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

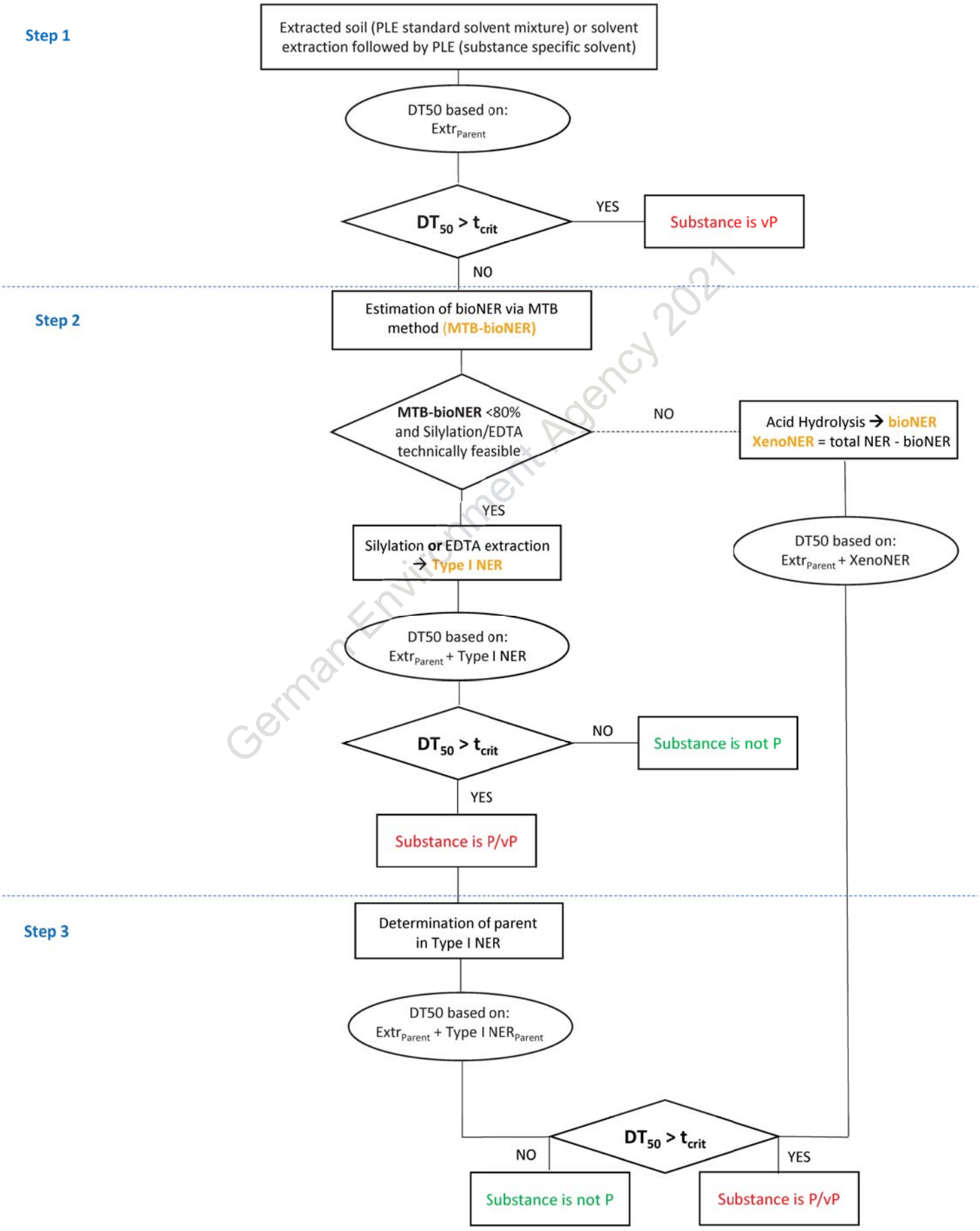
Degradation tests, e.g. in soil according to OECD 307, are carried out in the framework of persistence and environmental risk assessment of active substances. The calculated half-lives are relevant for further environmental assessment. To obtain complete mass balances in simulation tests isotope-labelled test substances should be used (preferably ^{14}C-label). Generally, the soils are spiked with the isotope-labelled substances and incubated under 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.

