

## Abstract

The OECD 301F Guideline 'Manometric Respirometry Test' has been a standard method in determining if a test substance is 'readily biodegradable' for several decades. If a test substance exposed to a dilute inoculum generally comprised of activated sludge achieves 60% biodegradation equivalent to 60% of its organic carbon being converted to CO<sub>2</sub> within a 10-day window (once reaching 10% CO<sub>2</sub> evolution) during the 28-day test, the test substance can be labeled 'readily biodegradable.' However, based on the method in its current form with starting concentrations at 100 mg/L, some compounds that could potentially be biodegradable may fail this test if they are inhibitory at this relatively high concentration, which may be above many compounds' Predicted Environmental Concentrations (PEC).

In this experiment, we modified the 301F study design by conducting the prescribed test with one of the standard reference substances, sodium benzoate, but at lower than guideline concentrations. This test was conducted to determine the sensitivity of the method at much lower and more environmentally relevant concentrations. The concentrations used in this experiment are 100, 40, 20, 10, 5, 2, and 1 mg/L. The results have demonstrated that this method is appropriate at concentrations lower than the test concentration of 100 mg/L, currently stated in the OECD 301F Guideline.

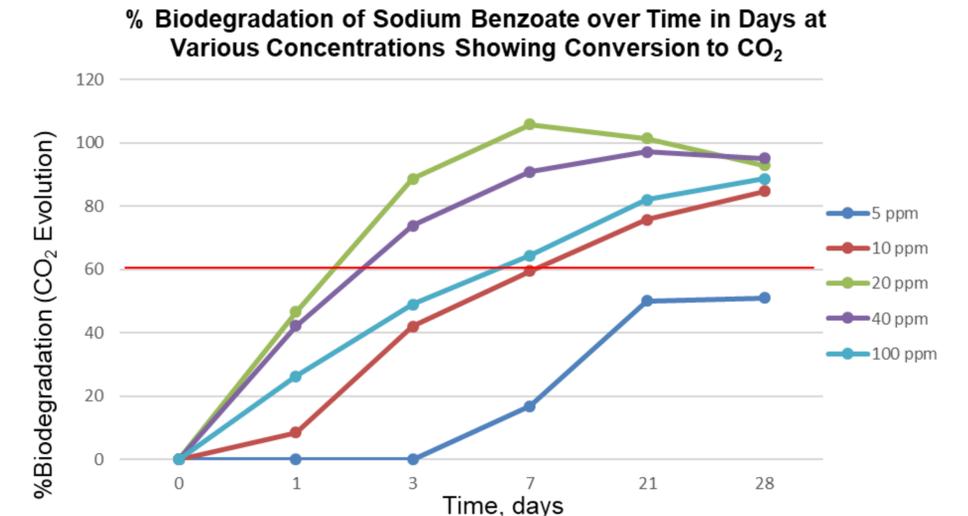
## Methods: Conduct of the OECD 301F Biodegradation Test

Test Design: This test was set up following the OECD 301F Guideline and used sodium benzoate (C<sub>7</sub>H<sub>5</sub>O<sub>2</sub> · Na) at seven concentrations in duplicate.

- Goal: assess the detection limit and sensitivity of the test guideline
- Mineral medium (164 mL), inoculated with a local activated sludge, at solids concentration of 30 mg/L, was used at pH 7.4
- Two additional vessels were used as inoculum blank controls
- All vessels were tightly capped with the respirometer heads and left to stir on multi-position stir plates for 28 days at 22 °C
- Inoculum source: activated sludge (Wareham, Massachusetts, USA)
- Respirometer and CO<sub>2</sub> Trap: Oxi-top respirometer and KOH pellets
- Sampling and Analysis: Samples monitored for CO<sub>2</sub> evolution
- Test concentration: 100, 40, 20, 10, 5, 2, and 1 mg/L and corresponding theoretical oxygen demand (ThOD) values 167, 67, 33, 17, 8, 3, 1.7, respectively

# Starting LOQ can be an order of magnitude lower for the OECD 301F Ready Biodegradation Test

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Note: This graph displays selected data points from the continuous data set. The 5 ppm day 21 point was a mean of the day 14 and day 28 value, due to some variability in some of the individual daily data points.



Example of the OECD 301F test vessels using the Oxi-Top Respirometer

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