**Biogenic, non-extractable residues of pesticides**

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After the application of pesticides, residues are formed in plants and soil, which cannot be extracted with organic or in-organic solvents without destruction of the matrix and/or the chemical compound. While quantification of non-extractable residues (NER) is feasible by use of radioactively labelled compounds, direct analysis of the chemical nature of NER is not possible. Because microorganisms use carbon both directly from the contaminants and indirectly by fixation of CO2 released from the xenobiotic, parts of the 14C‑labelled carbon atoms may be incorporated into microbial biomass. After cell death, dead biomass is incorporated in SOM (soil organic matter), thus, forming biogenic NER (bioNER) which is unlikely to pose any risk to the environment. The formation of bioNER is not yet measured routinely in environmental fate studies of xenobiotics due to a lack of methodology (Kaestner et al, 2014). This investigation focuses on proteinaceous carbon analysis because proteins make up the largest portion of the bacterial cells, i.e., 50‑55 % of the microbial mass.

The main goal of the experiments was to develop a rapid and reproducible method for quantifying bioNER based on 14C-analyses. Amino acids are known as microbial markers, which represent most of the analytically detectable biogenic components of SOM. Quantification of the magnitude and kinetics of the formation of biogenic residues during microbial degradation of the model substance bromoxynil in soil was examined. The 14C-distribution within the system was analysed in order to establish a mass balance comprising CO2 formation, solvent-extractable residues, total NER and bio-NER.

In soil treated with 14C-bromoxynil after 57 days of incubation significant amounts (approx. 60 % of applied) of NER are formed. Mineralisation amounted up to 23% of the applied radioactivity. For further characterisation of the NER fraction amino acids were extracted, purified, separated by two-dimensional TLC, and visualised by staining with ninhydrin. Radioactively marked amino acids which had been formed during incubation were visualised by bioimaging (BioImager, Fuji). Identification of TLC spots was obtained by GC-MS. For bromoxynil after 28 days of incubation the TLC analysis revealed, that about 62 % of the 14C‑label of the eluate was incorporated in amino acids. Extrapolating this content with the amount of protein in the biomass, in total about 10 % of the NER represent biogenic residues without any environmental relevance.

Reference:

Kästner M, Nowak KM, Miltner A, Trapp S, Schäffer A (2014). Critical Reviews in Environmental Science and Technology 44, 2107-2171