We present two approaches for discussion to determine the relevant fractions of NERs 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**$_{Parent}$)') 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.

Proposal 2



Proposal 2 (as shown above) represents a 'realistic-case approach', which sets the focus in trying to derive half-lives of substances as realistic and simple as possible, that can then be used for persistence assessment but also for risk assessment *(the latter needs further investigation before implementation will be possible).* Starting from a 'best-case scenario' in which it is considered that no fraction of non-extractable residues will become mobilised again, a chemical analysis of the extracts to determine the parent substance in the extracts is conducted (**Step 1**). In this first step the DT_{50} is derived based on extractable parent substance after PLE **[DT50 based on: $Extr_{parent}$]**. When this DT_{50} exceeds the trigger value (t_{crit} = 180 d) for vP in soil, then no refinement is necessary (substance is vP even in a best-case scenario, thus persistency would further increase, if fractions of potentially remobilisable NER would also be considered). A refinement and further characterization of the NER in soil should be considered, in case in the first step, no vP trigger value is reached and for all cases in which the registrant wants to derive a more realistic value for DT_{50} (which considers all the relevant and potentially remobilisable residues). The following step (**Step 2**) consists in extraction of the soil containing Total NER through silylation or EDTA depending on the technical feasibility of the two methods for the test item. After extraction, the remaining soil contains type II NER, which have a low potential for remobilisation. The silylation or EDTA extracts (EDTA/silylation) consists of type I NER, which is deemed to be highly remobilisable and thus of relevance from a regulatory point of view. To clarify if the type I NER may contain physically entrapped parent compound ($EDTA/Silylation_{Parent}$), the silylation or EDTA extracts have to be chemically analysed (**Step 3**). For the calculation of the DT_{50} with respect to type I NER, two options are possible: if chemical analysis of silylation or EDTA extracts is possible, DT50 should be calculated on the basis of $Solvent_{Parent}$, PLE_{Parent} and $EDTA/Silylation_{Parent}$ **[DT50 based on $Extr_{parent}$ + Type I Parent]**. If it is shown that the chemical analysis of silylation or EDTA extracts is technically not feasible, DT_{50} should be calculated on the basis of Sol_{Parent}, PLE_{Parent} and EDTA/silylation (i.e. the whole extract) **[DT50 based on $Extr_{parent}$ + Type I NER]**.

An alternative way in considering the environmental relevant parts of NER in the half-live calculation is using the acid hydrolysis method instead of Silylation or EDTA extraction. The microbial turnover to biomass (MTB) approach as a tool for the estimation of type III NER (bioNER) should be used to decide which method, acid hydrolysis or silylation/EDTA, should be performed. If the type III is predicted to be high based on the MTB approach, in **Step 2** acid hydrolysis of Total NER is recommended. In fact, type III NER can be experimentally quantified with the help of the purified HCl extract after acid hydrolysis as proxy for the amount of amino acids, amino sugars and other biomolecules. Finally, the experimentally quantified type III NER is deducted from Total NER to obtain the xenobiotic derived NER (XenoNER), which in principle includes both Type I and Type II NER. The DT_{50} with respect to the XenoNER should be calculated on the basis of Sol_{Parent}, PLE_{Parent} and XenoNER **[DT50 based on Extr. + (Total NER – bioNER) = Extr. + XenoNER]**. However, the use of the XenoNER for half-life modelling can lead to an overestimation of the persistence, since XenoNER consists of Type I NER and Type II NER, the latter having a low potential of being released from soil/sediment. Furthermore, the XenoNER evaluation does not allow for analysis of the parent only, which means that in the case of high percentage of transformation products in the XenoNER, these would not be subtracted to obtain DT_{50} only for the parent.

Nevertheless, the DT_{50} values obtained from EDTA/silylation extraction but also from acid hydrolysis represent the most realistic scenarios. If DT_{50} does not exceed the trigger value for P/vP at this stage, then the persistence of the test substance can definitely be excluded.

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.