Cefic LRI – Concave workshop, 27. Sep. 2018, Helsinki
Poster Summary and Feedback

GREEN POST-ITS

Ideas that could support P assessments in the near term

- Methods, tools and data are available now

ORANGE POST-ITS

Ideas that could support P assessments in the future

- Validation of methods is needed

BLUE POST-ITS

Ideas that provide context that may not be directly applicable to P assessments (at least in the context of the REACH regulation)

1. *Redmann et al.*: Application of GCxGC to characterize biodegradation of crude oil using the hydrocarbon block method [12 Green, 1 Orange, 1 Blue]

- Advanced 2D gas chromatography can be applied for determining half lives of major aliphatic and aromatic chemical classes and carbon numbers in complex products. Block-wise half-lives were similar to available half-lives for representative single constituents

2. *Schäffer et al.*: Characterisation of different NER types 'NER and PBT assessment [9 G, 7 O, 2 B]

- Three NER types can be experimentally quantified: I sequestered (releasable), II covalent (hardly releasable), III biogenic. Type I NER is relevant for persistence assessment. Modelling (MTB) can be used to estimate the formation of biogenic NER.

3. *Ott et al.*: Findings from an international ring test for an improved marine biodegradation screening test [10 G, 2 O, 2 B]

- Modification of OECD 306 (more bacterial cells and longer test duration) leads to more reliable persistence assessment by inclusion of extended lag phases and better representation of bacterial diversity in environmental matrices.

4. *ECHA*: Integrated testing strategy for persistency [5 G, 1 O, 0 B]

- The updated, revised Integrated Assessment and Testing Strategy (ITS) is necessary to conclude on the persistence/non-persistence of substances (screening information → decision not P, not vP; for potential P/vP substances higher tier information is needed. The update also considers "difficult" substances (UVCB, impurities, additives, …)

5. *Hughes et al.*: Persistence assessment of phenanthrene: a case study [4 G, 3 O, 0 B]

- In contrast to Phenanthrene SVHC dossier ("is persistent"), presented OECD tests 301, 307, 308 indicate that Phe "is not persistent". Bioavailability is of similar importance for biodegradability as experimental conditions (O_2, inoculum, nutrients, …)

6. *Bonnomet et al.*: Steps needed for incorporating scientific developments into regulatory practice [2 G, 5 O, 0 B]

- Provides PBT/vPvB assessment guidance: Weight of evidence tools, difficult to test substances, use of QSAR, NER, interpretation of bioaccumulation data

7. *Nicholls et al.*: Temperature and exposure history strongly influence GEO biodegradation in groundwater [3 G, 5 O, 2 B]

- Biodegradation of gasoline ether oxygenates (GEO) like MTBE depends on temperat. (not Arrhenius-like) due to T-sensitivity of degraders and differs at uncontaminated and contamin. sites due to adaptation of degraders (gene copy numbers tested).

8. *Sjøholm et al.*: Temperature dependency of biodegradation kinetics in environmental surface waters and biodegradation testing [10 G, 2 O, 2 B]

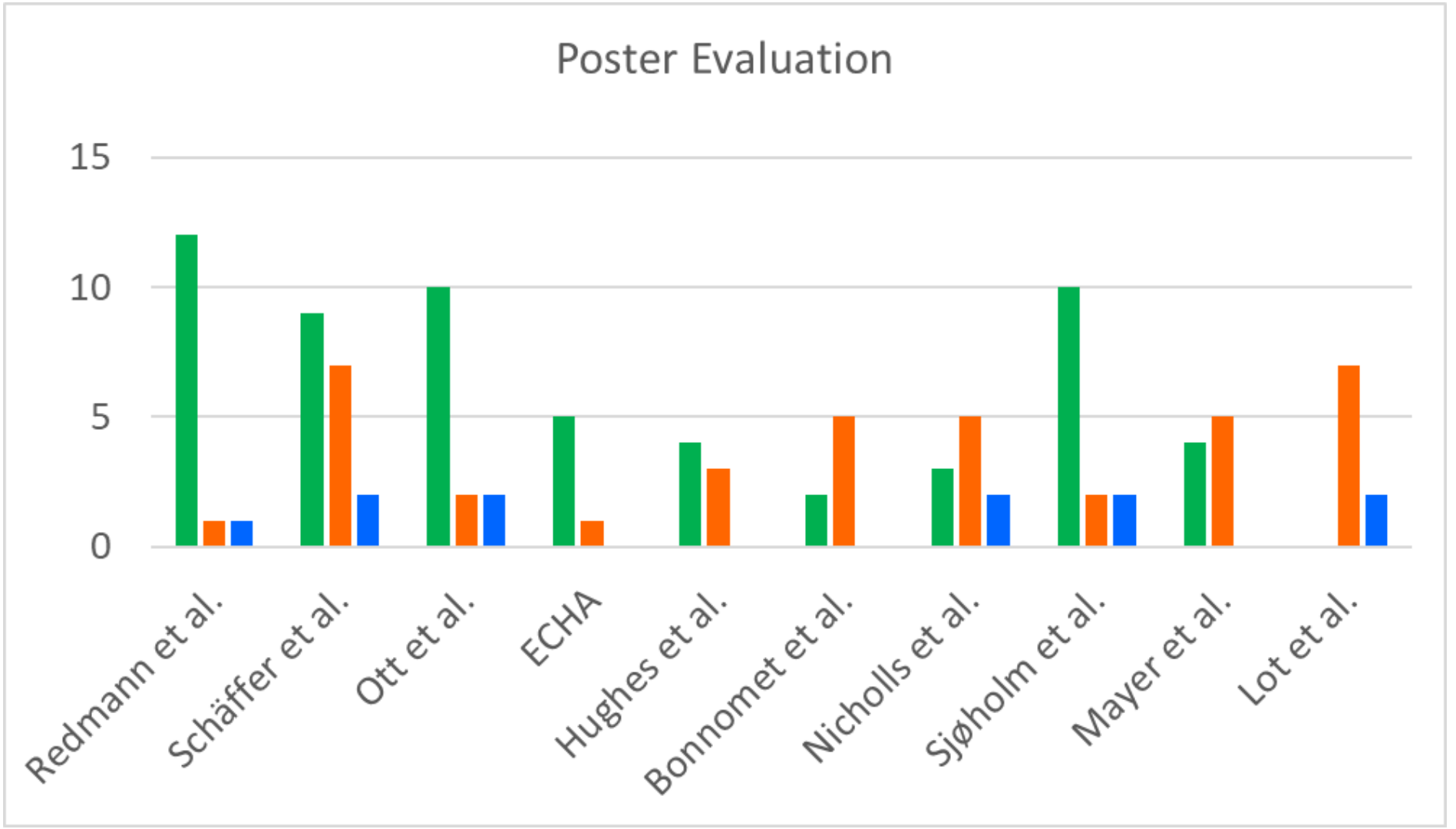
- Temperature dependence (both of test conditions and original inoculum temp.) of biodegradation kinetics for 30 chemicals and impact of test volume (# of degraders) can be tested by passive dosing.

9. *Mayer et al.*: UVCB fate-directed toxicity testing and risk assessment (UVCB-FATETOX) – Cefic LRI ECO 42 [4 G, 5 O, 0 B]

- Toxicity and bioaccumulation tests of persistent UVCB constituents can be determined by passive dosing at environmental relevant concentrations to develop an integrated risk assessment strategy of such complex products.

10. *Lot et al.*: Effluent Biodegradability Evaluation using Whole Effluent Approach [0 G, 7 O, 2 B]

- Whole waste water effluent approach: how to assess persistence (test only biodegrad. potential or representative environmental conditions)? Which inoculum? Compare effluent toxicity before and after biodegrad. rather than just testing biodegradation?


Poster Evaluation
15
10
5
0
Redmann et al.
Schäffer et al.
Ott et al.
ECHA
Hughes et al.
Bonnomet et al.
Nicholls et al.
Sjøholm et al.
Mayer et al.
Lot et al.

UVCB fate-directed toxicity testing and risk assessment (UVCB-FATETOX)

Philipp Mayer[1], Pim Leonards[2] & Matt MacLeod[3]
[1]Technical University of Denmark, Kongens Lyngby, Denmark. [2]VU University, Amsterdam, The Netherlands. [3]Stockholm University, Stockholm, Sweden.

Background

UVCBs are substances of unknown or variable composition, complex reaction products or biological materials. UVCB substances consist of many different chemical constituents, some which may be unknown.

Examples include petroleum substances, chlorinated paraffin's (CPs), flavoring agents, essential oils & their derivatives, natural oils and extractives, and biofuels.

Assessing the environmental and human risks posed by UVCB substances is a challenge currently confronting chemical industry and regulators.

Focus & Objectives

The **project focuses** at the toxicity and bioaccumulation testing of those UVCB constituents that are persistent.

The **objectives** are:

(1) to develop approaches for fate-directed ecotoxicity assessment of UVCBs based on new analytical methods, dosing methods, fate directed fractionation, toxicity testing and models

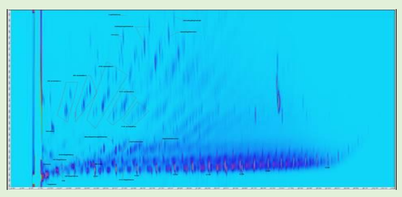
(2) to conduct a case study on selected UVCBs and develop a generic risk assessment strategy for UVCBs

(3) to cross fertilize and partially align ongoing research activities at three European research institutes.

Planned research and tasks

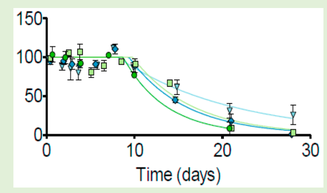
Work package 1: Combining State of the art dosing & analytical techniques with UVCB fate testing.

