The regulatory and scientific impact of LRI and ECETOC environmental persistence initiatives over the past ten years.

Dr Jason Snape

Principal Scientist AZ SHE

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Issues to be Addressed

- Environmental Persistence
- Pre-REACH initiatives
- Impact of CEFIC LRi, ECETOC and other science-based activities on REACH
- Post-REACH activities
- Concluding remarks
- Future needs

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Environmental Persistence

- The tendency of a chemical to remain in the environment without transformation or breakdown into another chemical form. Refers to the length of time a chemical is expected to reside in the environment and be available for exposure. http://www.thriftyfun.com/

- Persistence is the ability of a chemical substance to remain in an environment in an unchanged form. The longer a chemical persists, the higher the potential for human or environmental exposure to it. The individual environmental media for which a chemical's persistence is usually measured or estimated are air, water, soil, and sediment. http://www.pbtprofiler.net/

- Persistence is defined by environmental half-lives

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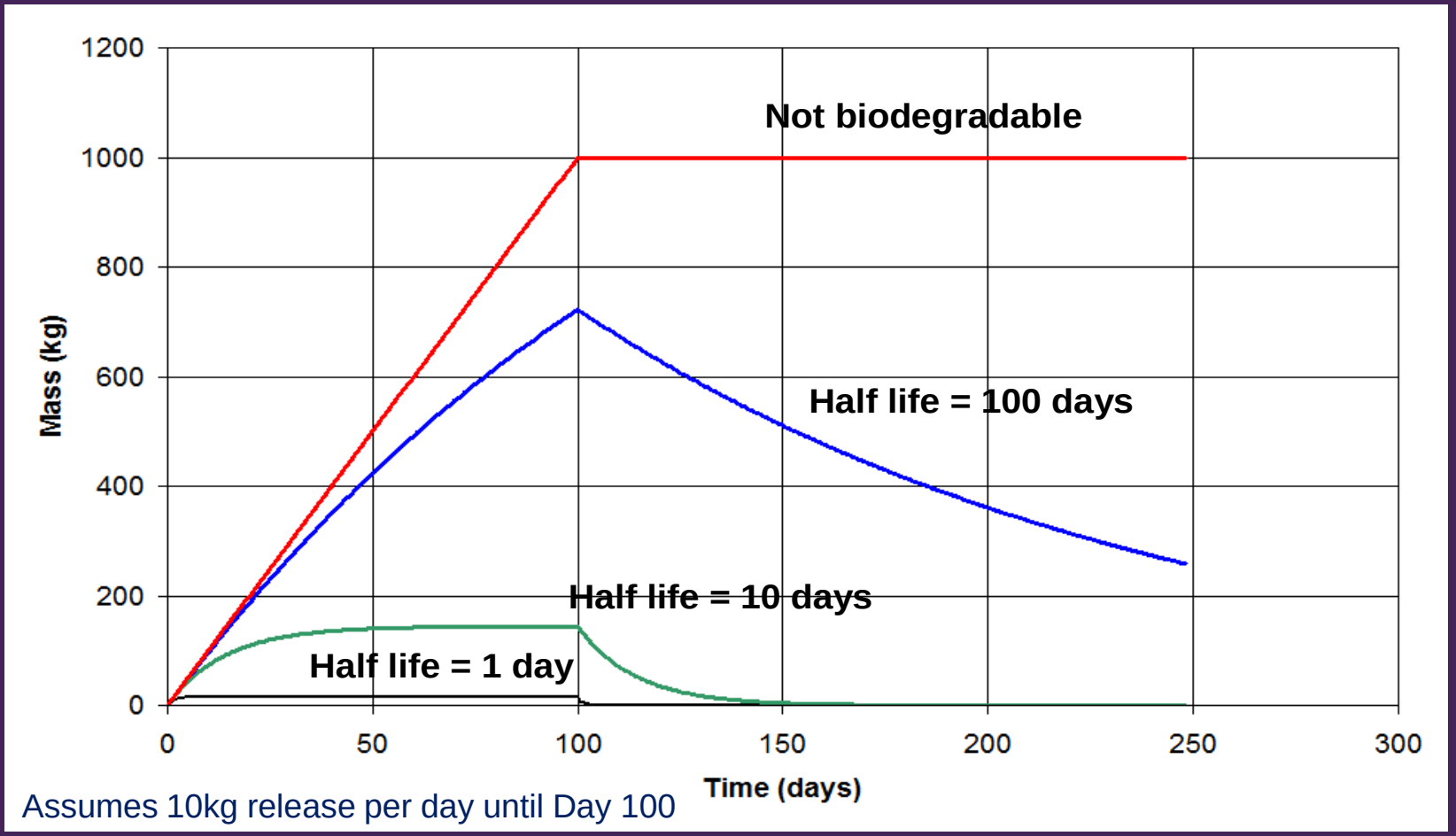
Persistence: Importance of Degradation



Assumes 10kg release per day until Day 100

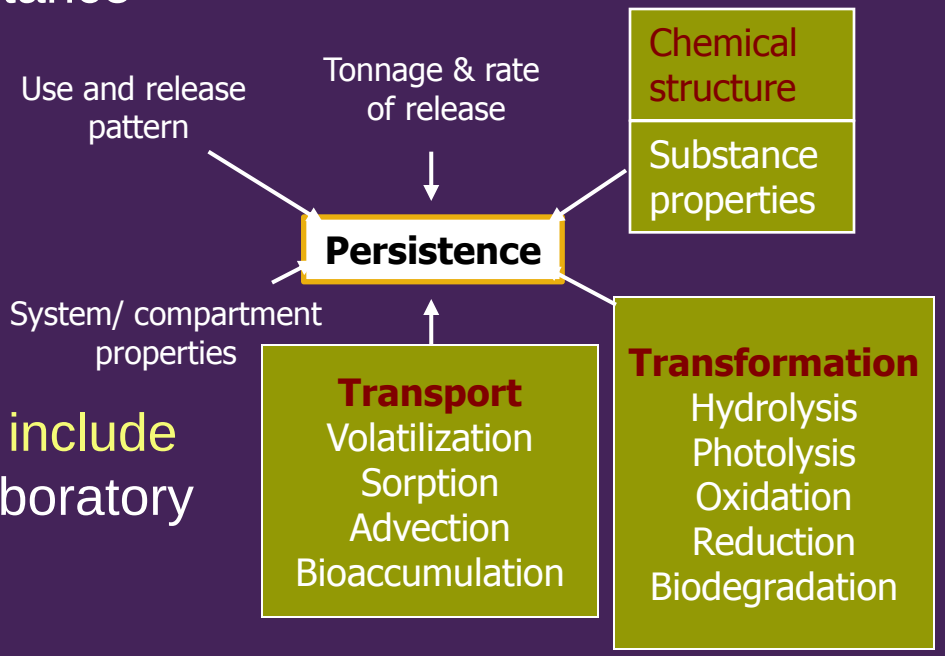
Substances that do not degrade or degrade slowly remain available to (potentially) exert toxic effects, bioaccumulate or be transported to other regions

Assessing Environmental Persistence

It is not straightforward

- Persistence cannot be directly measured
- Persistence is not an intrinsic substance property

Note: mere detection, by itself, is not evidence of persistence!



It can only be inferred. Evidence may include

- Lack of observed degradation in laboratory or field studies
  and/or
  predictions from models (e.g. SARs)

