
27 September 2018
Klaus K Hotel, Bulevardi 2/4,
Helsinki 00120 Finland
Cefic LRI – Concawe workshop
on recent developments
in science supportive to the
persistence/biodegradation assessment

08.30 – 09.00	***Registration and coffee***	
09.00 – 09.20	**Introduction by workshop co-chairs:** Graham Whale (Shell Health); Paul Van Elsacker (Federal Public Service Health, Food Chain Safety and Environment, Belgium) • Towards an improved understanding of persistence in the 21st Century • Objectives of the day	
09.20 – 09.45	**Persistence/biodegradation assessment from a regulatory point of view** Vincent Bonnomet (ECHA)	
09.45 – 11.30	**Session 1 - Role of microbial community in degradation testing (adaptation, variability, growth and cometabolism)** Moderator: Kees van Ginkel (AkzoNobel). Rapporteur: Markus Seyfried (Firmenich)	
	09.45 – 10.00	The effect of including environmentally relevant microbial diversity in biodegradation screening tests for persistence assessments – Cefic LRI ECO 11 Russell Davenport (Newcastle University)
	10.00 – 10.15	Implication of microbial adaptation for the persistency of emerging pollutants - Cefic LRI ECO 29 John Parsons (University of Amsterdam)
	10.15 – 10.30	Investigating mixture and concentration effects on biodegradation kinetics - DTU/Concawe project Rikke Hammershoj (Technical University of Denmark (DTU))
	10.30 – 10.45	Overview presentation on key issues around session theme Kees van Ginkel
	10.45 – 11.15	30 min Q&A/panel discussion Panellists: All presenters plus Anu Kapanen (ECHA) and Björn Hidding (BASF)

11.15 – 11.45	***Coffee and poster viewing***	
11.45 – 13.00	**Session 2 - Impact of environmental factors on bioavailability and degradation** Moderator: Russell Davenport. Rapporteur: John Parsons (University of Amsterdam)	
	11.45 – 12.00	Identifying strategies that will provide greater confidence in estimating the degradation rates of organic chemicals in water, soil, and sediment – Cefic LRI ECO 31 Philipp Dalkmann (Bayer AG)
	12.00 – 12.15	Environmental risk assessment of poorly soluble substances: Improved tools for assessing biodegradation, (de)sorption, and modelling – Cefic LRI ECO 32 Fabio Polesel (Technical University of Denmark (DTU))
	12.15 – 12.30	Overview presentation on key issues around session theme Russell Davenport
	12.30 – 13.00	30 min Q&A/panel discussion Panellists: All presenters plus Eric Verbruggen (RIVM)
13.00 – 14.15	***Lunch***	

14.15 – 15.45	**Session 3 - Interpretation of the OECD simulation test results and identified challenges** Moderator: Kathrin Fenner (Eawag). Rapporteur: Pippa Curtis-Jackson (UK Environment Agency)	
	14.15 – 14.30	Identifying limitations of the OECD water-sediment test and developing suitable alternatives to assess persistence - Cefic LRI ECO 18 Kathrin Fenner
	14.30 – 14.45	Limitations of OECD 307 and OECD 309 and recommendations for enhancements - Fraunhofer/Concawe project Dieter Hennecke (Fraunhofer IME)
	14.45 – 15.00	Biodegradation kinetics of hydrocarbons at low concentrations – Covering several orders of magnitude in hydrophobicity and volatility - DTU/Concawe project Heidi Birch (Technical University of Denmark (DTU))
	15.00 – 15.15	Overview presentation on key issues around session theme Kathrin Fenner
	15.15 – 15.45	30 min Q&A/panel discussion Panellists: All presenters plus Paul Van Elsacker, Eleni Vaiopoulou, (Concawe) and Chris Hughes, Ricardo Energy & Environment (on behalf of Concawe)
15.45 – 16.15	***Coffee and poster viewing***	
16.15 – 17.00	**Overview of key workshop outcomes and closing remarks/next steps** Moderators: Graham Whale and Paul Van Elsacker, and Andreas Schäffer (RWTH Aachen University) for the poster presentations	

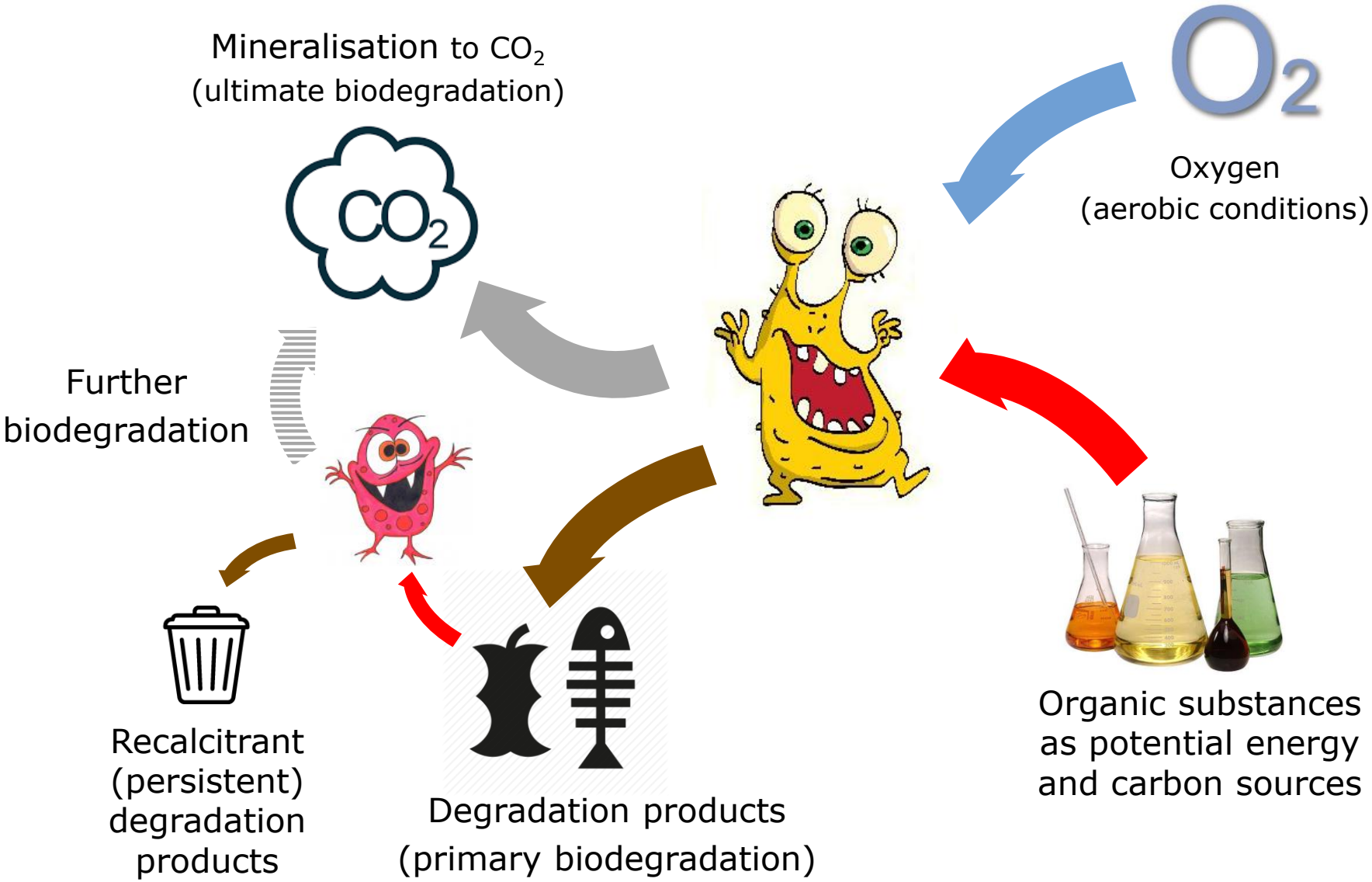
Cefic LRI - Concawe Workshop on recent developments in science supportive to the persistence/biodegradation assessment

Helsinki, 27 September 2018

Persistence/biodegradation assessment from a regulatory point of view

Vincent Bonnomet, European Chemicals Agency

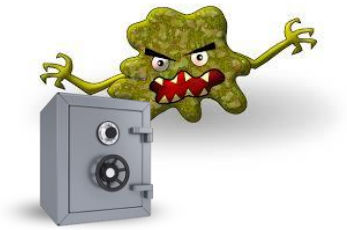
Biodegradation: basic principles



Biodegradation hindered by:

Improper environmental conditions
(temperatures, oxygen, pH, etc.)

Toxic chemicals



Low bioavailability
(low solubility, adsorption, volatility)

Very stable (persistent) molecules

Releases vs. (bio)degradation

Releases

High degradation

(But still potential problems caused by O_2 depletion)

Very high releases

High degradation

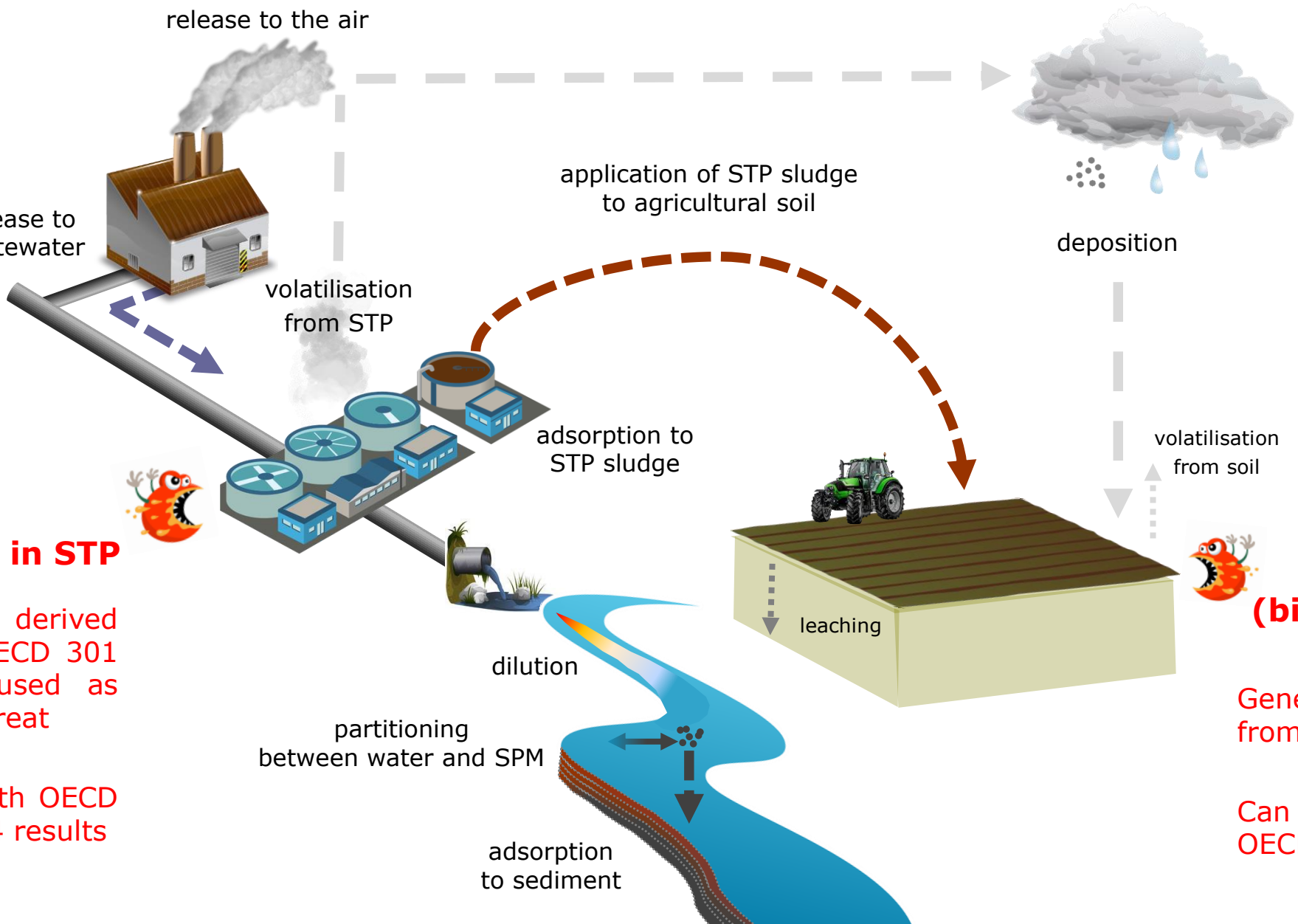
- Potential local risk
- O_2 depletion

Low releases

Very low degradation

- Potential large-scale risk but hardly predictable

Biodegradation in local risk assessment



biodegradation in STP

Generic k_{biodeg} derived from RB tests (OECD 301 series or 310) used as input into SimpleTreat

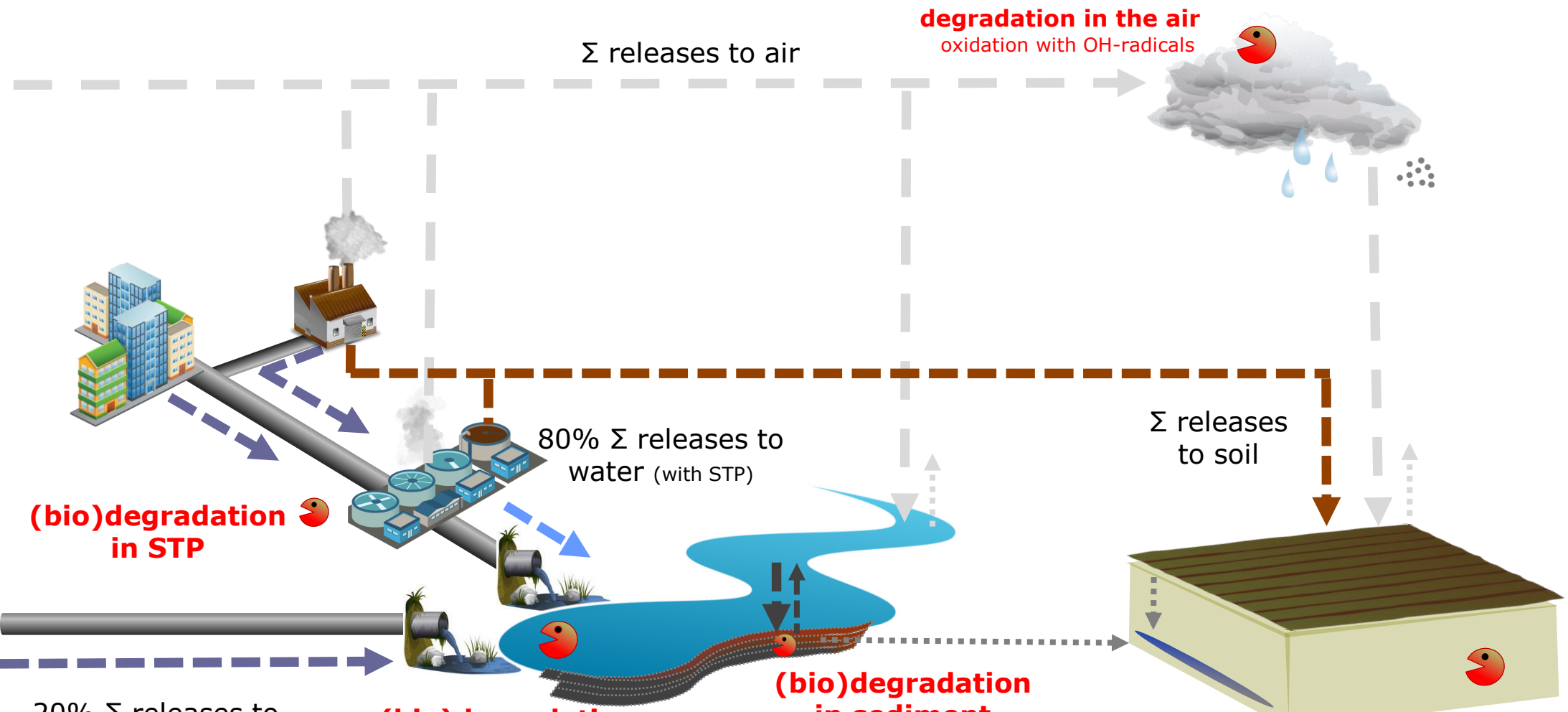
Can be refined with OECD 303A or OECD 314 results

(bio)degradation in soil

Generic k_{biodeg} derived from RB tests and Koc

Can be refined with OECD 307 results

Biodegradation in regional risk assessment



By default, generic value derived from RB tests

Can be refined with OECD 309

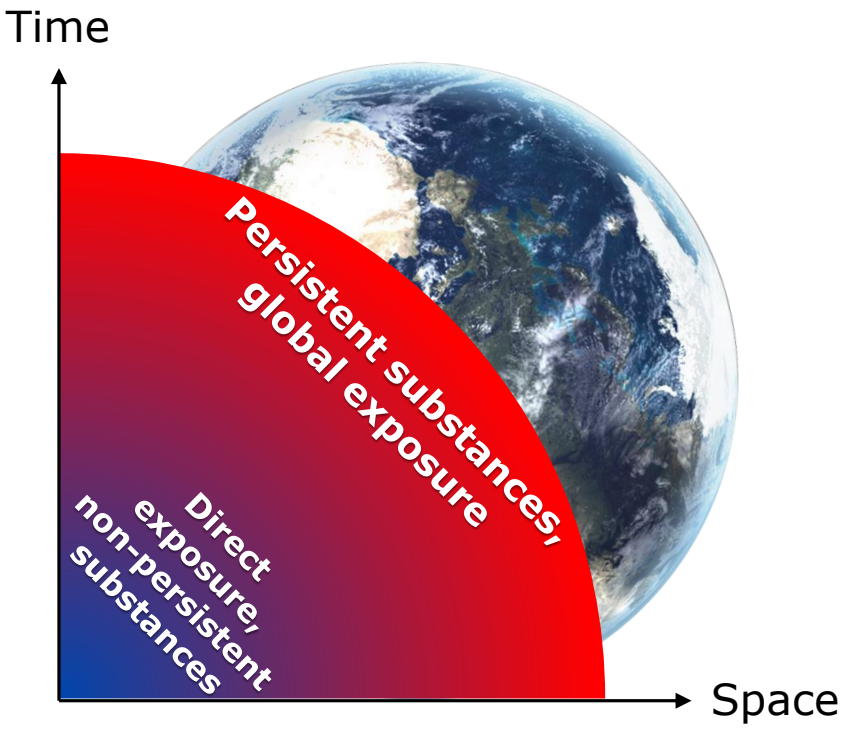
By default, assumed to be 10 times as slow as degradation in soil

Can be refined with OECD 308

By default, generic value derived from RB tests and Koc

Can be refined with OECD 307

Persistent substances: a potential global concern



PBT/vPvB are Substances of Very High Concern (SVHC)

Even low releases can be of concern.

Irreversible global contamination from SVHC must be avoided.

However, long-term fate (and effects) are hardly predictable for PBT/vPvB substances.

Risk assessment is not reliable for PBT/vPvB substances.

An ad hoc assessment framework is needed.

Standard information requirements for (bio)degradation

Tonnage band (t/y/registrant)	Required degradation data
1-10	Ready biodegradability
10-100	Ready biodegradability
100-1000	Ready biodegradability Hydrolysis Simulation of biodegradability in water[1] Simulation of biodegradability in sediment[2] Simulation of biodegradability in soil[3] Identification of degradation products
>1000	Ready biodegradability Hydrolysis Simulation of biodegradability in water[1] Simulation of biodegradability in sediment[2] Simulation of biodegradability in soil[3] Identification of degradation products Further testing shall be proposed if the CSA indicates a need for additional data on the degradation of the substance

[1]Not needed if the substance is highly insoluble in water and/or is readily biodegradable (see Section R.7.9.2)

[2]Not needed if the substance is readily biodegradable and/or direct and indirect exposure of sediment is unlikely (see Section R.7.9.2)

[3]Not needed if the substance is readily biodegradable and/or direct and indirect exposure of soil is unlikely (see Section R.7.9.2)

[4] Not needed if the substance has a low potential for bioaccumulation (for instance a log Kow <3) and/or a low potential to cross biological membranes and/or direct and indirect exposure of the aquatic compartment is unlikely.

Information requirements for (bio)degradation

Articles 10(b) and 14(1) of REACH, require a chemical safety assessment (CSA) to be conducted in accordance with Article 14(2) to (7) and with Annex I of REACH for every substance registered in quantities of 10 tonnes or more per year.

Annex I, Section 4 of REACH requires a PBT and vPvB assessment to be conducted as part of the CSA.

Furthermore, the "*guidance note on fulfilling the requirement of Annexes VI to XI*" laid down in Annex VI of REACH, explicitly indicates that "*in some cases, the rules set out in Annexes VII to XI may require certain tests to be undertaken earlier than or in addition to the standard requirements*".

Simulation tests can be required already for Annex VIII dossiers if there is a potential PBT/vPvB concern.

Annex XIII criteria

		Persistent (P)	very Persistent (vP)
Water	Freshwater & estuarine	> 40 days	> 60 days
	Marine	> 60 days	> 60 days
Sediment	Freshwater & estuarine	> 120 days	> 180 days
	Marine	> 180 days	> 180 days
Soil		> 120 days	> 180 days

These criteria correspond to degradation half-lives.