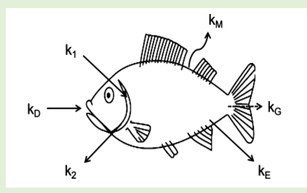
- *Passive dosing of UVCBs by polymer water partitioning and application to toxicity testing*
- *Aquatic biodegradation testing at environmental low concentrations coupled to advanced analytical instruments*
- *Bioaccumulation testing of UVCB mixtures coupled to Headspace-SPME and/or purge-and-trap*



Work package 2: Fate directed ecotoxicity and bioacumulation testing.

- *Literature review on fate-driven toxicity testing*
- *Aquatic toxicity testing with and without fate directed pre-treatment*
- *Bioaccumulation testing linked to biodegradation pre-treatment*
- *Linking toxicity to equilibrium concentrations in polymer and lipid – baseline versus excess toxicity*





Work package 3: Development of fate-directed hazard and risk assessment of UVCBs.

- *Case studies of fate-directed vPvB/PBT assessment of UVCBs*
- *Case studies of fate-directed risk assessment of UVCBs*
- *Development of an integrated risk assessment strategy for UVCBs*

Expected outcomes

Short-term: Scientific and technical progress that will support fate-directed toxicity testing of UVCBs, and a case study that will illustrate a fate-directed risk assessment methodology for selected UVCBs.

Long-term: More scientifically informed and higher quality testing of UVCBs, and thus an improved basis for future environmental risk assessment of UVCBs.

We thank CEFIC LRI and RIFM for the funding of this project (CEFIC LRI ECO 42, 2018-2020).

Persistence assessment of phenanthrene: a case study

Concawe Ecology Group
Concawe, Boulevard du Souverain 165, Brussels, Belgium
Corresponding e-mail address: chris.hughes@ricardo.com

Introduction

- Polycyclic aromatic hydrocarbons (PAH), such as phenanthrene (PHE, CAS: 85-01-8), are ubiquitous environmental contaminants with a range of natural and anthropogenic sources.
- PHE has been identified as a potential vPvB substance under REACH[1] and a public consultation launched for its inclusion on the candidate list.
- As part of REACH registration, Concawe has assessed the PBT/vPvB properties of petroleum substances and their constituents.
- This poster conducts a persistence (P) assessment of PHE according to the integrated assessment and testing strategy (ITS) detailed in ECHA guidance.[2]

Materials and methods

- Data compiled from OECD guideline (Table 1) and comparable tests covering water, soil and sediment. Non-standard test methods are described briefly below.

Table 1. Summary of OECD guideline biodegradation testing and results for PHE.

Media	Method	Result type	Result	P conclusion	Reference
Screening tests					
Freshwater	OECD 301C	BOD/ThOD (%)	67.2	Not P	Junker et al. 2016[3]
Simulation tests					
Activated sludge	OECD 314B	$DegT_{50}$ (d)	0.5	n/a	Meisterjahn et al. 2018[4]
Sediment	OECD 308, coarse sediment	$DegT_{50,system}$ (d)	116	Not P	Meisterjahn et al. 2018[4]
	OECD 308, fine sediment	$DegT_{50,system}$ (d)	116	Not P	Meisterjahn et al. 2018[4]
Soil	OECD 307	$DegT_{50}$ (d)	14	Not P	Meisterjahn et al. 2018[4]
	OECD 307	$DegT_{50}$ (d)	6.8	Not P	Meisterjahn et al. 2018[4]
	OECD 307	$DegT_{50}$ (d)	9.1	Not P	Meisterjahn et al. 2018[4]
	OECD 307	$DegT_{50}$ (d)	17.3	Not P	Meisterjahn et al. 2018[4]

Screening tests

- Water-Sediment Screening (WSST) and Soil Screening (SST) Tools developed based on the OECD 301C test method.[3] Results represent ultimate degradation (BOD/ThOD).

Simulation tests

Water

- Total of 17 biodegradation tests in environmental surface waters are available, covering a range of temperatures (5 – 22°C).[5–13]
- All tests used specific chemical analysis to measure degradation of PHE either in oil dispersions or solutions prepared by passive dosing. First order half-lives without lag phases preferred.

Sediment

- OECD 308 test using a flow-through design resulted in poor mass balance due to volatile losses. Tests run in static biometer systems with optical oxygen sensor.

Results and Discussion

Results for each compartment relative to P cut-off are presented in Figure 1.

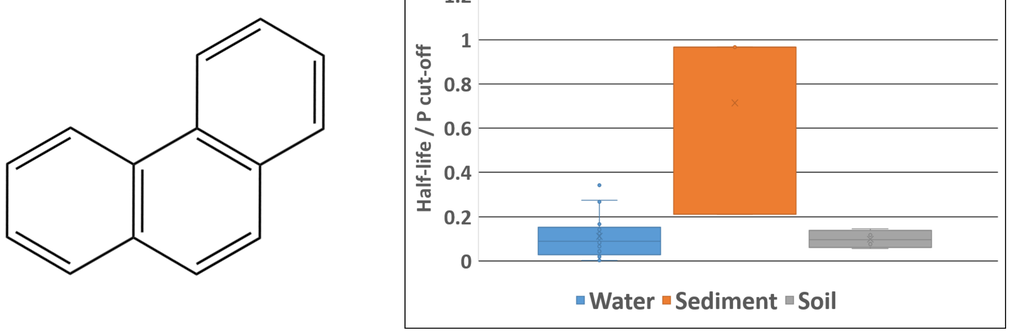


Figure 1: Structure of PHE and results of biodegradation testing relative to compartmental P cut-offs.

Screening tests

- In a ready biodegradability test (RBT), PHE achieved 67.2% mineralisation over 28 days. The 10-day window and all guideline validity criteria were met. The substance therefore meets the criteria for ready biodegradability.
- Due to the stringent nature of RBTs, positive results supersede negative results.[14]
- According to REACH Annex XIII and ECHA ITS, if a substance is readily biodegradable it is considered "not P".
- In WSST and SST tests, PHE achieved 51.3 and 39.8% mineralisation over 28-days, respectively. $DegT_{50,ultimate}$ for sediment was 25.3 days, which is below the P cut-off.[3]

Simulation tests

Water

- Measured half-lives cover a range 0.1 – 16 days, geometric mean: 2.9 days.[5–13] A correlation with temperature is not evident (Figure 2).
- Observed variability is characteristic of biodegradation experiments in general and influenced by external factors. Due to amount of data available, PHE could be a candidate for benchmarking of chemical persistence.[15,16]

Activated sludge

- Very fast degradation observed (half-life < 1 day) for tests using activated sludge as inocula.[4,5]

Sediment

- Half-lives in OECD 308 much longer than in WSST test. Likely due to differences in oxygen levels resulting from larger area at sediment-water interface and stirring of overlying water in WSST.
- OECD 308 test system likely to be particularly anoxic due to use of solvent and no flow of air in closed biometer setup.
- OECD 308 has previously been criticised as unrepresentative of environmental conditions due to high sediment:water ratio and lack of water flow velocity.[17]

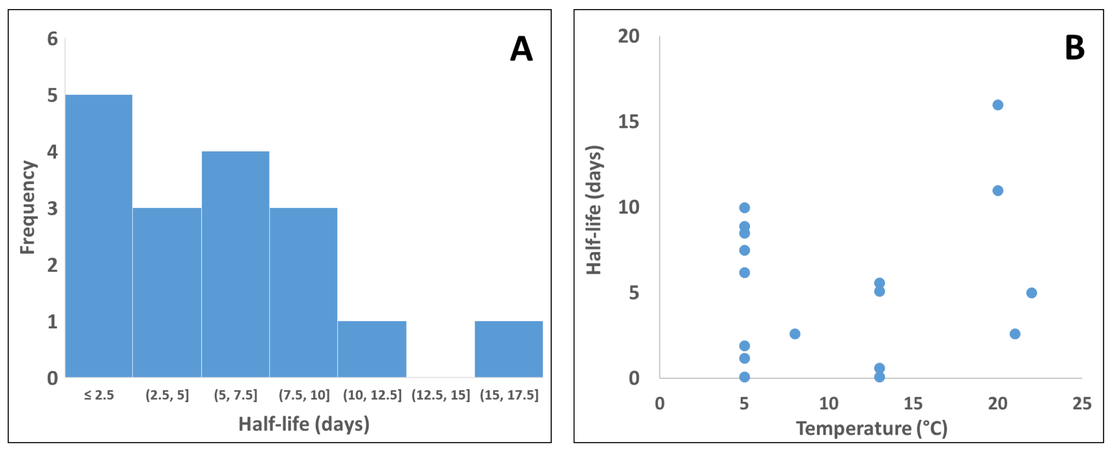


Figure 2: Distribution of experimental half-lives for phenanthrene in environmental surface waters (A) and test temperatures at which half-life was measured (B).

Soil

- In OECD 307, PHE demonstrated complete mass balance and half-lives of 6.8 – 17.3 days in four soils (geometric mean: 11.1 days), which are similar to results from aquatic exposures and comparable to those of other studies in soil.[18-20]
- Results contrast sharply with data in PHE SVHC dossier: 83-193 days and 5.7 years for laboratory and field experiments, respectively.[18,19] An important difference in these studies was that PHE was introduced via sewage sludge and may have been bound to particulate matter (e.g. soot) and not bioavailable.
- Use of archived samples in the field study may have introduced artefacts due to sample drying and storage.[21]
- Bioavailability of a substance is of similar importance to other environmental factors (e.g. nutrients, oxygen and competent degraders) in determining biodegradation rate, however is not explicitly addressed in framework of persistence assessment.
- A study on landfarming of dredged sediments was able to differentiate fractions of PAHs based on bioavailability and measure their individual degradation rates.[22]
- Soil simulation tests on PHE according to the standard information requirements, and upon which P criteria were developed, allow for consistent comparison with other substances and should be given high priority in an assessment based on weight-of-evidence.
- Substances which are not bioavailable are also not expected to pose a risk to human health or the environment. Bioavailable PHE is rapidly degraded by indigenous microbial populations.

