Session 'Bioavailability of organic chemicals'
Platform presentation
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Advances in the Development of Procedures to Establish the Toxicity of Non-Extractable Residues (NER) in soil

Joop Harmsen[1], Dieter Hennecke[2] Kerstin Hund-Rinke[2], Joost Lahr[1], John Deneer[1]

[1]Wageningen Environmental Research (Alterra), P.O.Box 47, 6700 AA Wageningen , The Netherlands
[2]Fraunhofer-IME, Applied Ecology, Auf dem Aberg 1, 57392 Schmallenberg Germany
E-mail contact: joop.harmsen@wur.nl

1. Introduction

There is already a long discussion around the bioavailability and ecotoxicological relevance of Non Extractable Residues (NER) in soil. Is NER formation a detoxification process or should it be considered to be a hidden hazard? The formation and presence of NER may be attributable to: 1) Association of the parent chemical or breakdown product with mineral and/or organic matter, 2) Mineralisation and incorporation of carbon into microbial biomass and carbonates.

NER can only be established using labelled compounds (e.g. 14C). Because it is non-extractable it cannot be measured with conventional chemical analytics. Regulations ask for understandable and measurable parameters. The approach of Ortega-Calvo et al., (2015) [1] is followed in this research project. Considered are both sides of the cell membrane, soil and organism:

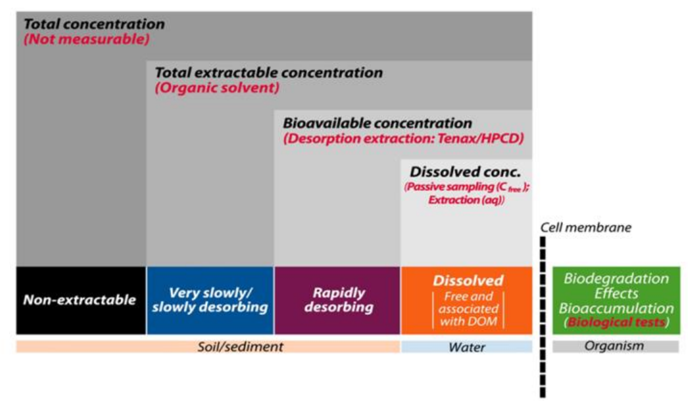

Figure 1: Bioavailability and NER according Ortega-Calvo et al., 2015

Left side of the cell membrane (soil)

- The water phase, in which concentrations/activity can be measured using passive sampling or extractions with 0.01 CaCl2 (Actual availability);
- A potentially available fraction in equilibrium with the water phase, measured using (ISO/ DIS 16751);
- The total extractable amount, to be measured with a (standard) method designed to measure the total substance amount;
- NER is considered, but mentioned as non-measurable and also non-bioavailable. All other fractions are measurable and bioavailable and non-bioavailable.

Right side of the cell membrane (organism)

- Measurement of the bioavailability using organism at the right site of the membrane.

In addition, the distribution of the chemical over the above fractions and the residual amount (still present after removing of the total extractable amount) was measured using ^{14}C-labelled compounds. With ^{14}C-experiments radio activity is measured, but the substance identity remains unknown.

2. Materials and methods

In ^{14}C-labeled experiments the distribution of ^{14}C in the soil was followed for 6 months. In the developed approach, toxicity was tested in both freshly spiked and aged soils. TNT, Cypermethrin and Carbendazim were use as test-chemicals. Three different soils were used. The soils were extracted using 0.01M $CaCl_2$, Tenax (ISO 16751) and Acetonitrile (total extractable). Radioactivity in the different fractions was measured. Effects on Earthworm avoidance, *Vibrio Fischeri* (Microtox), *Daphnia* and ammonium reducing micro-organisms were assessed in toxicity tests.

In a second experiment, non-labelled TNT was used. The TNT content in the different fractions was measured and also the toxicity before and after removal of the bioavailable fraction. Results for TNT are presented in this extended abstract.

3. Results and discussion

3.1. Radiolabelled experiment

It was shown, using ^{14}C-labelled TNT, that 6 months after spiking most of the TNT is present as NER (upper figure in figure 2). After spiking, most of the TNT is bioavailable, but this fraction decreases strongly during 6 months of aging. The amount mineralized is small and most of the TNT is present as NER (second figure in figure 1). Toxicity tests (lower tables in figure 1) show the large reduction in toxicity.

