

# Ring test to improve the OECD 306 marine biodegradation screening test

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## Introduction

A series of international standardised biodegradation screening tests (BSTs; OECD 301, 306 and 310) have been developed to measure the relative biodegradability of substances. In recent years, regulatory emphasis (e.g. REACH) has shifted from measuring biodegradation to prioritising persistent chemicals (ECHA, 2016).

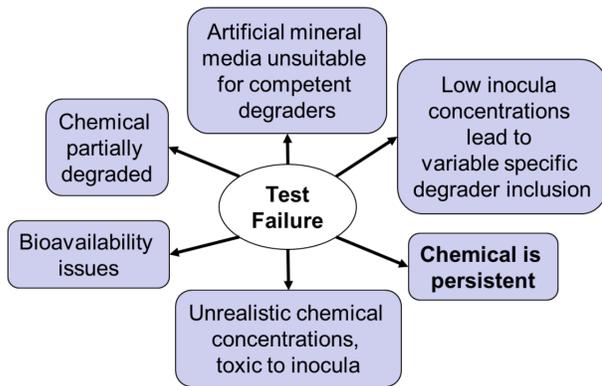


Fig. 1: Possible reasons for a failing of an OECD 306 test (adapted from Martin, 2014)

In their current guise, BSTs are ineffective as screens for persistency. They are subject to high amounts of variation and produce a large number of fails, many of which can be considered false negatives.

- OECD 306 biodegradation in seawater test**
- Marine water both as aqueous phase and source of microorganisms
  - Nutrients added to seawater
  - High concentration of test chemical

A substance may fail an OECD 306 test due to a range of reasons, only one of which is that the chemical is persistent (Fig. 1). One of the most important reasons for potential test failure is that degradation of a chemical is dependent upon the inclusion of competent degraders within the inoculum. Developing an improved OECD 306 test with a better representation of bacterial diversity is recognised as essential to allow the effective identification and prioritisation of persistent chemicals in the marine environment at the screening level.

## ECO11

### Results

The Cefic-LRi funded ECO11 project (ECETOC, 2013) investigated the impact of proposed modifications by REACH (ECHA, 2016) to existing screening studies, including effects of:

- Inoculum concentration
- Inoculum volume
- Extended study duration

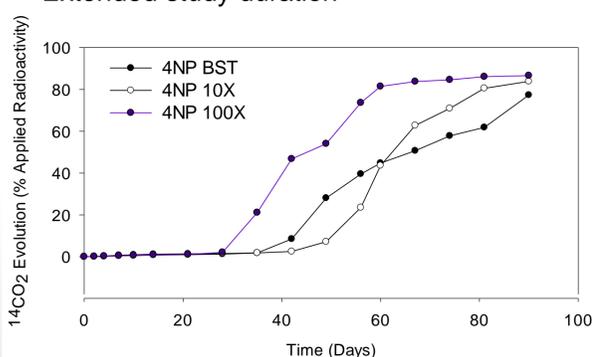


Fig. 2: <sup>14</sup>CO<sub>2</sub> evolution over time in marine tests with varying inoculum concentration (Martin, 2014)

## ECO11 recommendations for the OECD 306:

- x100 nominal concentration (Fig. 2)
- Volume of at least 500 mL
- Extended study duration (at least 60 days) as long lag phases occur in marine environment (e.g. Torang and Nyholm, 2005)

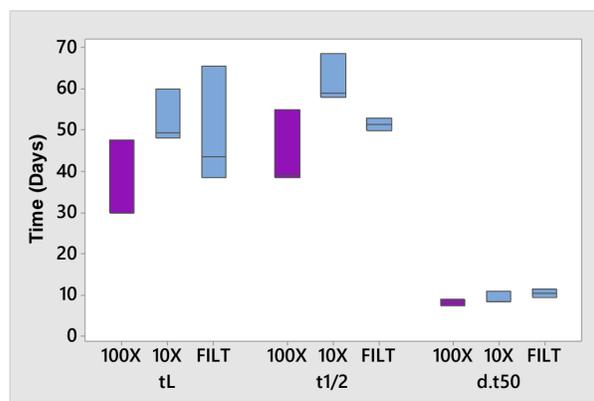


Fig. 3: Boxplots for  $t_L$  (lag time),  $t_{1/2}$  and  $\Delta t_{50}$  for marine tests with different biomass concentrations for 4-NP biodegradation (adapted from Martin, 2014)

## Workshop- 2015

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) hosted a two-day ECETOC/ Cefic workshop on 18- 19 February 2015 in Lowestoft, UK with 37 participants from academia, industry and regulatory bodies. Workshop paper in preparation to be published in a scientific journal.

