National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

PBT in progress

Overview developments in PBT/vPvB-assessment

Eric Verbruggen |
eric.verbruggen@RIVM.nl

Guidance update

- Latest update R.11: June 2017
- Non-extractable residues
  - Further guidance has been drafted
  - Guidance update is postponed

- Current R11 guidance document now being used
  - New experiences with
    - Test compound selection
    - Grouping of chemicals
    - Compartment for persistence
    - Non-extractable residues
    - Dietary bioaccumulation tests
    - Air-breathing organisms

Identified topics in 2011/2015 workshops

Topic	
Compartment of concern	P, B, and T
Temperature	Persistence
Non-extractable residue	Persistence
Hydrolysis	Persistence
Photolysis	Persistence
Field studies	Persistence
Multimedia modelling, overall persistence	Persistence
Dietary accumulation test	Bioaccumulation
Growth correction	Bioaccumulation
Half-life, metabolism	Bioaccumulation
Invertebrates	Bioaccumulation
Field data, trophic magnification	Bioaccumulation

Additional topics in R.11

Topic	
UVCBs	P, B, and T
Test item	
Known constituent	
Fraction	
Whole subtance	
Grouping of chemicals	P, B, and T
PFASs	
Air-breathing organisms	Bioaccumulation
Half-life, metabolism	

Test item for UVCBs

- Lively debate in recent substance evaluations
  - Known-constituents approach
  - Fraction profiling approach
    - Block method (PetCo Approach)
  - Whole-substance approach

- Advantages of multiconstituent testing
  - Data on many constituents
  - Screening for the critical constituents
    - Start broad testing for P (B),
    - Followed by narrowed testing on B (P) and T

Test item for UVCBs

- Disadvantages of multiconstituent testing
  - Multi-constituent testing is analytically complicated
    - Lower concentrations individual constituents
    - Separation and identification of individual constituents
  - Interference of constituents
    - Physical
      - Aquatic tests not suitable for large variability in solubility
        - Persistence: soil/sediment, Bioaccumulation: dietary
    - Biological
      - Certain constituents might induce metabolism (MFO)
        - Lower BCF values possible for other constituents

Test item for UVCBs

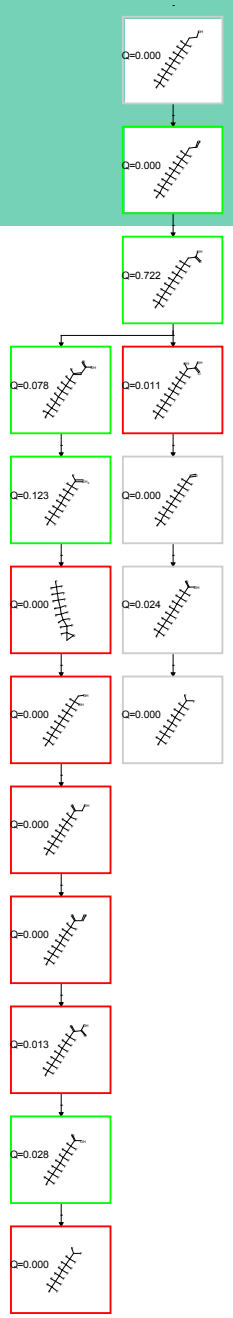
- Known-constituents approach
  - Applicable if worst-case constituents can be identified
- Whole-substance approach
  - Applicable if number of constituents is limited
  - All constituents are very similar
- Fraction profiling approach
  - Worst-case constituents can not be identified
  - Isolation of single constituents is not possible

- Toxicity testing requires single constituent testing
  - Unless all constituents are very similar

- Consequences for testing order?

Arrowhead approach (PFAS)

- Group approach for PFAS
- Reduce assessment of thousands of chemicals
- Only consider the ultimate metabolites (“arrowheads”)
- Predict metabolites of all chemicals with QSAR programs



That will not eliminate health concerns, because studies of the replacement PFAS chemicals that remain in AFFF show that they present **many of the same health concerns**. There are more than 4,000 PFAS chemicals, although the EPA estimates that **only 602** are actively used in commerce.