Degradation half-lives calculated from the results of simulation tests obtained under relevant conditions (e.g. temperature of 12° C – see later) are directly comparable to Annex XIII criteria (Annex XIII, Section 3.2: "assessment information").

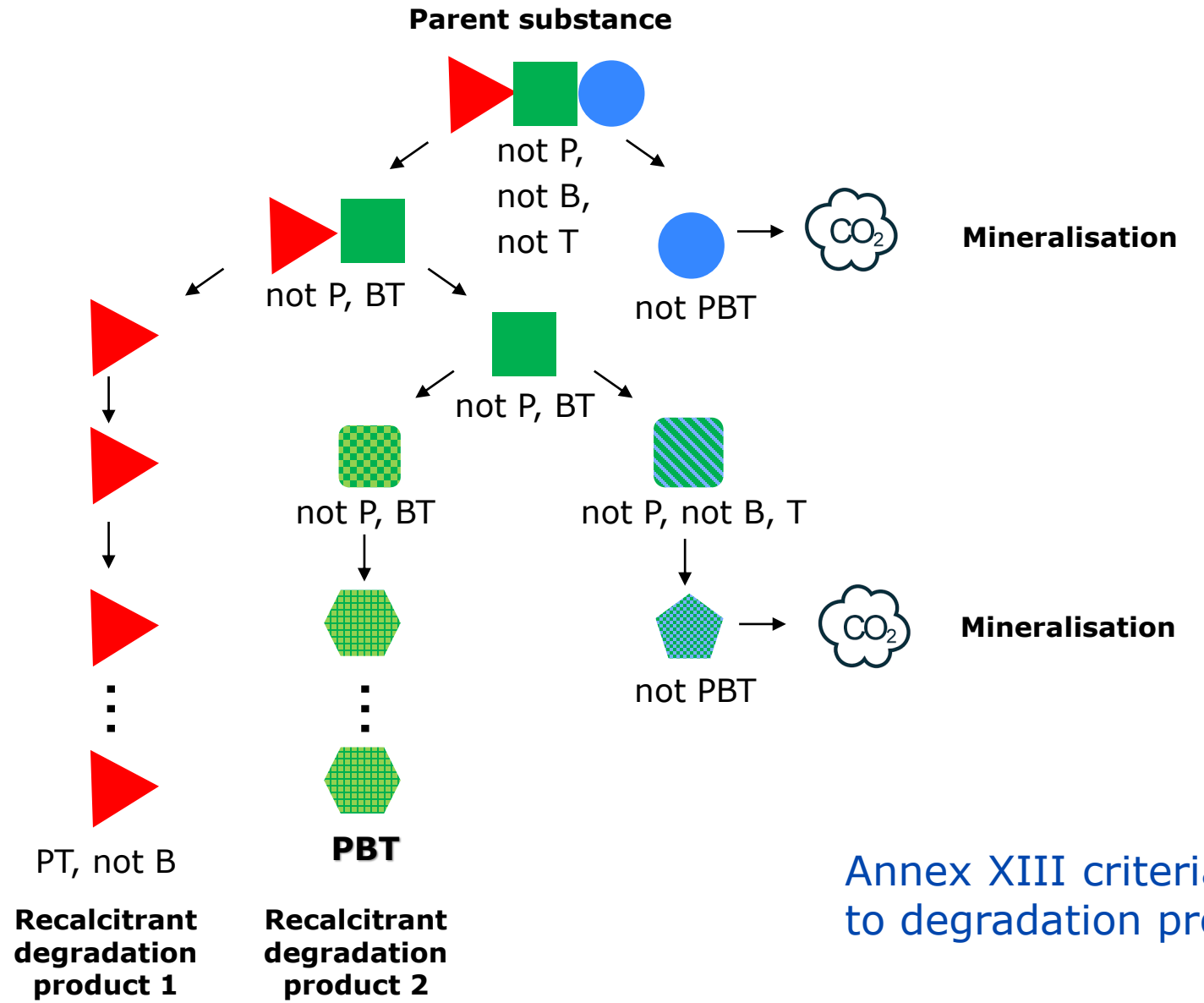
All main environmental compartments are covered (except the atmosphere)

These criteria apply to all constituents (including impurities and additives) reaching concentrations >0.1% of the total substance and to degradation products (as low as analytically possible).

Other information can be used as part of a weight of evidence approach: e.g. suitable and reliable monitoring studies, field studies.

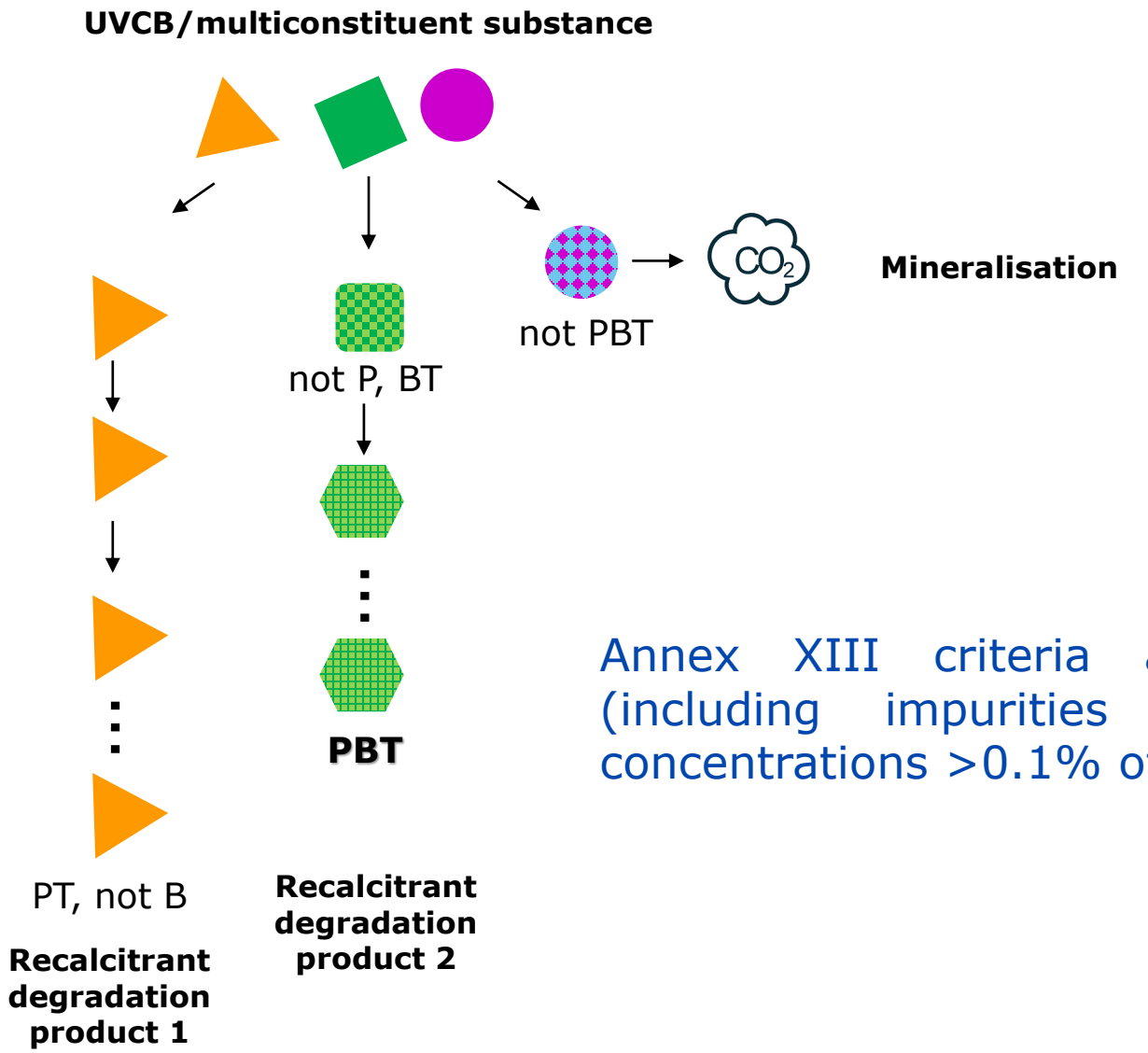
Screening information (Annex XIII, Section 3.1) can be used to conclude that a substance is not persistent (e.g. ready biodegradability tests, enhanced ready biodegradability tests, inherent biodegradability tests, QSARs).

Degradation products and degradation pathway(s)



Annex XIII criteria apply also to degradation products.

Degradation of UVCB/multiconstituent substances



Annex XIII criteria apply to all constituents (including impurities and additives) reaching concentrations >0.1% of the total substance

Inoculum

An ecosystem: a microbiome (bacteria, protozoa, fungi, viruses etc.) and a biotope (i.e. specific environmental conditions). Interactions between the different microorganisms (e.g. predation, parasitism, symbiosis) and between the microorganisms and their habitat.

A black box: difficult to control, difficult to harmonise:

- Source of the inoculum :
  - water (fresh water, brackish water, sea water),
  - sediment (freshwater, marine water, aerobic, anaerobic),
  - soil (soil type, aerobic, anaerobic),
  - activated sludge or sewage effluent (municipal, industrial)

- Diversity is dependent of environmental conditions.
  It may be altered by artificial adaptation to a substance.

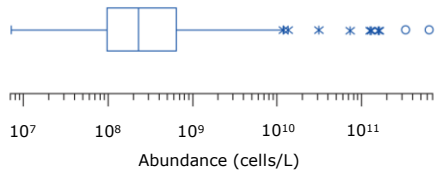
- Viability and activity, i.e. physiological state.
  Can be monitored by using positive controls with a reference substance.

- Density (can be controlled)

In the environment, only a (very) tiny fraction of microbes are expected to be actual degraders of the substance: e.g. Blok (2001) estimated for 137 aromatic substances that this fraction was varying between 10^{-2} – 10^{-7}

Recommended microbial densities in:
- OECD 301 & 310: 10^4 – 10^8 cells/L
- OECD 302: > 10^8 cells/L

Typical microbial densities in:
- Sea water: ≈10^5 cells/L
- Inland waters: 10^7 – 10^{11} cells/L



Abundance measurements from inland waters, as reported in the primary literature and summarized in Carr and Morin (2002).

- Activated sludge ≈ 10^{12} cells/L
- Sediment 10^8 – 10^{13} cells/kg

Adaptation of the inoculum

Potent organisms can grow and overtake other microbes as a result of a selective pressure caused by the substance: i.e. adaptation

In a regulatory context, artificial adaptation is generally not accepted (exception is for site-specific risk assessment when an industrial WWTP is present).

Artificial adaptation must be avoided for the P/vP assessment!

Indications that adaptation may have occurred during the course of a test:

- ⚠ Long test duration, long lag phase and sudden increase of degradation?
- ⚠ Origin of inoculum? (should not be from contaminated sites)

However, for substances with widespread, significant and long-lasting uses, adaptation may have already happened in STP, if not in the environment.

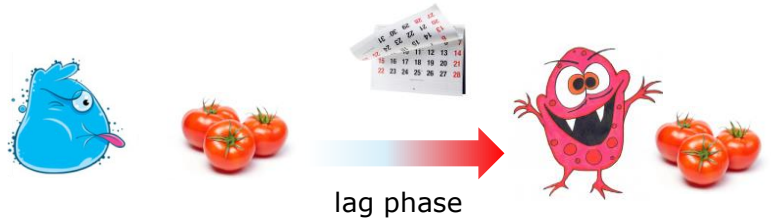
The test substance as substrate

Substance as primary/only substrate

(e.g. screening biodegradability tests)

The test concentration should be high enough.

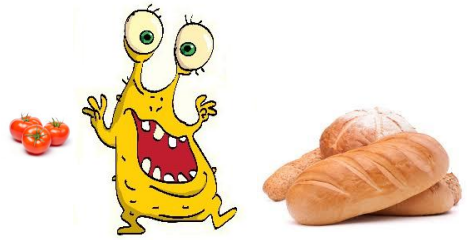
A lag phase (acclimation and/or adaptation) is to be expected



Diauxie

- Microorganisms tend to metabolise preferentially compounds on which they can grow faster. More easily degradable compounds will be used as a primary carbon and energy sources, in preference to the test substance.
- Concentration of the substance in the environment is generally low compared to other carbon and energy sources.

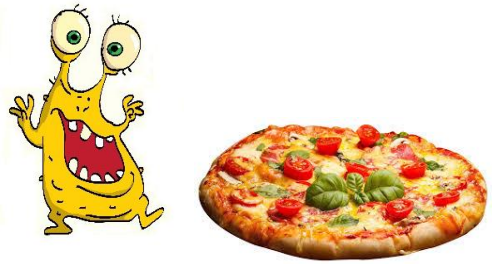
→ The substance will be used as carbon and energy source only after other more easily degradable substrates have been consumed.



Co-metabolism

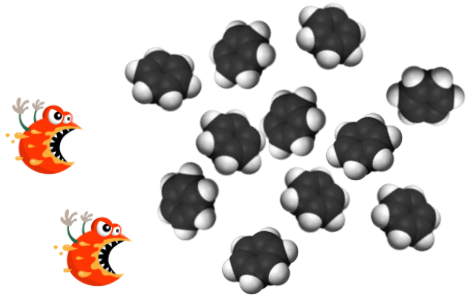
The test substance is not a primary substrate. Concentrations found in the environment often do not support the growth of degraders.

The substance is then a non-growth substrate which is degraded concurrently to another substrate (e.g. natural organic carbon), i.e. the primary substrate, which serves as primary carbon and energy source.



Inoculum density vs. substrate concentration

Respective substrate concentration and microbial density may induce very different degradation patterns



[C] of microbes << [C] of test substance

- The test substance can be used as energy source for microbial growth (high cell division)
- Incorporation into biomass
- Adaptation possible (after long-enough time)
- Long lag phase
- No first-order kinetics; not possible to calculate reliable half-lives

Typical of ready biodegradability tests

[C] of microbes > [C] of test substance

- The test substance is not used as energy source for microbial growth (low cell division)
- No or limited incorporation into biomass
- Adaptation unlikely
- Lag phase could be relatively short
- (pseudo)first-order kinetics; half-fives may be calculated

Typical of simulation tests and environmental conditions

The biodegradability tests

	Screening tests			Simulation tests
	Ready biodegradability tests (RBT)	Enhanced ready biodegradability tests (ERBT)	Inherent biodegradability tests (IBT)	
Conditions	Stringent	"Enhanced"	Favourable	More "realistic"
Medium	Water The amount of DOC in the test solution (due to the inoculum itsefl) should be kept as low as possible compared with the amount of organic carbon due to the test substance			• Water (with or without suspended solids) • Sediment (2 different sediments) • Soil (4 different soil types)
Test temperature	20-25°C			12°C recommended for half-lives 20°C for identification of deg. products
Inoculum	From activated sludge (domestic), sewage effluent (domestic), surface water, soil or mixture of them. Non-adapted		Activated sludge, sewage effluent Adaptation permitted (e.g. industrial effluent or sludge)	Sampled from the environment • No deliberate pre-adaptation, • not from contaminated sites
Inoculum concentration	High	High	Very high	Realistic (therefore potentially very diverse)
Test concentration	High (2 – >100 mg/L)			Low <1 – 10 µg/L for determination of half-lives <100 µg/L for identification of deg. products
Test substance	Primary substrate (sole carbon source) The test substance can be used as energy source for microbial growth → High cell division can be expected → Incorporation of into biomass possible			Secondary substrate (co-metabolism) Competition (diauxie) The test substance is hardly used as energy source for microbial growth → Low cell division → No or limited incorporation into biomass
Test duration	Short (28 days)	28 days, can be prolonged up to 60 days	Short (28 days)	Long: OECD 309: < 60 d (90 d for long lag time) OECD 308: < 100 d OECD 307: < 120 d
Kinetics	No first-order kinetics			(pseudo)first-order kinetics expected but other models possible (e.g. biphasic)
Use for the P/vP assessment	Not possible to calculate a half-life. Only screening, qualitative information: pass level met (positive result) or not (negative result)			Half-lives can be calculated in different environmental media and can be directly compared to criteria given in Annex XIII.
	RBT positive → not P RBT negative → ?	ERBT positive → not P * ERBT negative → ?	IBT positive → not P ** IBT negative → ?/P	

* Provided that the enhancements applied are valid for the PBT/vPvB assessment

** Only if pass levels specifically defined for the PBT/vPvB assessment are met

The ready biodegradability tests (1)

Standard information requirement (Annex VII)

Simple, cheap and quick screening tests for checking whether rapid degradation can occur.

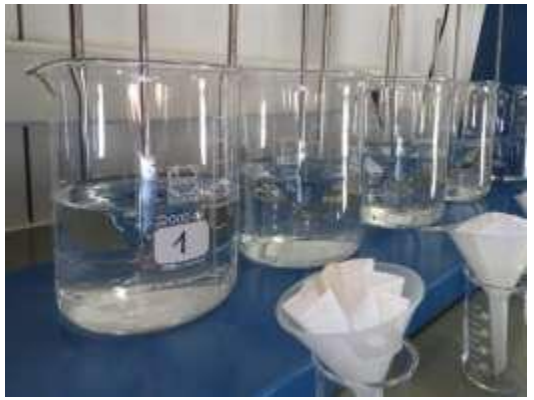
Stringent but highly artificial conditions.

Test substance as the only energy and carbon source: diauxie and cometabolism are ignored.

Measure mineralisation with non specific analytical methods:

- DOC removal,
- CO_2 production, or
- O_2 consumption

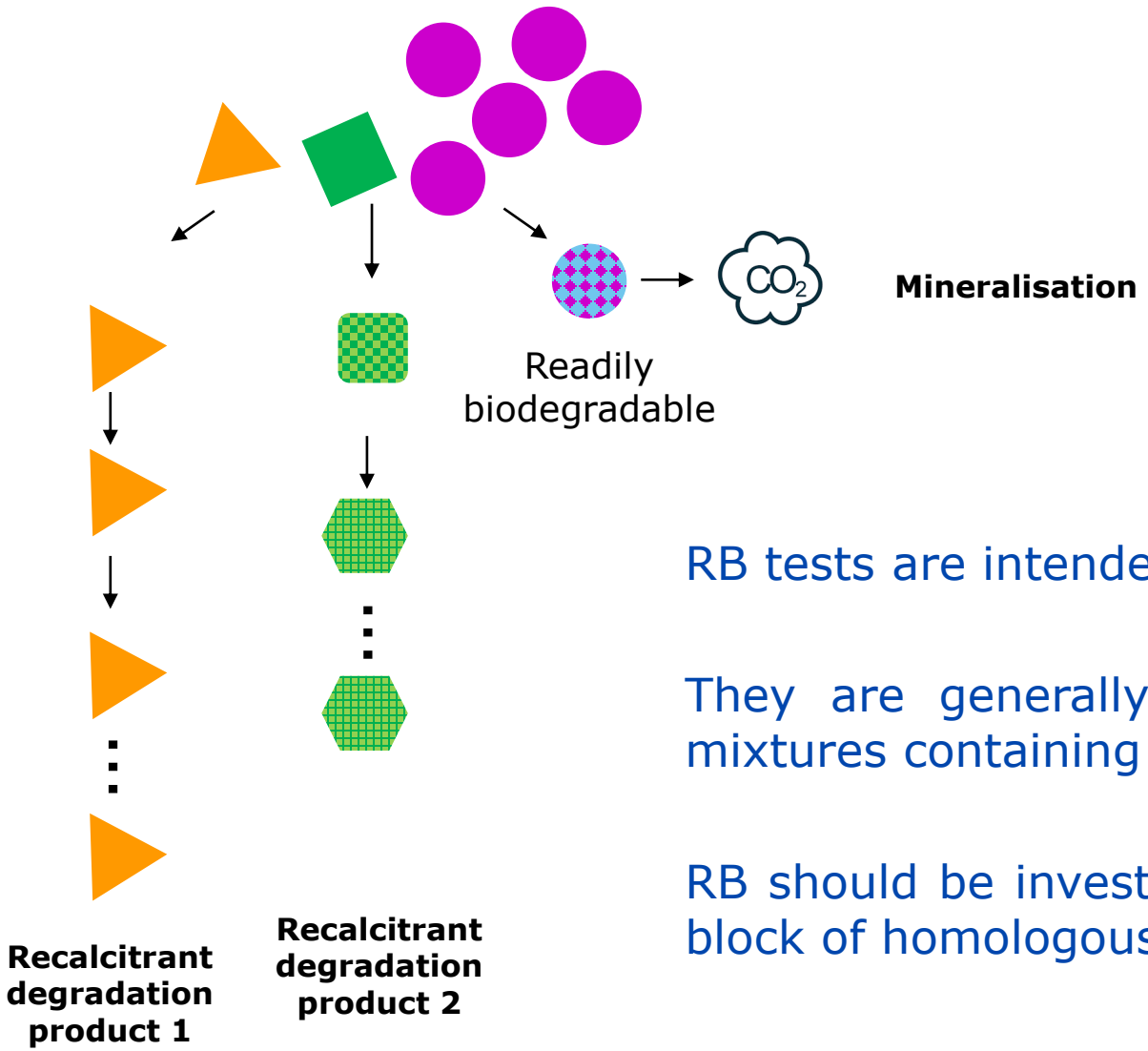
Specific chemical analysis can also be used to assess primary degradation of the test substance and to determine the concentration of any intermediate substances formed. This is usually only optional, except for the MITI method (OECD 301 C).



