Explanation of the proposed approaches for consideration of the NER in persistence assessment

Degradation tests, e.g. in soil according to OECD 307, are carried out in the framework of persistence and environmental risk assessment of active substances. The calculated half-lives are relevant for further environmental assessment. To obtain complete mass balances in simulation tests isotope-labelled test substances should be used (preferably ^{14}C-label). Generally, the soils are spiked with the isotope-labelled substances and incubated at standardised conditions.
Test vessels are regularly sampled, extracted and analysed. The non-extractable residues (NER) are partly reversibly bound to soil and might be remobilised over time, and thus should be included in the persistence assessment.

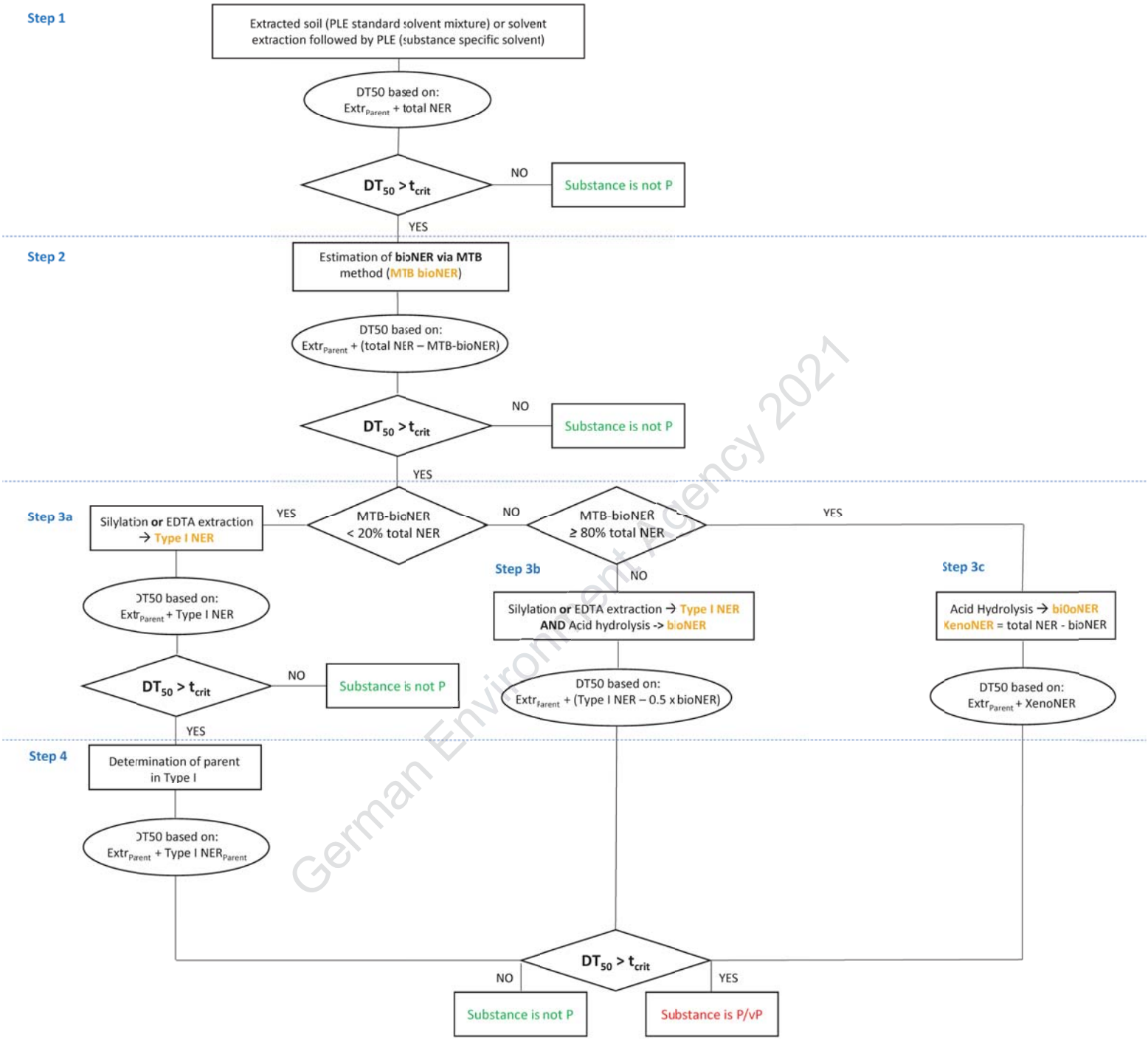
In the following we present two approaches to determine the relevant fractions of non-extractable residues and to consider them for the derivation of half-lives in the framework of persistency assessment (PBT/ vPvB) of these substances. The approaches are based on the results obtained in the UBA R+D project 'Consideration of non-extractable residues (NER) in PBT-assessment', FKZ 3718 65 407 0 and are in line with the expectation of ECHA for the handling of NER in the assessment of P, Persistence.

Both approaches show a common procedure for the first extraction steps for total NER quantification using either pressurized liquid extraction (PLE) with a standard solvent mixture (MeOH, acetone, water 50/25/25 at 100°C and 100 bar if the instrumentation allows) or conducting a solvent extraction followed by PLE with a substance specific solvent (depending on the efficiency of the two extraction procedures). This first step is able to distinguish between extractable residues ('Solvent+PLE extractable (**Extr**.)') and total non-extractable residues ('**total NER'**) by definition.

After this first step, the two proposed approaches differ in their strategy to refine the persistence assessment with respect to the relevant NER fraction.