Comments on the proposal to include PHE on the candidate list

- PHE itself is not registered under REACH and is present in low amounts (< 0.1%) in most petroleum substances.
- Incomplete combustion represents a major source of environmental exposure to PHE[23], which will not be addressed by its inclusion on the candidate list.
- Can the environmental risks posed by PHE be more effectively managed by other legislation (e.g. Water Framework Directive)?

Conclusions

- PHE is a data-rich substance, with information relevant to all environmental compartments and aspects of the ECHA persistence ITS.
- PHE is readily biodegradable, and therefore fulfils Annex XIII screening criteria for "not P".
- Simulation test data support a conclusion of "not P" for water, sediment and soil compartments.
- Aquatic half-lives demonstrate inherent variability in P data and support PHE as a candidate for benchmarking of chemical persistence.
- Standard flow-through OECD 308 sediment tests are unsuitable for PHE due to volatility. Test results heavily influenced by system dimensions and oxygen flux.
- Soil biodegradation data highlight the influence of bioavailability as an extrinsic environmental parameter.
- Consider whether environmental risks of PHE can be better addressed by other regulation.

References

1. EC 2006 – Regulation (EC) No 1907/2006 (REACH)
2. ECHA 2017 – R.11 Guidance Document (ref. ECHA-17-G-12-EN)
3. Junker et al. 2016 – Sci. Total Environ. 544 1020–1030
4. Meisterjahn et al. 2018 – Fraunhofer IME-AE, Germany (report in prep)
5. Birch et al. 2018 – Environ. Sci. Technol. 52 2143–2151
6. Brakstad et al. 2015 – Mar. Pollut. Bull. 93 144–152
7. Brakstad et al. 2018a – Mar. Pullut. Bull. 129 555–561
8. Brakstad et al. 2018b – Chemosphere 191 44–53
9. Concawe 2012 – Report no. 10/12
10. Loftus et al. 2018 – Chemosphere 206 465–473
11. Prince et al. 2008 – Chemosphere 71 1446–1451
12. Prince et al. 2013 – Chemosphere 90 521–526
13. Ribicic et al. 2018 - BMC Microbiology 18:83
14. OECD 2006 – https://doi.org/10.1787/9789264030213-en
15. ECETOC 2013 – Workshop Report No. 24
16. Zou et al. 2015 – Environ. Sci. Technol. 49 1646-1653
17. Shrestha et al. 2016 – Environ. Sci. Technol. 50 6856-6864
18. Wild et al. 1991 – Environ. Pollut. 72 141–157
19. Wild & Jones 1993 – Environ. Toxicol. Chem. 12 5–12
20. Sigmund et al. 2018 – J. Haz. Mat. 345 107–113
21. Northcott et al. 2001 – Environ. Sci. Technol. 35 1103–1110
22. Harmsen & Rietra 2018 – Chemosphere 207 229–238
23. Douben 2003 – PAHs: An Ecotoxicological Perspective – John Wiley & Sons Ltd

UVCB fate-directed toxicity testing and risk assessment (UVCB-FATETOX)

Philipp Mayer[1], Pim Leonards[2] & Matt McLeod[3]
[1]Technical University of Denmark, Kongens Lyngby, Denmark. [2]VU University, Amsterdam, The Netherlands. [3]Stockholm University, Stockholm, Sweden.

Background

UVCBs are substances of unknown or variable composition, complex reaction products or biological materials. UVCB substances consist of many different chemical constituents, some which may be unknown.

Examples include petroleum substances, chlorinated paraffin's (CPs), flavoring agents, essential oils & their derivatives, natural oils and extractives, and biofuels.

Assessing the environmental and human risks posed by UVCB substances is a challenge currently confronting chemical industry and regulators.

Focus & Objectives

The **project focuses** at the toxicity and bioaccumulation testing of those UVCB constituents that are persistent.

The **objectives** are:

(1) to develop approaches for fate-directed ecotoxicity assessment of UVCBs based on new analytical methods, dosing methods, fate directed fractionation, toxicity testing and models

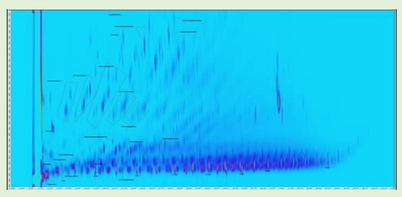
(2) to conduct a case study on selected UVCBs and develop a generic risk assessment strategy for UVCBs

(3) to cross fertilize and partially align ongoing research activities at three European research institutes.

Planned research and tasks

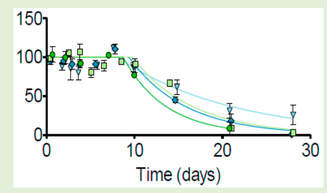
Work package 1: Combining State of the art dosing & analytical techniques with UVCB fate testing.

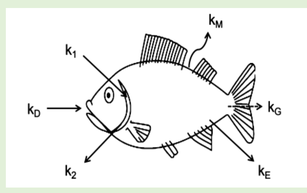
- *Passive dosing of UVCBs by polymer water partitioning and application to toxicity testing*
- *Aquatic biodegradation testing at environmental low concentrations coupled to advanced analytical instruments*
- *Bioaccumulation testing of UVCB mixtures coupled to Headspace-SPME and/or purge-and-trap*



Work package 2: Fate directed ecotoxicity and bioacumulation testing.

- *Literature review on fate-driven toxicity testing*
- *Aquatic toxicity testing with and without fate directed pre-treatment*
- *Bioaccumulation testing linked to biodegradation pre-treatment*
- *Linking toxicity to equilibrium concentrations in polymer and lipid – baseline versus excess toxicity*





Work package 3: Development of fate-directed hazard and risk assessment of UVCBs.

- *Case studies of fate-directed vPvB/PBT assessment of UVCBs*
- *Case studies of fate-directed risk assessment of UVCBs*
- *Development of an integrated risk assessment strategy for UVCBs*

Expected outcomes

Short-term: Scientific and technical progress that will support fate-directed toxicity testing of UVCBs, and a case study that will illustrate a fate-directed risk assessment methodology for selected UVCBs.

Long-term: More scientifically informed and higher quality testing of UVCBs, and thus an improved basis for future environmental risk assessment of UVCBs.

We thank CEFIC LRI for the funding of this project (CEFIC LRI ECO 42, 2018-2020).

New project:
Temperature dependency of biodegradation kinetics in environmental surface waters and biodegradation testing

Karina Knudsmark Sjøholm, Heidi Birch and Philipp Mayer
Technical University of Denmark, Lyngby, Denmark.

Background

- Extrapolation of biodegradation kinetics from laboratory experiments to environmental conditions is challenging.
- The biodegradation kinetics vary with the origin of test inoculum (Birch et al 2017) and realistic environmental temperatures deviate from standard test temperature.
- Biodegradation kinetics at different test temperatures for a large number of chemicals are needed to improve extrapolation between temperatures in risk assessment and predictive modelling.
- Increased test volumes will increase the number of degrader organisms, which in turn might shorten lag phases and increase rate constants.

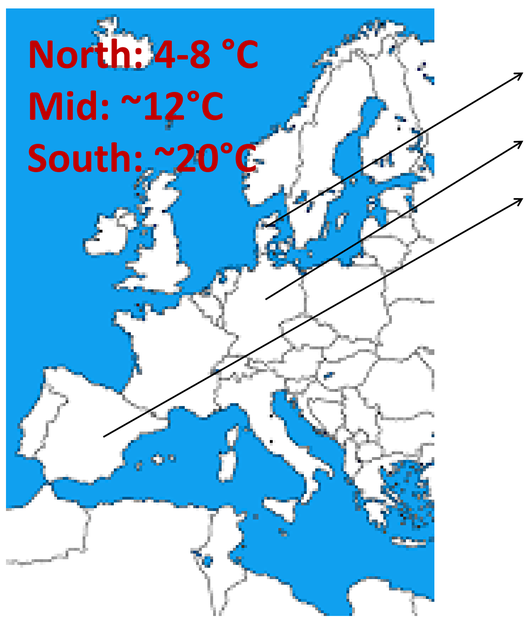
Hypothesis

1) Biodegradation kinetics depend on test temperature and inoculum origin temperature.

2) A test volume of 13,5 mL is sufficient for the biodegradation kinetic testing of most substances, but larger volumes might be necessary for some chemicals.

Methodology

European river sampling sites:



Passive dosing

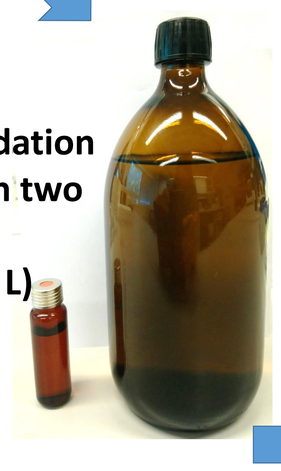
Composed mixture of 30-40 hydrocarbons will be loaded in silicone rods, and equilibrated with surface water.

Biodegradation at varying temperatures

Will be performed at site + standard test temperature

The water will be diluted (1:10) and will roll in gas-tight vials for up to 28 days. Concentration level: ng-µg/L. Azide poisoned abiotic controls. Characterization of the microbial population (DNA) is envisioned.

Test of biodegradation kinetics in two volumes (15 mL, 1 L)



Analysis

Fully automated SPME sampling will be carried out on each test system followed by GC-MS.

Birch et al 2018

Expected outcome

- Biodegradation kinetics of >30 chemicals at site and standard test temperature for 3 European rivers.
- Answers to the questions:

1) $k_{Tsite} \approx k_{Tstandard\ extrapolated\ to\ Tsite}$?
2) $k_{small\ test\ volume} \approx k_{large\ test\ volume}$?

We envision two scientific papers:

1) The temperature effects on biodegradation kinetics and biodegradation testing.

2) Comparison of the impact of (i) inoculum origin, (ii) test volume and (iii) test temperature on the obtained biodegradation kinetics.