The ready biodegradability tests (2)

	Endpoint	Suitability for substances which are:			Test concentration	Inoculum concentration (cells/L)	Biodeg. potential	Pass level	Remark
		Poorly soluble	Volatile	Adsorbing					
OECD 301 A DOC Die-Away	DOC removal	-	-	±	10 – 40 mg_{DOC}/L	10^7-10^8	++	70%	• Open system, and DOC measurement: not suitable for "difficult" test substances
OECD 301 B CO_2 Evolution	CO_2 evolution	+	-	+	10 – 20 mg_{TOC}/L	10^7-10^8	++++	60%	• Difficult to obtain a 10-d window (CO_2 in test medium slow to be released)
OECD 301 C MITI (I)	O_2 consumption	+	±	+	100 mg/L	10^7-10^8	++	60%	• Mixture of inoculums from 10 different sites • Continuous measurement in the same vessel • 10-day window not applicable • Specific chemical analysis
OECD 301 D Closed Bottle	O_2 consumption (dissolved O_2)	±	+	+	2 – 10 mg/L	10^4-10^6	+	60%	• O_2 from the test water only • For toxic/inhibitory and/or volatile test substances • Samples originate from different vessels • Difficult to obtain a 10-d window (nb of vessels)
OECD 301 E Modified OECD Screening	DOC removal	-	-	±	10 – 40 mg_{DOC}/L	10^5	+	70%	• Open system, and DOC measurement: not suitable for "difficult" test substances
OECD 301 F Manometric Respirometry	O_2 consumption	+	±	+	50 – 100 mg_{ThOD}/L	10^7-10^8	+++	60%	• Continuous measurement in the same vessel • 10-d window easy to derive
OECD 310 CO_2 Headspace test	CO_2 evolution	±	±	+	2 – 40 mg_{TOC}/L	10^6-10^7	+++	60%	• O_2 in the head-space • Samples originate from different vessels • Difficult to obtain a 10-d window (nb of vessels)

RB tests generally not applicable to UVCB/multiconstituent substances



RB tests are intended for pure chemicals.

They are generally not applicable to complex mixtures containing different types of chemicals.

RB should be investigated per constituent or per block of homologous constituents.

The enhanced ready biodegradability tests (1)

Historically, developed to improve environmental relevance without the need of performing simulation tests

For the P/vP assessment, can be used as screening information to assess the biodegradability of substances of low bioavailability

Pre-adaptation is not allowed!

No specific test guideline. They are based on ready biodegradability tests (OECD 301 or 310): mostly same test designs and same pass levels (e.g. 70% for CO_2 and BOD, 60% for DOC)

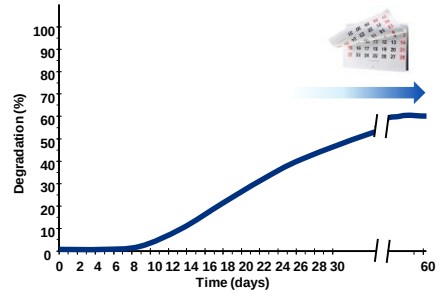
Only relevant for the P/vP assessment. Should not be used for risk assessment

Enhancements:

Prolongation up to 60 days

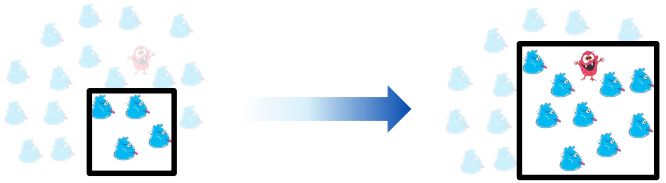
Test duration for RBT is normally 28 d, but it may be prolonged when the curve shows that biodegradation has started but the plateau has not been reached (e.g. for test substances with long lag phases or poor water solubility).

⚠ Should not be used to favour adaptation of the inoculum!

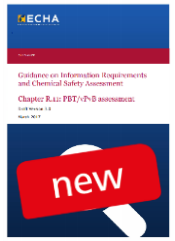


Use of larger test vessels

The absolute biomass is increased and therefore the probability of presence of competent degraders.



The enhanced ready biodegradability tests (2)



New in revised guidance:

- Inoculums from activated sludge or sewage effluent are now accepted (and even recommended). In the previous version of the guidance only inoculums from natural environmental media were allowed.

- ~~Increase biomass concentration and diversity~~
- ~~Low level pre-adaptation to the test item~~

are **no longer accepted** as valid enhancements for the P assessment.

They are deemed too much favourable and highly artificial.

- ~~Addition of co-substrate(s)~~ is **not allowed**– the test substance should be the only carbon source. The addition of a co-substrate may cause additional uncertainty and complicate the interpretation of results.

- Experimental modifications for improving the bioavailability of poorly water soluble substances such as the use of silica gel matrices, emulsifiers or solvents are not regarded as enhancements anymore since they are already proposed in Annex III of OECD 301. They are therefore considered to be part of "regular" ready biodegradability tests.

The inherent biodegradability tests (1)

	Endpoint	Suitability for substances which are:			Test concentration	Inoculum concentration (cells/L)	Remark
		Poorly soluble	Volatile	Adsorbing			
OECD 302 A Modified semi-continuous activated sludge test (SCAS)	DOC removal	-	-	±	20 – 50 mg_{DOC}/L	$>10^8$	Not relevant for P/vP assessment.
OECD 302 B Zahn-Wellens/EMPA test	DOC removal	-	-	±	50 – 400 mg_{DOC}/L	$>10^8$	
OECD 302 C MITI (II) test	O_2 consumption	+	-	+	30 mg/L	$>10^8$	• Similar to MITI I (OECD 301C) but MITI II has different test and inoculum concentrations to improve the biodegradation potential. • Specific chemical analysis

Not a standard information requirement

- High test concentration
- Very high inoculum concentration

→ increased ratio between the microbial biomass and the test concentration

→ high capacity for degradation

Pre-exposure of the inoculum (pre-adaptation) is allowed in the test guidelines, however pre-adaptation is not allowed for the P/vP assessment!

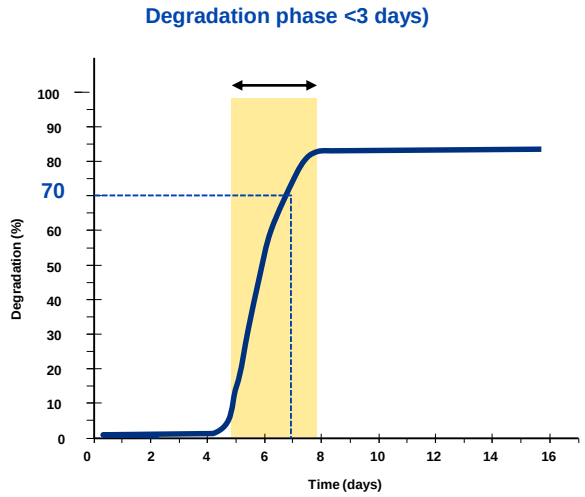
Ultimate and primary degradation can be measured.

Inherent biodegradability tests (2):
Screening information for the P/vP assessment

No pass-levels defined in the test guidelines.

The following pass-levels are defined in ECHA guidance for the P/vP assessment:

Zahn-Wellens test (OECD 302 B):
- No pre-adapted inoculum,
- ≥70 % mineralisation within 7 days,
- Degradation phase no longer than 3 days, and
- Removal before degradation occurs below 15%.



MITI II test (OECD 302 C):
- No pre-adapted inoculum,
- ≥70 % mineralisation within 14 days, and
- Degradation phase no longer than 3 days.

If those pass-levels are met, the substance can be concluded to be not P

If those pass-levels are not met, then no conclusion is possible.

However if an inherent test with very favourable conditions (e.g. with pre-adapted inoculum) is negative (no ultimate and no primary degradation), then the substance is likely to be persistent.

Simulation tests for degradability

Standard information requirement (Annex IX)

Simulation tests aim to simulate actual environmental conditions:

- Realistic environmental concentrations of the test substance
- Natural microbial community
- Bioavailability
- Temperature
- Redox potential
- pH
- Occurrence, concentration and impact of other available substrates

Simulation tests available for:

- Natural waters (OECD 309)
- Sediment (OECD 308)
- Soil (OECD 307)

Also exist for sewage treatment plants/wastewater, but these are generally not relevant for the P/vP assessment (only as part of a weight of evidence or if formation of specific degradation products, e.g. chlorination products).

Ultimate and primary degradation can be measured.
Radiolabelling generally used.

’Relevant’ conditions for simulation tests

Environmental conditions are highly variable.

However, for the purpose of REACH, it is impossible to simulate a set of too many conditions.

Annex XIII: “*the information used for the purposes of assessment of the PBT/vPvB properties shall be based on data obtained under relevant conditions*”.

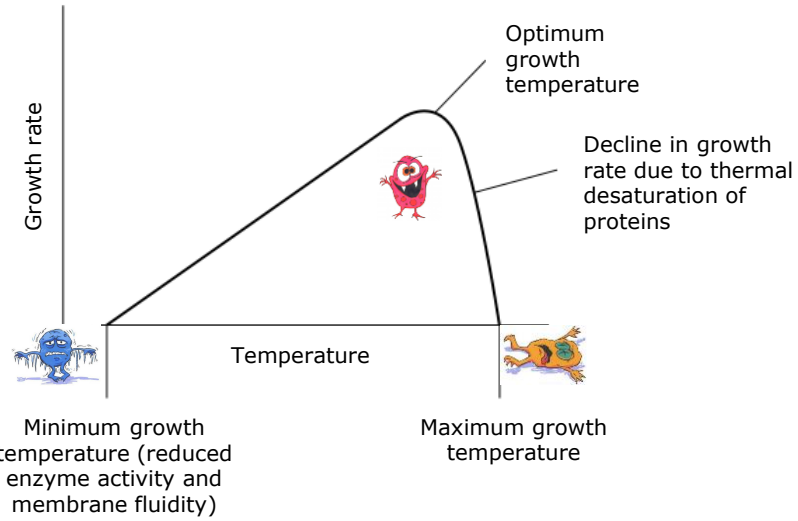
From a Board of Appeal Decision:

“*Annex XIII refers to* ‘relevant conditions’ *and not* ‘real life conditions’ *[…].*
‘relevant conditions’ *within the meaning of Annex XIII means those conditions that allow for an objective assessment of the PBT/vPvB properties of a substance and not the PBT/vPvB properties of a substance in particular environmental conditions”*.

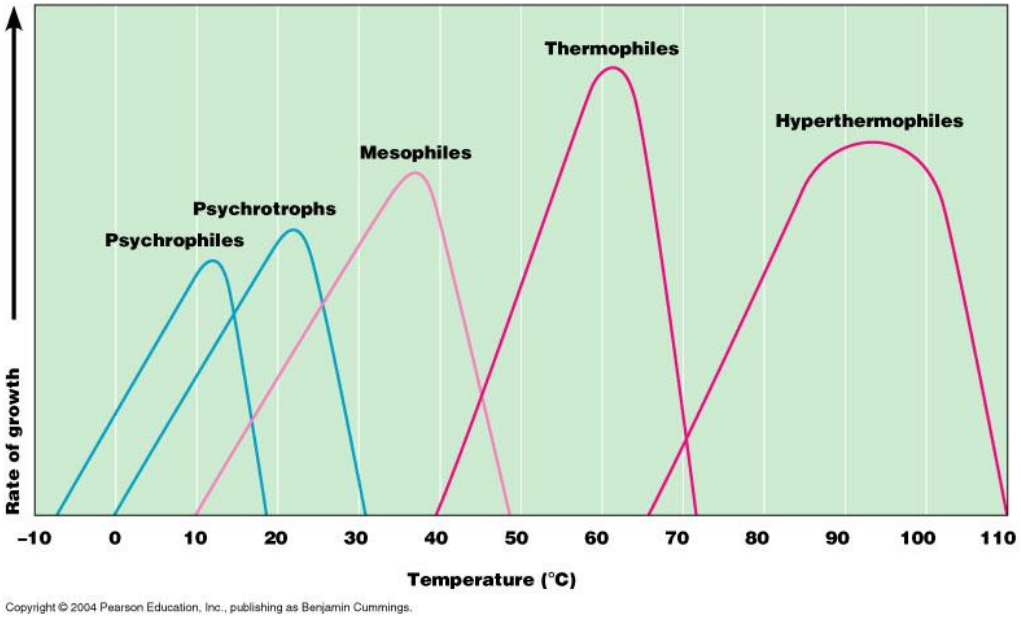
’Relevant’ conditions for simulation tests:
e.g. temperature

The reference temperature for the PBT/vPvB assessment and risk assessment was set to 12°C (285K), which is regarded as a reasonable representative temperature for the European Union.

Half-lives derived from simulation tests should therefore correspond to a temperature of 12°C.



Once the optimum temperature is passed, the loss of activity caused by denaturation of enzymes causes the rate of growth to fall away sharply



All microbes have optimum growth temperature, which can be quite different.

At 20°C a full class of microbes (psychrophiles) is potentially missing, whereas at 12°C the most common environmentally relevant classes can be present.

How to interpret non-extractable residues (NER)?

Two main principles for the P/vP assessment:

1. Mass balance must always be established

   ➡ **NER have to be quantified**

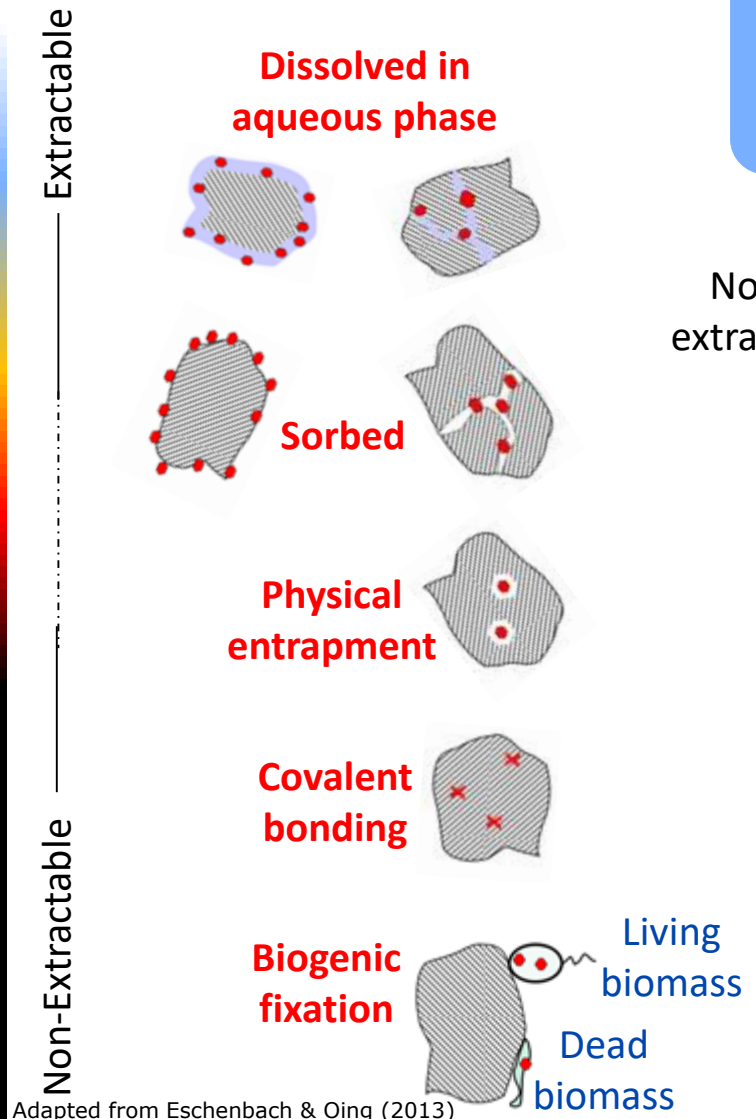
2. By default, NER have to be counted as non-degraded for the P/vP assessment, unless they are not remobilisable

   ➡ **Incentive for limiting the amount of NER: whenever technically possible, prefer OECD 309 to OECD 308 or 307 (see ITS)**

   ➡ **The milder the extraction, the higher the amount of NER. Incentive for using harsh extraction methods.**

   ➡ **Incentive for characterising NER according to their remobilisation potential**

Non *extractable* (extracted) residues (NER):
defined by the extraction method



Extraction with aqueous solutions
(Extraction of bioavailable, easily desorbable fraction)

Extracted

Non-extracted

Extraction with organic solvent mixtures
(Extraction of potentially bioaccessible, desorbable fraction)

Extracted

Non-extracted

Exhaustive extraction: Soxhlet, ASE (or PLE), SFE, MAE
(Extraction of total extractable, slowly desorbable fraction)

Extracted

Non-extracted

NER

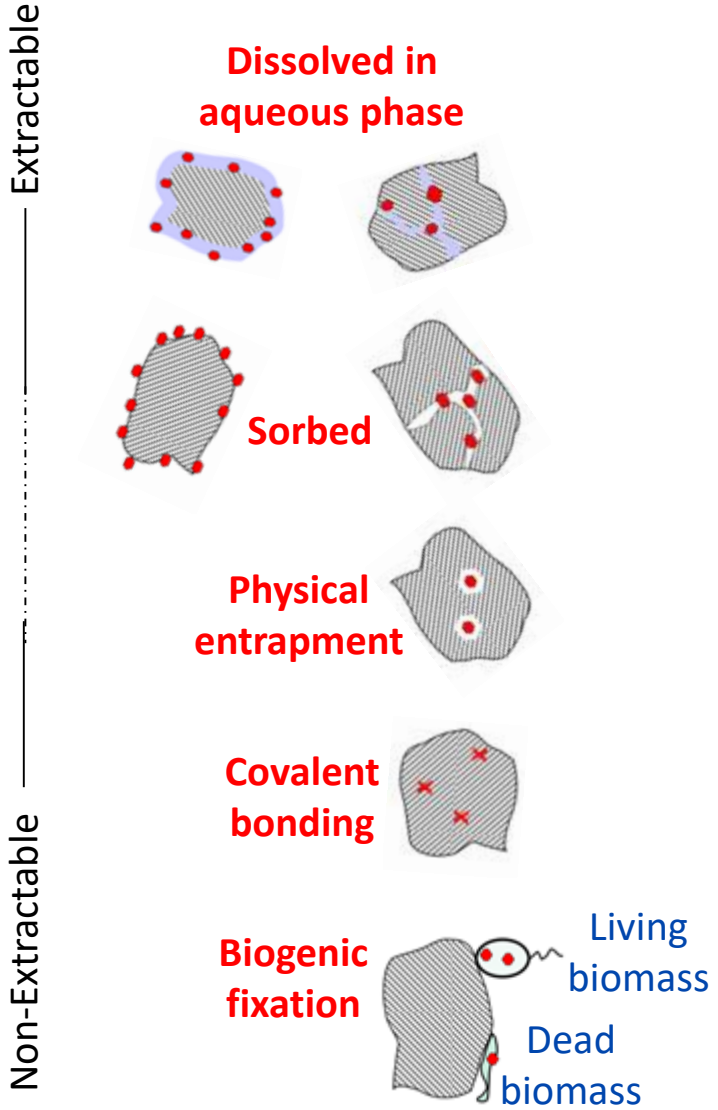
(Parent substance or transformation products)

EXTRACTED FRACTIONS:
(Parent substance or transformation products)

Adapted from Eschenbach & Oing (2013)

Proposal for a revision of Guidance R.11 (2019?):
further guidance on extraction methods and for the characterisation of NER

Based on Kästner, Trapp, Schäeffer , ECHA 2018*
See also poster from Schäeffer, Kästner, Trapp



1. Harsh extraction methods recommended in order to extract sorbed NER as much as possible

2. XenoNER should be regarded by default as non-degraded, but it depends on their remobilisation potential.

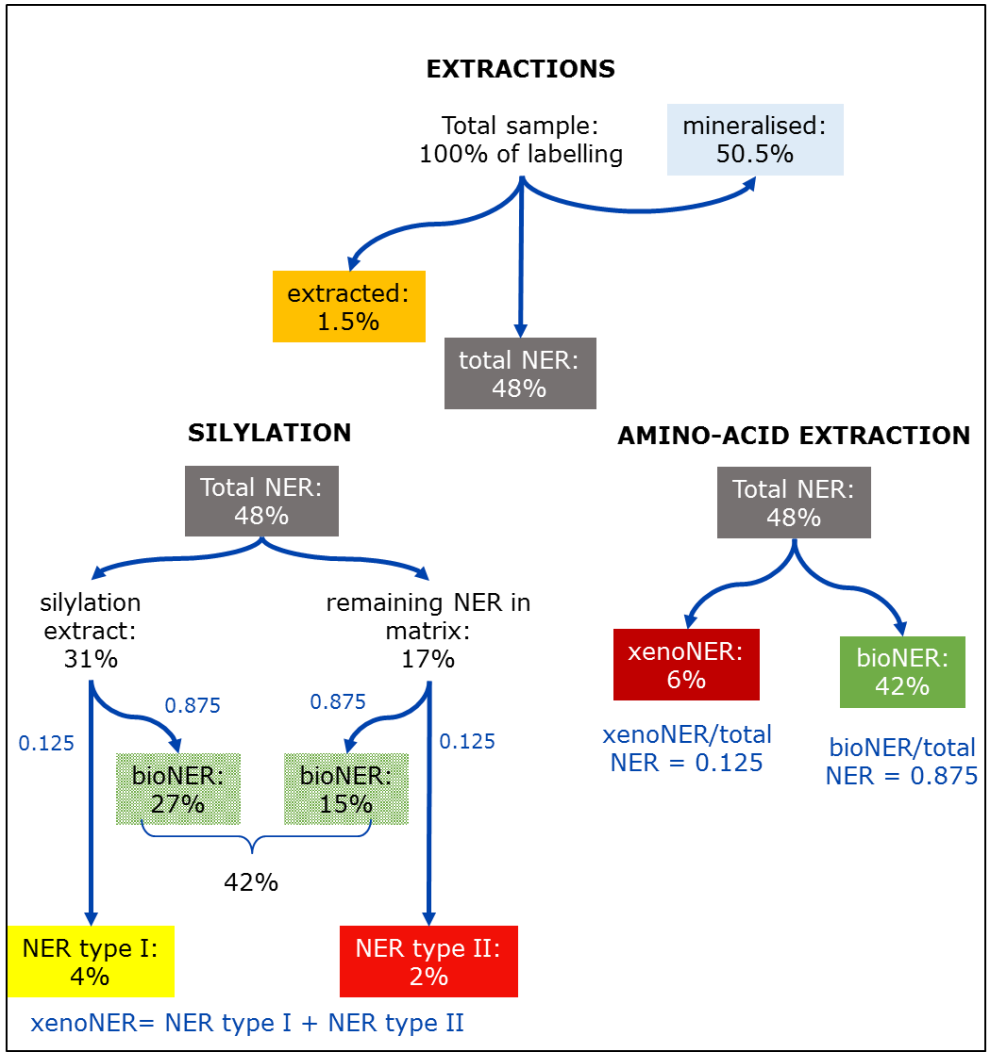
   Methodology to distinguish remobilisable xenoNER (e.g. physically entrapped NER – type I NER) from xenoNER for which remobilisation is deemed to be less likely (e.g. covalently bound NER – type II NER).