German Environment Agency 2021

Proposal 1



German Environment Agency 2021

The first plot shows a 'worst-case approach', which focuses mainly on the decision persistent/very persistent (P/vP) or not persistent (not P) with less relevance of the specific calculated DT_{50} values as these are not needed for further risk assessment.

The idea of the flowchart is a stepwise approach with increased data requirements at each consecutive step resulting in improvement of the reliability of the assessment. NER characterization in Step 3 with regard to type I NER and bioNER is only necessary if Steps 1 and 2 do not result in classification as non-persistent (not P).

In the first most conservative step (**Step 1**) it is assumed that all non-extractable residues (total NER) consist of unchanged parent test substance, which can be released over time. In this way, the worst-case half-life of the substance is evaluated by considering extracted and total non-extracted residues in the derivation of half-lives through kinetic evaluation **(DT_{50} based on: $Extr_{Parent}$ + total NER)**. If at this stage it can already be proven, that a substance is not persistent, then the persistence assessment can be stopped and no further extraction steps are needed. If proven otherwise a refinement is needed, i.e. if half-life is higher than the trigger value for persistency ($DT_{50} > t_{crit}$) which is the case when soil t_{crit} = 120 days (P) and 180 days (vP), then a refinement is needed.

As a first refinement (**Step 2**), the presented approach considers the fact that the degraded test substance is used for growth of biomass and forms biogenic NER or bioNER. The latter can be considered as a safe sink as it poses no risk to the environment and should not be considered in the derivation of the DT_{50} of test substances. BioNER can be estimated by the MTB Model („Microbial Turnover to Biomass"-Model) using the experimental mineralisation data of the soil degradation test. In this step, no additional experimental work on NER is required but only a calculation is conducted. In fact, to the experimentally determined total NER measured in the first step, the bioNER fraction can be subtracted. The remaining NER fraction is still assumed to consist of 100% potentially available parent test substance. The sum of extractable parent and total NER reduced by the amount of bioNER estimated by the MTB model is used for DT_{50} evaluation **[DT_{50} based on: $Extr_{Parent}$ + (total NER – MTB-bioNER)]**. If the calculated DT_{50} under this assumption does not exceed the P-trigger ($DT_{50} < t_{crit}$), the test substance is considered not persistent and no further refinement is needed.

If, after bioNER estimation by the MTB calculation, the DT_{50} of the test substance still exceeds the P trigger value, then in **Step 3** experimental characterisation of NER is required. The soil containing NER after the first extraction (**$Extr_{Parent}$**) should then be further extracted by silylation or EDTA extraction (depending on the substance properties and the feasibility for the respective substance) in order to obtain a fraction of potentially remobilisable NER (Type I NER) (**Step 3a**). This fraction can be added to the extractable parent obtained in the first step for deriving a refined DT_{50} **(DT_{50} based on: $Extr_{Parent}$ + Type I NER).** The strongly adsorbed, covalently bound NER (Type II NER) can instead be omitted when deriving the half-lives, since this fraction is assumed to be irreversibly bound and will not become available, even after many years. If still after this step $DT_{50} > t_{crit}$, it is strongly recommended to conduct a chemical analysis of the extracted Type I NER (**Step 4**), to differentiate between released parent test substance and other potential molecules carrying the isotope label which were released from the soil matrix. This is the least conservative step but also needs the maximum amount of additional laboratory effort. The sum of released parent substance by silylation together with the extracted parent will be used for the DT_{50} calculation (**DT_{50} based on: $Extr_{Parent}$ + Type I NER_{Parent}**).

Depending on the amount of estimated bioNER with the MTB Model (% of MTB-bioNER in the total NER) the choice whether to conduct silylation/EDTA extraction can be conducted a priori.

In the rare case that the amount of MTB-bioNER is equal to or more than 80% of total NER, then **Step 3c** should be conducted. Instead of refinement through silylation/EDTA a different refinement (after

German Environment Agency, 2021

measuring the amount of bioNER through acid hydrolysis) is possible. In this case the potentially harmful fraction of NER (the XenoNER) is considered, which is the fraction of total NER subtracted by the biogenic NER. BioNER can be estimated experimentally by conducting an acid hydrolysis of the soil containing total NER (**Step 3c**). The DT_{50} would then be calculated by considering the parent from the solvent extracts plus the total NER (from Step 1) minus the solid-phase extract eluate from the HCl extracts **[DT_{50} based on: $Extr_{Parent}$ + (total NER – bioNER) = $Extr_{Parent}$ + XenoNER].**

As seen before, if the estimated MTB-BioNER are ≥ 80% of Total NER, and thus the major portion of NER can be considered biogenic, the acid hydrolysis is recommended (Step 3c). If MTB-BioNER is < 20% of Total NER, then the silylation/EDTA extraction is recommended (Step 3a). If MTB-BioNER is ≥ 20% of Total NER and < 80% Total NER then it is recommended to derive the DT_{50} by considering the fact that also silylation could release BioNER and this part should also be subtracted (**Step 3b**). Since the amount of BioNER in the silylation extract is unknown, a default value of 50% of the BioNER will be subtracted from the NER in the silylation extract. In case there is any indication of another ratio of BioNER to be released by silylation, it should replace the default of 50%. Thus, the DT_{50} will be calculated by considering Extracted Residues + Type I NER – 0.5 x bioNER **(DT_{50} based on extr. + (Typ I NER – 0.5 x BioNER))**. If after all these steps, the P/vP-trigger is still exceeded, the substance is finally to be considered persistent or very persistent.

The final regulatory decision about persistency of a substance or non-persistency is based on the PBT/ vPvB criteria according to Annex XIII of the REACH regulation.

German Environment Agency 2021