How to characterise non-extractable residues (NER) in PBT assessment – development of a harmonised procedure to be used in routine testing

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Introduction

According to new developments in REACH guideline revisions (ECHA R.11, 2017) non-extractable residues (NER) have to be considered in PBT assessment. In June 2018, ECHA published the discussion paper "Consultancy services to support ECHA in improving the interpretation of Non-Extractable Residues (NER) in degradation assessment" where a first guidance is given on how to characterise NER and separate the different NER types in practical testing.
In parallel a project was conducted on behalf of the German Environment Agency (UBA) in order to develop a straightforward extraction procedure for NER characterisation for use in practical testing (BfG, FKZ 3713 63 413 1).

The procedures described in both approaches differ somehow and so the aim of the current study is to derive a harmonised procedure for use in regulatory routine testing. In September 2018 UBA initiated a project (FKZ 3718 65 407 0) in order to develop a procedure for practical testing based on both current approaches.

Next steps

- Soil degradation test in accordance to OECD 307 with one selected soil (silt loam)
- Application of extraction procedures as shown in Figure 1
- Complete mass balance for each sampling
- Chemical parent analysis in each extract; no metabolite identification
- Determination of NER-Type, comparison with data published in literature so far
- Application of the MTB modelling tool (prediction of bioNER based on measured mineralisation) and verification with experimental data
- Assessment of suitability of NER characterisation for persistency assessment

Conceptual differences of actual approaches and testing strategy

Both approaches for NER determination show conceptual similarities, both with a focus on a better assessment of NER for the approval of chemicals. However, the concepts follow different aims:

BfG approach [1]

- **pragmatic, fast extraction procedure for testing routine**
- **target on maximum extractable amount**
- **extractable part by definition "remobilisable"**
- **no further chemical analysis of "remobilisable" amount**
- **Non-extractable part by definition "safe sink"**

ECHA approach [2]

- **Focus on maximum information on NER speciation**
- **Chemical analysis after matrix disaggregation and differentiation of sequestered and covalently bound residues**
- **Direct determination of bioNER based on aminoacid analysis after hydrolysis**
- **Non-extractable (covalenly bound) part by definition "safe sink"**

Testing strategy: obtain a database on NER characterization for further decisions

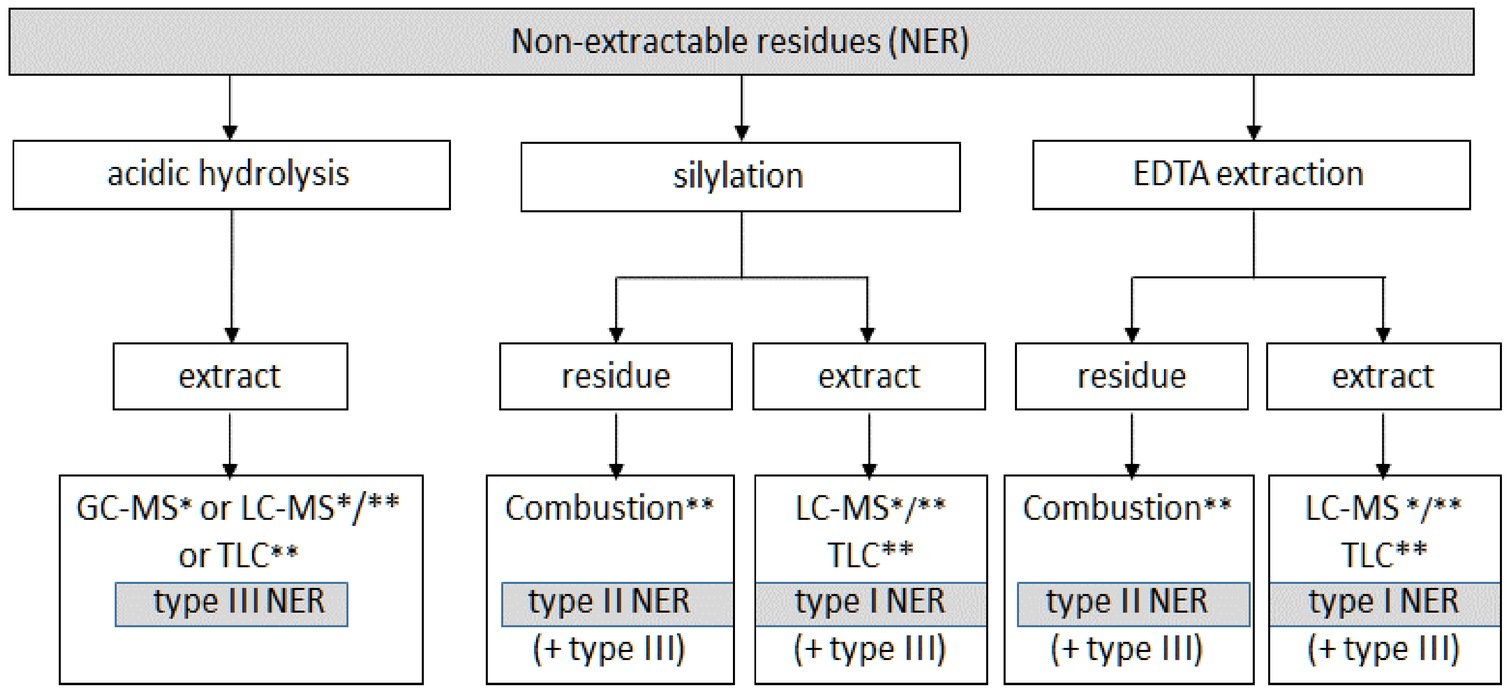
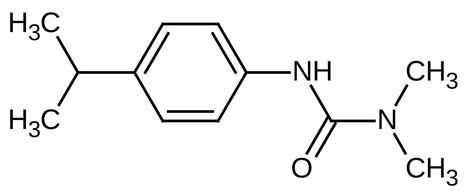