3. BioNER can be regarded as degraded.

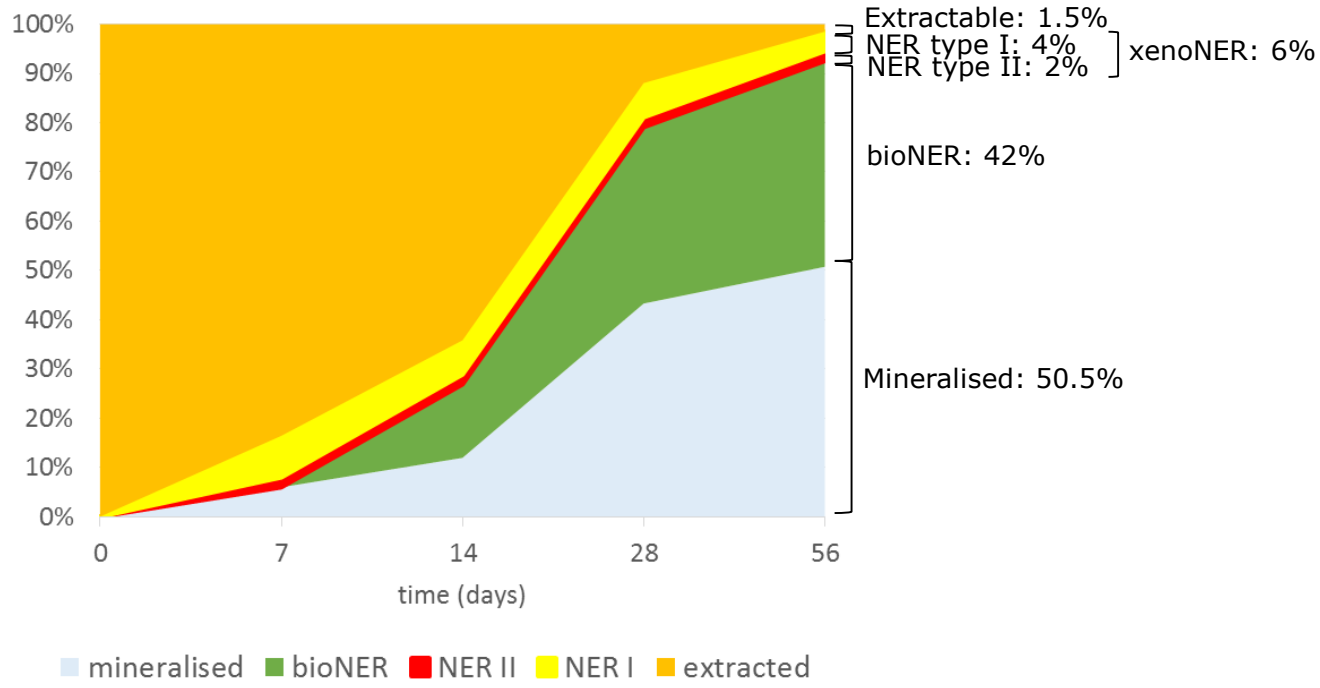
   Methodology to quantify bioNER.

* https://echa.europa.eu/documents/10162/13630/echa_discussion_paper_en.pdf/4185cf64-8333-fad2-8ddb-85c09a560f7c

Determination of mass balance
(E.G. At t=56 days)

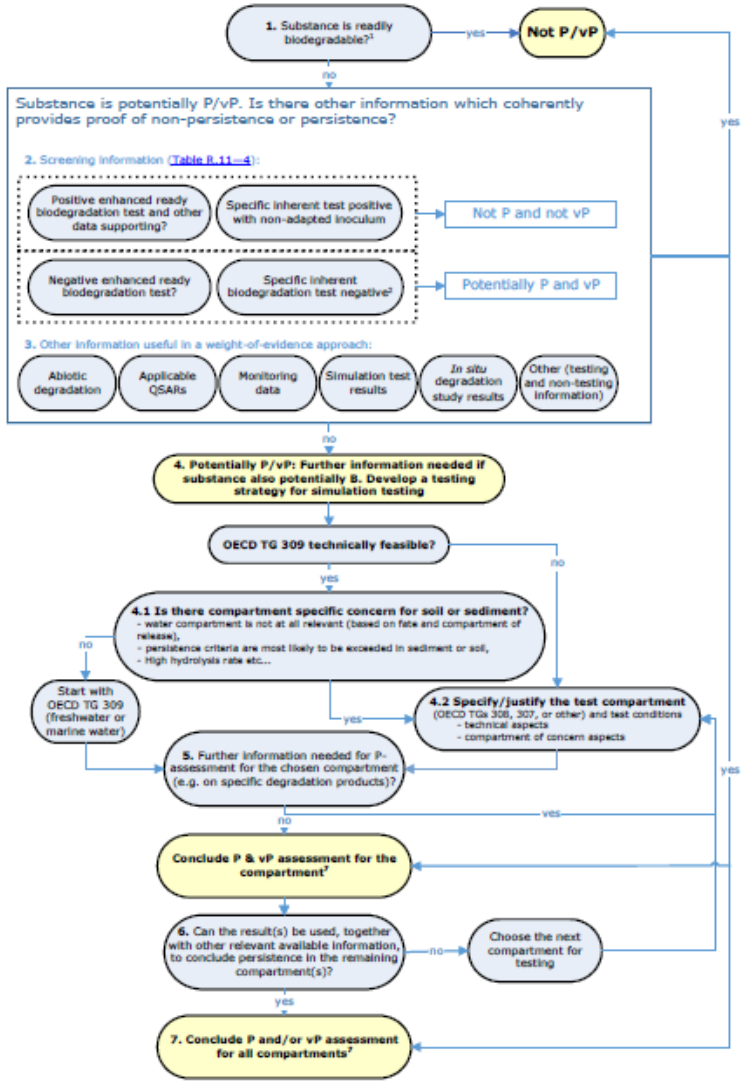


Total NER= type I NER + type II NER + bioNER



Revised integrated testing strategy (ITS) for persistence assessment

See ECHA's poster!



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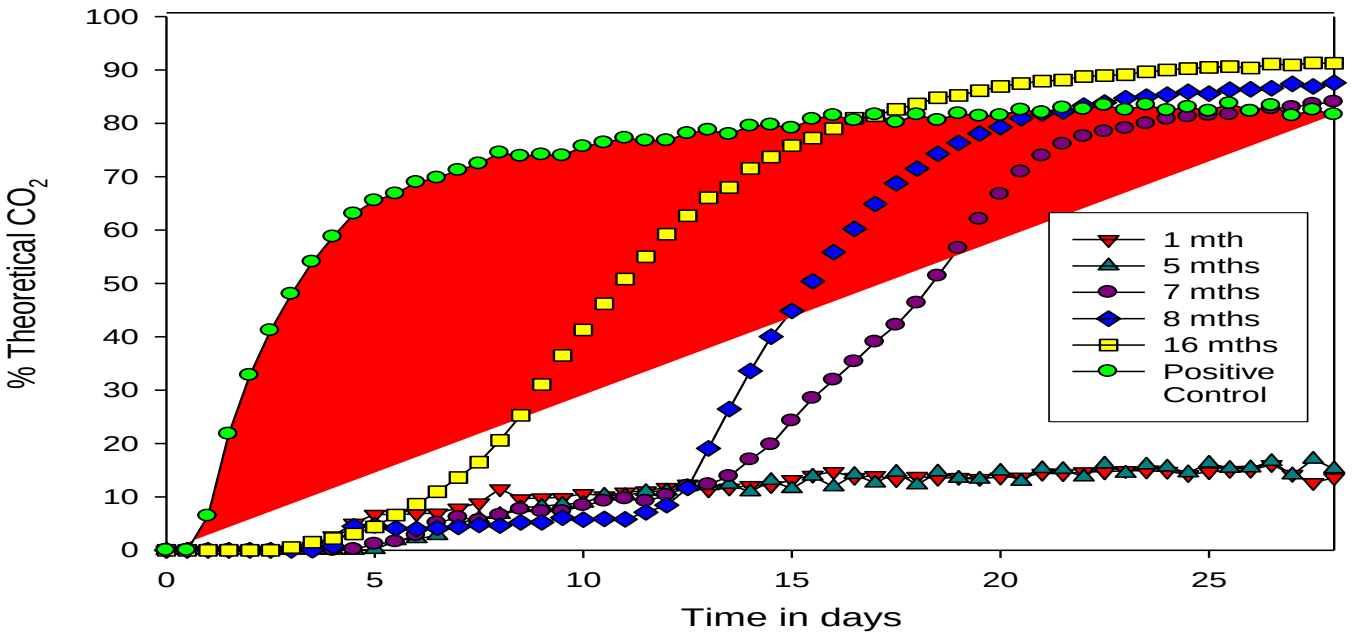
Application of chemostat systems to include adaptation of microbial communities in persistency testing (LRI ECO29)

John R. Parsons, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam

Influence of adaptation on biodegradation

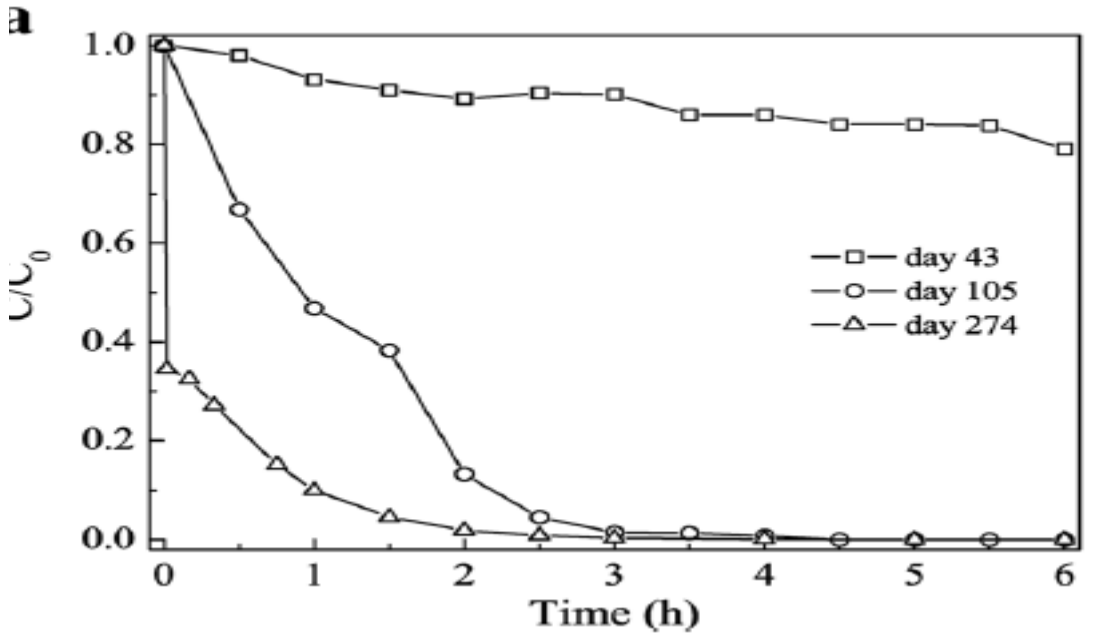
GLDA



Biodegradation as a function of time following initial shipment of product with L-GLDA

Itrich et al. (2015) *Environ. Sci. Technol. 49, 13314-13321.*

Atrazine

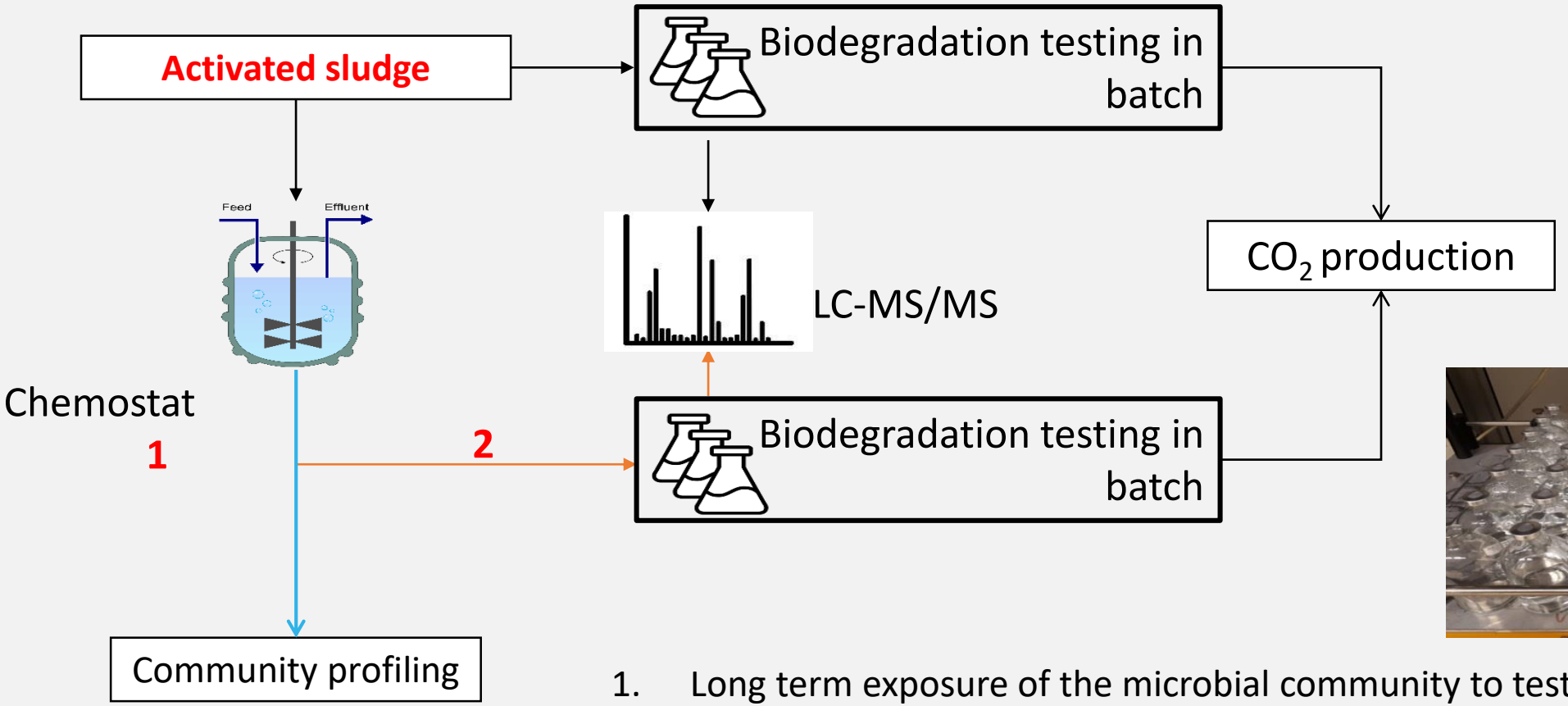


Comparison of the atrazine removal rates with (days 43 and 105) or without the addition of carbon and nitrogen sources (day 274).

Zhou et al. (2017) *Environ. Sci. Pollut. Res. 24, 22152-22157.*

- Current stringent protocols (such as OECE 310) underestimate biodegradability.
- Will incorrectly identify persistent chemicals if used for persistency determination

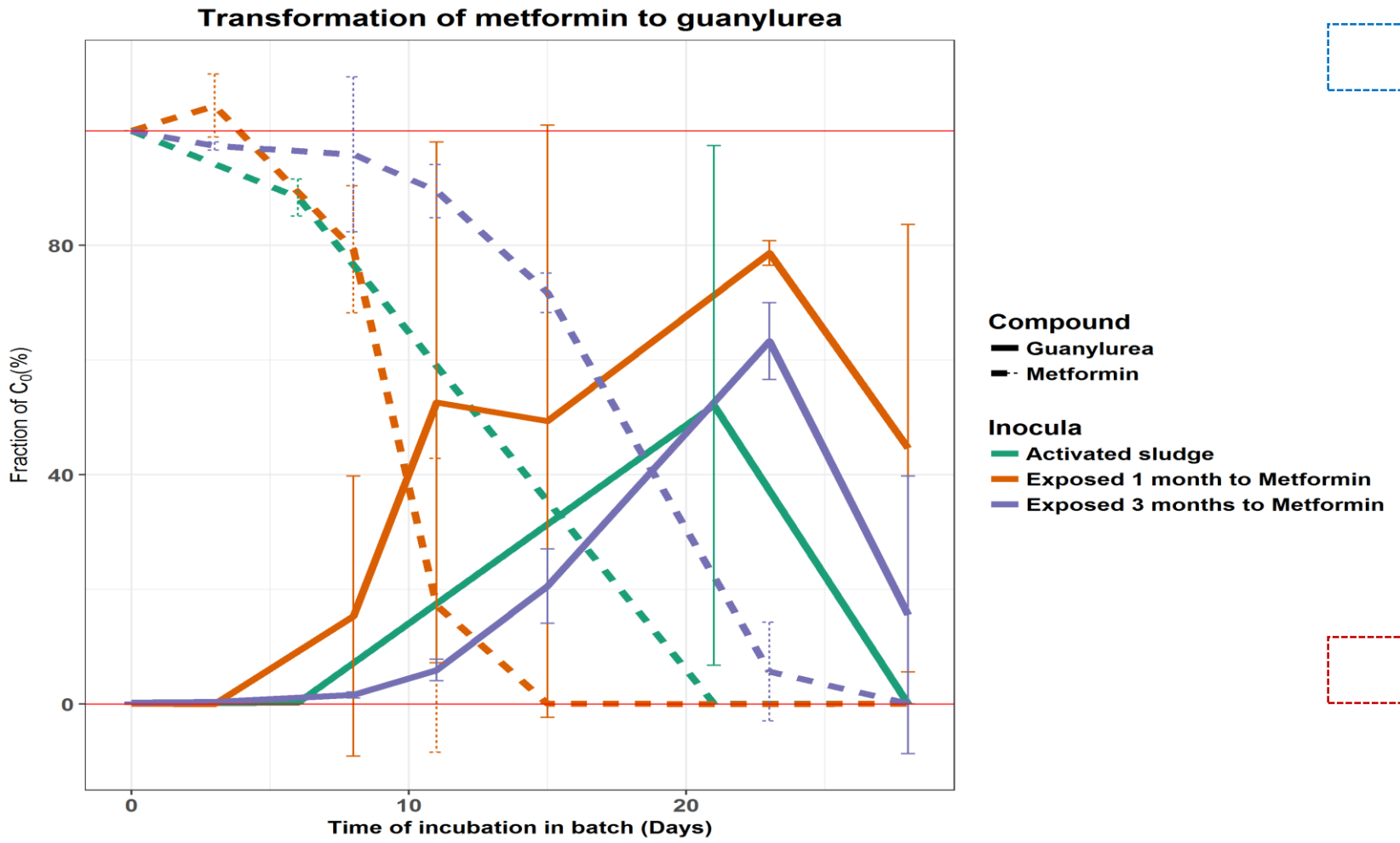
Experimental approach



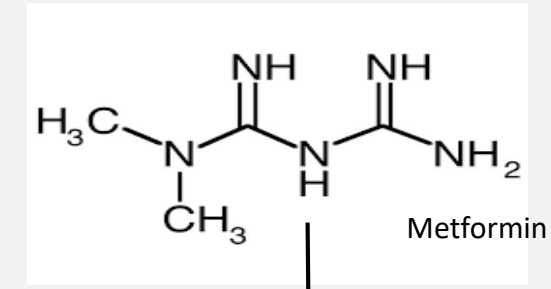
1. Long term exposure of the microbial community to test chemicals (4-chloroaniline, carbamazepine, metformin, N-methylpiperazine) in chemostat at 1.5 mg/L with 40 mg/L acetate

