# Summary

There is already a long discussion concerning the bioavailability and ecotoxicological relevance of non-extractable residues (NER) in soil. Is NER formation a detoxification process or should it be considered a hidden hazard?

NER can only be established using labelled chemicals (e.g. 14C) and cannot be measured with conventional chemical analytics. However, even using labelled compounds uncertainty exists about the identity of measured radioactivity. Do we measure: 1) Association of the parent chemical or breakdown product with mineral and/or organic matter, and/or 2) Mineralisation and incorporation of carbon into microbial biomass and carbonates?

Regulations ask for understandable and measurable parameters. The approach of Ortega-Calvo et al. (2015) has been followed, because this approach defines clear and measurable fractions. The only fraction not measurable is NER, but this can be considered as a residual fraction if all others are measured. Considered fractions are:

- Chemical present in the water phase, actually available (passive sampling or CaCl2-extraction);
- A potentially available fraction in equilibrium with the water phase (Tenax, ISO TS-16751);
- The total extractable amount, measured with a (standard) method;
- NER is considered, but mentioned as non-measurable and also non-bioavailable.

We applied a recently standardized method using Tenax (in accordance to ISO TS-16751) to remove the bioavailable fraction. Tenax is an extra solid phase that can be removed easily leaving the soil without a bioavailable fraction. Solvents can also be used to remove the bioavailable fraction, but these were not applied because residual solvent in the soil may cause toxicity, which would make it impossible to establish the role of NER.

We studied three NER-forming chemicals and followed their fate for a period of 6 months after addition. An important part of the study were experiments using $^{14}C$ chemicals. During these experiments, formation of non-extractable $^{14}C$ was observed for all chemicals.

Toxicity of NER-forming compounds is mostly low. Acute toxicity tests using Earthworm avoidance, Vibrio fischeri and Daphnia magna were found to be suitable for the selected chemicals, because they can be performed within 2 days, a period that NER-formation is still limited. Longer lasting chronic toxicity test were not applied. At the end of a chronic test, most of the spiked chemical can be present as NER, which makes interpretation of the results uncertain. A long-lasting test such as the earthworm reproduction test (incubation period 8 weeks) cannot answer the question whether toxicity is caused by the initial spiked concentration or by the bioavailable concentration at the end of the experiment.

For the chemical trinitrotoluene (TNT), NER-formation was reproducible and NER formation during aging removed toxicity. By removing the bioavailable fractions directly after spiking and after aging it was also possible to remove toxicity. The experiments with and without labelled TNT clearly showed that toxicity was caused by the bioavailable chemical and not by NER. With the other two selected chemicals, cypermethrin and carbendazim, results were less clear, because there was a large uncertainty in NER-formation. The degree of biodegradation was not reproducible for cypermethrin and unexpected losses occurred with carbendazim. This resulted in a very large uncertainty about NER-formation using non-labelled compounds. For these compounds, it is not possible to draw conclusions from non-labelled experiments only.

In conclusion, we developed a tool that can be used if the fate of the chemical including NER formation is well known. Additional experiments using labelled compound remain necessary if uncertainties about the fate of the chemical exist.