- Discussion of deficiencies in marine water biodegradation tests
- Presentation of ECO11 research
- Consideration of reliable and pragmatic improvements to OECD 306 method
- Practical demonstration of inoculum concentration with tangential flow filtration (TFF)

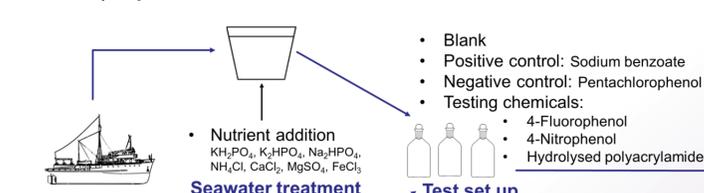
Agreement to evaluate the suggested revisions with a ring test to improve the reliability of the OECD 306 test

## Ring test- 2016- 2017

### Proposed design of the ring test

- 10  $\mu$ m pre-filtration
- 100x cell concentration by 0.22  $\mu$ m TFF

#### I) Improved 306 OECD



#### II) Standard 306 OECD

- Removal of coarse particles

Fig. 4: Schematic of the test protocol to revise the OECD 306 guideline

## Organisation

Project funded by Cefic, started January 2016 with following Involved parties:

- CROs from Europe (UK, Norway, Germany), North America (USA and Canada) and Japan
- Coordination and academic support from Newcastle University (UK)
- Regulators from the UK, Netherlands and Norway
- Industrial partners Shell and AstraZeneca

Expected test set up in spring 2017 and test results in autumn 2017.

## Protocol

- CROs conduct standard and improved OECD 306 test with defined test chemicals (Fig. 4)
- Newcastle University provides TFF unit including personal assistance for seawater concentration
- Comparison of community composition, diversity and total numbers of bacteria by next generation sequencing and total cell counts, conducted by Newcastle University

## Biodegradation measurement

- Increased oxygen background consumption for the 100x concentrated seawater limits the use of the closed bottle system
- Manometric respirometers will be used to measure the oxygen demand (~biodegradation) in the ring test

## Regulatory aspects- 2017+

This current proposal is to develop a method which is endorsed/accepted by OSPAR. In parallel, opportunities to engage the OECD will be sought, towards recognition of the improved test. However, there is still a requirement to establish better guidance on testing and interpreting the data obtained.

1. The enhancements for BSTs, suggested by REACH, are not currently recognised by OSPAR.
2. Enhancements in ECO11 did not change any classification of substances (ECETOC, 2013); however, current REACH guidelines still identify an improved OECD 306 to be only used to classify for persistency (ECHA, 2016).
3. Extended lag phases have to be correctly defined and interpreted.
4. Further guidance is needed to improve assessments of likely persistence if a substance shows partial degradation.
5. Other acceptable methods to improve marine biodegradation testing are needed.

### Chemical analysis

- T, pH, conductivity, salinity, TDS
- TOC, DOC
- Nitrogen, phosphorus, silicate

### Analysis

- Microbial analysis
- Microbial plate counting
- Total cell counts by flow cytometry
- DNA sequencing

## References

- ECETOC (2013). Assessing environmental persistence. Workshop Report No. 24. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.
- ECHA (2016). Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance. Version 3. European Chemicals Agency, Helsinki, Finland.
- Martin, T.J (2014). The influence of microbial inocula on biodegradation outcome towards enhanced regulatory assessments. PhD thesis. Newcastle University.
- Torang, L and Nyholm, N (2005). Biodegradation rates in adapted surface water can be assessed following a preadaptation period with semi-continuous operation. *Chemosphere*, 61: 1-10.