2. Exposed cultures used in biodegradation tests according to the OECD 310 guideline

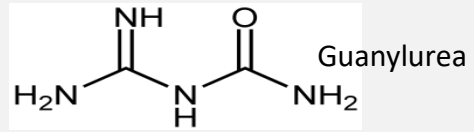
Biodegradation of metformin in OECD 310



Biodegradation of **metformin**



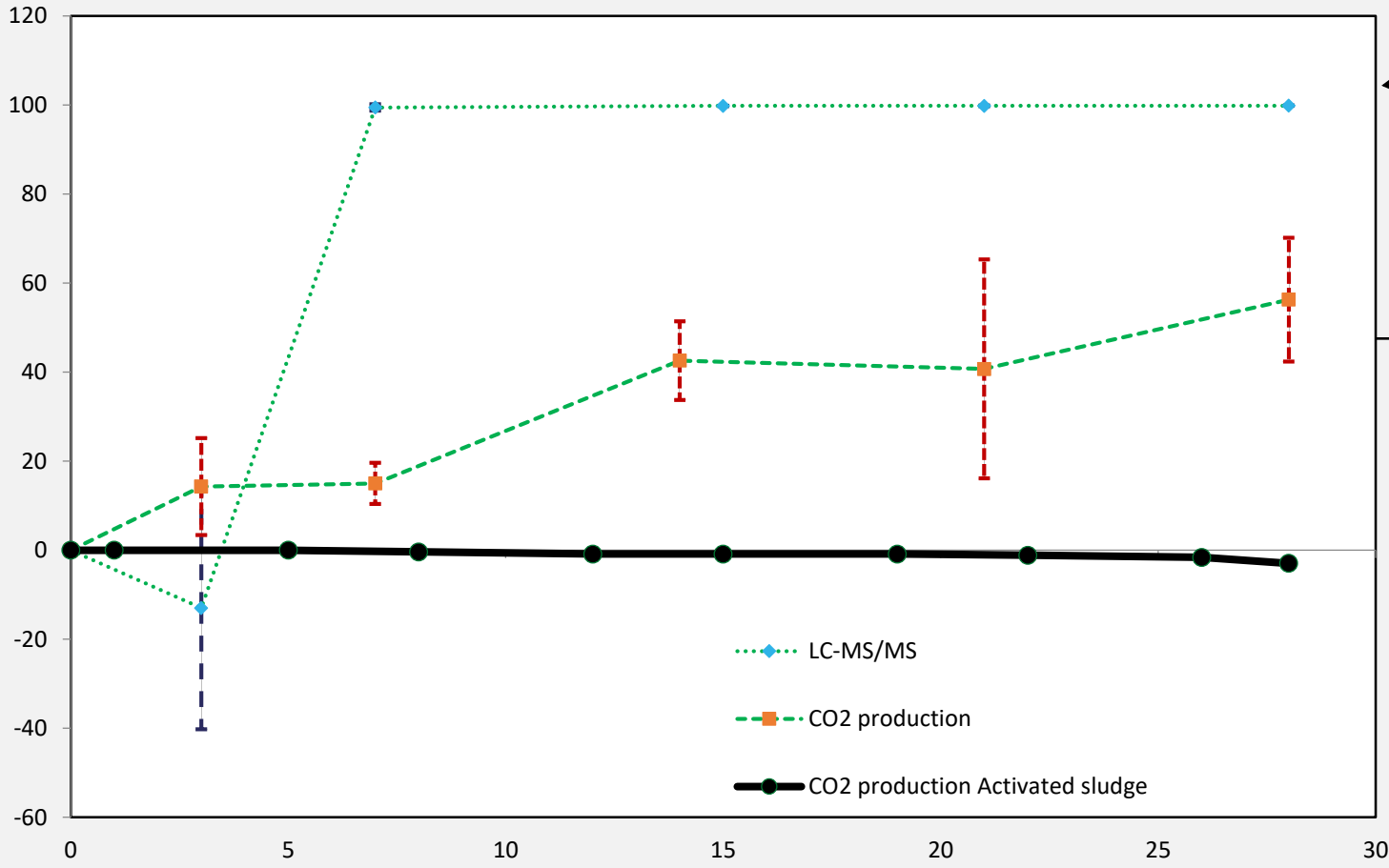
Transformation



Production of **guanylurea**

Guanylurea is eliminated from OECD 310 tests
- new transformation product? CO_2 production?

Biodegradation of N-methylpiperazine in OECD 310

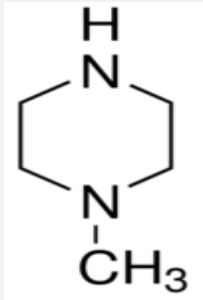


Primary biodegradation (LC-MS/MS)

Inoculum from chemostat after 9 months

CO_2 production

Inoculum from activated sludge (CO_2 production)



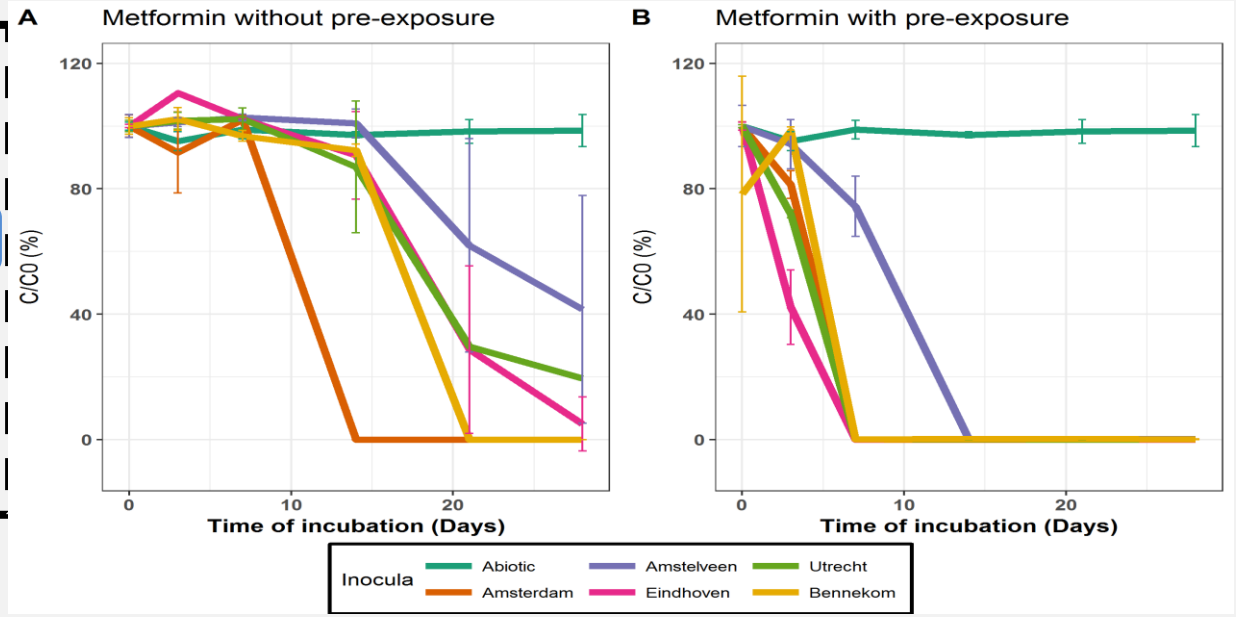
60% mineralisation of N-methylpiperazine by activated sludge exposed for 9 months

Impact of origin of inocula on biodegradation

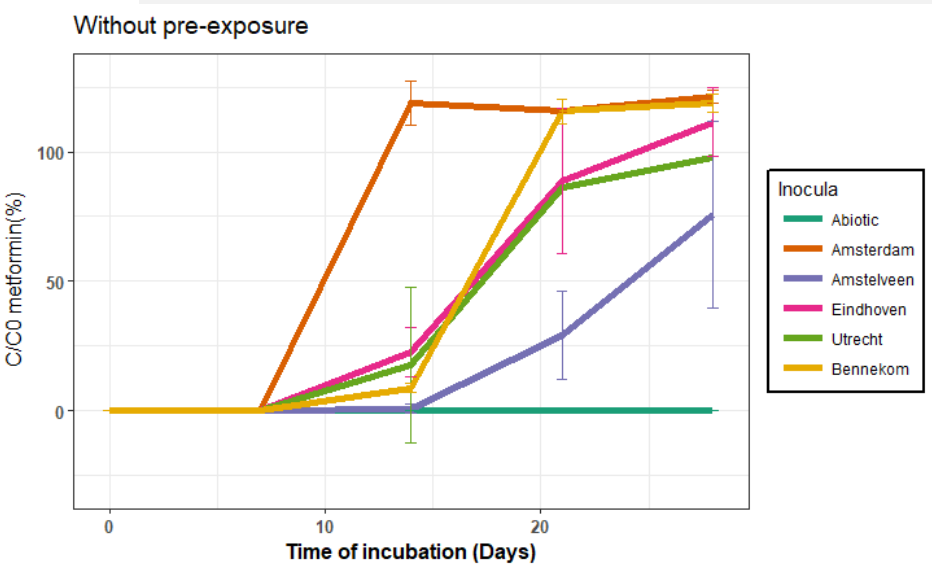
WWTP

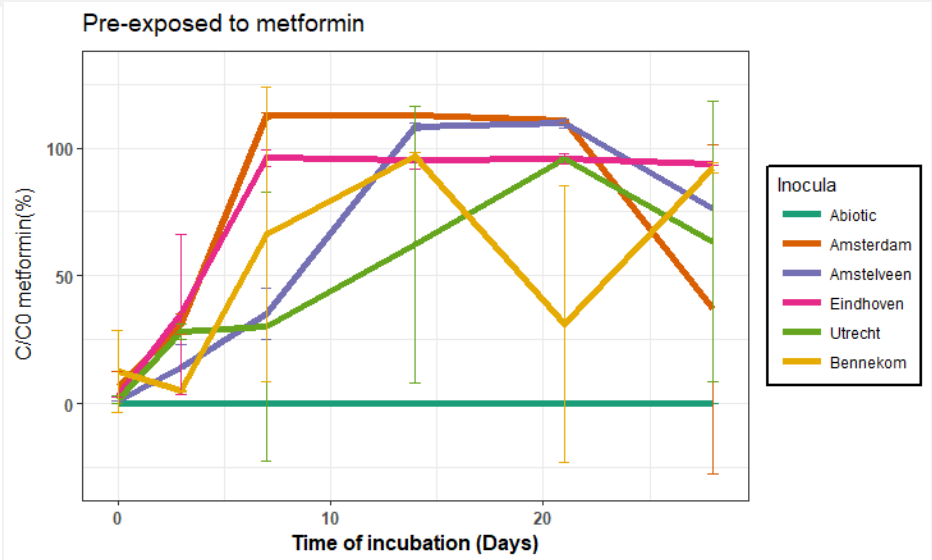
- Amsterdam
- Amstelveen
- Utrecht
- Bennekom
- Eindhoven

Metformin



Guanylurea





Biodegradability testing of 4-chloroaniline, carbamazepine, diclofenac, metformin, N-methylpiperazine

OECD 310 guideline with and without pre-exposure (6 d at 20 mg/L)

Conclusions

- The biodegradation capacity of microbial communities increases due to adaptation to pollutants during long term exposure, resulting in faster biodegradation of initially persistent chemicals
- Adaptation of microbial communities can be achieved under defined and realistic conditions in chemostat systems
- Taking adaptation into account in testing protocols will result in more realistic and reproducible assessment of biodegradability and persistency
  - Implementation in practical protocols for regulatory testing?

Thank you

Baptiste Poursat[1,2] ,Martin Braster[2], Rick Helmus[1], Pim de Voogt[1], Rob van Spanning[2]

[1] : Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.

[2] : Department of Molecular Cell Biology , Vrije Universteit, de Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands.

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Cefic LRI - Concawe Workshop on recent developments in science supportive to the persistence/biodegradation assessment

Helsinki, 27 September 2018

NEED FOR MORE ROBUST TESTS IN ADDITION TO OECD 301 SERIES, 310, 306, TESTS

CG van Ginkel AkzoNobel Specialty Chemicals

The Netherlands

(READY) BIODEGRADABILITY TESTS

Ready biodegradation tests (RBTs) are designed so that positive results lead to the conclusion that the test substance will undergo rapid degradation in the environment. RBTs are characterized by their inoculum (low number of micro-organisms (low endogenous respiration)), batch culturing for 28-day test period, "high" initial test substance concentrations, and a-specific end-points.

A RBT test result informs about the following aspects of biodegradation.

- ultimate (complete) biodegradation by competent microorganisms capable of utilizing the test substance as sole carbon and energy source (extent of oxygen consumption or carbon dioxide evolution).
- "rate" of biodegradation by microorganisms growing on the test substance (steepness of curve).
- the number and occurrence of competent microorganisms present in "unadapted" ecosystems and biological treatment plants (small inoculum size and lag phase).

Over five decades of experience with RBTs

Ready biodegradability tests only detect growth-linked biodegradation

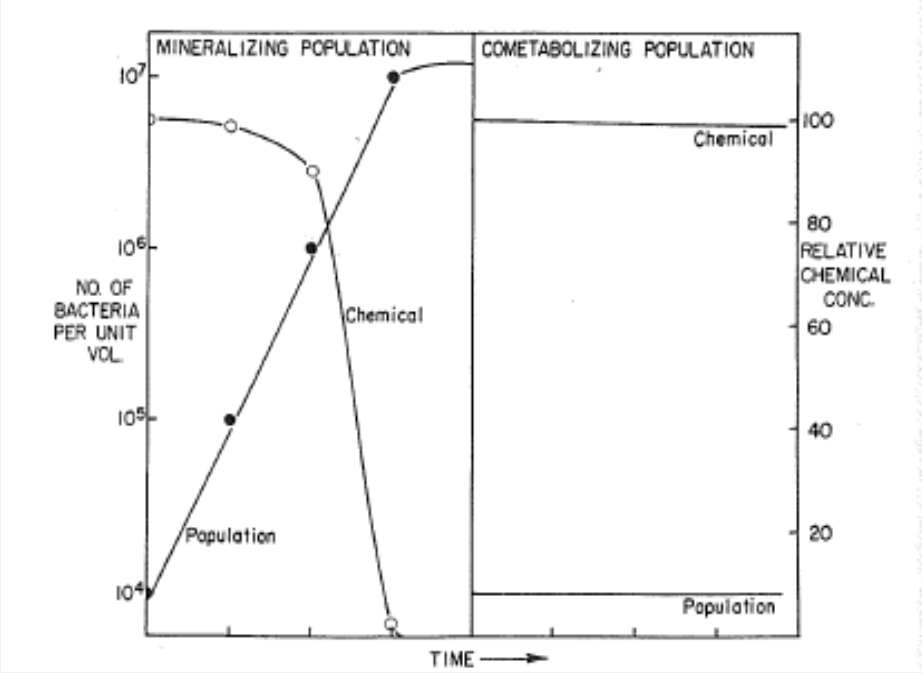


Figure Microbial population changes and disappearance of a chemical acted on (left) by bacteria growing logarithmically and using the compound as carbon and energy source or (right) bacteria co-metabolizing the chemical *(Alexander, 1981)*.

IMPORTANCE OF GROWTH-LINKED BIODEGRADATION

Growth-linked biodegradation is an autocatalytic process resulting in high biodegradation rates upon exposure of the microorganisms.

Other drawbacks of co-metabolism are conversion into (toxic) biodegradation products, dependent on availability of other substrates, competitive substrate inhibition.

Natural estrogens vs 17α-ethinylestradiol
Accelerated degradation of pesticides

Proposal for a science based approach; Non-persistency of chemicals based on biodegradation mechanisms increases in the following order; no degradation detected < co-metabolic (gratuitous) degradation (first order kinetics) < growth-linked biodegradation (enhanced tests) < growth-linked biodegradation (ready biodegradability tests).

ECO 11

The set-up of RBTs especially the prescribed inoculum limits the detection of growth-linked biodegradation. (Prolonged) RBTs are however a great start for a tiered approach.

Clearly demonstrated that RBTs are notoriously variable and unsuitable to assess persistence (ECO 11) unless ready biodegradability has been shown.

In the next tier, prolongation of RBTs is already used as enhancement. Other enhancements i.e. increasing biomass concentrations, larger vessels, more (different) inocula result in more reliable results (ECO 11).

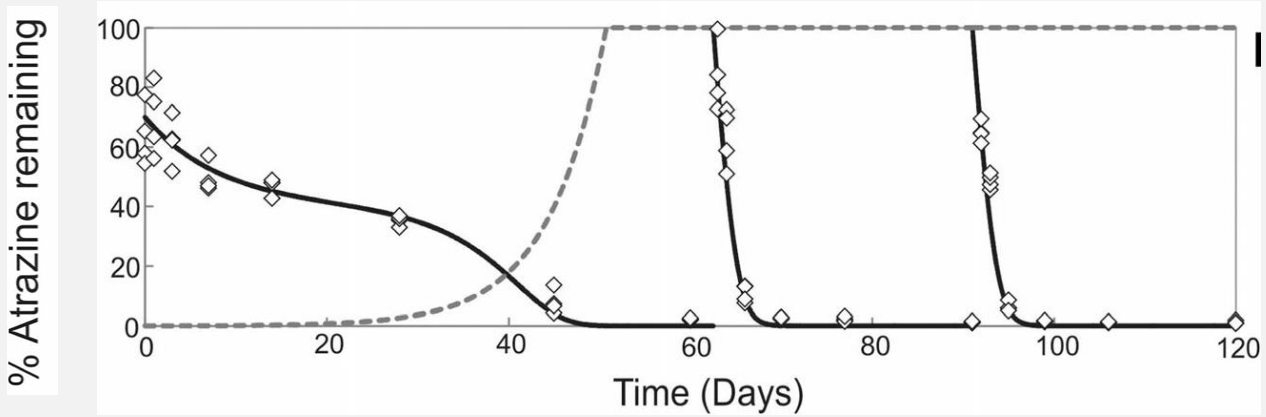
ECO 29

Adaptation of microbial communities present in ecosystems upon exposure of substance supporting growth has been described comprehensively (ECO 29).

This phenomenon is by far the most important process involved in the biodegradation of naturally occurring chemicals and should therefore be included in the testing scheme as an enhancement (prominent place).

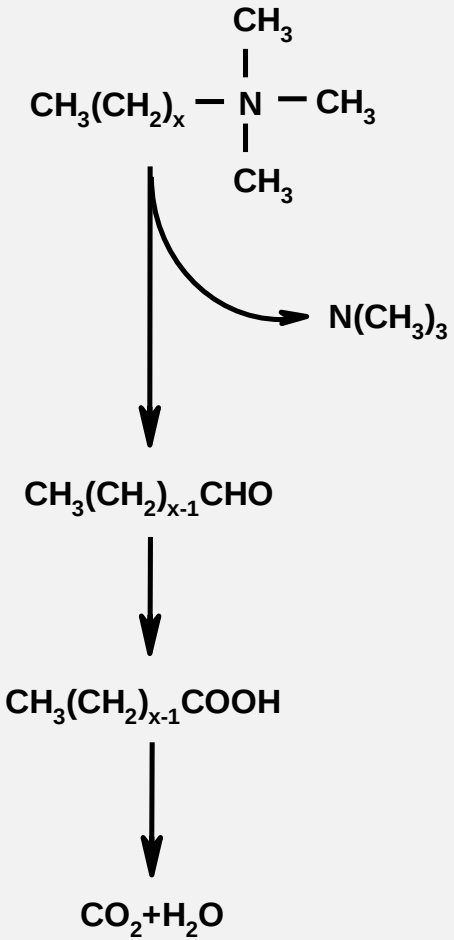
Growth-linked biodegradation does exclude the existence of an absolute biodegradation rate (half-lives). Use of categories or bins should therefore be considered as an alternative for half-lives for these chemicals.

Figure Dissipation of atrazine in soil over three applications (Yale et al, 2017)



CONCAWE

(Ready) biodegradability tests were NOT developed to assess biodegradation of multi-constituents. Specific analysis and/or microbial physiology may enable assessment of non-persistence of multi-constituents whether or not in combination with RBTs.



Specific analysis Biodegradation kinetics studies by Hammershøj* show that specific analysis in batch experiments is a useful tool to assess the non-persistency of multi-constituents. Determination of "rate" and occurrence but not of mineralization.