3.2. Non-labelled experiment

Toxicity was measured in a freshly spiked soil and after 5 months of aging. The two figures on the left in figure 3 showes the high toxicity directly after spiking and a lower toxicty after 5 months. NER is presented as 100% - measured fractions(%), to visualize the mass balance. With Tenax the bioavialable amounts have been removed. After this removal, no tocicity was left in the soil (figures on the right in figure 3).

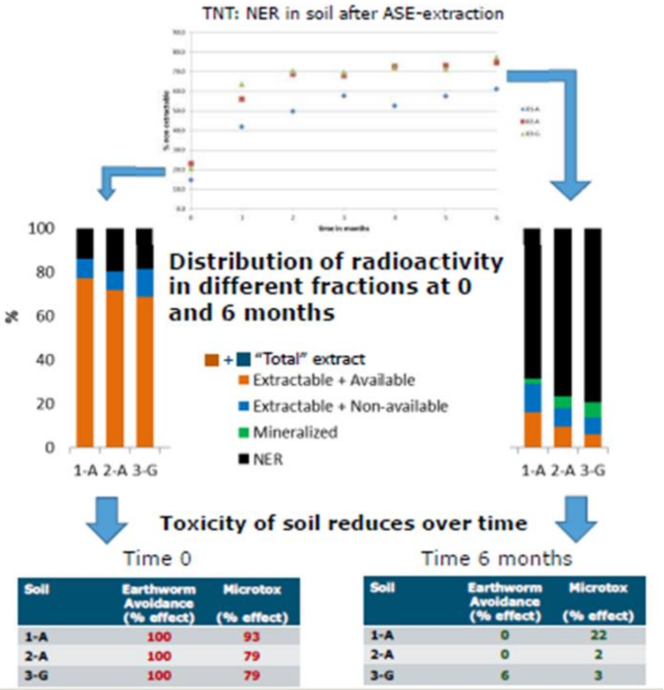

Figure 2: Distribution of radioactivity of radiolabelled TNT in freshly spiked soil and soil aged during 6 months.

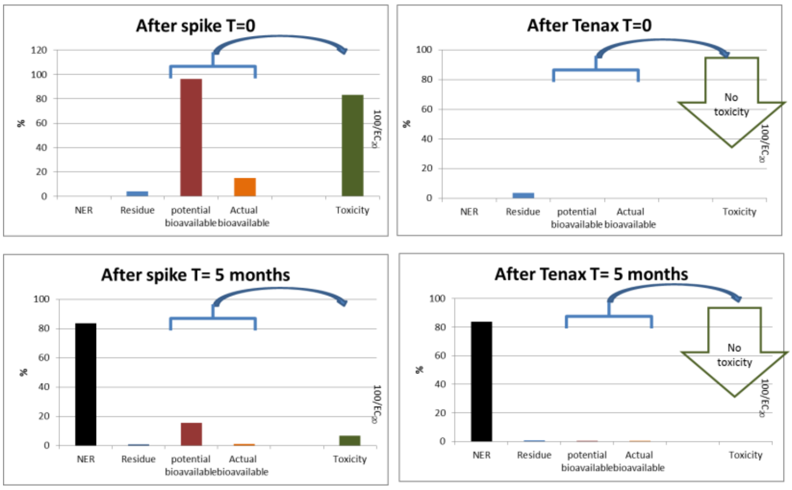

Figure 3: Distribution of TNT in different soil fractions versus observed soil toxicity by Microtox

4. Conclusions

Removal of the potential available fraction, using TENAX, removed also the toxicity in freshly spiked and aged soil. This potential available fraction explains the toxicity, whereas the non-measurable NER cannot be related to toxicity

In spite of the impossibility to measure NER directly in real environmental samples, it will be shown that the methods developed provides reliable indirect information that can be used to assess behaviour and toxicity of substances that are known to form Non Extractable Residues. The approach is logic and based on measurable parameters.

5. References

[1] Ortega-Calvo, J.J., J. Harmsen, J.R. Parsons, K.T. Semple, M.D. Aitken, C. Ajao, C. Eadsforth, M. Galay-Burgos, R. Naidu, R. Oliver, W.J.G.M. Peijnenburg, J. Römbke, G. Streck, B. Versonnen, 2015. From Bioavailability Science to Regulation of Organic Chemicals. Environ. Sci. Technol. 2015, 49, 10255−10264.

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