In combination with

- Continued presence of a substance in the environment

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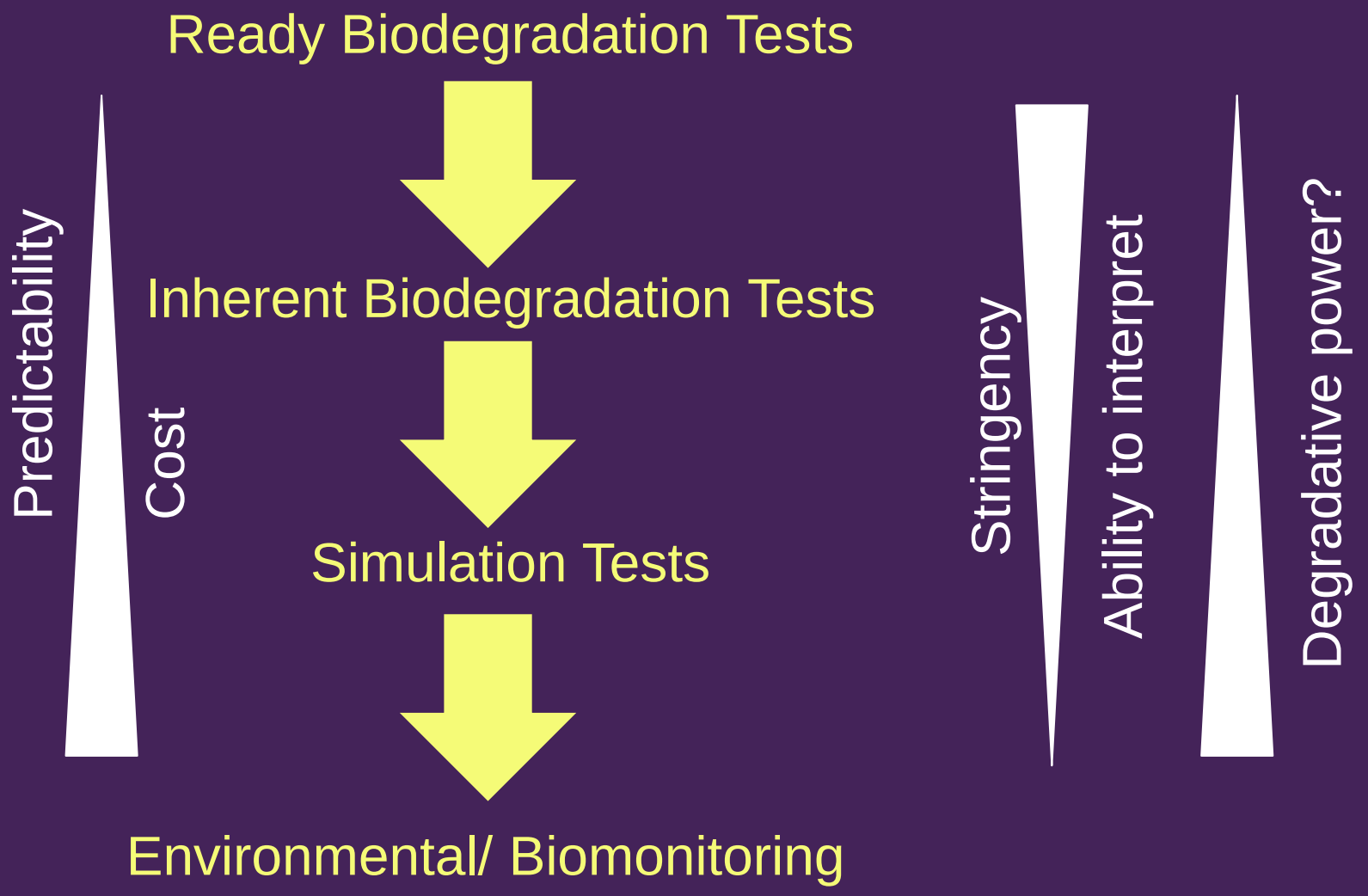
Current Standard testing Paradigm



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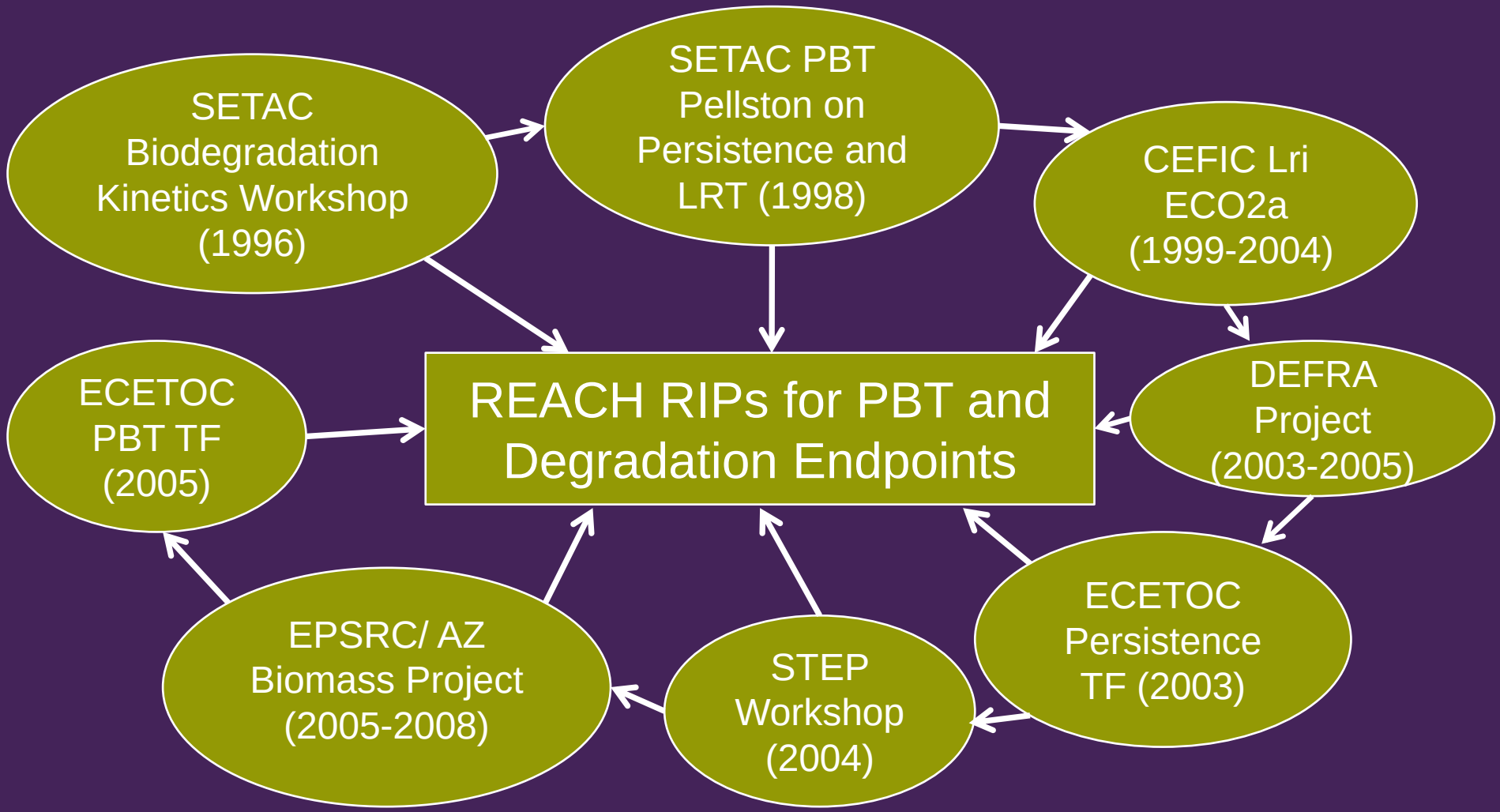
Pre-REACH Persistence Activities



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Conclusions of Pre-REACH Activities

- Challenged the current testing paradigm against persistency assessments
- Advocated the maximal use of all relevant data
  - Standard and non-standard
- Identified tiered approaches to persistence assessment
  - Screening phase and a targeted confirmatory phase
- Recognised that no single rate of degradation existed
- Recognised that freshwater studies with marine water would result in false negatives
- Advocated new approaches to persistency assessment
  - Test and model-based

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Challenging the Design and Use of Ready Biodegradability

- Designed to screen-out chemicals that will rapidly degrade in all environments through normal usage
  - extremely conservative/ stringent
  - cannot prioritise on persistency
- The 301 series are not truly standardised
  - preconditioning regimes differ
  - test apparatus differ (volumes, methods of aeration)
  - variable sources of inocula (sludge, soil, env. waters etc.)
  - different endpoints (CO_2, BOD, DOC)
  - standardised based test duration and temperature

High potential for conflicting & variable results

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Why the High Number of Fails?

- The chemical is persistent
- The chemical is only partially degraded (20-60% mineralization is observed)
- The chemical has been introduced at an initial concentration that is toxic to the inoculum
- The low level of microbial biomass results in the absence of competent microbial degraders
- The artificial phosphate-buffered medium is unsuitable for the competent degraders
- Bioavailability issues (mass transfer limitations)

