Consolidation of OECD tests for ready biodegradability on the example of OECD 310

Andrea Brunswik-Titze[1], Stefan Gartiser[1]

[1]Hydrotox GmbH, Freiburg, Germany

Introduction

Standardized test systems ensure constant test conditions and facilitate generating comparable and reproducible test results. The OECD 301 A-F/ OECD 310 series for testing ready biodegradability are considered equally valid. The tests are routinely used both for the investigation of ready biodegradability and for the identification of non-persistent substances within the PBT-assessment. However, the guidelines have not been revised for quite some time.

The last substantial revision was in 1992 for OECD 301 A-F and in 2006 for OECD 310. Revising, updating and consolidation of the current guidelines is therefore a recurring topic of discussion. This is a task that we are preparing on behalf of the German Environment Agency (project: Round Robin Test). In a further independent project, we assessed the applicability of the CO_2-Headspace test as an ultimate inherent biodegradability test.

Ready type CO_2-Headspace test OECD 310

Among the different ready type tests the OECD 310 represents a broad range of boundary testing conditions: The allowed test concentration range is from 2-40 mg/L TOC (20 mg/L TOC preferred), the allowed inoculum sources are activated sludge (4-30 mg d.s./L, 4 mg/L d.s. preferred), secondary effluent (up to 100 mL/L), surface water and soil. Among the points which require further clarification are:

- The inoculum concentration should result in 10^2 to 10^5 CFU/mL in the final mixture (**c**olony-**f**orming **u**nits), but there is no indication of the method for determining the CFU. Due to the inhomogeneous and flocculent structure of the activated sludge, CFU determination is critical.
- One validity criterion refers to the inorganic carbon allowed in the inoculum blank control which must be < 3 mg C/L at the end of the test. This criterion is only achievable when the standard concentration of 4 mg/L d.s. activated sludge is applied. The validity criterion should reflect both the test concentration and the inoculum concentration.
- The incubation temperature of the OECD 310 is 20°C ± 1°C, which is different from the incubation temperature of the OECD 301 tests (22°C ± 2°C)

Inherent type CO_2-Headspace test (no guideline)

So far, no standardized inherent biodegradability test using the unambiguous endpoint CO_2-evolution exists. The most often applied inherent test is the Zahn-Wellens test (OECD 302 B), which sometimes is carried out as combination test with parallel DOC and CO_2 determination [2].

Results:

The inherent CO_2-Headspace test proved to be a suitable method:

The mean CO_2-evolution in the inoculum blanks reached 135 mg/L (or 36.9 mg/L **i**norganic **c**arbon (IC)) within 28 days. The IC of the inoculum blanks corresponds to around 75% of the TOC introduced with the test item, but due to the low standard deviation (SD) of 0.3 mg/L, the IC is acceptable.

The CO_2-evolution in the inoculum blanks corresponds to that observed in the OECD 302 B combination tests with the same test- and inoculum concentrations (mean IC in the blanks from 9 tests was 42.9 mg/L IC within 28 day (table 1)).

The reference substance diethylene glycol was 81.1% biodegraded within 28 days, which is in the expected range (mean ultimate biodegradation in the OECD 302 B combination tests from 9 tests was 87.8% within 28 day (figure 1)).

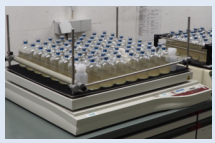
Materials and methods

Based on our experience from routine GLP CO_2-Headspace tests, the existing OECD 310 test guideline was carefully evaluated for ambiguous or contradictory phrases [1].

Further on, an inherent type CO_2 Headspace test was developed with the following test design:

Serum bottles	250 mL, liquid volume 125 mL (headspace 50%), sealed with butyl rubber septa for N20 neck with aluminum Bördel-caps,
Test concentration	50 mg TOC/L
Inoculum concentration	200 mg d.s./L activated sludge
Reference substance	50 mg TOC/L diethylene glycol
Test duration	28 days (up to 60 days)

The TOC- and inoculum concentrations correspond to that of the lower limit of the inherent type Zahn-Wellens test OECD 302 B. Data were compared with routine testing data from the OECD 302 B combination test with CO_2-evolution (test performance see [2]).

