Session: 3. Environmental chemistry and exposure assessment: analysis, monitoring, fate and modeling
3.01 – Advances in methods to evaluate environmental degradation and persistence
Co-chairs: Christopher Hughes, Dieter Hennecke, Pippa Kate Curtis-Jackson, Jacques Lharidon
Discussion session: Wednesday 5th May 2021; 16:20 PM; Virtual Room 6; Platform presentation, Control ID 62039

Analysis of non-extractable residues (NER) for use in chemical persistency assessment – proposal for a standardised testing procedure

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1. Introduction

In solid environmental media such as soil and sediment, the degradation of organic chemicals is often accompanied by the formation of non-extractable residues (NER). The extent of the NER formed can vary greatly between 10% and more than 90% of the applied amount, depending besides the extraction methods on the substance and medium properties. NER can still contain the parent substance and regulatory-relevant transformation products that can be remobilized and pose a risk, but also biogenically bound carbon, which represent a safe sink. As there is currently no agreed method for differentiating between NER types, there is much discussion about how to consider them in persistence assessment. Using test substances labelled with radioactive, e.g. ^{14}C -, or stable, e.g. ^{13}C – isotopes, three types of NER can be experimentally distinguished [1]: sequestered/entrapped residues (type I) having the potential to be released, and type II NER, i.e., residues covalently bound to organic matter in soils or sediments, the latter being considered to have very low remobilisation potential. Type III NER (bioNER) are formed after degradation of the xenobiotic chemical to residues which are anabolically used to form biomolecules like amino acids and other biomass compounds, and are, thus, of no ecotoxicological or regulatory relevance.

Due to the lack of a standardised method allowing the identification of the three NER types, NER are currently treated incoherently in European regulations. While REACH considers NER as non-degraded substance if not proven otherwise, others, e.g. the plant protection product regulation, up to now consider NER as degraded. As this might have significant impact on the degradation half-lives determined and thus on the outcome of the persistence assessment, the German Environment Agency (UBA) is working on a standardised and harmonised approach on NER assessment. Potential methods for NER characterization were tested after performance of soil incubation studies according to OECD 307 using a set of three different ^{14}C-labelled substances. For all test substances, additional studies were done also using ^{13}C label. Different exhaustive extraction procedures were compared to get the solid matrix containing only NER. We showed that NER characterization methods are reproducible and applicable for routine analyses [2]. Formation of individual NER types was predicted by the recent developed Microbial Turnover to Biomass (MTB) modelling tool [3] which uses CO_2 formation as indicator of microbial degradation and formation of biogenic NER. Modelled and experimentally determined data were compared.

2. Materials and methods

Differentiation of type I and type II NER: Two methods were compared, i.e., silylation and EDTA extraction. For silylation, the thoroughly extracted and dried soil is solved in dry chloroform with NaOH pellets and trimethylchlorosilane (TMCS). Since TMCS reacts with air moisture, samples are flushed with argon and kept in an inert gas atmosphere. In order to allow a pressure balance for the HCl gas produced in the reaction and to maintain the protective gas atmosphere during silylation, a gas bag filled with argon is connected to the Schlenk flask with a silicone tube (**Fehler! Verweisquelle konnte nicht gefunden werden.**). EDTA extraction of the thoroughly extracted soil was performed according to Loeffler et al. [4]. After clean-up samples can be analysed for the presence of parent substances and relevant transformation products.

Figure 1: Experimental silylation set-up

Determination of type III NER (biogenic NER): thoroughly extracted soil is hydrolyzed by 6 M HCl at elevated temperature and the extract is cleaned by ion-exchange chromatography. The method can be analyzed using

stable [5] and radioactive isotope labels [6]. The *Microbial Turnover to Biomass model (MTB)* uses the Gibbs free energies of products and educts, the molar mass, the empirical formula of the substance and the number of C-H bonds as input data [3].

3. Results and discussion

3.1. Characterization of non-extractable residues of bromoxynil, sulfadiazine, isoproturon

Both NER characterisation methods, silylation and EDTA extraction, were reproducible and can be used for routine analysis. Using ^{13}C- or ^{14}C-labelled bromoxynil, sulfadiazine, and isoproturon as test substances, all forming significant amounts of NER (all above 50% of the applied amounts), characterization of the non-extractables revealed that both EDTA extraction and silylation released considerable amounts of NER (between ca. 15% and 30%). However, only a minor fraction of the released radioactivity could be attributed to parent test substance representing sequestered type I NER. Results demonstrate that chemical analysis of the received extracts is crucial for a proper assessment.

MTB estimation, based on measured CO_2 and theoretical yields, predicts biogenic NER formation in the order sulfadiazine < bromoxynil < isoproturon. Preliminary measured data support the calculations.

3.2. Consideration of NER in the persistence assessment of chemical substances

A standardised approach for determination of total NER and identification of the different NER types is needed and should be implemented in regulatory guidance where it can help to converge European regulations in respect of dealing with NER. The potentially remobilisable NER pose a risk to the environment and should be considered in the persistence assessment (e.g. PBT, vPvB, POP), while the biogenic NER (type III) can be seen as safe sink and are no longer of regulatory relevance.

The different approaches to determine the NER types and to intergrate them into the persistence assessment have been compared with the objective to develop a refined proposal for routine testing. A harmonised concept needs to be established. One solution could be the consideration of parent and relevant transformation products from the potentially remobilisable NER fraction for the calculation of the DT_{50} values. Another option might be to establish a new persistence trigger for the reversibly bound NER fraction.

3.3. Dissemination of results

The results of an international workshop in February 2021 will be presented. Aim of the workshop was to propose and discuss a standardised and harmonised concept for NER characterisation as basis for the inclusion of NER in the regulatory persistence assessment.

4. Conclusions

The final aim of NER research is to have a unified approach to be implemented into the persistence assessment strategies for REACH chemicals and biocides, human and veterinary pharmaceuticals and pesticides, irrespective of the different regulatory frameworks. For this, a reliable methodology to quantify type I, type II and type III NER is of utmost importance.

5. References

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Acknowledgement - The authors thank all presenters and participants of the workshop, to be held in February 2021, for the valuable input and discussion.