Persistency is only one cause of failure to degrade

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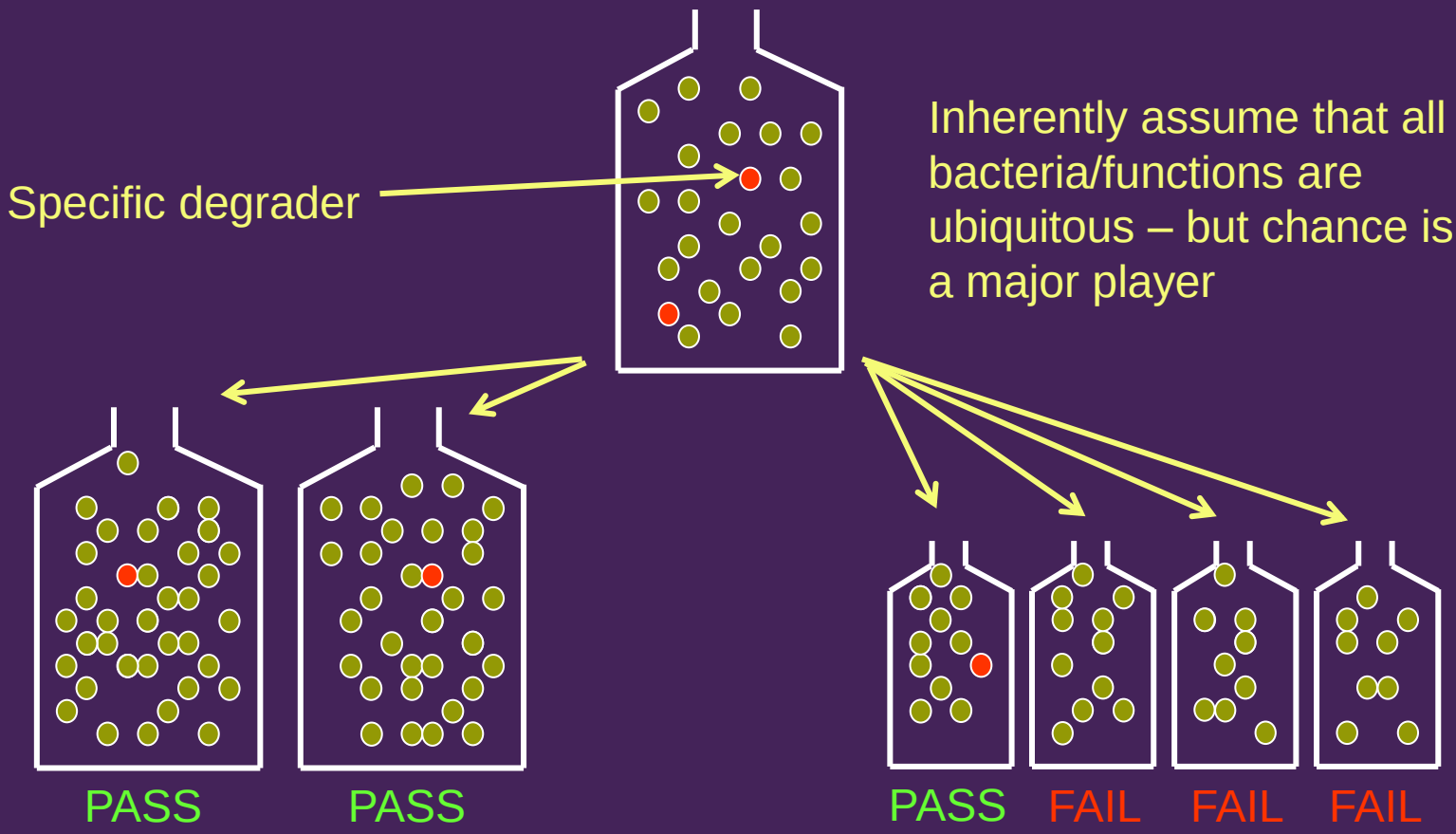
Lab-based Biodegradation Screening Tests are a Lottery

The odds are against you!!



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CEFIC LRi ECO2a Persistence in marine and terrestrial environments

Key outputs:

- Direct replacement of freshwater with marine water in biodegradation tests would:
  - Increase replicate variability/ noise masking other variables
  - Increase stringency of P assessments
  - Increase the number of false negatives
- Attributed to low biomass densities and low test volumes
- Attracted additional funding from Defra
  - Generated guidance for poorly solubles and marine assessment (Defra) – *Modified Studies Now in REACH*
- Used Stable Isotope Probing in the field (^{13}DNA)

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CEFIC LRi ECO2a Persistence in marine and terrestrial environments

Key outputs:

- Looked at ranges of rates within tests with high noise (early use of distributions)
- New methods tested and recommended:
  - Increased test volumes *(enhanced method now in REACH)*
  - Included semi-static marine studies *(now in REACH)*
  - Extended test durations for persistency assessments *(now in REACH)*
- AZ carried on funding density-based work with Newcastle (co-funded with EPSRC)
  - Proof of concept formed basis for method *now in REACH*
  - Team now awarded ECO11

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ECETOC TR90 Persistence of chemicals in the environment (2003)

Key Outputs:

- Together with ECO 2a it had a major impact on REACH
- Robust ‘intelligent’ persistence scheme was proposed
  - Effective use of existing data
  - The use of relevant data (compartment)
  - Scheme forms basis of P ITS in REACH
- Integrated model assessments earlier in the process
  - Prioritise and refine lab-based P assessments
- Identified a probabilistic approach with no single persistence half-life
- Promoted new test systems

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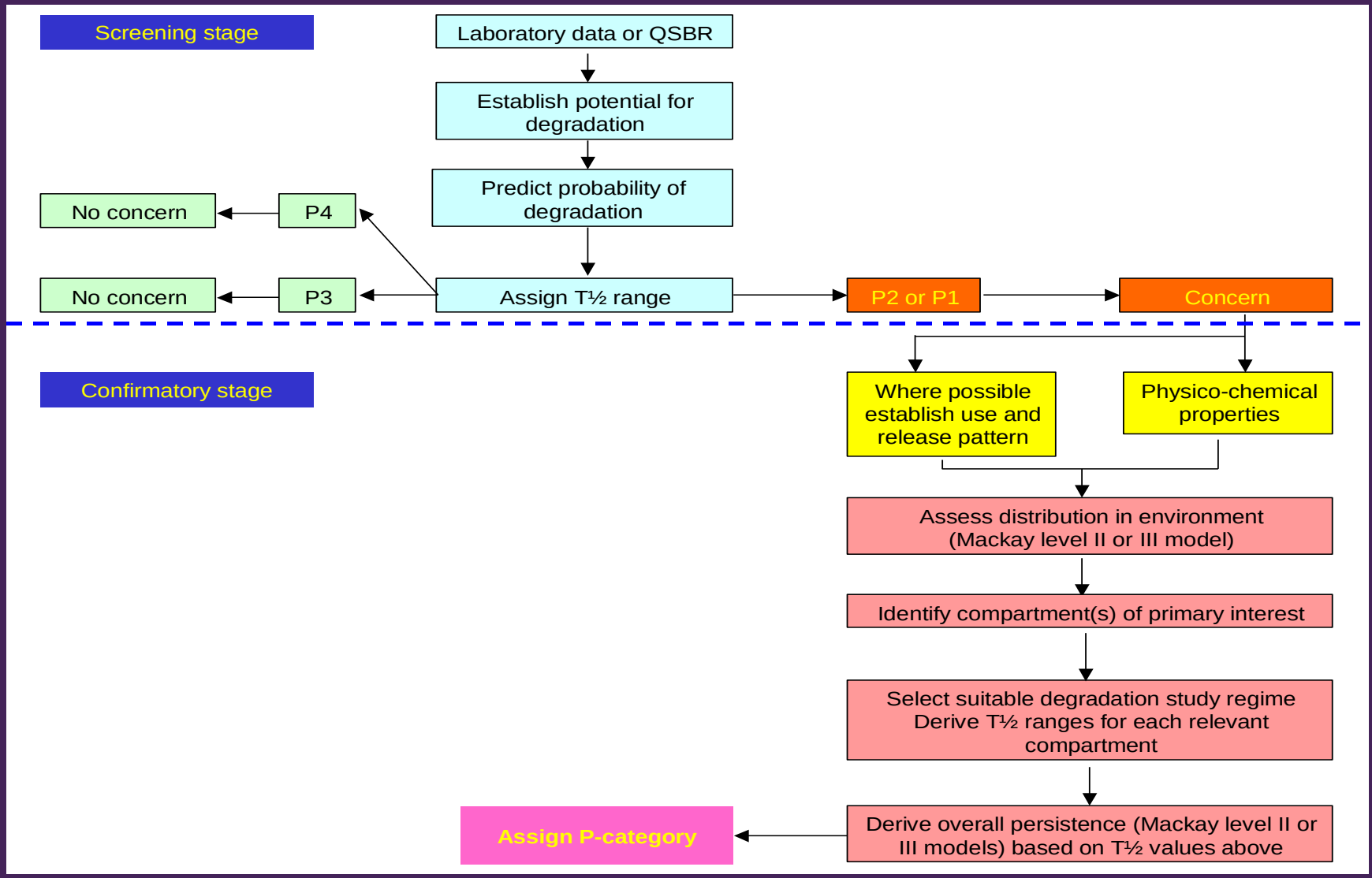
ECETOC Approach to Evaluating Persistence (2003)



Impact of these Science-Based Activities on REACH

- Challenged the information requirements outlined in REACH w.r.t. degradation for each tonnage trigger
- Data requirements excluded:
  - Sewage treatment simulations (OECD 303)
  - Inherent biodegradability tests (OECD 302 series)
  - Marine biodegradability tests (OECD 306)
  - Anaerobic biodegradability (OECD 311)
- Ensured maximum use of all available standard and non-standard data in persistency assessments
- Identified two new tiers of biodegradation assessments
  - Modified ready biodegradation tests
  - Enhanced biodegradation screening tests

