Round Robin Test: consolidation of OECD ready biodegradability tests

Ringtest zur Vorbereitung einer Konsolidierung der Tests auf leichte biologische Abbaubarkeit nach OECD

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SETAC Europe 36th Annual Meeting, Maastricht, 17-21 May 2026

Background

For the environmental assessment of substances internationally accepted and standardised test systems are applied, such as those of the OECD. Although the tests for ready biodegradability (RBTs) according to OECD 301 A-F and 310 differ considerably in terms of their methodology and historical background, the results of these test systems are nevertheless considered equivalent. However, experience shows that the results obtained in these tests can sometimes differ considerably. Further on, the available testing approaches and the equipment have evolved since the last revision in 1992. The German Environment Agency (Umweltbundesamt, UBA) therefore intends to examine whether the existing OECD test systems for ready biodegradability could be adopted to a more uniform test system to enable more reliable results. This R&D project, launched by the UBA in 2024, aims to prepare a revision of the OECD tests and builds on research projects, expert reports and a workshop conducted over the last 10 years. For this purpose, a round robin test with 11 GLP certified laboratories[4] from 7 countries belonging to 3 OECD regions (EU, USA, Japan) is being organised.

Test Setup

- Respirometer test based on OECD 301 F/C
- Different respirometers allowed
- Activated sludge from municipal STP, 30 mg^{-1} d. s
- Additional DOC-analysis and (optionally) N-analytics (NH_4, NO_2, NO_3)
- Characterisation of the inoculum (CFU, flow cytometry, DNA-sequencing, …)
- Two independent series ➔intra-laboratory variability.
- Descriptive statistics ➔ preliminary results ➔ this poster
- Ring test statistics DIN ISO 5725-2 ➔repeatability and reproducibility

Table 1: Test and reference items

Test- and reference items	CAS	sum formula		$ThOD_{NO3}$ [mg O_2 mg^{-1}]	Water solubility [mg L^{-1}]
1 Potassium hydrogen phthalat	877-24-7	$KC_8H_5O_4$		1.18	80,000
2 Diisobutyl hexahydrophthalate	70969-58-3	$C_{16}H_{28}O_4$		2.36	18.3
3 Acesulfame potassium	55589-62-3	$C_4H_4KNO_4S$		0.95	237,000
4 Aniline	62-53-3	C_6H_7N		2.41	34,000

Outlook

- 2026 Completion of the test phase
- 2027 International workshop
- 2027 Project end
- 2028 Proposal to revise the OECD RBTs

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Preliminary results

Table 2: Interim evaluation of round robin tests (≈ 90% of results)

1st series	Lag phase [d]			[%] degradation end 10 d-window		[%] degradation test end 28 d		ready *)	ultimate **)
	n	Mean	SD	Mean	SD	Mean	SD	n	n
1 Potassium hydrogen phthalat	11	2,3	0,5	86,2	4,7	92,4	3,7	11	11
2 Diisobutyl hexahydrophthalate	11	7,6	5,5	18,3	5,7	30,2	19,0	0	1
3 Acesulfame potassium	11	8,2	1,3	57,0	4,7	60,9	3,2	1	8
4 Aniline	11	4,4	1,1	68,0	9,6	82,1	11,0	9	11
2nd series									
1 Potassium hydrogen phthalat	11	2,2	0,4	86,4	3,6	90,7	4,0	11	11
2 Diisobutyl hexahydrophthalate	10	8,1	6,8	20,3	10,9	32,2	21,6	0	1
3 Acesulfame potassium	11	8,2	1,1	56,5	4,7	60,8	5,4	1	8
4 Aniline	11	4,2	0,8	69,5	11,1	82,5	12,4	9	10

*) No. of labs with >60% $ThOD_{NO3}$ end of 10-d-window
** No. of labs with >60% $ThOD_{NO3}$ end of test (28 d)

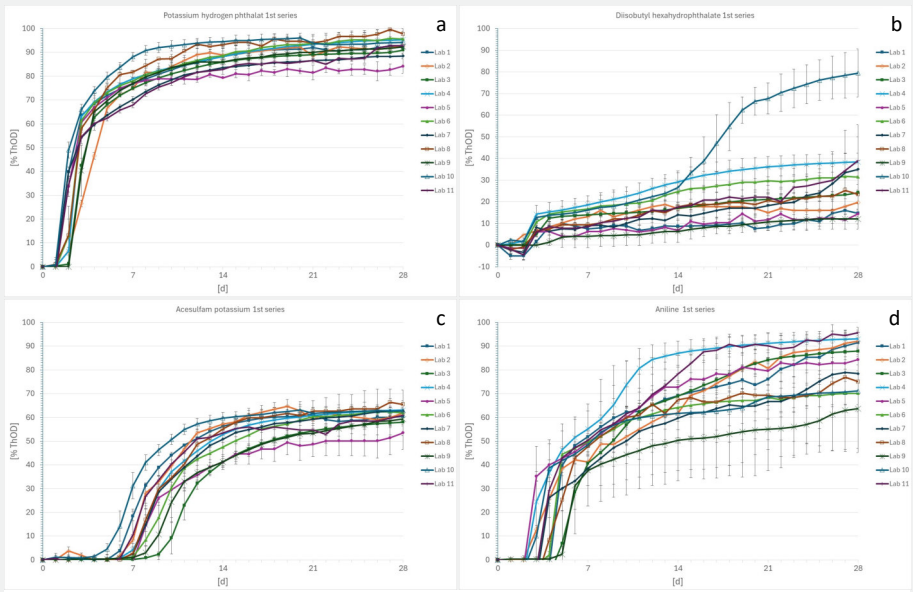

Figure 2 a-d: Preliminary degradation graphs (mean values) with SD of replicates

Preliminary conclusions

- Overall good reproducibility between 1st and 2nd series.
- Very good reproducibility for readily biodegradable Potassium hydrogen phthalat (suitable reference substance).
- Diisobutyl hexahydrophthalate after lag phase of ≈ 8 d not readily or ultimately biodegradable. Potentially stable transformation products (confirmed by additional DOC analysis) ➔ Median DOC-elimination 44% of initial ThTOC.
- Acesulfame potassium after lag phase of ≈ 8 d not readily -, but ultimately biodegradable (confirmed by DOC analytics) ➔ Mean DOC-elimination 98% of initial ThTOC. Thus, Acesulfame, previously known as recalcitrant, is not persistent, probably due to acquired adaptation of activated sludge.
- Aniline is readily biodegradable, but with high variability between replicates and laboratories. Nitrification has a major influence (confirmed by N-analytics NH_4, NO_2, NO_2). Two laboratories, failing the pass level 60% $ThOD_{NO3}$, clearly passed 60% $ThOD_{NH4}$. Further potentially impacts caused by inhibition and de-adaptation: Aniline is acute toxic H400 and a known nitrification inhibitor.

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