Acknowledgement: This 2 year project is funded by Concawe

Birch, H, Hammershøj, R, Comber, M & Mayer, P (2017): Biodegradation of hydrocarbon mixtures in surface waters at environmentally relevant levels – Effect of inoculum origin on kinetics and sequence of degradation. *Chemosphere* (184) 400-407
Birch H, Hammershøj R & Mayer P (2018): Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of $K_{ow}$and K_{aw}. *Environ Sci Technol* (52) 2143-2151.

Persistence assessment of phenanthrene: a case study

Concawe Ecology Group
Concawe, Boulevard du Souverain 165, Brussels, Belgium
Corresponding e-mail address: chris.hughes@ricardo.com

Introduction

- Polycyclic aromatic hydrocarbons (PAH), such as phenanthrene (PHE, CAS: 85-01-8), are ubiquitous environmental contaminants with a range of natural and anthropogenic sources.
- PHE has been identified as a potential vPvB substance under REACH[1] and a public consultation launched for its inclusion on the candidate list.
- As part of REACH registration, Concawe has assessed the PBT/vPvB properties of petroleum substances and their constituents.
- This poster conducts a persistence (P) assessment of PHE according to the integrated assessment and testing strategy (ITS) detailed in ECHA guidance.[2]

Materials and methods

- Data compiled from OECD guideline (Table 1) and comparable tests covering water, soil and sediment. Non-standard test methods are described briefly below.

Table 1. Summary of OECD guideline biodegradation testing and results for PHE.

Media	Method	Result type	Result	P conclusion	Reference
Screening tests					
Freshwater	OECD 301C	BOD/ThOD (%)	67.2	Not P	Junker et al. 2016[3]
Simulation tests					
Activated sludge	OECD 314B	$DegT_{50}$ (d)	0.5	n/a	Meisterjahn et al. 2018[4]
Sediment	OECD 308, coarse sediment	$DegT_{50,system}$ (d)	116	Not P	Meisterjahn et al. 2018[4]
	OECD 308, fine sediment	$DegT_{50,system}$ (d)	116	Not P	Meisterjahn et al. 2018[4]
Soil	OECD 307	$DegT_{50}$ (d)	14	Not P	Meisterjahn et al. 2018[4]
	OECD 307	$DegT_{50}$ (d)	6.8	Not P	Meisterjahn et al. 2018[4]
	OECD 307	$DegT_{50}$ (d)	9.1	Not P	Meisterjahn et al. 2018[4]
	OECD 307	$DegT_{50}$ (d)	17.3	Not P	Meisterjahn et al. 2018[4]

Screening tests

- Water-Sediment Screening (WSST) and Soil Screening (SST) Tools developed based on the OECD 301C test method.[3] Results represent ultimate degradation (BOD/ThOD).

Simulation tests

Water

- Total of 17 biodegradation tests in environmental surface waters are available, covering a range of temperatures (5 – 22°C).[5–13]
- All tests used specific chemical analysis to measure degradation of PHE either in oil dispersions or solutions prepared by passive dosing. First order half-lives without lag phases preferred.

Sediment

- OECD 308 test using a flow-through design resulted in poor mass balance due to volatile losses. Tests run in static biometer systems with optical oxygen sensor.

Results and Discussion

Results for each compartment relative to P cut-off are presented in Figure 1.

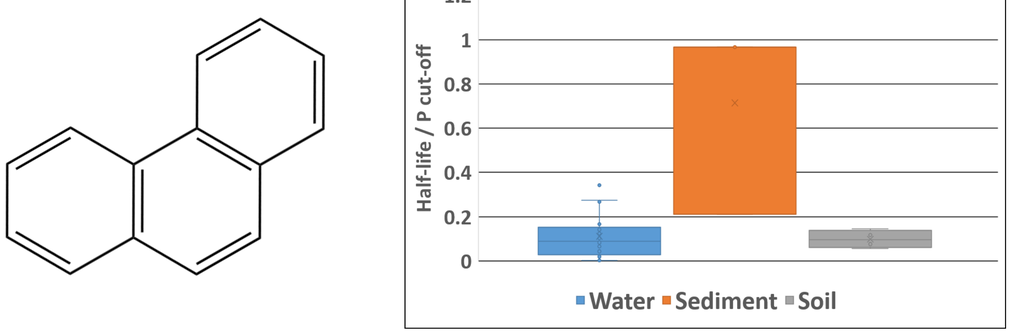


Figure 1: Structure of PHE and results of biodegradation testing relative to compartmental P cut-offs.

Screening tests

- In a ready biodegradability test (RBT), PHE achieved 67.2% mineralisation over 28 days. The 10-day window and all guideline validity criteria were met. The substance therefore meets the criteria for ready biodegradability.
- Due to the stringent nature of RBTs, positive results supersede negative results.[14]
- According to REACH Annex XIII and ECHA ITS, if a substance is readily biodegradable it is considered "not P".
- In WSST and SST tests, PHE achieved 51.3 and 39.8% mineralisation over 28-days, respectively. $DegT_{50,ultimate}$ for sediment was 25.3 days, which is below the P cut-off.[3]

Simulation tests

Water

- Measured half-lives cover a range 0.1 – 16 days, geometric mean: 2.9 days.[5–13] A correlation with temperature is not evident (Figure 2).
- Observed variability is characteristic of biodegradation experiments in general and influenced by external factors. Due to amount of data available, PHE could be a candidate for benchmarking of chemical persistence.[15,16]

Activated sludge

- Very fast degradation observed (half-life < 1 day) for tests using activated sludge as inocula.[4,5]

Sediment

- Half-lives in OECD 308 much longer than in WSST test. Likely due to differences in oxygen levels resulting from larger area at sediment-water interface and stirring of overlying water in WSST.
- OECD 308 test system likely to be particularly anoxic due to use of solvent and no flow of air in closed biometer setup.
- OECD 308 has previously been criticised as unrepresentative of environmental conditions due to high sediment:water ratio and lack of water flow velocity.[17]

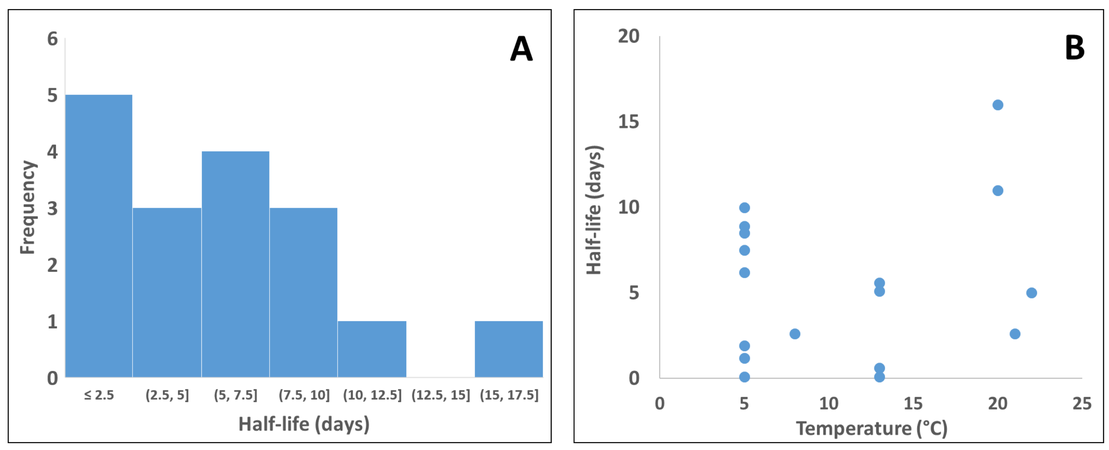


Figure 2: Distribution of experimental half-lives for phenanthrene in environmental surface waters (A) and test temperatures at which half-life was measured (B).

Soil

- In OECD 307, PHE demonstrated complete mass balance and half-lives of 6.8 – 17.3 days in four soils (geometric mean: 11.1 days), which are similar to results from aquatic exposures and comparable to those of other studies in soil.[18-20]
- Results contrast sharply with data in PHE SVHC dossier: 83-193 days and 5.7 years for laboratory and field experiments, respectively.[18,19] An important difference in these studies was that PHE was introduced via sewage sludge and may have been bound to particulate matter (e.g. soot) and not bioavailable.
- Use of archived samples in the field study may have introduced artefacts due to sample drying and storage.[21]
- Bioavailability of a substance is of similar importance to other environmental factors (e.g. nutrients, oxygen and competent degraders) in determining biodegradation rate, however is not explicitly addressed in framework of persistence assessment.
- A study on landfarming of dredged sediments was able to differentiate fractions of PAHs based on bioavailability and measure their individual degradation rates.[22]
- Soil simulation tests on PHE according to the standard information requirements, and upon which P criteria were developed, allow for consistent comparison with other substances and should be given high priority in an assessment based on weight-of-evidence.
- Substances which are not bioavailable are also not expected to pose a risk to human health or the environment. Bioavailable PHE is rapidly degraded by indigenous microbial populations.

Comments on the proposal to include PHE on the candidate list

- PHE itself is not registered under REACH and is present in low amounts (< 0.1%) in most petroleum substances.
- Incomplete combustion represents a major source of environmental exposure to PHE[23], which will not be addressed by its inclusion on the candidate list.
- Can the environmental risks posed by PHE be more effectively managed by other legislation (e.g. Water Framework Directive)?

Conclusions

- PHE is a data-rich substance, with information relevant to all environmental compartments and aspects of the ECHA persistence ITS.
- PHE is readily biodegradable, and therefore fulfils Annex XIII screening criteria for "not P".
- Simulation test data support a conclusion of "not P" for water, sediment and soil compartments.
- Aquatic half-lives demonstrate inherent variability in P data and support PHE as a candidate for benchmarking of chemical persistence.
- Standard flow-through OECD 308 sediment tests are unsuitable for PHE due to volatility. Test results heavily influenced by system dimensions and oxygen flux.
- Soil biodegradation data highlight the influence of bioavailability as an extrinsic environmental parameter.
- Consider whether environmental risks of PHE can be better addressed by other regulation.