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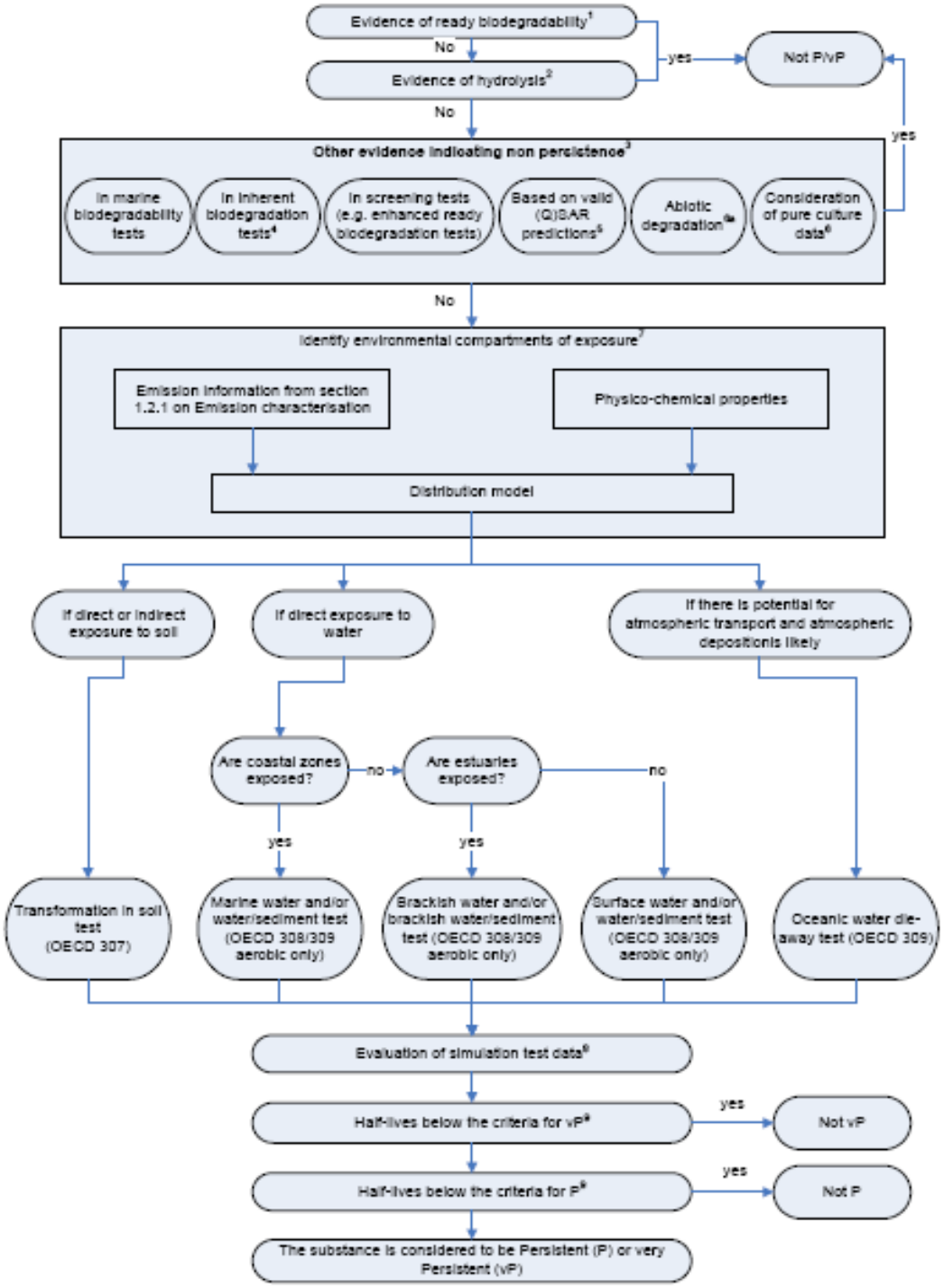

Screening Phase

Confirmatory Phase

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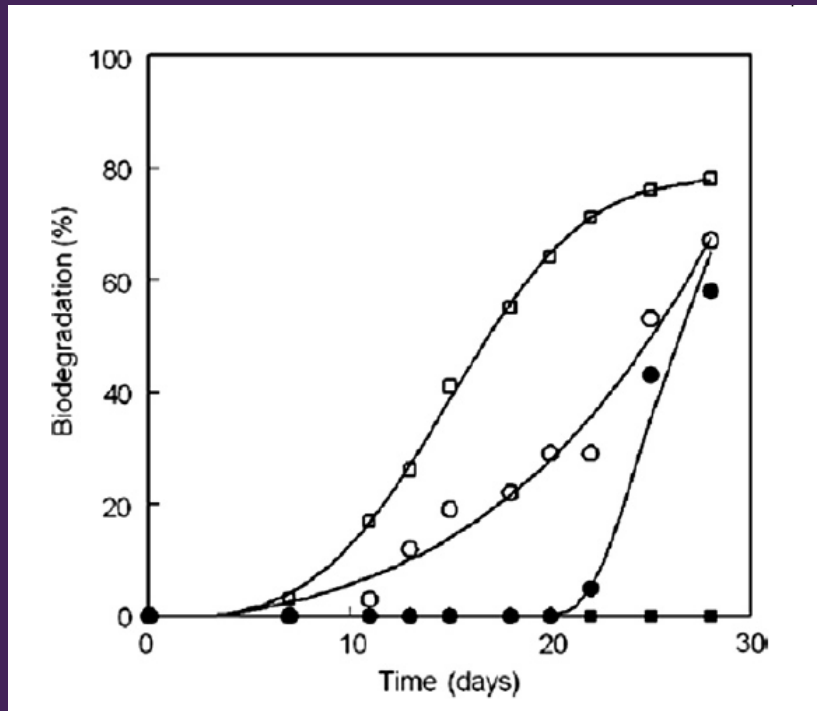
Modified Biodegradation Tests

Two simple modifications not considered to reduce the stringency of the ready test philosophy (can be used in Classification, CSA and P assessments)

- Testing at low substance concentration due to toxicity
- Use of solvents, emulsifiers, carriers etc. for poorly water soluble substances

Biodegradation of octadecyltrimethylammonium chloride
direct addition (closed square)
2 g silica per bottle (open square)
2.0 mg/l lignosulphonic acid (closed circle)
2.0 mg/l humic acid (open circle)



Van Ginkel et al. (2007)

Enhanced Testing

Only for use in Persistence assessments and not to be considered as ready biodegradable.

- Increased test duration beyond 28 days
- Increased vessel size or inoculum density derived from environmental samples not previously exposed
- Semi-continuous test systems with environmental samples (adaptation or enrichment from low initial densities)
- Running two ready tests in series (single subculture)

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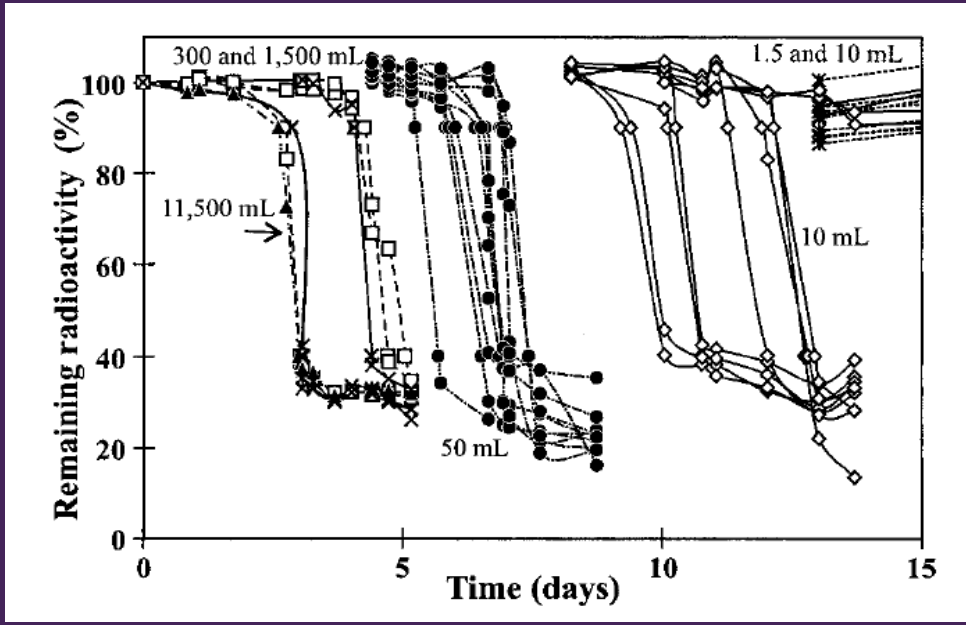
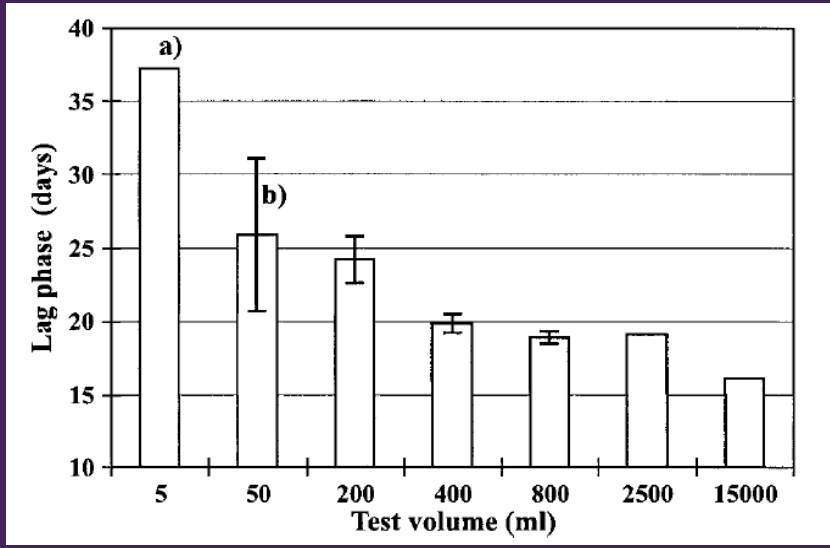
Increased Vessel Size