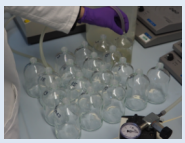


Table 1: Comparison of CO_2-evolution in different inherent tests

Days	7	14	21	28
a) Inherent CO_2-Headspace test OECD 310				
Mean ICin Blank control (Inoculum) [mg/L IC] from 2-3 replicate flasks	22,0	30,0	34,7	36,9
Standard Deviation (2-3 replicates within one test)	0,4	0,4	0,6	0,4
Mean Blank with 200 mg d.s./L (CO_2 [mg/L])	80,8	110,2	127,1	135,2
Corresp. Blank for 30 mg d.s./L (CO_2 [mg/L])	12,1	16,5	19,1	20,3
b) Inherent CO_2-Combination test OECD 302 B				
Mean Blank control (inoculm) with 200 mg d.s./L (CO_2 [mg/L]) from 9 tests	88,5	120,8	136,9	157,4
Standard Deviation (mean values from 9 independent tests)	13,4	18,1	21,2	36,9
Corresp. Blank for 30 mg d.s./L (CO_2 [mg/L])	13,3	18,1	20,5	23,6

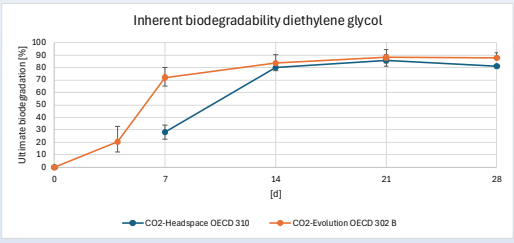


Figure 1: Biodegradation in the inherent CO_2-Headspace Test

Conclusions:

- The CO_2-Headspace test is an appropriate and variable test system allowing biodegradability testing under both ready and inherent type conditions.
- The existing OECD 310 guideline should be revised, and some contradictory and ambiguous phases should be adopted.
- An inherent CO_2-Headspace test was also proposed by CONCAWE in 1999 (draft OECD 302 D) for lubricants, but was not accepted by OECD, probably because of the use of preadapted inoculum sources, which were pre-exposed to the test substance [3], [4].
- To date, no standardized inherent test based on CO_2-evolution exists. The applicability of DOC-based tests (OECD 302 A and 302 B) is restricted to water soluble and non-adsorbing test items. The endpoint oxygen consumption used in the MITI(II) test (OECD 302 C) is influenced by nitrification. The combination test OECD 302 B with additional CO_2-evolution has not been standardized yet and is not suitable for volatile test substances [5].
- Therefore, the inherent CO_2-Headspace test would close a gap in the existing screening tests for biodegradation.

[1] OECD TG 310: Ready biodegradability - CO_2 in sealed vessels (Headspace test). 23 March 2006, corrected: 26 Sept. 2014

[2] Gartiser, S. et al. (2007): Ultimate biodegradation and elimination of antibiotics in inherent tests. Chemosphere 67, p. 604–613

[3] Battersby, N. S. et al. (1999): A test method to assess the 'inherent' biodegradability of oil products. Concawe report no. 99/59, Brussels, October 1999

[4] Beran, E. (2008): Experience with evaluating biodegradability of lubricating base oils. Tribology International 41(2) 2008, Pages 1212-1218

[5] Gartiser S. et al. (2017): Assessment of environmental persistence: regulatory requirements and practical possibilities – available test systems, identification of technical constraints and indication of possible solution. German Environment Agency Texte 10/2017,

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Corresponding author: Andrea Brunswik-Titze, phone: +49 761-45512-0, e-mail: brunswik@hydrotox.de