)
Parameter: Abundance of test species plus various other biological parameters (e.g. microbial biomass, feeding rate, litter decomposition)

In addition, together with the screening of macrofauna, litter decomposition and soil fertility (Hanagarth et al. 2000) at selected agroforestry sites an extrapolation of the results of the two lower investigation level laboratory and microcosm will be tried. This work is characterized by the following key elements:
Method: Application of one (?) pesticide at some of the screening field plots
Substrate: Autochthonous field soil (the same as in the laboratory test)
Organisms: Evaluation of main macrofauna groups (e.g.

Fig. 2: Description of the bait-lamina tests system (von Törne 1990): Left: Individual test sticks showing the holes which contains the feeding material; right: bait-lamina sticks in the litter of a primary forest soil

earthworms, isopods)

Conditions: No control of abiotic factors

Substance: The same pesticides as used in the laboratory tests (incl. Rapid Kits)

Parameter: Abundance and dominance spectrum of soil animals plus functional parameters (e.g. feeding rate, litter decomposition).

It will not be possible in this work step to cover field situations in general, since they can be very different (i.e. concerning soil type or anthropogenic impacts). However, the experience gained here can be used for two purposes:

- firstly, whether an extrapolation of laboratory data to the field is possible at all;
- and secondly, how a field monitoring program evaluating side-effects of pesticides can be organized.

3.4 Inclusion of functional endpoints on the microcosm and field level

The testing of functional endpoints would be an important improvement of the environmental assessment of the side-effects of pesticides since a potential impact of these substances would be measured directly on the ecosystem level (i.e. the extent of extrapolation would be much smaller than e.g. in the case of structural laboratory tests). It might include the bait-lamina test (measuring the feeding rate of the macrofauna; VON TÖRNE 1990; Fig. 2) and the litter-bag test (measuring the decomposition rate of organic material). The first has the advantage of being very quick and easy, but it is of less ecological relevance in comparison to the litter-bag method (KULA & RÖMBKE 1998). In the laboratory, only bait-lamina could be used just by adding them to the test substrate in the effect tests running anyway. In the microcosms and in the field, potentially both methods could be used with either natural (Vismia ?) or artificial (cellulose ?) organic material (HÖFER et al. 2000).

3.5 Formulation of test guidelines (according to OECD standards)

Since approximately 20 years, international organizations like OECD or ISO have been publishing standardized ecotoxicological test guidelines. There is no complete agreement on the principles that should be applied for the formal development and validation of test methods, but the recommendations by OECD (OECD 1996) are probably those most widely accepted. With the introduction of test methods into test guidelines as the ultimate goal, it is often argued that validation is more important than test development (which is probably true in terrestrial ecotoxicology). The practical aspects of this whole process can be classified as follows (BALLS et al. 1995):

I. Test Development (laboratory of origin)
Purpose and need for the test; derivation of the method; application to appropriate chemicals and development of a protocol

II. Prevalidation (informal inter-laboratory study)
Assessment of the inter-laboratory transferability and optimisation of the protocol

III. Validation (formal inter-laboratory study)
Study design; selection of laboratories; selection and distribution of chemicals; data collection and analysis and assessment of results

IV. Independent assessment of study and protocols
Assessment of ring-test results (data, experiences) by peer review (incl. authorities)

V. Progression toward regulatory acceptance
Further discussions with international authorities, publications, etc.

In the project presented here the first one will be done for two terrestrial effect tests under tropical conditions, leading to two draft guidelines according to current OECD standards.

4 Summary and outlook

The whole work planned for the new project SHIFT ENV-52 II can be summarized as follows:

1. Modification of existing ecotoxicological test methods for tropical conditions
2. Performance of tests using two different chemicals under laboratory, semi-field and field conditions
3. Use of these data to formulate recommendations for pesticide usage as part of a sustainable management of decomposition processes in agroforestry
4. Draft guidelines according to OECD standards for ecotoxicological tests under tropical conditions
5. Improvement of the risk assessment of pesticides in the tropics

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