References

1. EC 2006 – Regulation (EC) No 1907/2006 (REACH)
2. ECHA 2017 – R.11 Guidance Document (ref. ECHA-17-G-12-EN)
3. Junker et al. 2016 – Sci. Total Environ. 544 1020–1030
4. Meisterjahn et al. 2018 – Fraunhofer IME-AE, Germany (report in prep)
5. Birch et al. 2018 – Environ. Sci. Technol. 52 2143–2151
6. Brakstad et al. 2015 – Mar. Pollut. Bull. 93 144–152
7. Brakstad et al. 2018a – Mar. Pullut. Bull. 129 555–561
8. Brakstad et al. 2018b – Chemosphere 191 44–53
9. Concawe 2012 – Report no. 10/12
10. Loftus et al. 2018 – Chemosphere 206 465–473
11. Prince et al. 2008 – Chemosphere 71 1446–1451
12. Prince et al. 2013 – Chemosphere 90 521–526
13. Ribicic et al. 2018 - BMC Microbiology 18:83
14. OECD 2006 – https://doi.org/10.1787/9789264030213-en
15. ECETOC 2013 – Workshop Report No. 24
16. Zou et al. 2015 – Environ. Sci. Technol. 49 1646-1653
17. Shrestha et al. 2016 – Environ. Sci. Technol. 50 6856-6864
18. Wild et al. 1991 – Environ. Pollut. 72 141–157
19. Wild & Jones 1993 – Environ. Toxicol. Chem. 12 5–12
20. Sigmund et al. 2018 – J. Haz. Mat. 345 107–113
21. Northcott et al. 2001 – Environ. Sci. Technol. 35 1103–1110
22. Harmsen & Rietra 2018 – Chemosphere 207 229–238
23. Douben 2003 – PAHs: An Ecotoxicological Perspective – John Wiley & Sons Ltd

Findings from an international ring test for an improved marine biodegradation screening test

Amelie Ott[1] (a.i.g.ott2@ncl.ac.uk), Tim Martin[1], Graham Whale[2], Bob Rowles[3], Nik Robinson[4], Ian Still[4], Jason Snape[1,5], Bruno Hubesch[6], Russell Davenport[1]

1 Newcastle University, Newcastle-upon-Tyne, UK, 2 Shell Health, Shell Centre, London, UK, 3 Centre for Environment, Fisheries and Aquaculture Science (Cefas), Lowestoft, UK, 4 European Oilfield Speciality Chemicals Association (EOSCA), Aberdeen, UK, 5 AstraZeneca Global Environment, Alderley Park, UK, 6 European Chemical Industry Council (Cefic), Brussels, Belgium

Bringing the OECD 306 test in the 21st century

1992 *2018*

1992 — **OECD 306 "Biodegradability in Seawater"**

The OECD 306 is a standard biodegradation screening test (BST) for assessing biodegradation of chemicals in the marine environment[1]. It is part of a series of standardised BSTs, developed to measure the relative biodegradability of chemical compounds.

2007 — **EU chemicals Regulation REACH enters into force**

- Philosophical shift in regulatory emphasis from measuring biodegradation to assessing persistence;
- BSTs exhibit high levels of variation[4-2];
- 20-80% of BST fails may be considered false negatives[2,4], potentially requiring further persistence, bioaccumulation and toxicity (PBT) testing (←→ 3R's principle for humane animal research[5]);
- Effective persistence assessments may save upwards of 600 fish and $75K per chemical reliably screened out earlier in risk assessment process[6];

2009-11 — **Cefic LRI ECO11 project**

2015 — **Cefic-LRI workshop on the improvement of the OECD 306 screening test**

Based on previous regulatory recommendations[7], research findings (Cefic LRI ECO11[8]) and discussions from a Cefic-LRI workshop with industry, regulatory bodies and academia[9], two key methodological modifications to potentially improve the marine BST (OECD 306) were high lighted:

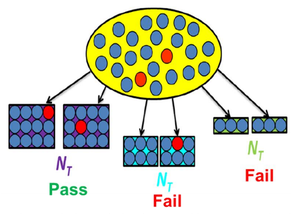


Fig. 1: Biodegradation lottery with N_T = sample size

- Increasing bacterial cell concentrations to better represent the bacterial diversity inherent in the sampled environments (Fig. 1);
- Increasing test durations to investigate extended lag phases observed in marine assessments;

Evaluating the improved method across 13 laboratories

2016 — **Ring test protocol development**

- Ring test compared 3 marine biodegradation tests (Fig. 2);
- Organising committee consisted of stakeholders from academia, industry and regulatory bodies;
- Ring test coordination followed SOLNA/ OECD 34 principles[10];
- SOPs developed in close collaboration with multiple contract research organisations (CROs) and regulatory bodies;
- Several review and discussion papers composed for method selection decision:

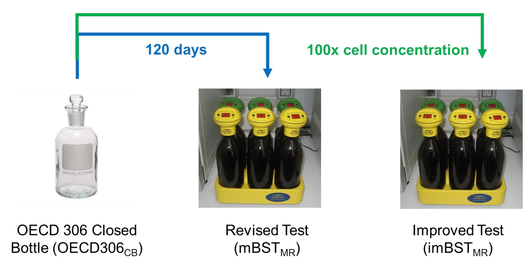


Fig. 2: Test set-ups for the ring test

- Use of manometric respirometers for revised and improved test; similar to OECD 301F;
  + less water required for test set-up,
  + headspace provides more O_2;
- Tangential flow filtration (TFF**)** to increase bacterial cell concentrations in seawater; previously optimised in ECO11 (Fig. 3);
- Comparison of test set-ups using 5 reference compounds with range of biodegradation potentials (Fig. 4);

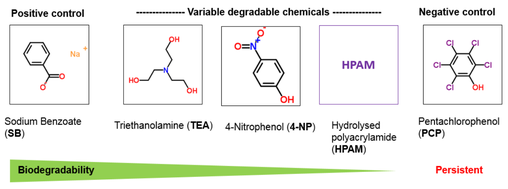


Fig. 3: Reference compounds assessed in the ring test

Fig. 4: TFF set up to concentrate bacteria in seawater

2017 — **Ring test across 13 CROs in Norway, UK, Germany, Italy, Canada, USA and Japan**

Results

Bacteria concentrations (flow cytometry)

- Raw seawater bacteria concentrations varied highly from 10^3 to 10^6 total cells/mL (Fig. 5)
- Highest total cell concentrations in raw seawater = lowest total cell concentrations in 100-fold concentrated seawater

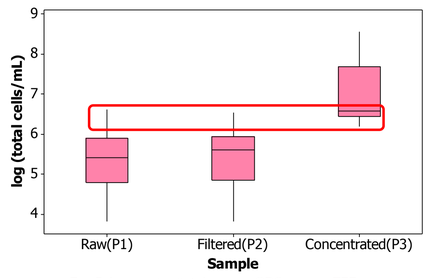


Fig. 5: Log total cells/mL across CROs where TFF was applied

Biodegradation plots

- Long lag phases were followed by rapid degradation (Fig. 6);
- Longer test durations provided a more reliable persistence assessment for variable compounds (Fig. 7, 8);

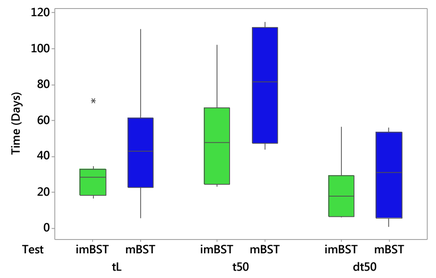


Fig. 6: Boxplot showing t_L (time to 10% degradation), t50 (time to 50% degradation) and dt50 (t50-t_L) for TEA across all CROs

Comparison of the 3 tests

- Improved test correctly characterised 69% of replicates based on REACH non-persistency criteria t50<60 days (Tab. 1);
- Standard closed bottle test correctly characterised 39% of replicates based on OECD 306 pass criteria (Tab. 1);

Microbial community analysis (DNA sequencing)

- Concentrated samples represented well the microbial communities in raw seawater (Fig. 9);
- Microbial composition varied significantly between locations (Fig. 9);
- Source community composition appears to be more important than total number of cells for delivering a reliable screening test

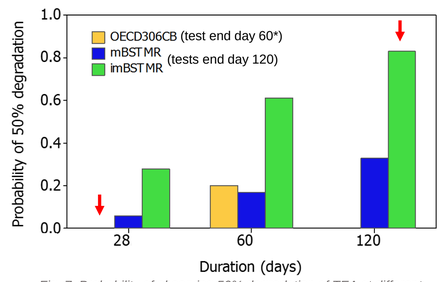


Fig. 7: Probability of observing 50% degradation of TEA at different days for three test set ups across all CROs; probability is based on frequency of observed degradation across all CROs;

*OECD306$_{CB}$ extended beyond 28 days for one measurement at day 60 following par. 14 OECD 306

Tab. 1: Comparison between test systems showing likelihood of fulfilling the relevant degradation criteria in replicates

	imBSTMR	mBSTMR	OECD306CB
OECD 306 Closed Bottle 60% in 28 days for rapid biodegradation	51%	44%	39%
REACH t50 < 60 days for non persistency	69%	51%	45%*
Proposed threshold dt50 < 40 days for non persistency	73%	54%	44%*

2018 — **Cefic-LRI workshop agrees to put improved method forward as a new test guideline for persistence assessment**

The improved test under REACH

Let's discuss:

- How does the improved test fit into REACH considering the recent change in REACH R.7b v4 to remove the principle of "increasing the biomass concentration" as an improvement for enhanced biodegradation screening tests?
- How can we correctly define and interpret lag phases?
- Should we consider a test design including multiple environmental sources for a more reliable chemical assessment?
- Should we consider a toolbox approach for marine biodegradation testing (cf. OECD 301 method)?