*Co-metabolic transformation in batch cultures with low initial concentrations can not be ruled out. A lag period followed by a decrease of the test item is evidence of growth-linked biodegradation.

Microbial physiology Another approach to assess non-persistence of multi-constituents (UVCBs) is to demonstrate that all constituents are channeled into the same biodegradation pathway by a single microorganism.

Biodegradation of ready biodegradable substances in aquatic environments being faster at the lower substrate concentrations typical of the environment, as compared to the 15-day half-life default value is again demonstrated (Hammershøj ; ECETOC report no 129).

STATEMENTS

Growth linked degradation has several advantages over co-metabolic degradation. Therefore, when assessing persistency, greater emphasis should be placed on tests (experiments) detecting only growth-linked biodegradation in comparison to those also determining biodegradation through co-metabolic transformation.

Environmental half-lives of substances supporting growth do change constantly because the number of competent organisms (catalysts) vary with the availability of the substance . Assessing half-lives should therefore be replaced by assigning substances to categories or bins.

Robustness and applicability of tests (OECD 301 series and OECD 310) detecting only growth linked biodegradation should be increased in a tiered assessment approach by allowing longer test periods, improved (e.g. more concentrated, diverse) inocula, and adaptation (pre-exposure).

Assessment of persistence should be allowed to enter the 21st century .

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Helsinki, 27 September 2018

Identifying strategies that will provide greater confidence in estimating the degradation rates of organic chemicals in water, soil, and sediment

Philipp Dalkmann, Bayer AG

on behalf of

Yuxin Wang[1], Kathrin Fenner[2], and Damian Helbling[1]
[1]Cornell University; [2]Eawag

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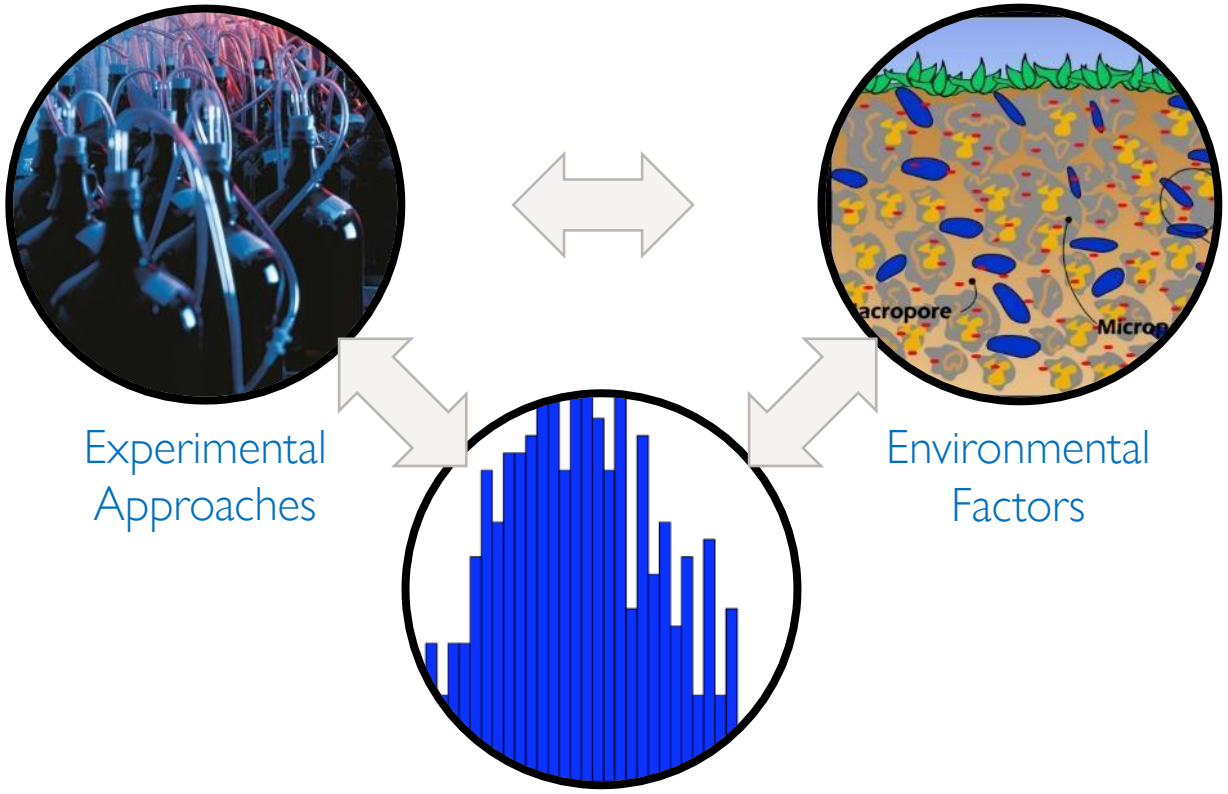
THERE WERE TWO MAJOR RESEARCH OBJECTIVES FOR ECO31

Objective 1: Review state-of-the-science on chemical degradation and persistence assessment



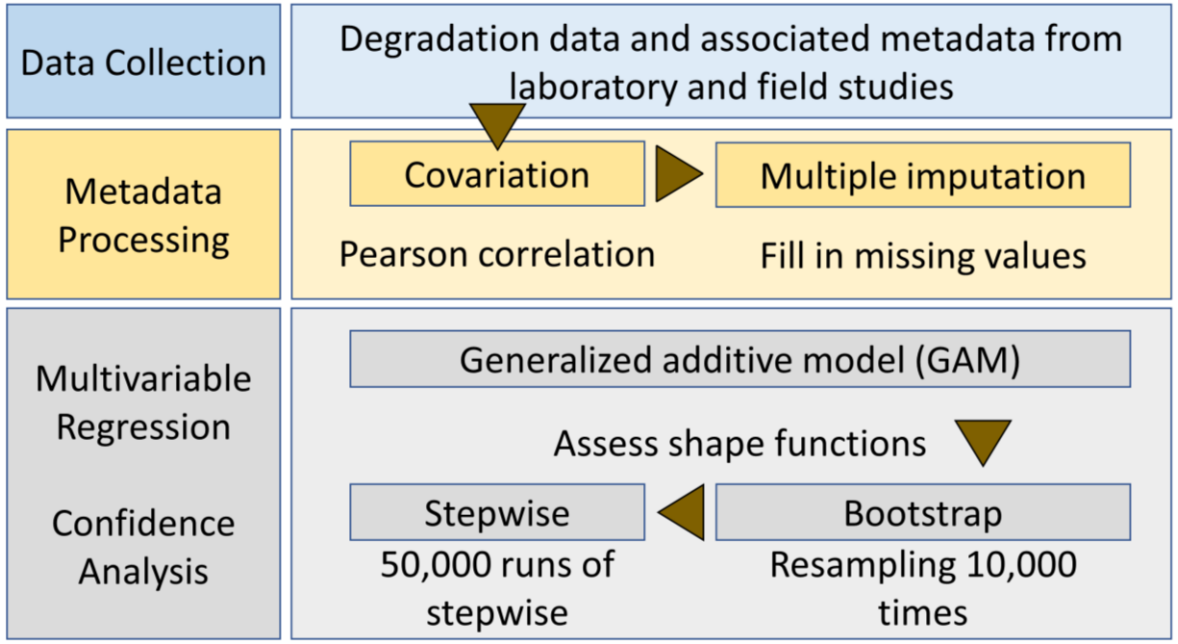
- Reviewed and summarized regulatory (i.e., OECD) and non-regulatory approaches for estimating half lives of chemicals;
- Considered experimental and model-based approaches;
- Reviewed degradation data sources and collected half lives of chemicals in different environmental compartments;
- Provided a theoretical discussion on environmental factors that contribute to chemical degradation in different environmental compartments.

THERE WERE TWO MAJOR RESEARCH OBJECTIVES FOR ECO31

Objective 2: Provide an evidence-based evaluation of the key factors that drive chemical degradation rates

- Workflow developed for aerobic biodegradation half lives of pesticides in soil;
- First considered data for atrazine from 95 laboratory and 65 field studies;
- Then applied workflow to laboratory data for 10 additional pesticides;
- General workflow valid for any chemical and any degradation process, aerobic biodegradation of pesticides is soil selected based on data availability.

Schematic of the Multivariable Workflow



Wang et al. *Chemosphere* (2018)
DOI: 10.1016/j.chemosphere.2018.06.077

WHAT WE LEARNED FROM OBJECTIVE 1 OF ECO31 – REVIEW OF DEGRADATION AND PERSISTENCE

- Environmental factors specified in OECD tests are rarely reported in non-regulatory settings and are infrequently included in databases that summarize the results of OECD regulatory tests;
- The environmental factors specified in OECD tests also likely do not adequately cover the space of environmental factors that are important for degradation;
- Well-curated metadata related to the environmental conditions under which rate constants were estimated are essential for improving our understanding of variable degradation. We strongly recommend improved reporting of all experimental metadata, whether they are explicitly specified in the OECD test or not;
- These data should be stored in publically accessible electronic databases for efficient access.

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WHAT WE LEARNED FROM OBJECTIVE 2 OF ECO31 – THE MULTIVARIABLE WORKFLOW

- We found that the main factors that drive atrazine degradation in laboratory studies are the same factors that drive atrazine degradation in the field - atrazine application history and soil texture;
- We found that chemical application history and biomass concentration were important factors for all of the chemical substances for which data was available;
- We noted that half-life dependencies on pH were inconsistent and not particularly strong for most pesticides, reflecting that pH is a parameter whose influence on aerobic biodegradation half-lives is difficult to predict;
- We found that the organic carbon content of the soil was a key factor driving degradation rates for more soluble and hydrophilic chemical substances;
- We found that factors related to soil sampling depth are key factors driving degradation rates of pesticides with higher organic carbon-water partition coefficients (K_{OC}).

Wang et al. *Chemosphere* (2018)
DOI: 10.1016/j.chemosphere.2018.06.077

LIMITATIONS OF STUDY AND FURTHER CONSIDERATIONS

- Our comparison of laboratory and field data for atrazine degradation confirm the utility of multivariable workflows for identifying key factors driving degradation half lives, but it is possible that the smaller datasets available for some other pesticides (and for other chemicals in general) may lead to less reliable results;

- There may be other environmental factors that are important for variable degradation that have not yet been considered in either our theoretical discussion or in our multivariable framework;

- Future work should couple multivariable analyses with careful laboratory experiments that systematically explore the effects of the factors identified as key variables in determining degradation rates;

- Results of these synergistic studies can be used to inform the future evolution of OECD and similar guidelines to control for and record the most important environmental factors that contribute to the magnitude of degradation rates. These guidelines may need to be malleable as the role of each factor may depend also on intrinsic chemical properties.

Thank you

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Helsinki, 27 September 2018

Identifying limitations of the OECD water-sediment test and developing suitable alternatives to assess persistence - Cefic LRI ECO 18

Kathrin Fenner, Eawag & University of Zurich

Collaborators: D. Hennecke, P. Shresta, S. Hahn (Fraunhofer IME/ITEM), M. Honti (Hungarian Academy of Sciences), Th. Junker (ECT Oekotoxikologie GmbH)

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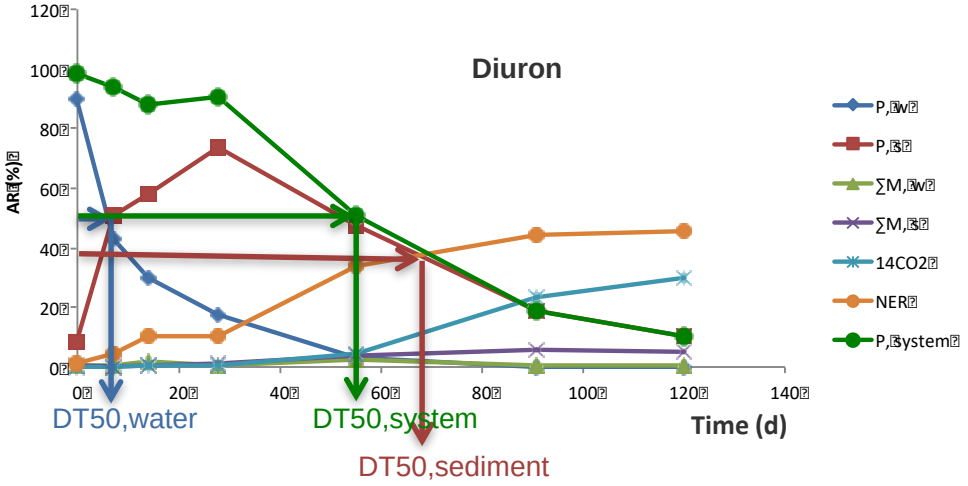
Starting point of ECO18 (2012-2016)

Experimental issues OECD 308

- Large experimental effort (vessels ≥ 60; labelled compounds), very expensive
- High sediment:water ratio shifting mass distribution excessively towards sediment
  - ➢ Not representative of most exposure situations
  - ➢ Sorption often dominant process, “masking” degradation
  - ➢ Extensive NER formation: Relevance in natural systems?
- Redox gradient within sediment layer

Data interpretation issues OECD 308



- ➢ Dynamic partitioning between solid aerobic/anaerobic phase and water during incubation
- ➢ $DT_{50,water}$ and $DT_{50,sediment}$ confound degradation and phase transfer; not suitable for comparison to P cut-off values or exposure modeling
- ➢ $DT_{50,system}$ to some extent system-dependent

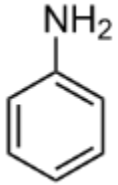
Honti M, Fenner K. 2015. *Env Sci Technol* 49: 5879-5886

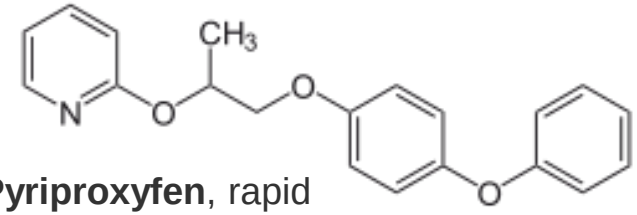
Alternative experimental systems



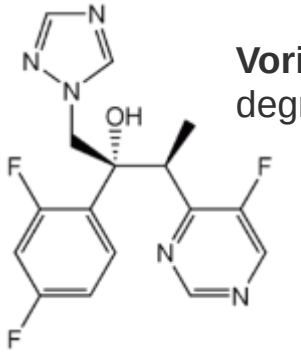

Test system	OECD 308 (standard)	OECD 308 modified	OECD 309 modified	OECD 309 (standard)
Compartments	stratified	stratified, H_2O stirred	mixed, stirred	mixed, stirred
Water [mL]	150	500	300	300
- height [mm]	60	42	-	-
Sediment [g dw]	50	50	3	0.3
- height [mm]	20	5	-	-
- interfacial area [cm^2]	22	90	-	-
- Volume [mL]	45	45	-	-
Water:Sediment (w/w)	3:1	10:1	100:1	1000:1
Water:Sediment (v/v)	3.3:1	11.1:1	-	-

Aniline, ready degradability, weak sorption

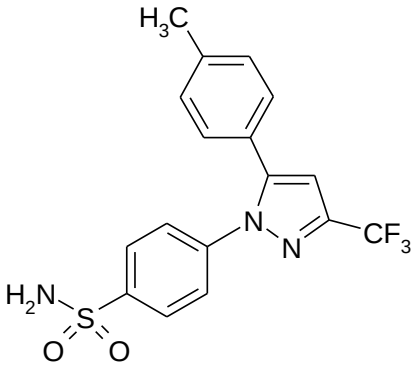




Pyriproxyfen, rapid degradability, strong sorption



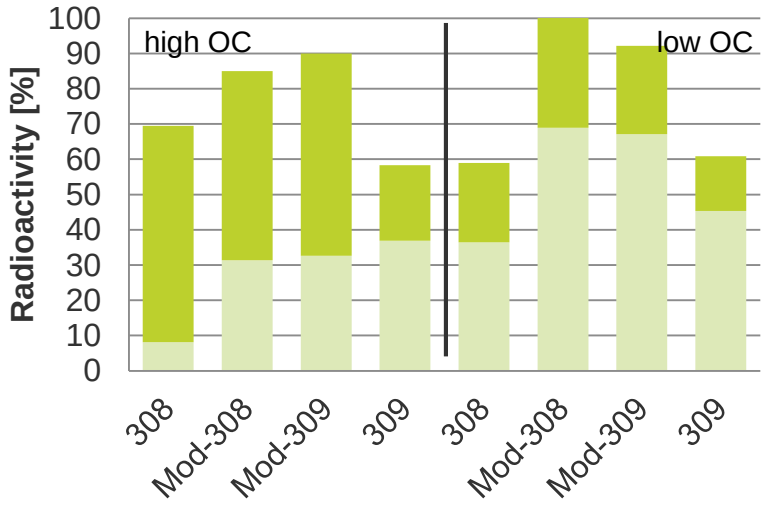
Voriconazole, low degradability, weak sorption



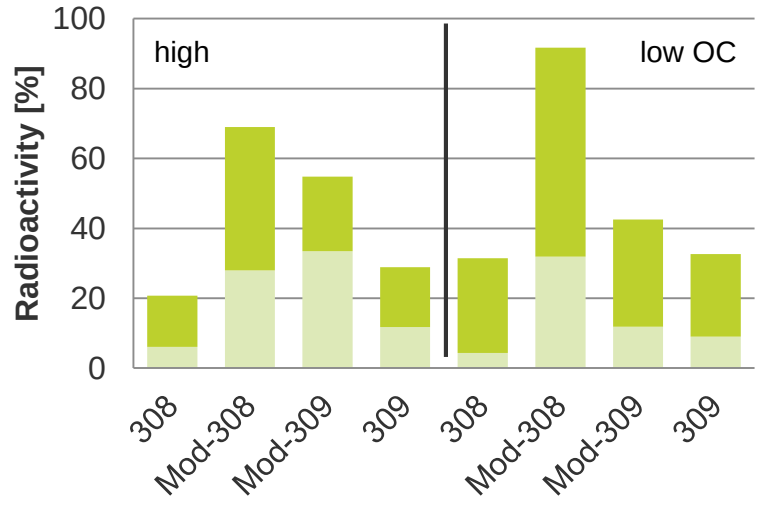
Celecoxib, low degradability, strong sorption

Alternative systems – Results & recommendations

Aniline



Pyriproxifen



Results

- Increased mineralization in modified systems, but mostly coincides with increased NER formation
- Deeper oxic sediment layer due to thinner sediment layer and stirring of water
- No systematically improved reproducibility or completeness of mass balance in 309 vs 308

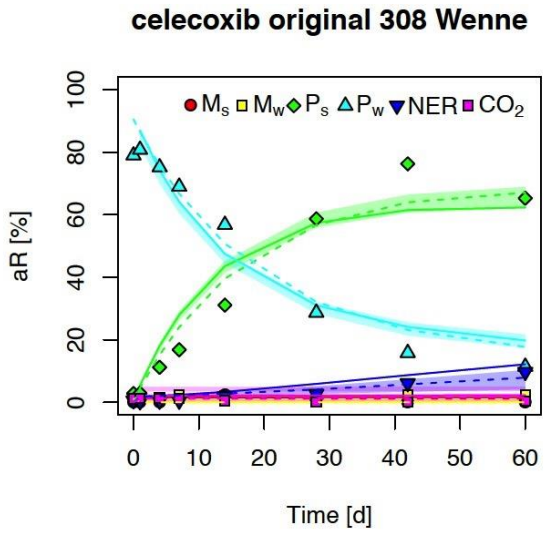
Recommendations

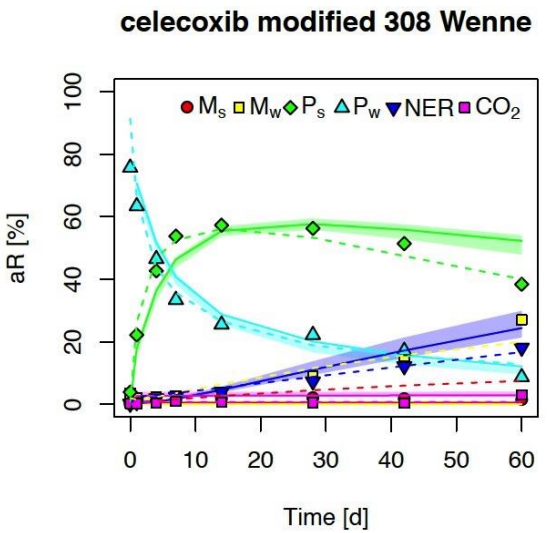
- Large flexibility of experimental options in 309 (e.g., amount of sediment, stirred/shaken, light/dark, sediment sampling) leads to high variability in outcomes → Need for further standardization
- Modified 309 with increased sediment concentration might be simple, representative system to test transformation at water-sediment interface

