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Options to address non-extractable residues in regulatory persistence assessment

1. Background

Besides being subject to various degradation and transport processes, all chemicals that enter environmental matrices potentially form non-extractable residues (NER) in varying amounts (Barriuso *et al.*, 2008; Kästner *et al.*, 2014). Amount and characteristics of NER may have a significant impact on the derivation of degradation half-lives and hence on the regulatory conclusions with respect to persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) assessments.

The methodology currently presented in ECHA's Guidance offers a conservative interpretation of NER, i.e. assumption by default that NER are not biodegraded, unless further information demonstrates the contrary. In cases where NER may affect the outcome of the persistence assessment, it would be useful to have an option for refinement of the assessment by differentiation between remobilisable and irreversibly bound NER fractions, to make the default approach less conservative as far as available information and scientific knowledge permit. While the irreversibly bound part (e.g. biogenically bound) can be assessed as a removal pathway, the remobilisable fraction (strongly sorbed, physical inclusion) poses a potential risk.

As a follow-up to the Topical Scientific Workshop on Soil Risk Assessment[1] and discussions at the PBT Expert Group on the interpretation of NER in persistence assessment, characterisation of NER was identified as one of the priority topics for further development. On that basis, in 2017 ECHA launched a project to identify means to characterise and quantify different types of NER. The work took into account both, scientific developments and regulatory work carried out by the Member States, as well as work done by The European Chemical Industry Council/ The European Centre for Ecotoxicology and Toxicology of Chemicals (CEFIC/ECETOC). As a result, a discussion paper on improving the interpretation of NER was published in June 2018 as a technical report on the ECHA website[2].

To ensure a common understanding of the topic, the NER project steering group, the PBT Expert Group and relevant ECHA committees (The Member State Committee (MSC) and Biocidal Products Committee (BPC)), as well as European Food Safety Authority (EFSA) and European Medicines Agency (EMA) experts, were consulted during drafting of the discussion paper. Additionally, members of the NER project steering group, PBT Expert Group, MSC and BPC were asked to comment on draft text proposed by ECHA Secretariat for updating ECHA's Guidance, in particular *Chapter R.11* of the REACH *Guidance on Information Requirements & Chemical Safety Assessment (IR&CSA)*, related to the assessment of PBT/vPvB properties, and Chapter R.7b, related to persistence assessment. Since Chapter R.11 is also used for assessment of biocidal substances (referred to in the BPR Guidance), any update is also relevant for the assessment of biocides.

The PBT Expert Group, MSC and BPC aknowledged that there is still limited experience on the

[1] See https://echa.europa.eu/view-article/-/journal_content/title/topical-scientific-workshop-on-soil-risk-assessment for more details

[2] *"Consultancy services to support ECHA in improving the interpretation of Non – Extractable Residues (NER) in degradation assessment" June 2018; Discussion paper published in ECHA website;* https://echa.europa.eu/publications/technical-scientific-reports; https://echa.europa.eu/documents/10162/13630/echa_discussion_paper_en.pdf/4185cf64-8333-fad2-8ddb-85c09a560f7c

characterisation of the different NER types and the applicability of the proposed methodologies, which are not standardised (cf. silylation and amino acid extraction). Different concepts with regard to the consideration and interpretation of NERs are implemented under the REACH/Biocides Regulations and the Plant Protection Products Regulation (PPPR). Under PPPR, NER can be assumed to be degraded residues of no environmental concern (DG_SANCO_2012; FOCUS, 2014) if the amount of NER is below a certain threshold or the mineralisation rate is high enough. On the contrary, under the REACH/Biocides Regulations, NER are regarded as potentially bioavailable and as non-degraded residues ("parent substance") if no other information is available (EC_1907_2006; ECHA_2017_R.7b; ECHA_2017_R.11). In other words, NER are considered either a `safe sink´ or a potential risk (remobilisable repository) depending on which regulatory context applies.

ECHA management has decided to adjourn the update of Chapter R.11 of the guidance beyond 2019. This allows for further clarification of the options to assess NER in the REACH regulatory context, based on the state of the art of the scientific methodologies, the regulatory acceptance in line with the current guidance and the experience so far. Until the guidance is updated, the present ECHA note is intended to inform duty holders about acceptable approaches to refine assessment of NER in the regulatory context of the REACH and Biocidal Products Regulations.

2. Quantification and characterisation of NERs to refine the persistency assessment

2.1. Quantification of NERs

In a simulation test, concentrations of the parent substance and the transformation products should be analysed in appropriate time intervals. For solid matrices, this requires extraction methods to be used. However, some analytes are so tightly bound to the matrix that they cannot be extracted. Such residues are called non-extractable residues (NER). This is especially relevant for simulation tests in soil and in sediment but also for simulation test with surface water which may also lead to the formation of NER depending on the concentration of suspended particulate matter (SPM).

