MCCP REACH Consortium

Compilation and Summarization of Outcomes from OECD 301D Closed Bottle Tests for Medium Chain Chlorinated Paraffins (MCCPs) and Select Constituents
July 2020

Introduction

Between 2009 and 2018, multiple OECD 301-D Closed Bottle tests were conducted on MCCP mixtures and constituents with single paraffin chain-lengths, that varied in their chlorination levels. These tests included 25 separate experiments with 11 distinct test materials and the results are contained in 11 separate study reports. Nineteen of the experiments were inoculated with secondary activated sludge derived from a plant treating predominantly domestic wastewater (Nieuwgraaf WWTP in Duiven, The Netherlands), and six were inoculated with Rhine River water collected near Heveadorp, The Netherlands. Every study was conducted by Nouryon (formerly Akzo Nobel) under GLP.

The Closed Bottle Test (CBT), is one of the original OECD 301 methods for evaluating ready biodegradability. It determines the level of biodegradation based upon oxygen consumption using the following principles:

- Chlorinated Paraffin + oxygen $\rightarrow$ carbon dioxide + water + Cl^- + biomass
- % Biodegradation = O_2 consumption corrected for a blank control/Theoretical O_2 Demand for the Chlorinated Paraffin

Since the OECD 301-D quantifies only the fraction of theoretical oxygen demand that is respired and not the fraction that is incorporated into biomass, a biodegradation percentage of 100% is virtually impossible during the study duration. Given this fact, the OECD guideline considers $\geq$ 60% biodegradation as evidence for complete biodegradation of a test substance.

In comparison to other OECD 301 ready biodegradability tests methods, the Closed Bottle Test (CBT, OECD 301-D) has long been considered the most stringent. While it involves the lowest test substance concentration, which may benefit substances which inhibit microbes, it has by far the smallest inoculum, with an estimated 10 to 100- fold lower concentration of bacteria than that used in other OECD 301 ready tests ()[1]. Gericke and Fischer (1979)[2] conducted a systematic comparison of seven screening tests that were candidates to be ready tests by comparing test results for 44 test chemicals in each test. They found that two tests, the closed bottle and the MITI often yielded low results when the others seemed to indicate sufficient or even ready biodegradation. The poor performance of the MITI test has been attributed to the procedure for preparing and pretreating the inoculum. In this study, only soluble test substances were included due the known issues associated with bioavailability substances with poor water solubility in screening tests.

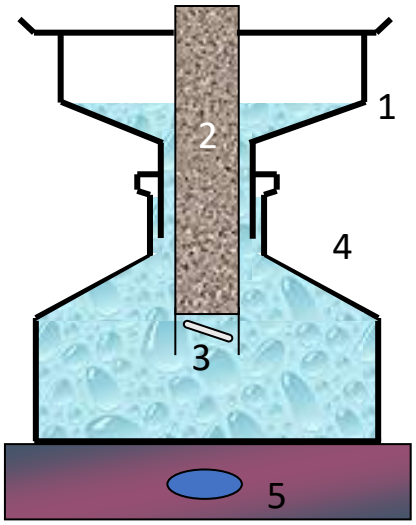
[1] OECD (1992), *Test No. 301: Ready Biodegradability*, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070349-en.

[2] Gerike, P. and W.K. Fisher. 1979 A correlation study of biodegradability determinations with various chemicals in various tests Ecotoxicol Envrron Saf 3 159-173.

Methods

The tests were conducted in 300 ml glass stoppered BOD bottles, and the test substances were emulsified with a poorly biodegradable polyalkoxylated alkylphenol surfactant (Agnique BP NP 1530). Dosing solutions were prepared by sonicating equal parts test substance and surfactant together in distilled water. Dissolved oxygen was determined using an oxygen electrode inserted into the top of the bottle. Replicate test systems were sacrificed at each sample point. Beginning in 2014, an innovative approach was utilized to prolong the study duration by measuring the oxygen decrease in day 28 test systems using a special funnel. This funnel fitted exactly into the BOD bottle opening and the oxygen electrode was inserted in the BOD bottle to measure the oxygen concentration. The medium displaced by the electrode was collected in the funnel. After withdrawal of the electrode the displaced medium flowed back into the BOD bottle, which was resealed following removal of the funnel. See diagram below:

Schematic representation of the funnel (1) and inserted electrode (2) with an integrated stirrer bar (3) in a BOD bottle (4) on a magnetic stirrer (5) used to non-destructively sample and prolong the Closed Bottle test.



Results

There are two important endpoints derived from this type of study that have important regulatory implications:

- Does the test substance meet the criteria for being ready biodegradable? Each test substance was a mixture of structurally similar chemicals. Hence, the criteria for ready biodegradation involves meeting or exceeding 60% biodegradation within 28 days, since the 10-day window is not applicable for such mixtures.[3]

[3] OECD (2006), *Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3*, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264030213-en.