Larger volume
- same cell density
- greater biodiversity



Ingerslev, 2000

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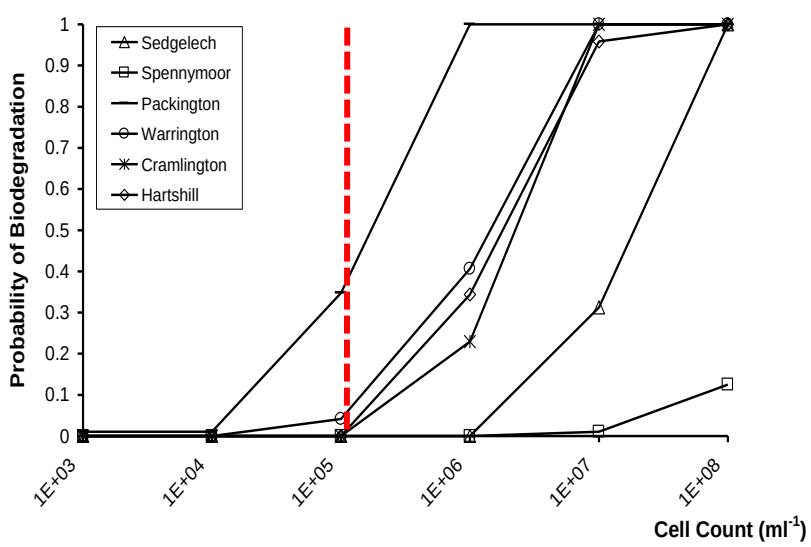
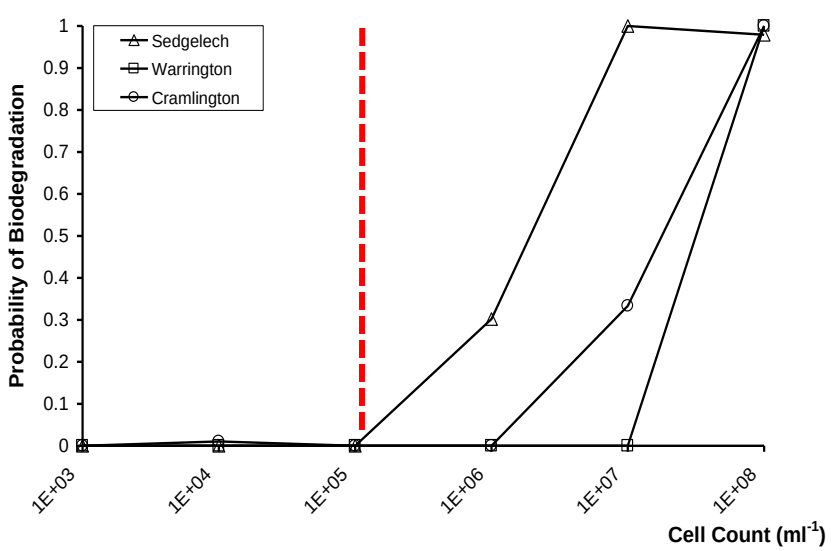
Enhanced Biomass Tests

Chemical X



Chemical Y



Thouand et al. (1995)
ECETOC (2003)
Goodhead et al. (2007, 2008, 2009; 2010)
Martin et al. (2010)

Fixed volume
- greater cell density
- greater biodiversity

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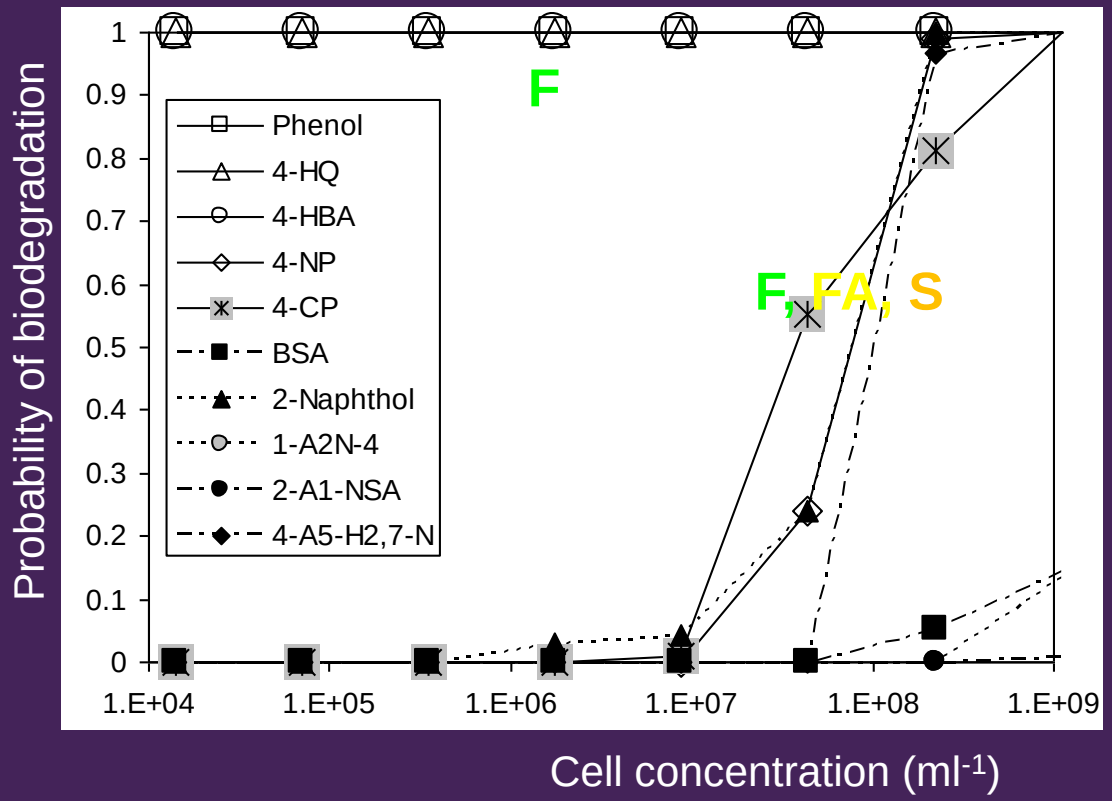
Chemical Screening & Prioritisation



BIODEG result:
F, Fast; FA, Fast with acclimation;
S, Slow; SA, Slow with acclimation

Howard *et al .*, 1987 *Env. Tox. Chem.*

Phenol (**F**)

4-HQ: 4-hydroquinone (**F**)

4-HBA: 4-hydrobenzoic acid (**F**)

4-NP: 4-nitrophenol (**F**, **FA**, **S**)

4-CP: 4-chlorophenol (**FA**, **S**)

BSA: Benzene sulfinic acid (nd – no data)

2-Naphthol (**F**, **FA**)

1-A2N-4: 1-amino-2-naphthol-4-sulfonic acid (nd)

2-A1-NSA: 2-amino-1-naphthalene sulfonic acid (nd)

4-A5-H2,7-N: 2-amino-5-hydroxy-2,7 naphthalene sulfonic acid (nd)

- High throughput – equivalent of ~ 59,700 individual BST
- Analysis is cheap, rapid and automatable

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Challenges posed by REACH

- Evaluation of higher tiered test data e.g. Water-sediment test
  - Data rich studies
  - Dissipation versus degradation
  - ½ lives for degradation & the end-point (1y or ultimate)
  - ½ live for total system??
  - Bound material – removal??
  - Temperature correction??
  - Metabolite ERA - practicalities
  - Conflicting results (e.g. two sediments)
- Validation of enhanced biodegradation studies