Persistence: Simulation testing

- Many new tests are OECD 309 (water only) performed at 12 ºC.
  - Aerobic test
  - Limited confounding issues with e.g. NER

- Technical reasons not to perform OECD 309
  - Low water solubility
  - UVCB with varying solubilities

- Some additional questions since guidance completion
  - Spiking method
  - Influence of test Concentration

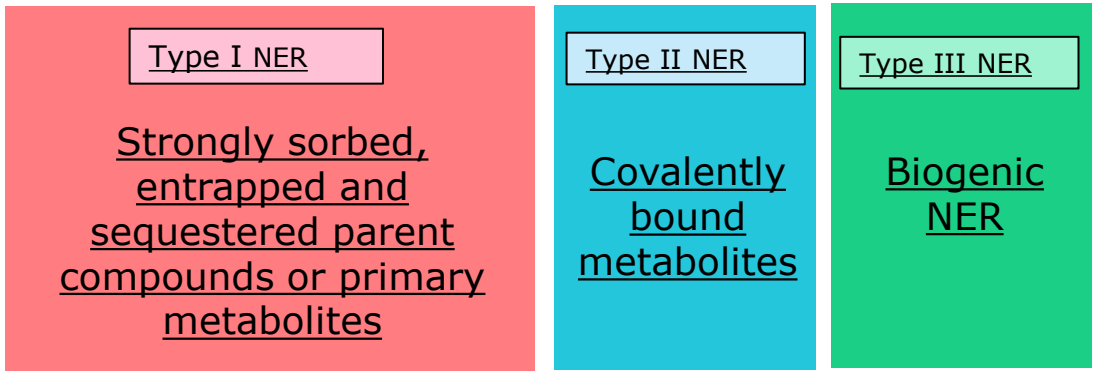
- Further guidance for non-extractable residues in OECD 307/308

Non-extractable residues (NER)

- Current guidance document: NER is parent compound unless…

- New developments: scoping paper and guidance
  - Guidance update on hold
  - https://www.echa.europa.eu/nl/publications/technical-scientific-reports