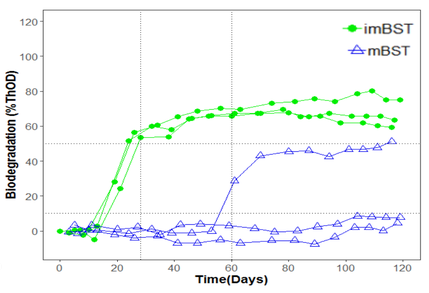


Fig. 8: TEA degradation over time in imBST$_{MR}$ and mBST$_{MR}$ tests at one CRO (three replicates per test)

New guideline

Ring test findings in a nutshell

- Better persistency assessment with longer test duration
- Successful cell concentration with tangential flow filtration
- Cell concentration did not significantly change microbial composition within sampling location
- Improved test is more reliable than OECD 306 test
- Improved test is less variable than OECD 306 test

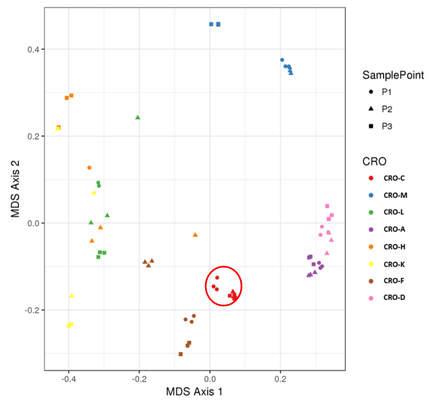


Fig. 9: Multidimensional scaling plot showing similarity between CRO microbial communities based on Bray-Curtis resemblance at genus level

Reference: **1.** OECD, Guidelines for the testing of chemicals, section 3, degradation and accumulation. Test No. 306: Biodegradability in seawater. 1992. **2.** Martin et al., Environmentally relevant inocula concentrations improve the reliability of persistent assessments in biodegradation screening tests. Environ. Sci. Technol. 2017, 51, (5), 3065-3073. **3.** ECETOC Persistence of chemicals in the environment. Workshop Report No. 90; 2003. **4.** ECETOC, Workshop on biodegradation and persistence. Workshop Report No. 10. 2007. **5.** NC3Rs National Centre for the Replacement Refinement and Reduction of Animals in Research. http://www.nc3rs.org.uk/the-3rs (16/12/2015). **6.** Martin et al., A high-throughput biodegradation screening test to prioritise and evaluate chemical biodegradability. Environ. Sci. Technol. 2017. **7.** ECHA, Guidance on information requirements and chemical safety assessment, chapter R.7b: endpoint specific guidance, version 3. 2016. **8.** CEFIC-LRi ECO11-UNEW: Towards rationally designed hazard, risk and persistency assessment: Putting the "bio" back into biodegradability tests. http://cefic-lri.org/projects/eco11-towards-rationally-designed-hazard-risk-and-persistency-assessment-putting-the-bio-back-into-biodegradability-tests/. **9.** ECETOC Improvement of the OECD 306 screening test. Workshop Report No. 34.; 2017. **10.** OECD, Series on testing and assessment, number 34, guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. 2005.

This work has been funded by Cefic-LRI and EOSCA

Temperature and exposure history strongly influence GEO biodegradation in groundwater

Henry Nicholls[1*], Emma Mallinson[1], Stephen Rolfe[2], Steven Thornton[1]

[1] Groundwater Protection & Restoration Group, University of Sheffield, United Kingdom

[2] Animal & Plant Sciences, University of Sheffield, United Kingdom

*Contact email: h.nicholls@sheffield.ac.uk

Introduction

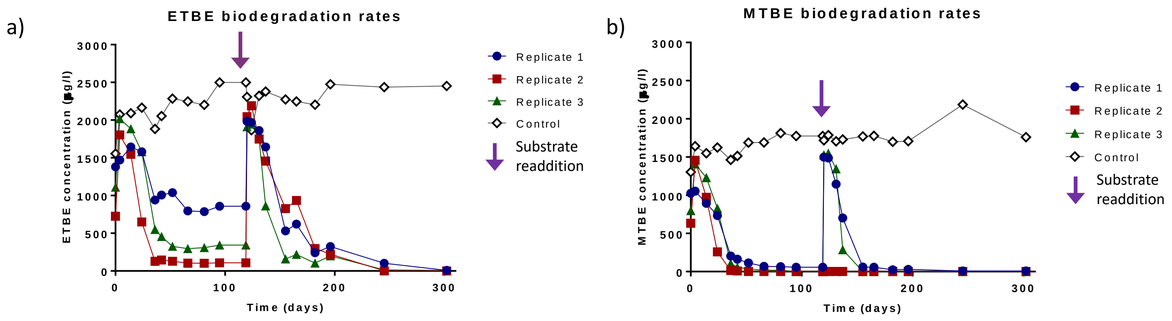
Gasoline Ether Oxygenates (GEOs) are used to increase the octane content of gasoline, improve combustion and reduce vehicle emissions. Several GEOs are used in European fuel blending, including methyl *tert*-butyl ether (MTBE), *tert*-amyl methyl ether (TAME) and ethyl *tert*-butyl ether (ETBE). In this study the biodegradation of GEOs in groundwater was investigated in: i) laboratory microcosm experiments incubated at *in situ* groundwater temperature (12°C) and laboratory room temperature (20°C), to assess the effect of temperature on biodegradation rates and examine the validity of the Arrhenius equation often used to estimate the effect of temperature on biodegradation rates, and ii) microcosms constructed with aquifer material and groundwater sampled from a dissolved contaminant plume containing GEO and upgradient of the GEO plume, to assess the effect of exposure history on the microbial community and GEO biodegradation rates. Dynamic responses in the microbial community during the incubation period were assessed using qRTPCR to measure changes in copy numbers of known MTBE and ETBE degrading genes (*mdp*A, *eth*B respectively[1,2]), and work is ongoing to profile the microbial community using 16S-rRNA sequencing. Isolates from the microcosms will be investigated to determine their biodegradative capabilities under different conditions (e.g. availability and concentration of GEO substrates, temperature, nutrient status).

Results

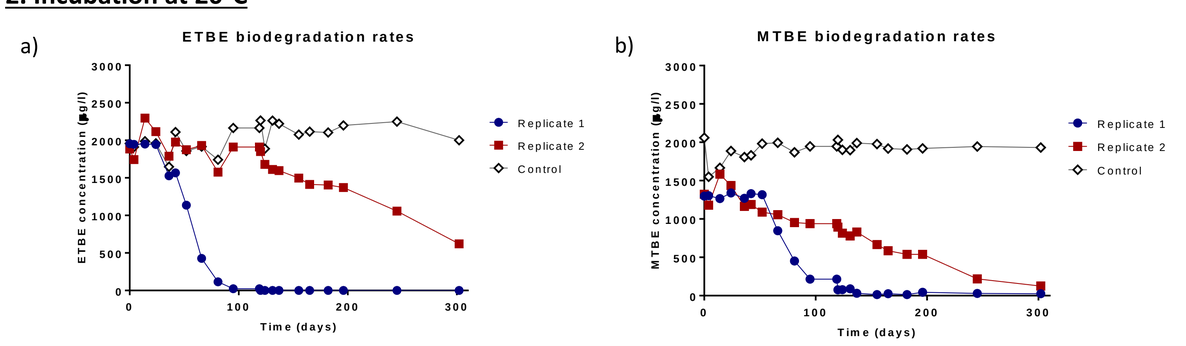
1 – Effect of temperature on GEO biodegradation

The effect of temperature was assessed using microcosms composed of uncontaminated aquifer material inoculated with GEO-impacted groundwater (from a site with a historical release of MTBE and TAME). ETBE and MTBE were added and the microcosms incubated at *in situ* aquifer (12°C) or room temperature (20°C).

1. Incubation at 12°C



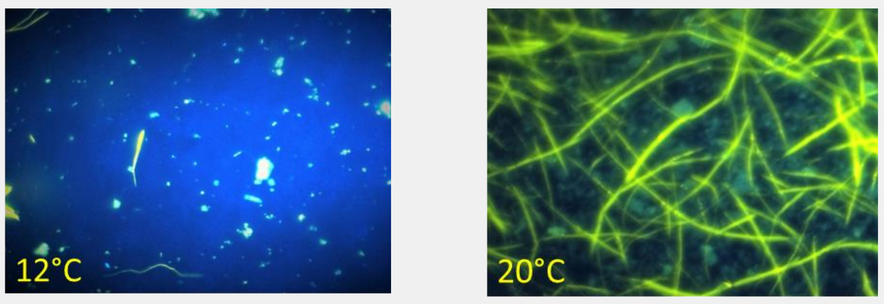
2. Incubation at 20°C



3. Detection of GEO degrading genes by PCR

12°C microcosm (Replicate)	ETBE *eth*B (copy number/g sed)	MTBE *mdp*A (copy number/g sed)	20°C microcosm (Replicate)	ETBE *eth*B (copy number/g sed)	MTBE *mdp*A (copy number/g sed)
1	✓ ($4.7x10^3$)	✓ ($6.6x10^4$)	1	✓ ($2.1x10^3$)	×
2	✓ ($1.4x10^4$)	✓ ($2.3x10^5$)	2	×	×
3	✓ ($1.9x10^3$)	✓ ($7.6x10^2$)			