Shrestha, P., *et al*. 2016. Env Sci Technol **50**: 6856 - 6864

Data interpretation – Bridging across OECD 308 & 309

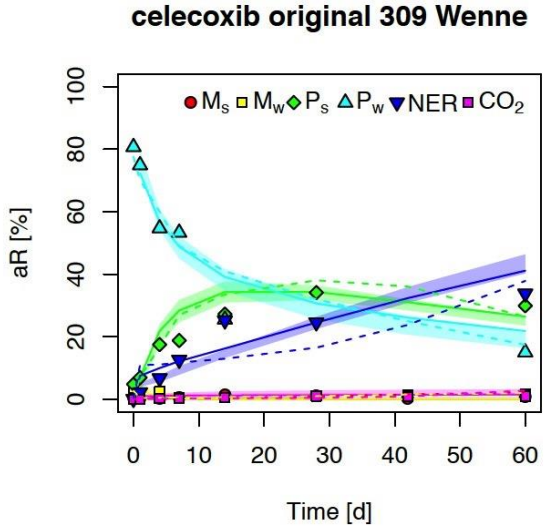
OECD 308

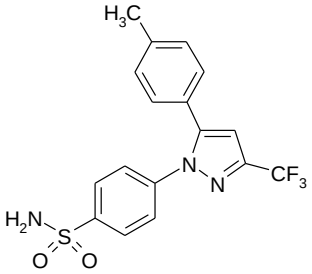


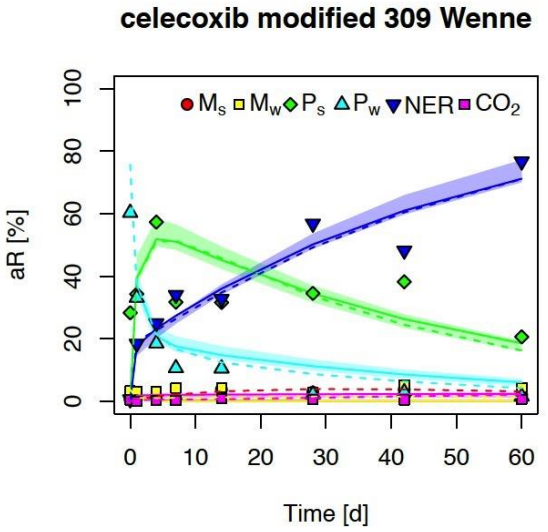


mod. OECD 308

OECD 309







mod. OECD 309

Data interpretation – Results

Hypothesis: A bioavailability- and biomass-normalized k'_{bio} parameter can unify observations from different water-sediment test system setups

Organic Carbon: proxy for biomass and sorbent

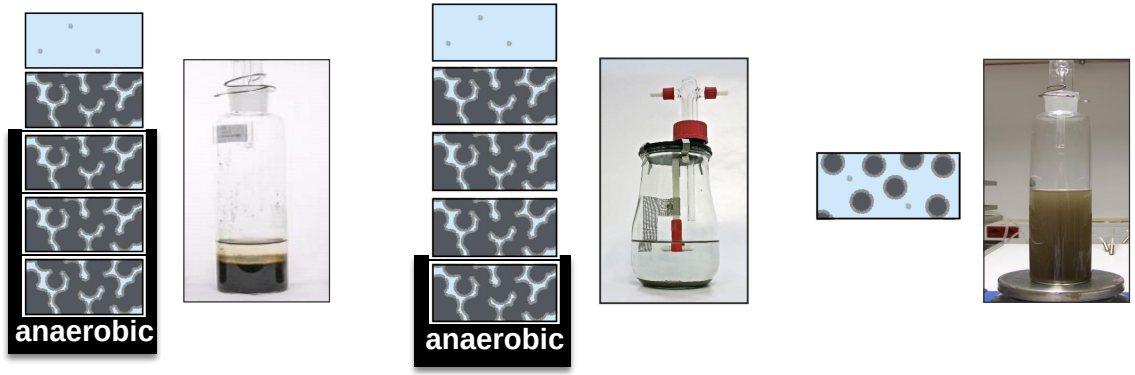
Transformation rates: $k_{\mathrm{deg,I-III}} = k'_{\mathrm{bio}} \mathrm{TOC} f_{\mathrm{aq}}$

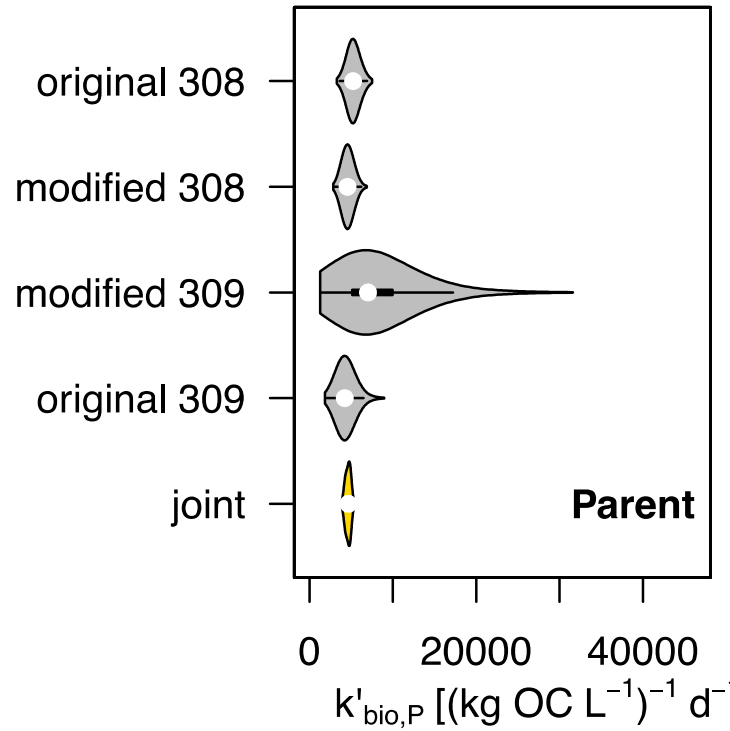
Bioavailability: $\frac{1}{1 + K_{\mathrm{oc}} \cdot \mathrm{TOC} \frac{V_{\mathrm{tot}}}{V_{\mathrm{aq}}}}$

$$k_{\mathrm{deg,I}} = k'_{\mathrm{bio,P}} \mathrm{DOC} \frac{1}{1 + K_{\mathrm{oc}} \mathrm{DOC}}$$

$$k_{\mathrm{deg,II}} = k'_{\mathrm{bio,P}} \left(\mathrm{DOC} + f_{\mathrm{oc,sed}} \mathrm{TSS}\right) \frac{1}{1 + K_{\mathrm{oc}} \left(\mathrm{DOC} + f_{\mathrm{oc,sed}} \mathrm{TSS}\right)}$$

$$k_{\mathrm{deg,III}} = k'_{\mathrm{bio,P}} f_{\mathrm{oc,sed}} (1-\theta) \rho_{\mathrm{solid}} \frac{1}{1 + K_{\mathrm{oc}} f_{\mathrm{oc,sed}} \rho_{\mathrm{solid}} \frac{(1-\theta)}{\theta}}$$





Honti, M., *et al*. 2016. Env Sci Technol **50**: 6865 - 6872

Data interpretation – Recommendations

Recommendations

- A bioavailability- and biomass-normalized k'_{bio} value can be found, which
  - can be derived with acceptable precision if data from two test systems are available (e.g., two 309 tests with different amounts of suspended sediment → simpler test protocol)
  - could be used as a test system-independent indicator of biotransformation in aerobic sediments
  - can also be converted into an *aerobic* P_{sed} value for comparison against the sediment cut-off criterion (using sediment composition default values).
  - can readily be used in exposure modeling

Further research needs

- Assessment of NER (see Cefic LRI ECO24 & ECO25)
- Development and validation of improved method to measure active biomass
- Further validation of conceptual soundness and applicability of k'_{bio} values with additional data sets

Honti, M., *et al*. 2016. Env Sci Technol **50**: 6865 - 6872

Thank you

Upcoming conference:
Transcon2019.ch

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Cefic LRI - Concawe Workshop on recent developments in science supportive to the persistence/biodegradation assessment

Helsinki, 27 September 2018

Limitations of OECD 307 and OECD 309 and recommendations for enhancements

Dieter Hennecke, Fraunhofer IME

OECD Guidelines for the Testing of Chemicals, general

Since 1981, in recognition of the advantages of internationally agreed test methods, OECD member and partner countries have developed the OECD Guidelines for the Testing of Chemicals in order to:

- *enhance the validity and international acceptance of test data;*
- *…*

"OECD Test Guidelines may be applicable to and may be required for different types of chemicals, e.g. mono-constituent or multi-constituent substances, mixtures of chemicals, pesticide formulations, cosmetic products etc., depending on the legislation and depending on whether they provide relevant results for the intended regulatory purpose."

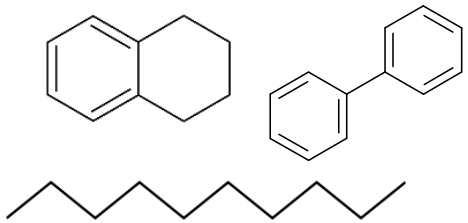
http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

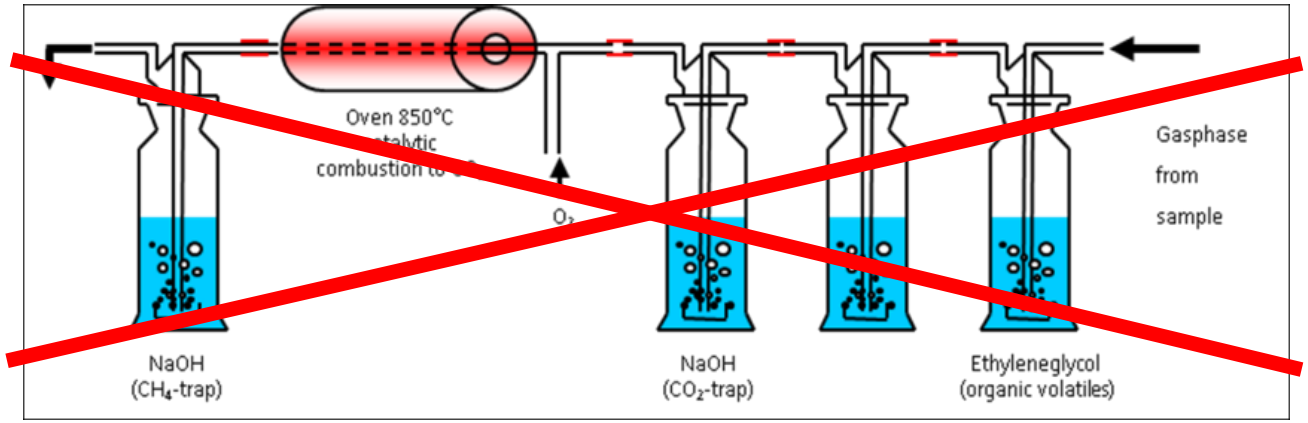
…may be applicable…

…may NOT be applicable…

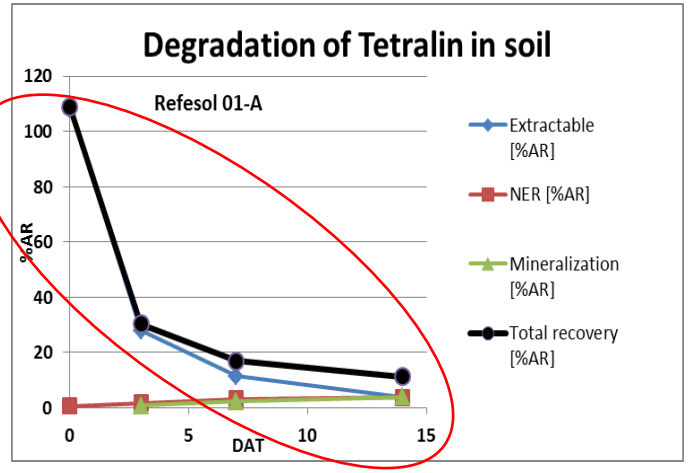
Testing of volatiles with OECD Guidelines

Standard OECD guidelines unsuitable for testing volatile chemicals





Flow-through designs result in incomplete mass balances which can be misinterpreted as degradation if test conducted without ^{14}C-radiolabelled substance

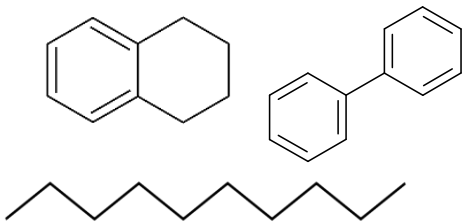


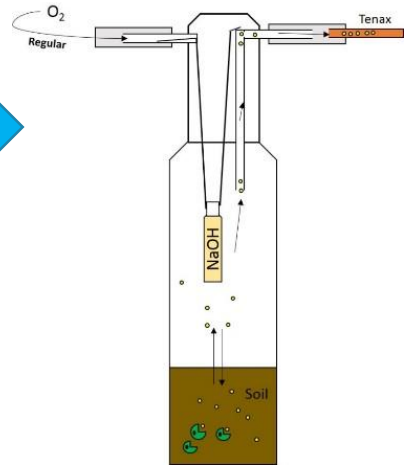
Fraunhofer IME

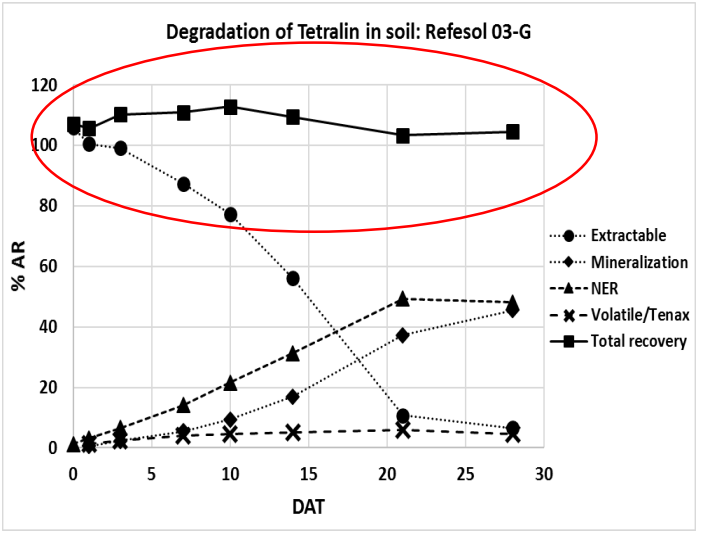
Testing of volatiles with OECD Guidelines

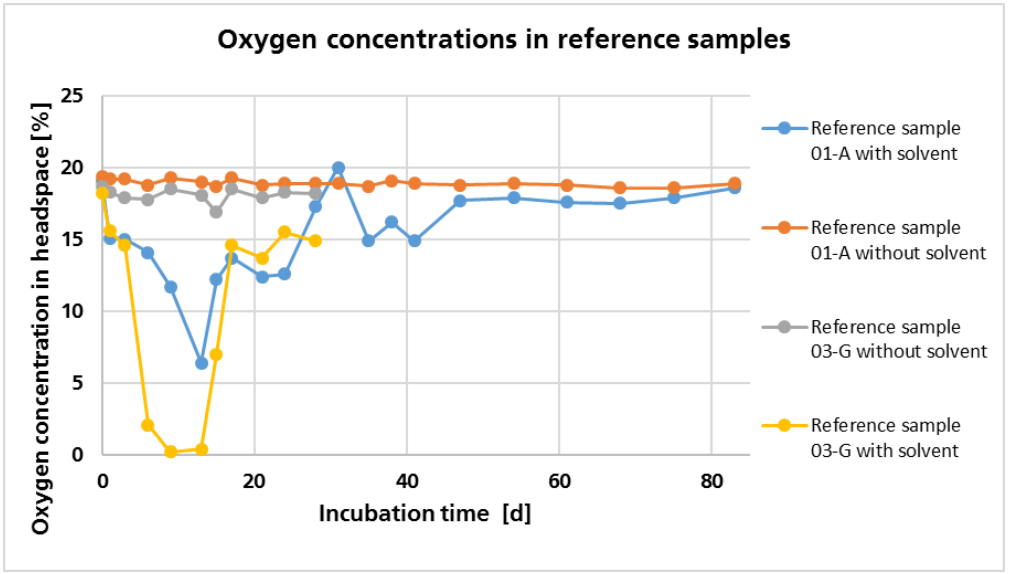
OECD 307

The use of closed systems improves mass balances and enables to quantify also volatilized fractions









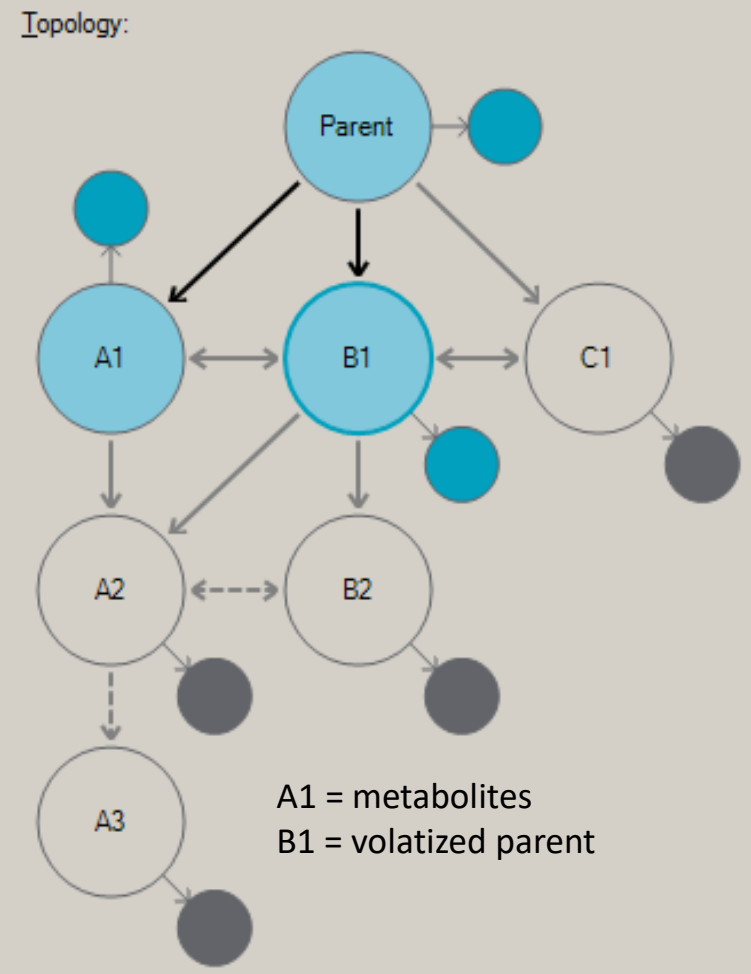
Closed systems must be monitored for oxygen consumption to maintain aerobic conditions.

Recommendation: optical O_2-measurement

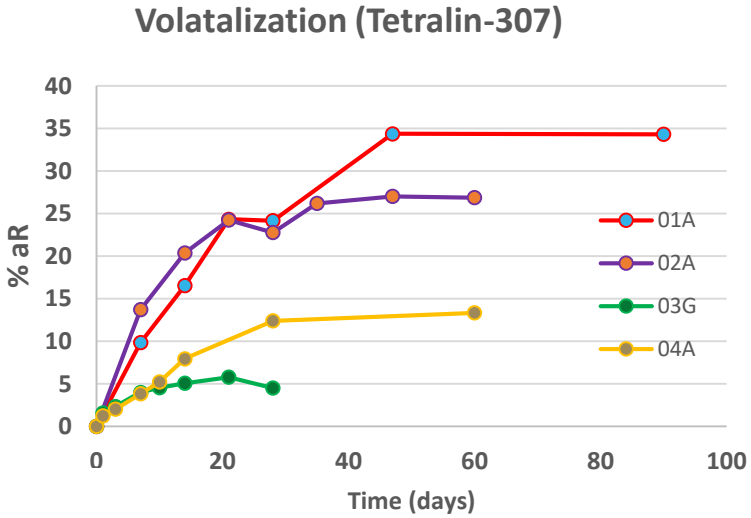
Testing of volatiles with OECD Guidelines

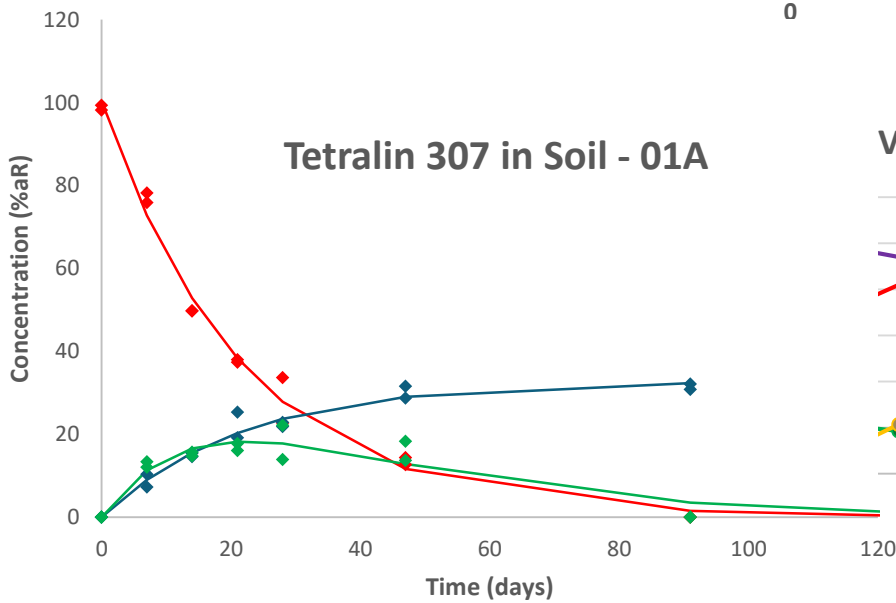
OECD 307

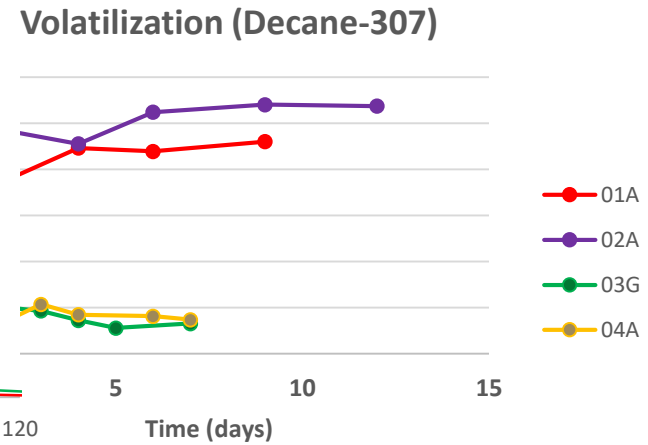
But even in closed test setup:
competition between sorption, degradation and volatilization!



lependent on soil properties.
not considered.