- NER divided in three categories

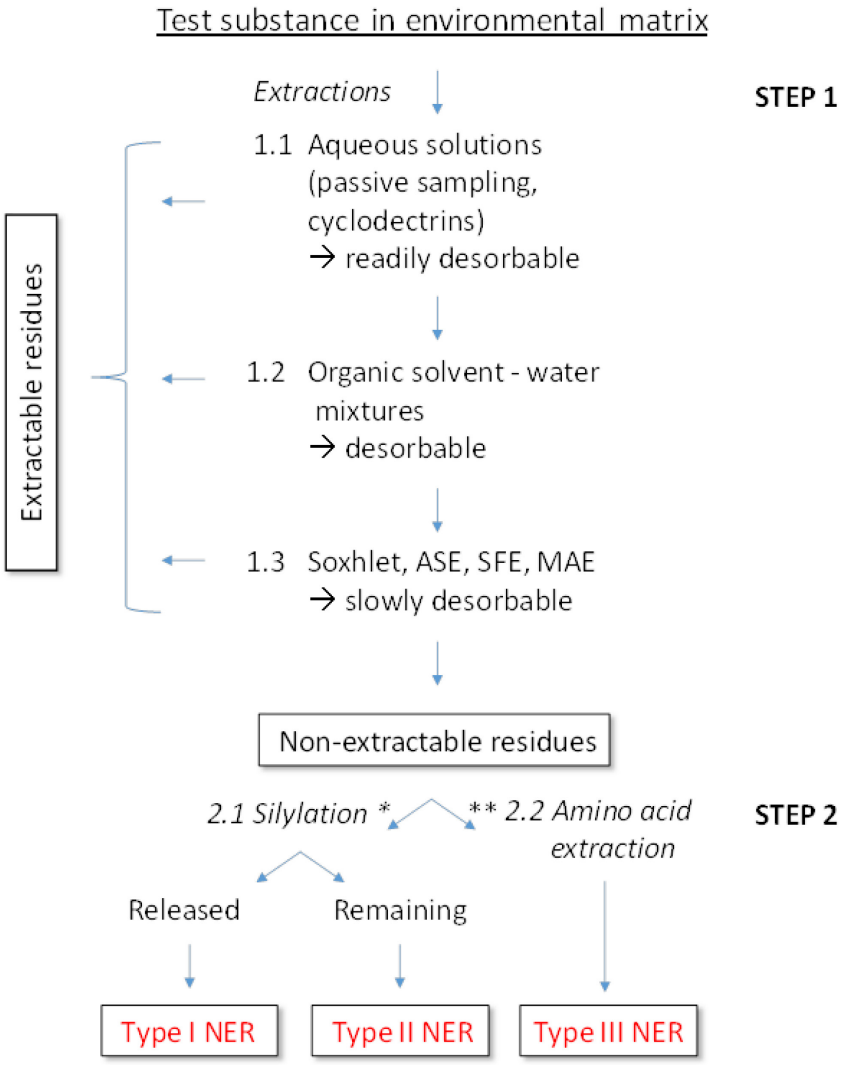


NER	Type I	Type II	Type III
Properties	sorbed, entrapped and sequestered	covalently bound	label conversion to biomass
Formation from	parent and transformation products	parent and transformation products	ultimate degradation and mineralisation
Evidencing	identification of parent compounds and transformation products	identification of cleavage products (very difficult)	label in biomarkers
Formation processes	van der Waals, hydrophobic interactions, etc.	C-C, C-N, C-O-C, ester covalent bonds in general	microbial metabolism
Stability	low to high	high	not relevant
Release probability of parent compounds or transformation products	high	low	not possible

Extraction scheme

(Kästner, Trapp, Schäffer, ECHA 2018)

- Harsh extraction scheme to minimise NER



- Determination of non-extractable residues (NER) types I and II by silylation
- Extraction of amino acids (NER type III)

Microbial Turnover to Biomass (MTB)

- Tool to predict bioNER formation
  - BioNER / CO_2= Yield / (1-Yield)

- Indicative
  - BioNER formation is dependent on labelling position

- Useful for assessing whether an observed amount of NER is possibly bioNER

- Only an estimate, but useful to identify likely cases of type I+II
  - In accordance with current guidance
    - "For example, NER formation might be an indication of degradation only if the NER level decreases concurrently with gradual increase in mineralisation or metabolite formation."

Non-extractable residues in R.11

- Remarks about NER focus on avoiding NER in simulation tests
  - e.g. no stirring but shaking
  - OECD 309 instead of OECD 307 and 308
  - Exhaustive extraction schemes

- Some qualitative remarks about the possible interpretation of NER
  - Fast NER formation: parent compound entrapped
  - NER formation concurrent with CO_2 evolution: degradation
  - Difference between sterile and non-sterile matrices

- Not contradictory with scoping paper

Sediment spiking: Is there an influence?

- OECD 308: Spiking of the overlying water
- D4 sediment simulation test: Surface spiking
- Draft R.11 Guidance document: dry spiking: drying part of the sediment, spiking and mixing
- Other bulk spiking methods
  - Spiking wet sediment and mixing
  - Spiking by coated glass walls