4. Morphology of microorganisms in microcosms

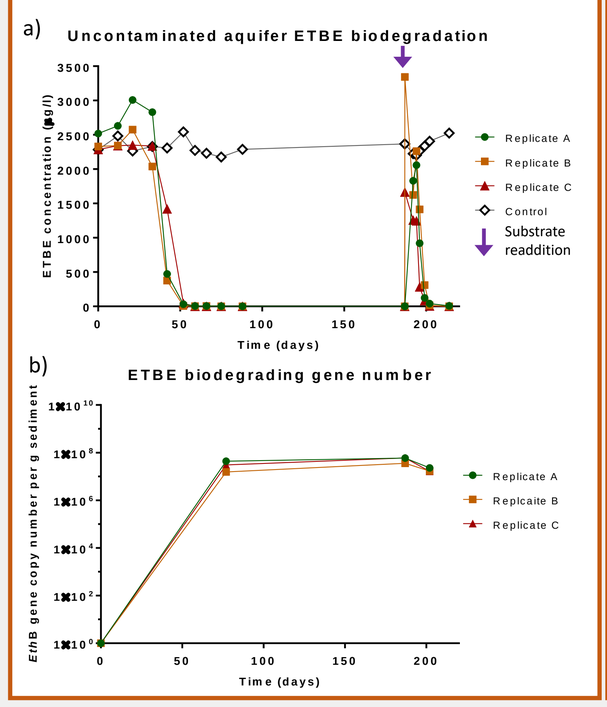


- At 12°C, ETBE and MTBE were degraded relatively rapidly in all microcosms (1a, b), whereas at 20°C degradation was slower (2a, b)
- At 12°C the *eth*B gene was detected in all cases where rapid ETBE degradation occurred, whereas at 20°C *eth*B was not detected in microcosm 2 where ETBE degradation was slow (section 3 table)
- The morphology of microorganisms at 12°C and 20°C were different (section 4)
- Microbial communities will be adapted to different temperatures and a change in temperature will alter community activity (and therefore community function), often in an unpredictable manner – indicated by results above

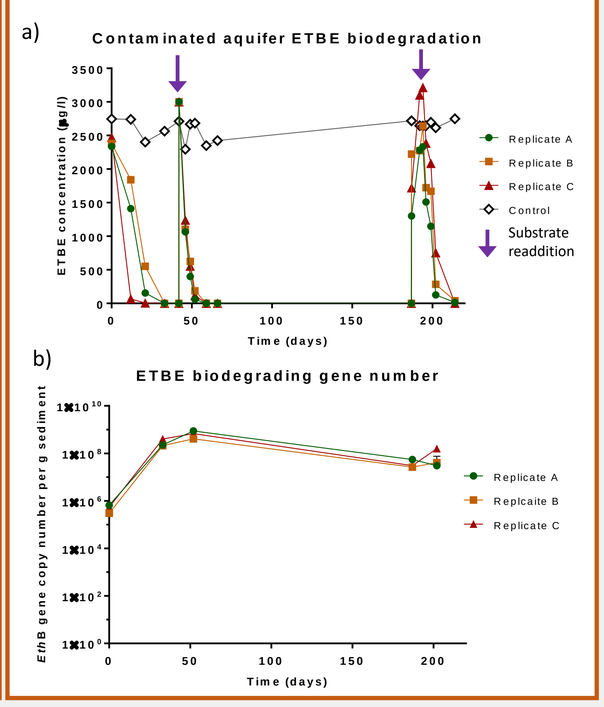
2 – Adaptation of ETBE biodegrading microorganisms

The timescale for ETBE biodegradation related to exposure of aquifer microorganisms to ETBE was assessed in microcosms containing aquifer material and groundwater from uncontaminated (upgradient of source) and contaminated (ETBE plume) locations at a site with a historical ETBE release.

1. Uncontaminated aquifer



2. Contaminated aquifer



- The microcosms established with uncontaminated groundwater responded slowly to ETBE, with a lag of approximately 40 days (1a), whereas ETBE was biodegraded without a lag in groundwater previously exposed to this GEO (2a)
- The presence of ETBE degrading genes (*eth*B) was measured. These genes were not detectable initially in the uncontaminated microcosms, but increased rapidly to 10^7 copies per g after exposure to ETBE (1b)
- An increase in the *eth*B gene was also observed in contaminated microcosms, starting at 10^5 and increasing to 10^8 copies per g (2b)
- ETBE biodegraders (as evidenced by detection of genes) remained within the community, even after a long period (100+ days) with no ETBE available, which corresponded with subsequent rapid degradation in both series of microcosms

Conclusions

- Temperature had a profound effect on GEO degradation rate and *eth*B and *mdp*A gene copy numbers, which may be due to temperature sensitivity of the dominant GEO degraders
- The use of the Arrhenius equation to estimate biodegradation rates at different temperatures is not justified by the results from this study
- A longer lag was observed for the background (uncontaminated) aquifer but the biodegradation rate for both pre-exposed and background locations was similar when biodegradation started, reflecting adaptation of the microbial community to ETBE, most likely the development of a viable population of ETBE-degraders
- The microbial community retained the ability to biodegrade ETBE, even after prolonged absence of ETBE, implying a robust population of ETBE-degraders

References

1. Schmidt, R. et al., 2008. Involvement of a novel enzyme, MdpA, in methyl tert-butyl ether degradation in Methylibium petroleiphilum PM1. Applied and Environmental Microbiology, 74(21), pp.6631–6638.

2. Chauvaux, S. et al., 2001. Cloning of a genetically unstable cytochrome P-450 gene cluster involved in degradation of the pollutant ethyl tert-butyl ether by Rhodococcus ruber. Journal of Bacteriology, 183(22), pp.6551–6557.

GPRG Groundwater Protection & Restoration Group

The University Of Sheffield.

Concawe

Effluent Biodegradability Evaluation using Whole Effluent Approach

Marie-Claire LOT[1], Paul THOMAS[2], Caroline CROUZY[2], Patrick BALDONI-ANDREY[1], Clémentine GELBER[1]
[1]TOTAL SA, Pôle d'Étude et de Recherche de Lacq Pôle Économique 2 – BP 47 64170 Lacq – France
[2]CEHTRA SAS, 23 rue du Creuzat, 38080 L'Isle d'Abeau - FRANCE

PERL Séparations Physico-chimie Environnement

CEHTRA

Context

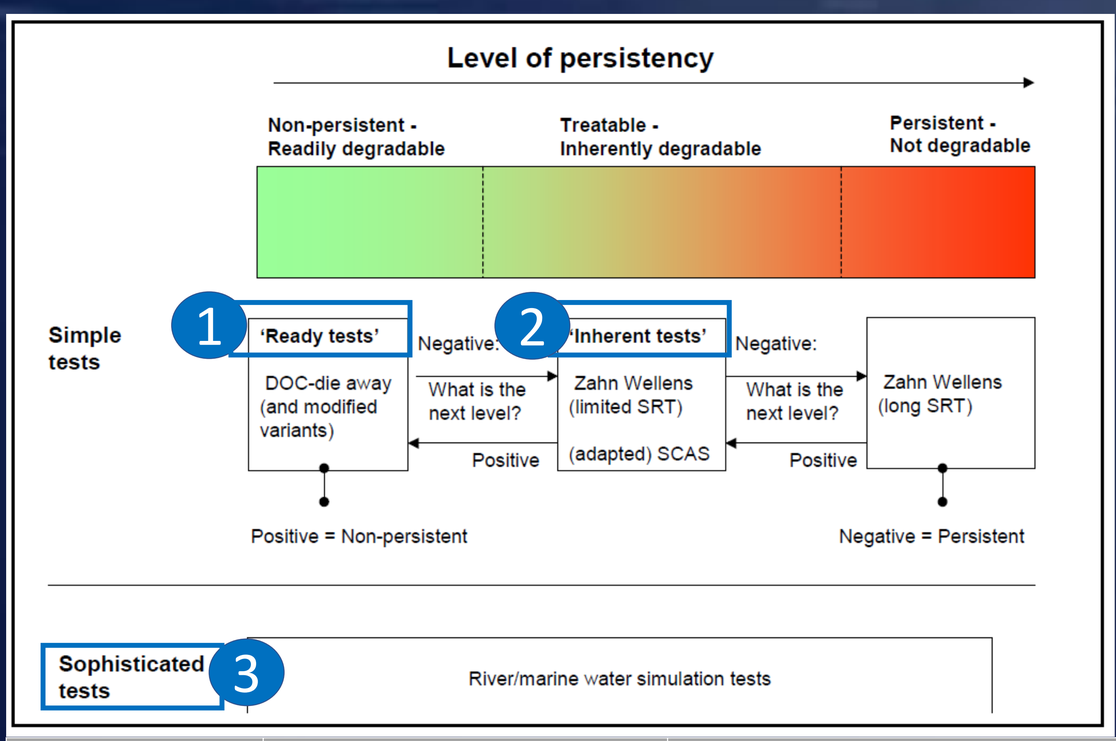
- ✓ For the management of Produced Water discharges from offshore installations, the Oslo and Paris Commission (OSPAR) Recommendation 2012/5[1] includes the **Whole Effluent Assessment (WEA)** in its **Risk-Based Approach (RBA)**.
- ✓ WEA approach improves the understanding of the combined effects of substances in complex mixtures like effluents. The advantage of WEA approach compared to Whole Effluent Toxicity (WET) approach is that WEA approach gives more information on the fate of the effluent components with the characterization of **P (Persistency)** and **B (Bioaccumulation) criteria.**
- ✓ A **WEA practical guidance document[2]** from OSPAR Commission includes some **persistency tests** (table 2) **but without giving any instruction about their interpretation.**

Existing methods & Discussion

	Ready Biodegradability	Inherent Biodegradability	Simulation tests
Aim	To determine if rapid mineralisation in wasterwater treatment plant (WWTP) and environment is likely to happen	To demonstrate potential of toxicants to degrade in the technosphere **(and not in the environment)**	To demonstrate the potential for toxicants to degrade in specific environmental compartments and simulate « environmentally realistic » situations (in terms of toxicants concentrations and environmental media employed)
Characteristics	• Low inoculum concentrations • Do not allow use of sludge preadapted to the test material • Do not allow significant time for adaptation of sludge to the test material (28 day test duration) • Must respect stringent criteria (level of biodegradation and 10-d window)	• High inoculum concentrations similar to WWTP • Study duration allows adaptation to test material	• Low inoculum concentrations and not adapted to test material • Degradation rate generally follows first order kinetics allowing determination of a half-life • Must respect stringent half-life criteria
Standards	OECD 301 Series A to F OECD 310	OECD 302 (A, B and C)	OECD 303A (simulation in WWTP) OECD 306 OECD 309 ISO 10708