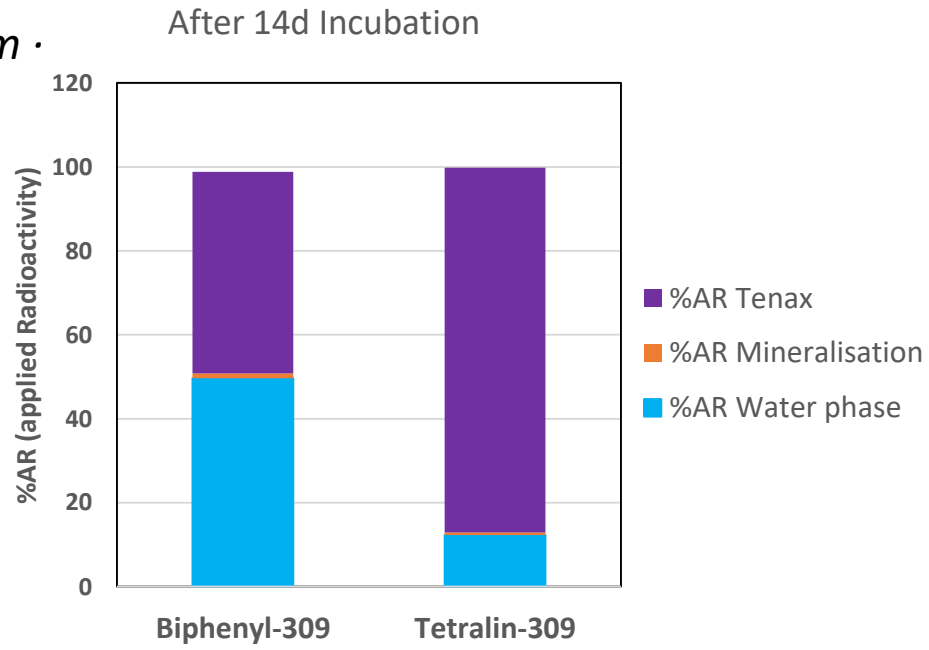


Fraunhofer IME

Testing of volatiles with OECD Guidelines

OECD 309

Section 7: *"Using closed flasks with a headspace, it is possible to test slightly volatile substances (with Henry's law constants **<100 Pa · m³/mol** or <10^{-3} atm · m^3/mol) without losses from the test system."*



Current studies: Pelagic test with marine water in new setup

- Biphenyl: K_H 31.2 Pa m^3/mol
- Tetralin: K_H 138 Pa m^3/mol

Results:

- other than in 307, volatilization as dominating process
- *thin* microbial inoculum results in low degradation rate
- no solid phase for sorption to keep substance in system

Further research needed for 309 setup. Suspended sediment setup might change results significantly

Fraunhofer IME

Conclusions

- OECD-Guidelines are used in REACh often out of their scope regarding substance properties
- Standard test setup will lead to false data! Special care has to be taken.
- Specific test setup has been developed. Enables complete mass balances for 307!
- O_2 monitoring in closed flask tests necessary! New challenge.
- For OECD 309 further research needed. Current setup not satisfying.
- ^{14}C labelled test substance avoids wrong interpretation and shows pathways;
  for upcoming NER-assessment ^{14}C-label necessary!
- Extended model considers volatilization.
  Simple and pragmatic for generating the degradation kinetics with a good fit

There are new challenges by new substances but it is possible to overcome it by modification of existing test methods.
Most important: awareness, that available guidelines might not work without modifications in new regulations!

Thank you

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Cefic LRI - Concawe Workshop on recent developments in science supportive to the persistence/biodegradation assessment

Helsinki, 27 September 2018

Biodegradation kinetics of hydrocarbons at low concentrations – Covering several orders of magnitude in hydrophobicity and volatility

Heidi Birch, Technical University of Denmark (DTU)

Introduction

New experimental platform developed for biodegradation measurements

Similarities to simulation tests (OECD 309)

- Environmentally relevant concentrations (ng-µg/L)
- Environmentally native microorganisms
- Closed test systems applicable to (semi)volatile chemicals

New platform applicable for

- Non-labelled substances (Primary biodegradation)
- Multi Constituent Mixtures
- Hydrophobic chemicals
- Volatile chemicals

Cost-efficient

Expanding domain

Experimental

Simulation test adapted from OECD 309 (substrate depletion)

Passive dosing

Silicone rod loaded with a mixture of test chemicals

Stock solution 1:10

Degradation

Biodegradation in gas-tight vials at 20°C

ng-µg/L concentrations

Abiotic controls

Analysis

Automated SPME sampling on each test system followed by GC-MS

Chemosphere 174 (2017) 716–721

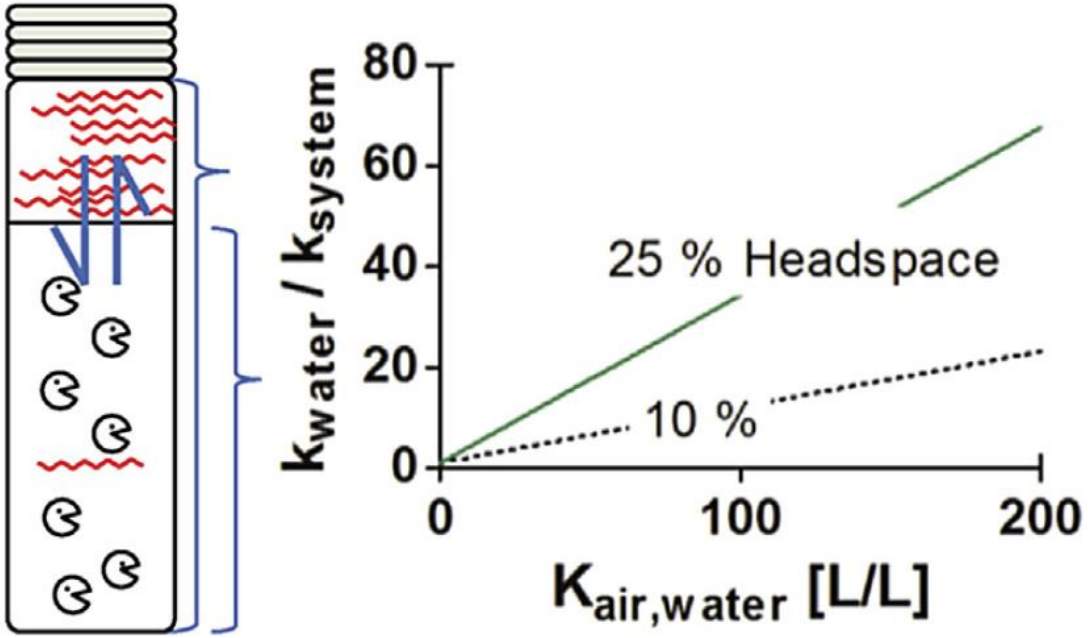
Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Biodegradation testing of chemicals with high Henry's constants – Separating mass and effective concentration reveals higher rate constants

Heidi Birch [a, *], Henrik R. Andersen [a], Mike Comber [b], Philipp Mayer [a]



k_{water} : first order biodegradation rate constant in the water phase

k_{system}: first order biodegradation rate constant for the test system

Chemosphere 184 (2017) 400–407

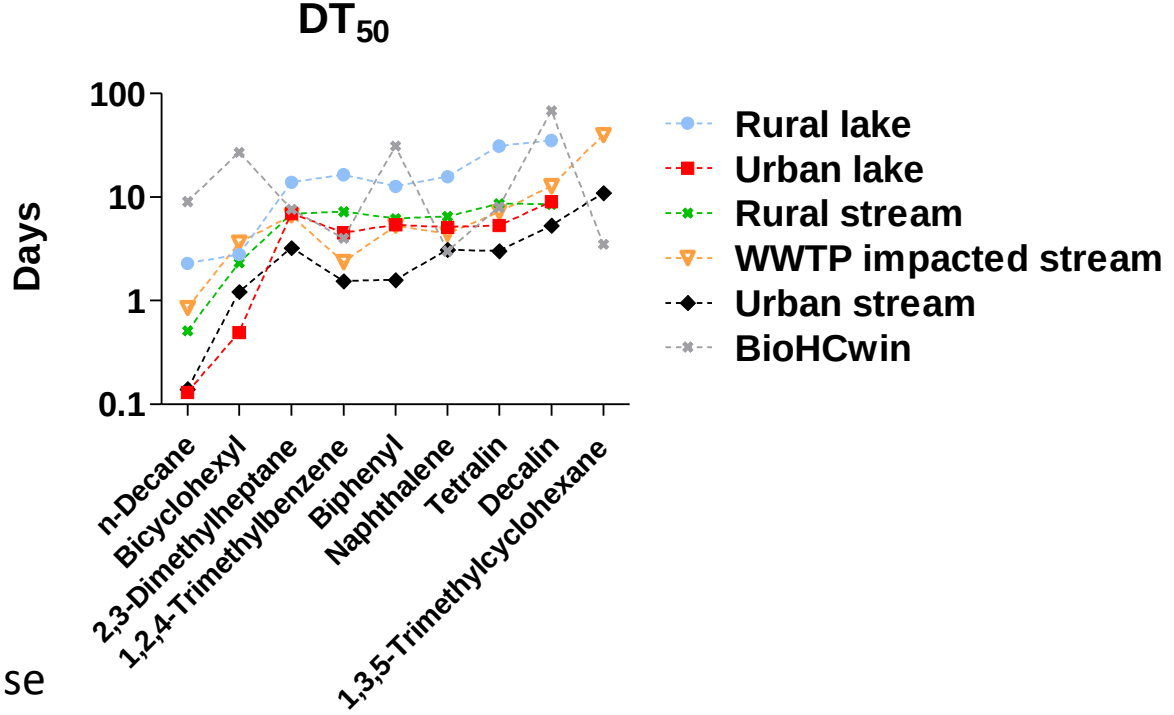
Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Biodegradation of hydrocarbon mixtures in surface waters at environmentally relevant levels – Effect of inoculum origin on kinetics and sequence of degradation

Heidi Birch [a, *], Rikke Hammershøj [a], Mike Comber [b], Philipp Mayer [a]



Environmental Science & Technology

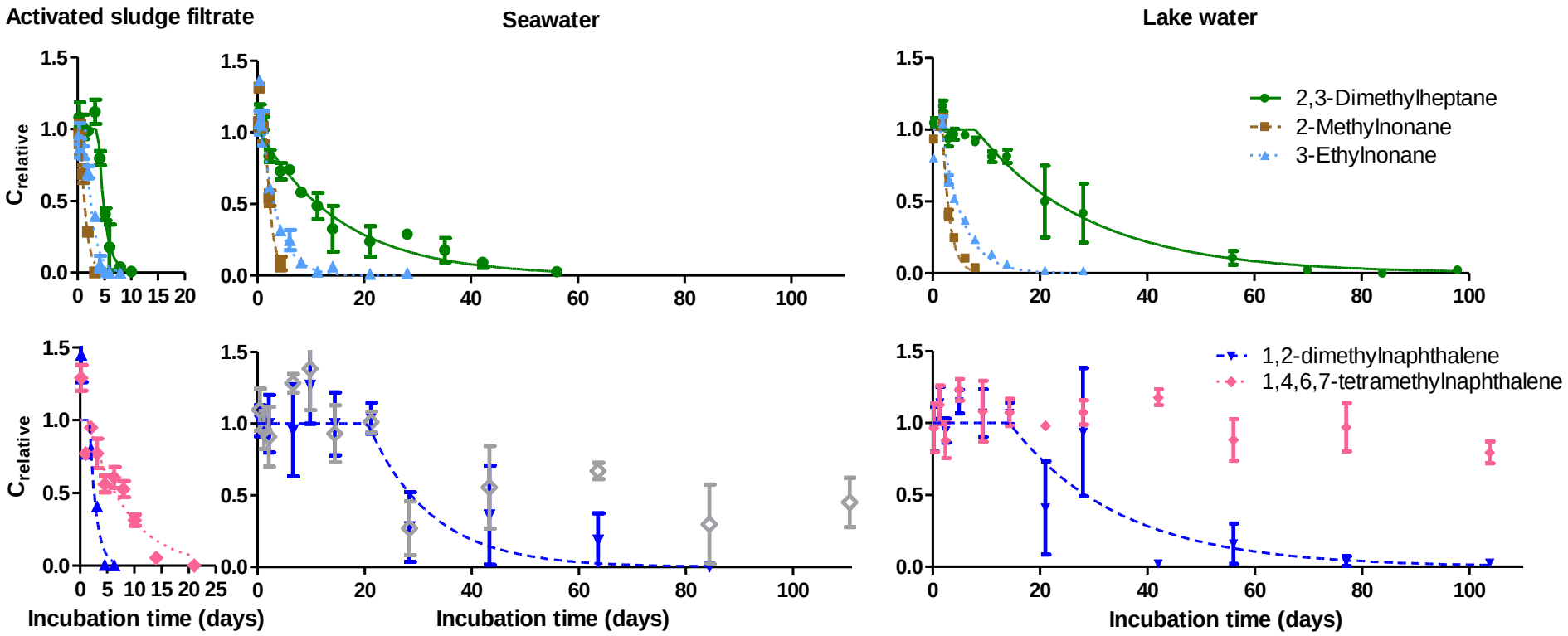
Article

Cite This: Environ. Sci. Technol. 2018, 52, 2143−2151

pubs.acs.org/est

Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of K_{ow} and K_{aw}

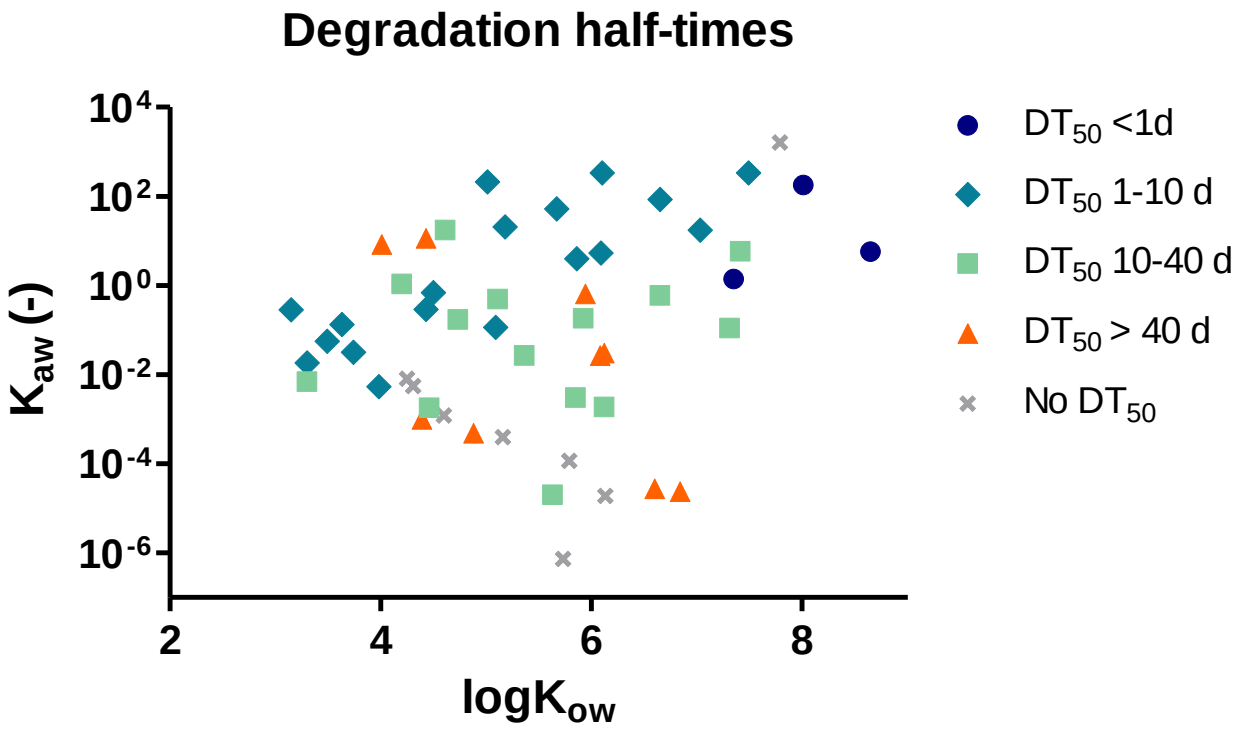
Heidi Birch,* Rikke Hammershøj, and Philipp Mayer

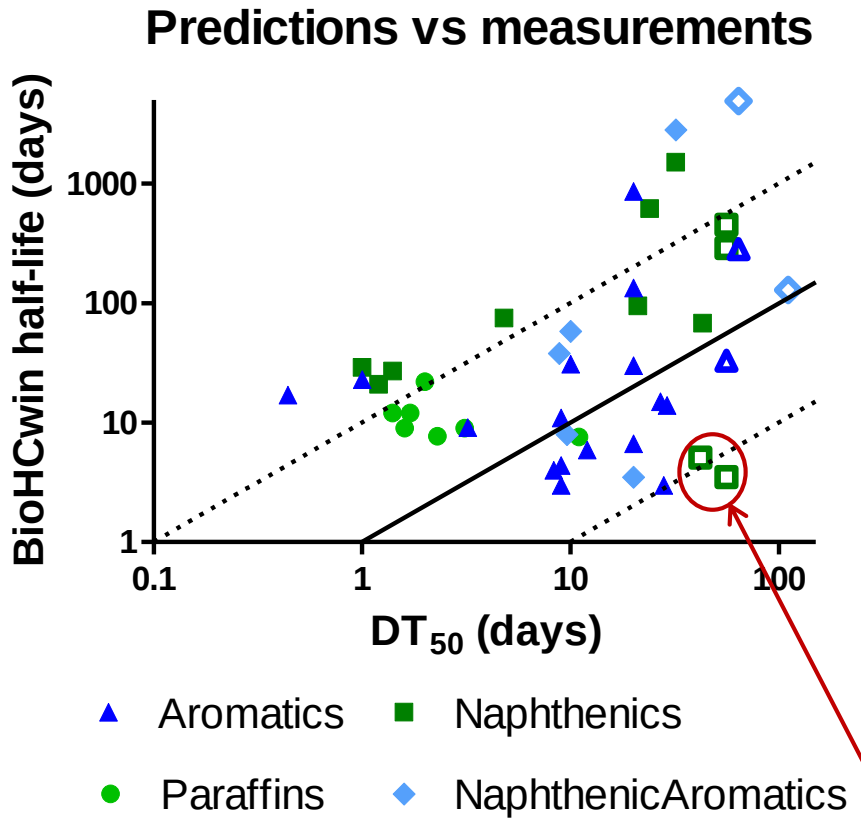


Most hydrocarbons were degraded within the test duration

- 4 hydrocarbons with limited/no degradation in all three water types

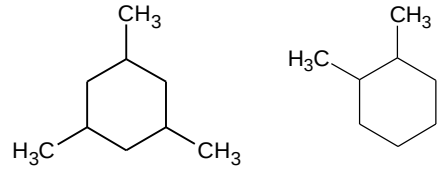
Biodegradation of hydrocarbons, seawater





Half-times did not correlate with logK$_{OW}$, K$_{AW}$ or hydrocarbon blocks

Most chemicals within a factor of 10 of BioWin predictions or faster degradation than predicted, except:



Conclusions

Advantages

- Testing multi-constituent mixtures can yield large sets of well aligned data
- Native microorganisms exposed to relevant concentrations
- Test substance losses minimized: (a) gas tight test system, (b) liquid handling with glass syringes & (c) automated SPME
- Extended applicability domain for high K_{OW} and K_{AW} substances

Limitations

- Based on substrate depletion, thus limited to primary degradation
- Limited to aqueous media
- 13.5 mL can be insufficient at low degrader densities

Acknowledgements

- We thank Concawe for financial support, Hanne Bøggild for technical assistance and Lynetten wastewater treatment plant for providing activated sludge

Thank you

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