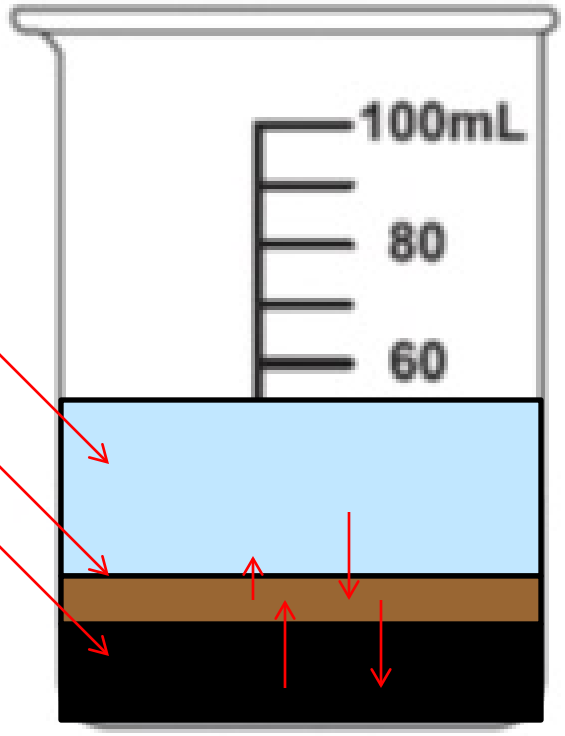
- Solvents
  - Solvents introduced in the system except for
    - Dry spiking: solvent evaporated before mixing with rest
    - Coated spiking: solvent evaporated before addition of sediment

Multi-compartment system

Water spiking

Surface sediment spiking

Bulk sediment spiking



Simulation testing in sediment/soil

- Is there an influence of concentration?

  - General assumption
    - Lower concentration -> higher degradation
      - In water
        - Inhibitory action
        - Non first-order kinetics

  - Non-linear sorption
    - Lower concentration -> higher sorption -> lower availability
      - In sediment and soil
        - Relevance for the environment?
        - Relevance for toxicity?

Fish bioaccumulation test

- Revised OECD TG 305 (2012)
  - Many new aqueous exposure tests
    - With growth correction
    - With lipid normalisation
  - Dietary test less frequently used
    - Discussion on interpretation
      - BCF calculated with estimated uptake rate constant (k_1)
        - BCF with all available models for k_1 estimates: factor 2.7
      - BMF in a comparative way ('benchmarking')
        - Benchmarking only for data obtained under similar conditions, i.e. Feeding rate, Food lipid content, Fish size and species
        - No single BMF value can be set as criterion

Developments of alternatives for OECD 305

- *In vitro* metabolism for fish
  - OECD guidelines and guidance document completed in 2018
    - OECD319A: Hepatocytes
    - OECD319B: S9
  - › BCFs more uncertain than BCFs from dietary exposure or minimised test
    - Additional step: Extrapolation from *in vitro* to overall *in vivo* degradation rate
    - Fish model also requires estimated k_1, k_2 etc.

Developments of alternatives for OECD 305

- Bioaccumulation test with *Hyalella azteca*
  - CEFIC-LRI ECO 40 (Fraunhofer)
  - Invertebrate test
  - Aqueous bioconcentration tesy
  - *In vivo* test
  - Comparable to fish?
    - Differences in metabolism (e.g. BaP)?
    - Lipid normalisation?

Air-breathing organisms and terrestrial food webs

- More emphasis on octanol-air as a measure for potential bioaccumulation
  - Log K_{oa} > 5 indicates the possibility for magnification in air-breathing and terrestrial organisms if log K_{ow} > 2
    - › Only screening values

- Toxicokinetic data for mammals
  - Laboratory studies with experimental animals
    - › Toxicokinetics for rats

- Half-life in (exposed) humans
  - Basis for B assessment of PFASs
    - › Protein binding

Conclusions

- Guidance was updated in 2017
- Most of topics identified in earlier workshops addressed in updated draft REACH guidance
- Progress on NER has been made
  - Actual guidance update postponed
- Some new topics identified, mainly on UVCBs and persistence
- Focus on persistence in REACH discussions
  - Consequence of testing order?