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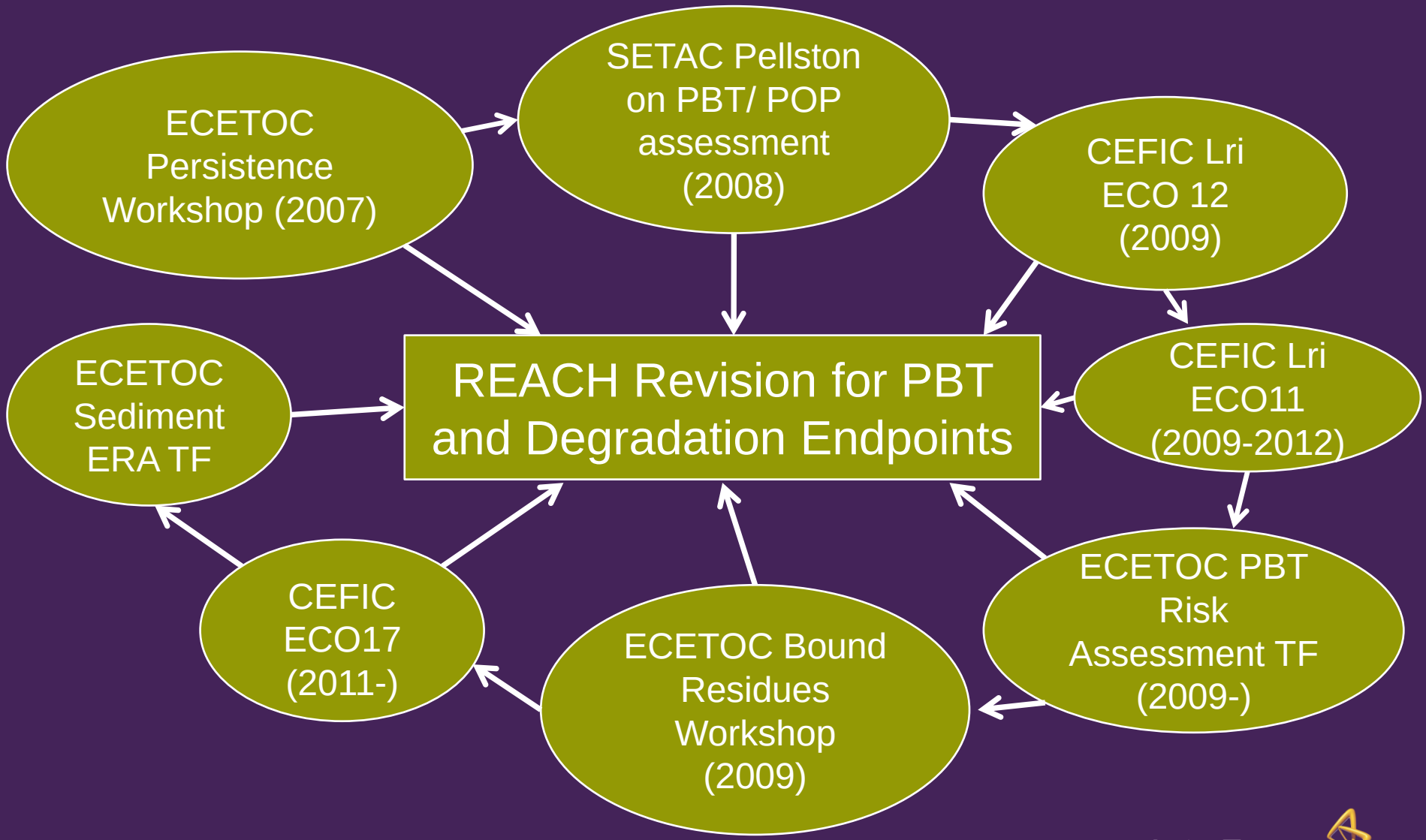
Post-REACH Persistence Activities



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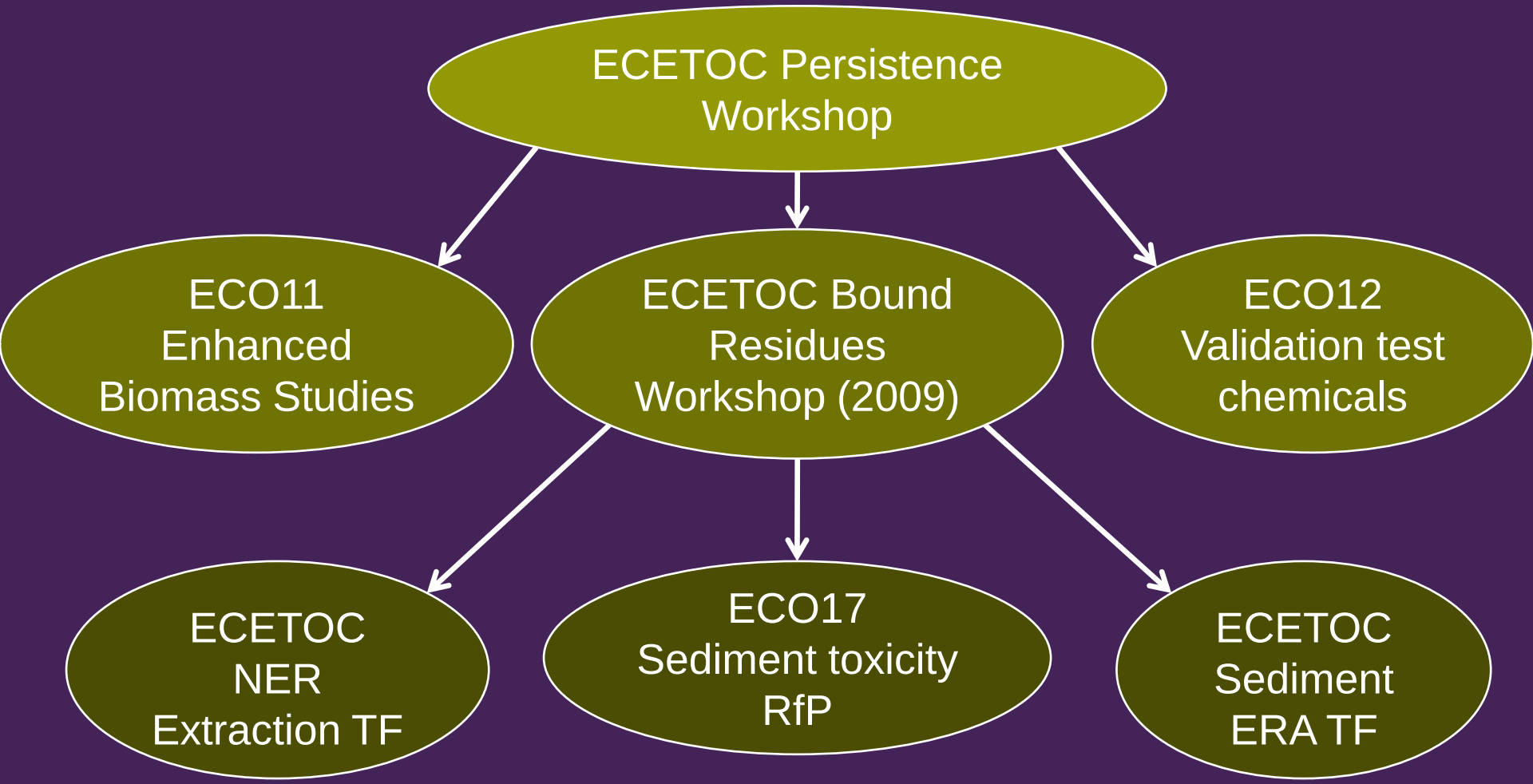
ECETOC Persistence Workshop - Actions



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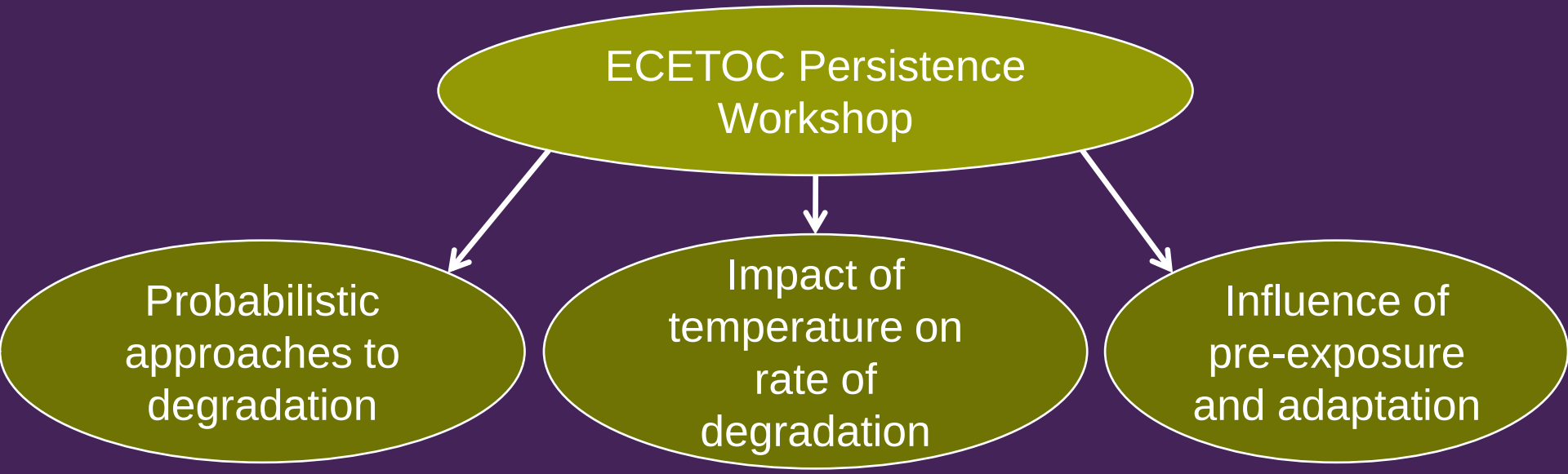
ECETOC Persistence Workshop - Parked