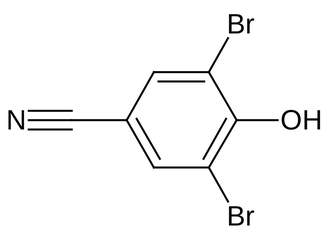
*Figure 1: testing strategy, schematic (incubation with ^{13}C * or ^{14}C ** labelled test substance)*

Reference substances to be used within the project

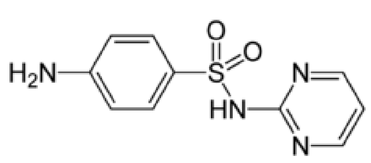
1. Substance: **Isoproturon** ^{14}C and ^{13}C, Urea herbicide, DT_{50soil}: 12 d



2. Substance: **Bromoxynil** ^{14}C and ^{13}C, HBN Herbicide, DT_{50soil}: 1 d



3. Substance: **Sulfadiazine**,^{14}C and ^{13}C Sulfonamide antibiotic, DT_{50soil} 1-3 d



Furthermore, two hydrocarbons will be tested in a connected projected funded by Concawe. Data will be made available for the current project.

Open questions

- Can the ECHA approach be simplified with a more practical NER specification and is this still suitable to improve regulatory assessment?
- Can analysis for NER type speciation be added to the BfG approach, e.g., just EDTA separation, amino acid hydrolysis and label analysis?
- Is it possible to analyse all ^{14}C- or ^{13}C-labeled amino acids as a summary parameter in the "hydrolysis soup" and how good does this represent bioNER?
- How to quantify bioNER in the silylation- and EDTA-treatment and what is remaining in the matrix?
- Does PLE performed at 100°C already change the soil matrix for further NER analysis by denaturation of biomolecules and does it already extract bioNER?
- How to consider NER types in PBT assessment / environmental risk assessment?

References:
[1] Eschenbach A, Oing K.: Erarbeitung eines gestuften Extraktionsverfahrens zur Bewertung gebundener Rückstände. UBA Draft report, FKZ 360 01 070, unpublished
[2] Kästner M, Trapp S, Schäffer A. Consultancy services to support ECHA in improving the interpretation of Non-Extractable-Residues (NER) in degradation assessment. ECHA Report 2018: 76.

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In degradation testing in soils and water/sediment systems for regulatory purposes according to OECD standard guidelines, very often a fraction of the test substance is observed, that cannot be released from the solid matrix with non-destructive extraction methods. These so called Non- Extractable Residues (NER) can be detected only, if isotope labelled test substance is used, which is mandatory in regulatory framework if technically feasible. The importance of NER in the persistency assessment has been more or less neglected in the past. But according to new developments e.g. in REACH guideline revisions (ECHA R.11, 2017) the NER has to be considered as they may potentially be remobilised as parent or transformation product. One approach is to consider NER as 100% potentially available parent substance if not proven otherwise. This worst case assumption might be misleading because NER can also represent residues or products of degradation without any environmental relevance as they are irreversibly bound or transformed into biomass. Therefore, the ECHA published in June 2018 the discussion paper "*Consultancy services to support ECHA in improving the interpretation of Non-Extractable Residues (NER) in degradation assessment*" where guidance is given on how to characterize NER and the different NER types in practical testing. However, the discussion paper clearly states that discussions on NER assessment are still ongoing and the current paper represents a state of the art report only. In parallel and on behalf of the German Environment Agency (UBA) a project was conducted in order to develop a straightforward extraction procedure for NER characterisation for use in practical testing (FKZ 3713 63 413 1). The project results differ to some extent from the procedures described in the discussion paper and the question came up, how an acceptable method for use in regulatory routine testing can be derived. Thus, in September 2018 the German Environment Agency (UBA) initiated a project (FKZ 3718 65 407 0) in order to develop a harmonised procedure for practical testing taking into account both current approaches and proving the applicability of the harmonized approach experimentally using a set of reference substances. The project results will be discussed with regulators, scientists and stakeholders in an international workshop at the end of the project and it is intended to set the standard for future consideration of NER in chemicals persistency assessment.