- When the OECD 301D ready test is prolonged, does biodegradation meet or exceed 60% within 60 days. In ECHA's Integrated Assessment and Testing Strategy for Persistence Assessment, a positive enhanced ready test provides proof that a substance is not persistent.[4]

In compiling and inspecting the data *in toto* from the various studies, it was apparent that the ability for a substance to meet the criteria above was more impacted by a substance's chlorine content rather than chain-length or whether activated sludge or river water was used as the inoculum. In fact, the ability of an MCCP (C14-17) mixture to meet these criteria corresponded with the ability of pure chain-length materials with the same or similar chlorine content to meet them. This finding indicates that a "Whole Substance" approach based on chlorine content rather than a "Fraction Profiling" approach is appropriate for assessing the persistence of MCCPs.

Given the key role of chlorine content in determining the outcome, the results of all the 301D experiments are sorted in the table below in a hierarchal manner based on chlorine content, chain-length and inoculum.

Conclusions

- OECD 301D Closed Bottle tests were conducted on full-range MCCP products and single chain-length paraffins in the MCCP range with a wide range of chlorination levels.

- The ability to emulsify these substances as permitted and recommended within the OECD 301guideline made it possible to determine the intrinsic biodegradability of these sparingly soluble substances at 2 mg/L, which was orders of magnitude above their actual water solubility limits (4-10 µg/L for MCCP).

- There was a clear, inverse, relationship between chlorination level and the ability of the test substances to meet the criteria for ready biodegradability and for providing proof for non-persistence when studies were prolonged into enhanced ready tests.

- In the case of tests with substances with chlorine content equal to or less than 45.6%, 6 of 8 experiments met the criteria for ready biodegradability, and the substances that did not meet the criteria in one experiment did so in another.

- All experiments with substances with chlorine content equal to or less than 50% met the criteria needed to provide evidence that the substances were not persistent.

[4] European Chemicals Agency, Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment Version 3.0 June 2017

Summary of OECD 301D Biodegradation Study on MCCP

% Chlorine by wt	Chain Length	Inoculum	Biodegradation >/= 60% in </= 28 days	Biodegradation >/= 60% in </= 60 days	Max Observed % Biodegradation in </= 60 days	Report Date	Reference
41.3	C14	Act. Sludge	Yes	Yes	74% (Day 56)	12/2010	van Ginkel (2010e)
41.3	C14	Act. Sludge	Yes	Yes	83% (Day 56)	12/2010	van Ginkel (2010e)
41.3	C14	River Water	Yes	Yes	65% (Day 56)	12/2010	van Ginkel (2010e)
45.0	C14	Act. Sludge	Yes	Yes	67% (Day 42)	5/2010	van Ginkel (2010a)
45.5	C14	Act. Sludge	No	Yes	74% (Day 56)	12/2010	van Ginkel (2010e)
45.5	C14	Act. Sludge	Yes	Yes	73% (Day 56)	12/2010	van Ginkel (2010e)
45.5	C14	River Water	No	Yes	70% (Day 56)	12/2010	van Ginkel (2010e)
45.6	C14-17	Act. Sludge	No	Yes	63% (Day 42)	5/2010	van Ginkel (2010b)
50.0	C14	Act. Sludge	No	Yes	63% (Day 56)	12/2010	van Ginkel (2010e)
50.0	C14	Act. Sludge	No	Yes	78% (Day 56)	12/2010	van Ginkel (2010e)
50.0	C14	Act. Sludge	No	Yes	61% (Day 60)	5/2018	van Ginkel (2018a)
50.0	C14	River Water	No	Yes	63% (Day 56)	12/2010	van Ginkel (2010e)
51.0	C15	Act. Sludge	No	Yes	63% (Day 60)	4/2014	van Ginkel (2014a)
51.0	C15	Act. Sludge	No	No	40% (Day 60)	5/2018	van Ginkel (2018d)
51.0	C15	River Water	No	No	57% (Day 60)	4/2014	van Ginkel (2014b)
51.7	C14-17	Act. Sludge	No	No	57% (Day 60)	1/2010	van Ginkel (2010c)
55.0	C14	Act. Sludge	No	No	58% (Day 84)	12/2010	van Ginkel (2010e)
55.0	C14	Act. Sludge	No	No	57% (Day 56)	12/2010	van Ginkel (2010e)
55.0	C14	Act. Sludge	No	No	15% (Day 60)	5/2018	van Ginkel (2018b)
55.0	C14	River Water	No	No	39% (Day 56)	12/2010	van Ginkel (2010e)
60.0	C14	Act. Sludge	No	No	13% (Day 42)	5/2018	van Ginkel (2018c)
60.2	C14	Act. Sludge	No	No	40% (Day 56)	12/2010	van Ginkel (2010e)
60.2	C14	Act. Sludge	No	No	49% (Day 56)	12/2010	van Ginkel (2010e)
60.2	C14	River Water	No	No	4% (Day 56)	12/2010	van Ginkel (2010e)
63.2	C14-17	Act. Sludge	No	No	10% (Day 60)	4/2010	van Ginkel (2010d)