The quantification and identification of extractable residues (parent substance and transformation products) of the tested substance is a core part of performing a standard simulation test (OECD TG 307, TG 308 and TG 309), and is always needed. By default, the residues remaining in the matrix after these extractions (total NER), should be regarded as non-degraded substance[3]. It is therefore important that the fraction of NER is minimised, e.g. by using extraction methods that are so harsh that they just do not modify the physicochemical nature of the extracted compounds. I.e. the extraction method has to be tailored to the substance tested and its degradation products, so that modification of parent substance and degradation products is avoided. In addition to extraction methods described in ECHA Guidance R.11, section on Non-extractable residues, a more detailed description of different extraction regimes is presented in the Report on non-extractable residues (2018)[2].

When new studies are performed, harsh extraction methods such as Soxhlet (reflux), microwave assisted extraction (MAE), ultra-sonication and Accelerated Solvent Extraction (ASE) (also named Pressurised Liquid Extraction (PLE)) (Nießner *et al.* 2017, Lariviere *et al.* 2017, Lindholm-Lehto *et al.* 2017, Dimpe *et al.* 2016, de Morais *et al.* 2012, Tadeo *et al.* 2012a, Tadeo *et al.* 2012b, Tadeo *et al.* 2010) should be used in order to minimise the fraction of NER. When quantifying NER, the extraction methods used and their efficiencies as well as

[3] Without further analyses, it is indeed not possible to distinguish whether NER consist of unmodified parent substance or of transformation products.

the analytical methods and corresponding detection limits should always be reported (including the results achieved with the extraction methods employed, even if they were unsuccessful). For derivation of degradation half-lives, determination of total NER should be performed for all sampling points.

2.2. Characterisation of NERs

Characterisation of the NER may also be attempted. NER could be differentiated between remobilisable (therefore still of potential concern) and irreversibly bound fractions (which could be regarded as of low or no concern). Characterisation of the NER is not a standard information requirement, but may be considered as opportunity to refine the persistence assessment. In some cases it is not useful, for example when the extractable fraction already shows that the substance is (very) persistent.

ECHA Guidance R.11 indicates that even in the absence of a systematic methodology, a case-by-case refinement is possible if it can be reasonably justified or analytically demonstrated that part of the NER is irreversibly bound. The guidance does not however define any methodologies to achieve this. Methods to quantify and differentiate the remobilisable and irreversibly bound fractions have recently been introduced[4].

An approach to experimentally discriminate **three separate types** of NER in environmental matrices has been proposed by Schäffer *et al.,* 2018. The approach entails silylation to differentiate NER type I and II and extraction of amino acids to quantify bioNER (NER Type III).

NER Type I: adsorbed or physically entrapped into the matrix, contain the parent substance, transformation products or both. NER Type I have the potential to be remobilised, and therefore should be regarded as non-degraded substances when calculating the half-life. If chemical analyses are conducted, it may be possible to distinguish whether NER Type I consist of unmodified parent substance or of transformation products.

NER Type II: residues that are **strongly bound** to the matrix (i.e. to humic matter) in surface water, soils or sediments and that are considered to have low remobilisation rates. Unless there are indications from the available literature or monitoring data regarding their potential remobilisation, strongly bound residues may be regarded as irreversibly bound.

NER Type III: incorporated into biomass (biogenic NER, also called **bioNER**), NER Type III result from the anabolic formation of biomolecules (amino acids, phospholipids, and other biomass compounds) from the degradation products of the parent substance. Dead biomass, and therefore biogenic NER, are eventually fixed in organic matter derived from decaying microbial biomass. NER Type III are considered to be of no concern.

The information on the quantity of NER types (I, II, III) can be used for refining the half-life. The half-life to be compared to the persistent/very persistent criteria may be calculated using the sum of the concentrations of the parent substance, transformation products and remobilisable NER (NER Type I) (see example in Kästner *et al.* (2018)). Biogenic NER (bioNER = NER Type III), and strongly bound NER (Type II) if there are no indications on the contrary, can be regarded as removed for the PBT/vPvB assessment.

[4] *"Consultancy services to support ECHA in improving the interpretation of Non – Extractable Residues (NER) in degradation assessment" June 2018; Discussion paper published on ECHA website;* https://echa.europa.eu/publications/technical-scientific-reports; https://echa.europa.eu/documents/10162/13630/echa_discussion_paper_en.pdf/4185cf64-8333-fad2-8ddb-85c09a560f7c

For existing studies, it is expected that a mass balance of the labelled test item is presented, which should thus imply that the amount of NER is reported, irrespective of the extraction method(s) used. In this case, as explained above, by default NER should be regarded as non degraded. A prediction of the quantity of bioNER with tools such as the Microbial Turnover to Biomass (MTB) model[2] can be helpful, especially for existing cases, where information on NER types is usually not available. The likelihood of NER being biogenic (bioNER) or not could be very useful in the interpretation of the results.

3. References

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