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WP1

Sample collection and preparation
- Fresh, estuarine and sea water; activated sludge
- Six locations to encompass variations in diversity

High throughput BST
- All samples, 60 day test
- 1 × cell concentration method
- 3 × test compounds of varied persistency

Molecular analyses
- Community fingerprinting (DGGE)
- Pyrosequencing
- Total cell counts

Milestone I

WP2

Cell concentration methods
- Filtration and centrifugation
- Tangential flow filtration
- Sandwich filtration
- Glass bead colonisation

High throughput BST
4-nitrophenol for 60 days

Milestone II

WP3

DOC removal experiments
- Washing with phosphate buffered saline
- Pre-incubation for natural DOC depletion

Scaled-up 60-day tests (up to 3L)
- Best concentration method
- Samples each of fresh, estuarine and sea water
- 3 × ^{14}C-labelled test compounds of varied persistency
- $^{14}CO_2$ & parent compound analysis over time to measure mineralisation and half-lives; DOC before and after test

↑ **inoculum & test volume** ↔ **compound concentration**
i.e. varied F:M

↑ **inoculum volume & compound concentration** ↔ **test volume**
i.e. F:M maintained

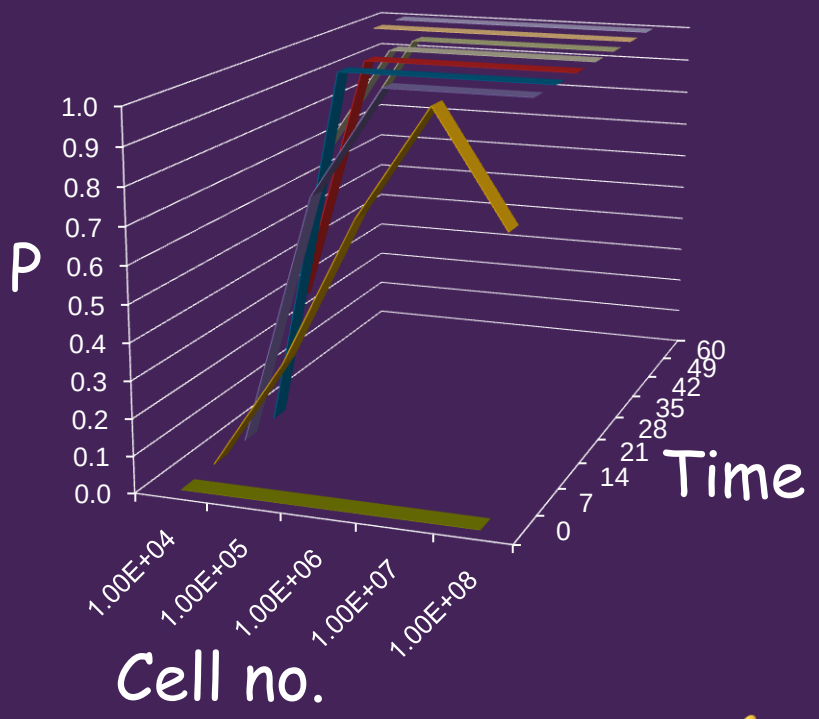
Milestone III

WP4

Method validation
Best method; reference chemicals for persistence benchmarking (ECO12)

Milestone IV: Guideline publication

CEFIC ECO11
- Newcastle
- AZSHE
- Pfizer
- Danish EA



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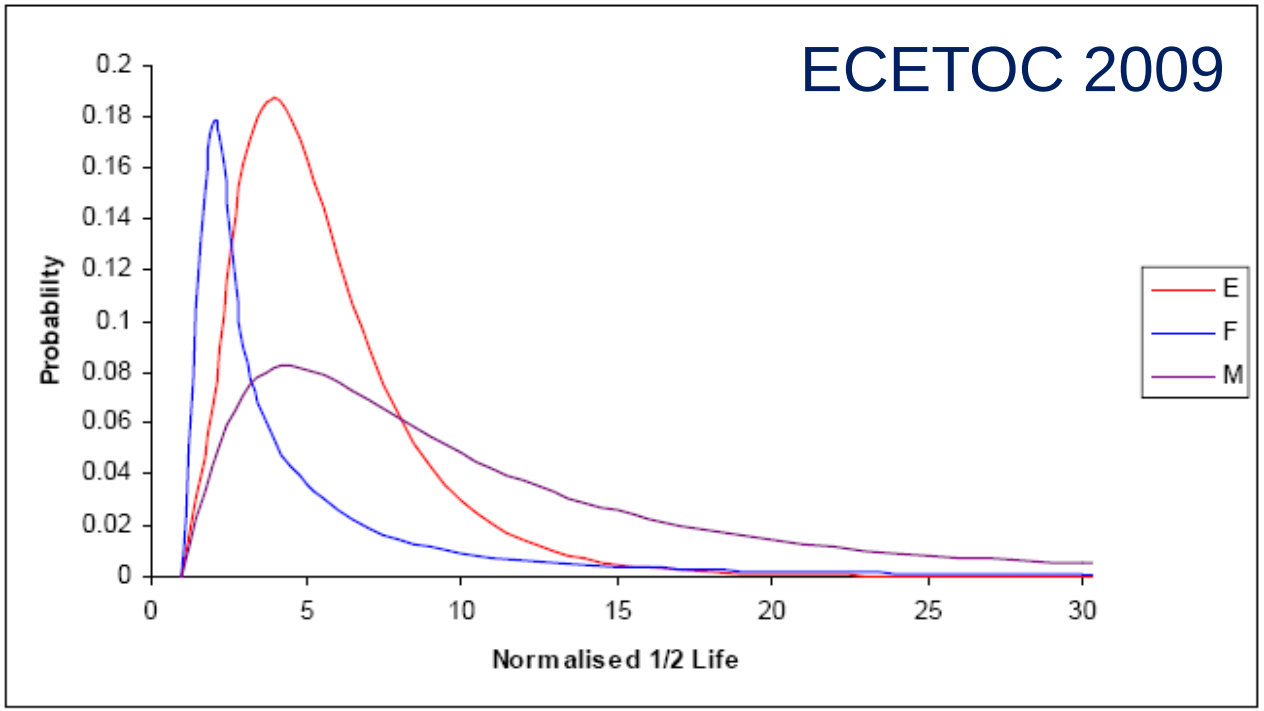
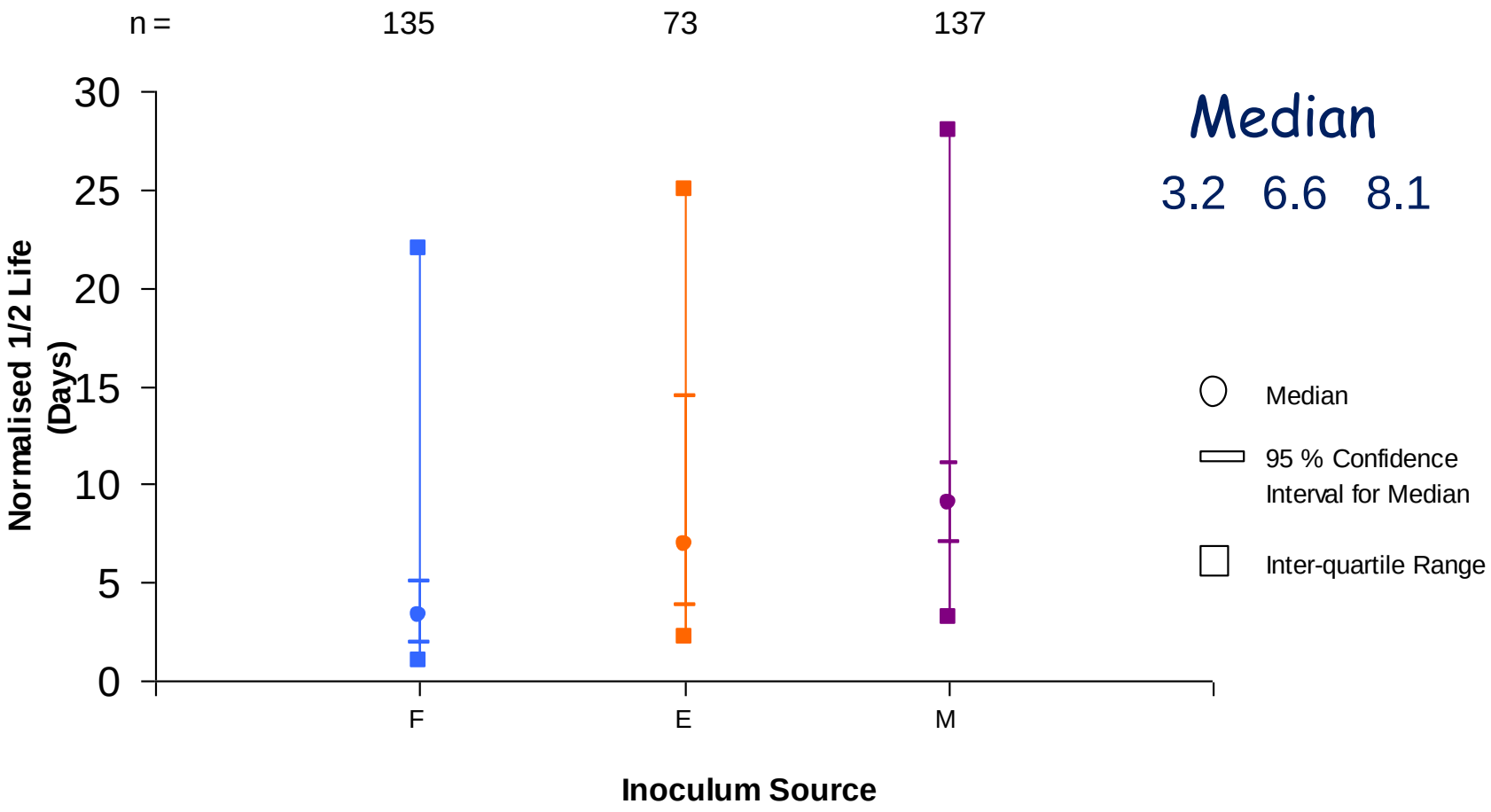
ECETOC Biodegradation Kinetics TF