Drawbacks for mixtures	• Tests designed for individual substances. Not clear how the tests can be applied to complex mixtures.
	• Unknown substances in mixtures → issues calculating biodegradation rate
	  • CO_2 production and O_2 consumption cannot be used for theoretical calculation
	  • Chemical Oxygen Demand (COD) and Dissolved Organic Carbon (DOC) or Total Organic Carbon must be used
	• No data interpretation or classification for mixtures
	• Biodegradation results do not indicate whether some substances remaining in the mixture are potentially persistent → need for complementary chemical analysis		

Table 1 : Existing standard persistency/biodegradation tests (WWTP : WasterWater Treatment Plant)



DOC-die away	Zahn Wellens	Biodegradability in seawater
OECD 301 A OECD 301 E	OECD 302B, DIN EN 29888 ISO 9888	OECD 306, ISO 16221

Table 2 : Recommended persistency/biodegradation tests in WEA guidance document (OSPAR Commission, 2007) ; *1 → 3 : chronology of persistency tests*

Conclusion

- **No biodegradation method completely adapted to test persistency mixtures**
- **Some questions are not addressed in WEA guidance and remain open as :**
  - ✓ **What kind of biodegradation test do we need? A biodegradation test potential? A biodegradation measurement representative of environmental conditions?**
  - ✓ **Which kind of inoculum do we have to use? Should it be adapted to toxicants? Which ratio between toxicants and inoculum?**
  - ✓ **How to interprate effluent biodegradation results?**
- **It may be interesting to compare effluent toxicity before and after biodegradation rather than to make judgement about the biodegradability of the mixture itself. For exemple, 60% biodegradation of the mixture within 28 days is a good sign but tells you nothing about the remaining 40%.**

References:
[1] OSPAR Commission (2012). *OSPAR Recommendation 2012/5 for a risk-based approach to the Management of Produced Water Discharges from Offshore Installations (Annex 18)*
[2] OSPAR Commission (2007). *Practical Guidance Document on Whole Effluent Assessment*

TOTAL

Poster presented at Cefic-LRI-Concawe Workshop on persistency/biodegradation assessment , 27th Sept 2018, Helsinki (Finland)

Characterisation of NER types - NER and persistence assessment

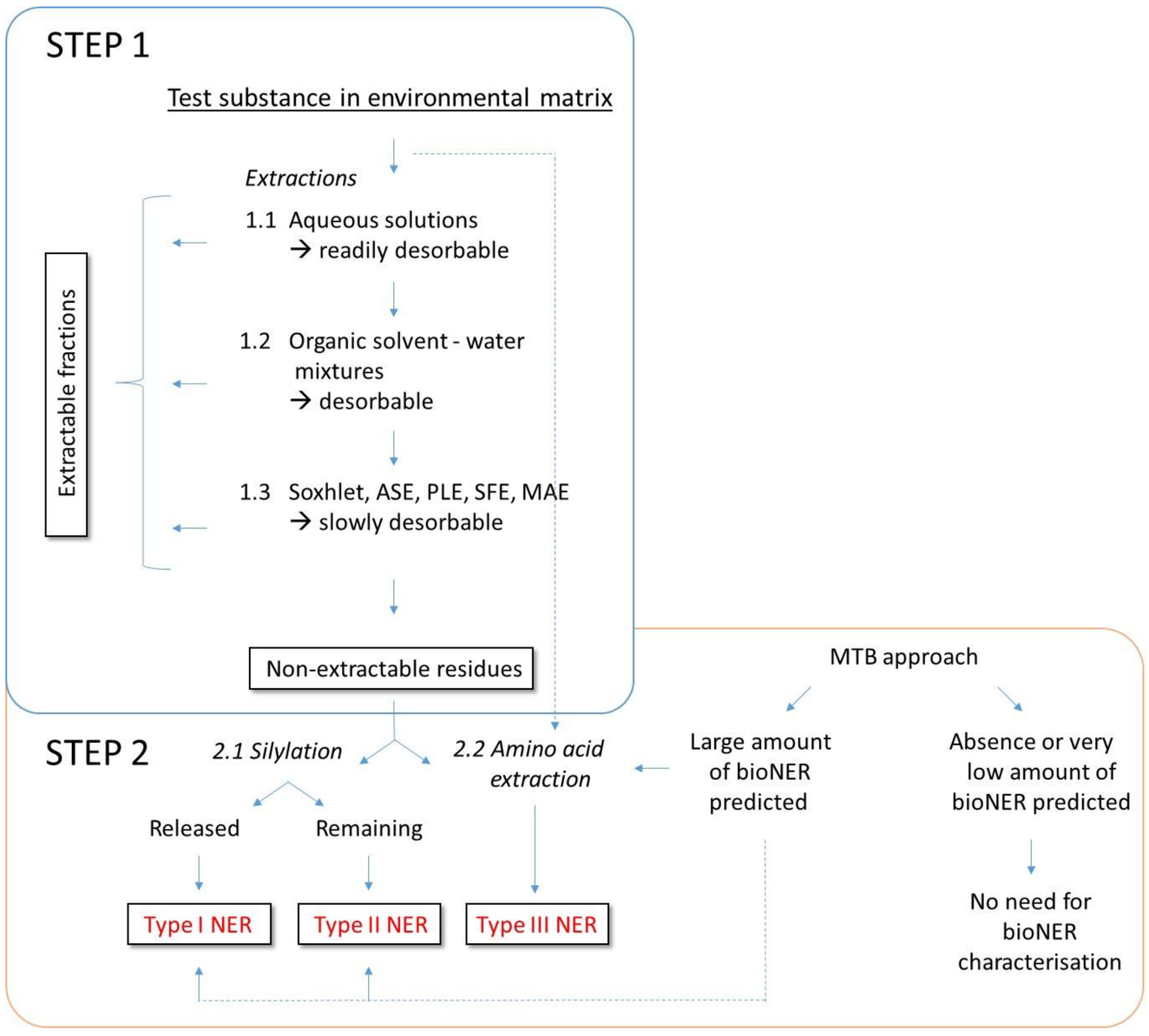
Andreas Schäffer[1], Matthias Kästner[2], Stefan Trapp[3]
[1]RWTH Aachen University, Institute for Environmental Research
[2]Helmholtz-Centre for Environmental Research – UFZ, Department of Environmental Biotechnology
[3]Technical University of Denmark, Department of Environmental Engineering

• Introduction

REACH chemicals, veterinary drugs and plant production products have to undergo a PBT assessment. According to the ECHA guidance document R.11, NER after exhaustive extraction should be differentiated in remobilisable and irreversibly bound fractions [1].
In degradation tests, non-extractable residues (NER) are always formed to a certain extent. Recent research indicated that three types of NER of chemicals in environmental matrices can be experimentally discriminated [2]: type I (sequestered, entrapped), type II (covalently bound), and type III NER (biogenic, bioNER).

• NER discrimination

NER is determined by chemical extractions (Steps 1 and 2) and can be prospectively calculated from microbial true yields, the Microbial Turnover to Biomass (MTB) model [3].



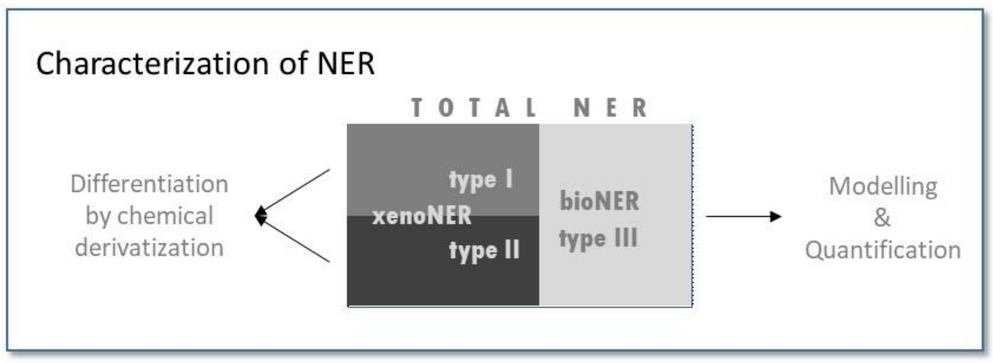
• Discussion

Potential treatments for assessment of the remobilisation potential for NER types I and II:

physical: simulation of heavy rain events, hot water extraction, freeze/thaw cycling, grinding, wet/dry cycling; chemical: pH changes, long term Tenax extraction, changes in ionic strength, hydrolysis in the presence of $Na^{18}OH$ or $H_2{}^{18}O$; biological: application of oxidative and other enzymes with release potential like peroxidases, laccases, and gluthathione-S-transferases, treatment with soil feeding organisms.

Research needs:
* Standardisation of differentiation methods;
* Further analysis of remobilisation potential;
* Further validation of bioNER modelling

• Conclusion



bioNER are of no environmental concern and, therefore, can be assessed as such in persistence assessment.

Type I NER and type II NER should be considered as potentially remobilisable residues in persistence assessment but the probability of type II release is much lower.

The total amount of NER minus bioNER should be considered as the amount of xenoNER, type I + II. If a clear differentiation of type I and type II is possible, type I NER are considered as not degraded parent substance or transformation product(s)for DT50 calculation. On the contrary, type II NER may generally be considered as (at least temporarily) removed. However, providing the proof for type II NER is the most critical issue in NER assessment and requires additional research.

If no characterisation and additional information on NER is available, it is recommended to assess the total amount as potentially remobilisable.

References
[1] ECHA_2017_R.11. Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11: Endpoint specific guidance (PBT/vPvB assessment), version 3.0, June 2017; update expected.
[2] Kästner M, Nowak KM, Miltner A, Trapp S, Schaeffer A. Classification and Modelling of Nonextractable Residues (NER) Formation of Xenobiotics in Soil - A Synthesis. Critical Reviews in Environmental Science and Technology 2014; 44: 2107-2171.
[3] Trapp S, Brock AL, Nowak K, Kästner M. Prediction of the formation of biogenic non-extractable residues during degradation of environmental chemicals from biomass yields. Environmental Science & Technology 2018; 16: 663-672.