- Only test once?
- How confident are we in the rate measured?
- False negatives?



½-Life Distribution by Compartment



5 days 14.4 days 11 days

Upper 95% Confidence limit

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ECETOC Biodegradation Kinetics TF

Recommending changes to the default rate constants assigned to biodegradation screening tests

	Freshwater[1]	Estuaries[4]	Other marine environments[5]
Degradation in marine screening test	N.a.	15	50
Readily degradable[2]	15	15	50
Readily degradable, but failing 10-day window	50	50	150
Inherently degradable[3]	150	150	150
Persistent	∞	∞	∞

Proposed

	Freshwater[1]	Estuaries[4]	Other marine environments[5]
Degradation in marine screening test	N.a.	15	Measured rate
Readily degradable[2]			
Readily degradable, but failing 10-day window	5	15	11
Inherently degradable[3]	150	150	150
Persistent	∞	∞	∞

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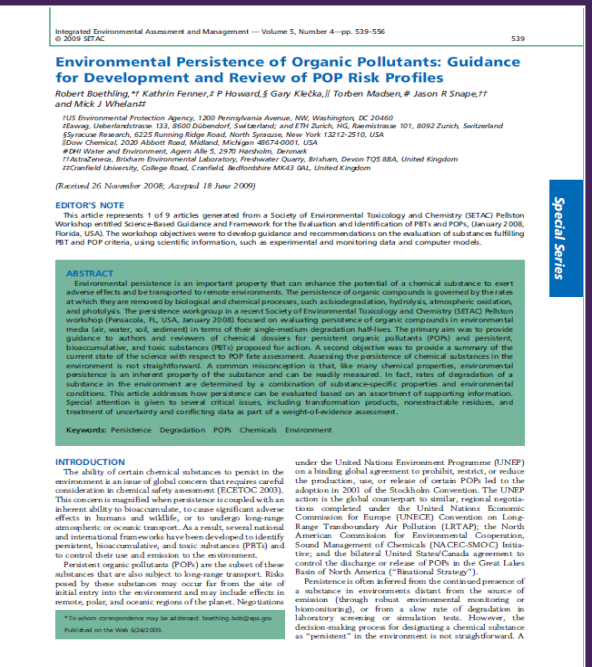
SETAC PBT/ POP Pellston

- Overview of Persistence discussions in IEAM Boethling et al (2009)
  - Temperature correction not required
  - Bound residues need careful consideration (covalently bound?)
  - Higher tiered tests are not simulations
  - More biologically and environmentally relevant tests needed



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Environmental Persistence of Organic Pollutants: Guidance for Development and Review of POP Risk Profiles

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EDITOR'S NOTE

This article represents 1 of 9 articles generated from a Society of Environmental Toxicology and Chemistry (SETAC) Pellston Workshop entitled Science-Based Guidance and Framework for the Evaluation and Identification of PBTs and POPs, (January 2008, Florida, USA). The workshop objectives were to develop guidance and recommendations on the evaluation of substances fulfilling PBT and POP criteria, using scientific information, such as experimental and monitoring data and computer models.

Special Series

ABSTRACT

Environmental persistence is an important property that can enhance the potential of a chemical substance to exert adverse effects and be transported to remote environments. The persistence of organic compounds is governed by the rates at which they are removed by biological and chemical processes, such as biodegradation, hydrolysis, atmospheric oxidation, and photolysis. The persistence workgroup in a recent Society of Environmental Toxicology and Chemistry (SETAC) Pellston workshop (Pensacola, FL, USA, January 2008) focused on evaluating persistence of organic compounds in environmental media (air, water, soil, sediment) in terms of their single-medium degradation half-lives. The primary aim was to provide guidance to authors and reviewers of chemical dossiers for persistent organic pollutants (POPs) and persistent, bioaccumulative, and toxic substances (PBTs) proposed for action. A second objective was to provide a summary of the current state of the science with respect to POP fate assessment. Assessing the persistence of chemical substances in the environment is not straightforward. A common misconception is that, like many chemical properties, environmental persistence is an inherent property of the substance and can be readily measured. In fact, rates of degradation of a substance in the environment are determined by a combination of substance-specific properties and environmental conditions. This article addresses how persistence can be evaluated based on an assortment of supporting information. Special attention is given to several critical issues, including transformation products, nonextractable residues, and treatment of uncertainty and conflicting data as part of a weight-of-evidence assessment.

Keywords: Persistence Degradation POPs Chemicals Environment

INTRODUCTION

The ability of certain chemical substances to persist in the environment is an issue of global concern that requires careful consideration in chemical safety assessment (ECETOC 2003). This concern is magnified when persistence is coupled with an inherent ability to bioaccumulate, to cause significant adverse effects in humans and wildlife, or to undergo long-range atmospheric or oceanic transport. As a result, several national and international frameworks have been developed to identify persistent, bioaccumulative, and toxic substances (PBTs) and to control their use and emission to the environment.

Persistent organic pollutants (POPs) are the subset of these substances that are also subject to long-range transport. Risks posed by these substances may occur far from the site of initial entry into the environment and may include effects in remote, polar, and oceanic regions of the planet. Negotiations under the United Nations Environment Programme (UNEP) on a binding global agreement to prohibit, restrict, or reduce the production, use, or release of certain POPs led to the adoption in 2001 of the Stockholm Convention. The UNEP action is the global counterpart to similar, regional negotiations completed under the United Nations Economic Commission for Europe (UNECE) Convention on Long-Range Transboundary Air Pollution (LRTAP); the North American Commission for Environmental Cooperation, Sound Management of Chemicals (NACEC-SMOC) Initiative; and the bilateral United States/Canada agreement to control the discharge or release of POPs in the Great Lakes Basin of North America ("Binational Strategy").

Persistence is often inferred from the continued presence of a substance in environments distant from the source of emission (through robust environmental monitoring or biomonitoring), or from a slow rate of degradation in laboratory screening or simulation tests. However, the decision-making process for designating a chemical substance as "persistent" in the environment is not straightforward. A

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Concluding Remarks

- CEFIC Lri and ECETOC initiatives have had a major impact on REACH degradation and persistence technical guidance
- Stranglehold has been broken on the ready biodegradation test
  - Room for test-based innovation to conclude on persistence
  - Enhanced and modified methods (time, cost and value benefits)
- Constructive dialogue with regulatory stakeholders during the drafting of REACH guidance has been maintained
  - ECETOC Persistence Workshop
  - ECO12 (Validation test chemicals)
  - ECETOC Bound Residues Workshop
  - SETAC PBT and POPs Pellston

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Future Issues

- Concerns re. OECD Water-Sediment study
  - Dissipation and not a degradation study
  - Interpretation issues
    - Bound versus available
    - Concluding on half-lives for a two phase system
    - Temperature correction
    - Half-lives for mixtures/ preps?
  - How robust is the OECD review process for fate?
- Lack of data for non-readily and inherently biodegradable chemicals in freshwater and marine test systems
- Test systems that can in give confidence in ½ life values
- PBT assessment and ERA of Transformation Products

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REACH 3.3-2 EWG 9 (Degradation)

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ECETOC Persistence Workshop (Organising Committee)

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ECETOC Biodegradation Kinetics TF

K van Ginkel, M Holt, N Rhedman, T Wind, M Galay-Burgos & J Snape (Chair)

SETAC PBT/ POP Workshop

B Boethling (Co-Chair), J Snape (Co-Chair), M Whelan, K Fenner, T Madsen & P Howard

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