

21-22 January 2025 | Brussels

OVERCOMING CHALLENGES AND ADVANCING (BIO) DEGRADATION GUIDELINES: OECD TG309 REVISITED



Date and Venue: 21-22 January 2025, The Hotel (Boulevard de Waterloo 38) Brussels, 26th Floor.

Workshop Format: 1.5-day F2F + online

Background and objectives: The OECD 309 test guideline (Aerobic Mineralisation in Surface Water), last updated in April 2004, plays a pivotal role in understanding the environmental fate of chemicals and is generally the first test requested under REACH for non-readily biodegradable substances. Given the anticipated update in the OECD work programme, this workshop aims to unite experts in environmental chemistry, toxicology, and regulatory science to discuss the improvement of the guideline.

Objectives:

- Discuss and address current challenges and limitations in conducting and interpreting degradation simulation studies.
- Define/align on the key updates needed for the OECD 309 guideline.
- Define roadmap to ensure that the prioritised updates flagged would be addressed in time for the OECD 309 TG (test guidelines) update.
- Identify research needs and potential methodological advancements to improve this and other guideline's relevance and applicability.

Participants: The workshop seeks to bring together 30 participants from regulatory bodies, academia, industry, and environmental NGOs. Inclusion of specialists in (bio)degradation, persistence assessment and environmental modelling is particularly encouraged.

Funded by: ECETOC

Workshop Reporting: A publication detailing the workshop discussions, outcomes, and proposed roadmap for the OECD 309 guideline update will be co-authored by OC members. Consideration will also be given to preparing a supplementary workshop report, subject to OC agreement.

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Workshop programme

Day 1: 21 st January 2025		
12:30 – 13:00	Arrival and registration (26 th Floor)	
13:00 – 14:00	Welcome Lunch	
14:00 – 14:15	Welcome, introduction and workshop objectives	Dr. Blanca Serrano Ramòn
14:15 – 15:00	Scene setting keynotes: <ul style="list-style-type: none"> i. Lessons learned in ISO ring test in 1998-99 for OECD TG309 – (20 minutes) ii. Advancing Environmental Assessment: Revising OECD Test Guidelines with Insights from the German Environment Agency – (25 minutes) 	Dr. Lars Toräng Dr. Ulrich Jöhncke
15:00 – 15:30	Coffee break	
15:30 – 16:30	Robustness pillar (1 hour duration): <ul style="list-style-type: none"> i. Main presentation on key challenges – (20 min) ii. Short follow-on presentations on selected issues (20 min) <ul style="list-style-type: none"> a) <i>Chris Hughes</i> b) <i>Marie Collard</i> c) <i>Fola Ogungbemi</i> iii. Questions, research needs, discussion points, and additional topics raised by the audience. (20 min) 	Dr. Carolin Seller
16:30 – 17:30	Implementation pillar (1 hour duration): <ul style="list-style-type: none"> i. Main presentation on key challenges – (20 min) ii. Short follow-on presentations on selected issues (20 min) <ul style="list-style-type: none"> a) <i>Jason Snape</i> b) <i>Heidi Birch</i> c) <i>Philipp Mayer</i> iii. Questions, research needs, discussion points, and additional topics raised by the audience. (20 min) 	Dr. Dieter Hennecke
19:00	Evening event	

Day 2: 22 nd January 2025		
9:30 – 9:45	Welcome and setting the day	
9:45 – 10:45	Relevance pillar (1 hour duration): <ul style="list-style-type: none"> i. Main presentation on key challenges – (20 min) ii. Short follow-on presentations on selected issues (20 min) <ul style="list-style-type: none"> a) <i>Marie Collard</i> 	Dr. Michael McLachlan

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	b) <i>Martin Brüggemann</i> iii. Questions, research needs, discussion points, and additional topics raised by the audience. (20 min)	
10:45 – 11:00	Coffee break	
11:00 – 12:30	Breakout Sessions Part 1 (<i>Only In-person participants</i>)	
12:30 – 13:30	Lunch	
13:30 – 15:00	Breakout Sessions Part 2 (<i>Only In-person participants</i>)	
15:00 – 15:30	Coffee Break	
15:30 – 16:15	PLENARY: Overview of Breakout Sessions (15 minutes/ pillar)	
16:15 – 16:30	Conclusion & Close	

Abstracts and Speakers' bios

Lessons learned in ISO ring test in 1998-99 for OECD TG309 Lars Toräng, Ph.D.	
<p>First batch simulation test of surface water was drafted by Niels Nyholm, Technical University of Denmark and Udo Pagga, BASF in "ISO/DIS 14592 Part 1 - Water quality - Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 1: Shake flask batch test with surface water or surface water/sediment suspensions" under ISO/TC 147/ SC5/ WG4 Biodegradability.</p> <p>Batch simulation tests aim at estimating degradation rates, conducted in a laboratory system with conditions that are realistic for the particular environmental compartment. Simulation tests should mimic the actual environmental conditions such as redox potential, pH, temperature, microbial community, concentration of test substance, and occurrence and concentration of other substrates.</p> <p>Seven laboratories participated and the main results and important considerations behind the method will be presented. Selected model compounds were aniline and 4-chloroaniline in the concentration range 0.5-500 µg/L. All laboratories used ¹⁴C-radiotracer technique with ¹⁴C supplied from recognized sources, and none used specific analysis. In addition to the mandatory pelagic tests with water, two included tests with sediment spiking. Only one laboratory carried out optional biodegradation studies with aniline and 4-chloroaniline after a pre-adaptation step with semi continuous operation.</p>	
	<p><i>Regulatory Affairs manager at Bjørn Thorsen A/S (2022-) and Agilent Technologies (2017-2022), REACH expert at Sun Chemical (2007-2017), Post Doc. about Equilibrium Sampling Through Membranes (2004-2007) at National Environmental Research Institute (2004-2007), and Ph.D. (1998-2003) at Technical University of Denmark.</i></p> <p><i>Analyzed data and author of the first batch simulation ring test back in 1998-99. My Ph.D. was about understanding adaptation and biodegradation of organic compounds in batch simulation tests. For direct</i></p>

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interpretation of measured rates, it is important to keep the concentrations of simulation studies as low as the actual concentrations, or sufficiently low, to ensure the same kinetics as in the environment. Biodegradation rates obtained in degradation studies at high concentrations (> 100 µg/L) may grossly overestimate the actual rates at environmental relevant concentrations, even when investigated in the same environment.

Advancing environmental Assessment: Revising OECD Test Guidelines with Insights from the German Environment Agency **Ulrich Jöhncke, UBA**

The talk will give information on the Research and Development project “Review of the OECD Test Guidelines relevant to environmental assessment with regard to the state of the art in science and technology” done in behalf of the German Environment Agency. This project investigated the revision needs of OECD test systems from 2020 to 2023. Background and procedure chosen by the consultants which led to identification of the OECD TG 309 identified as the TG in most need of revision will be described. A compilation of the suggestions received will be given. In addition, experiences that the German Environment Agency made with and views on the test depending on the assessment concerned will be presented from the perspectives of sections which operate under different legal frameworks. This will be accompanied by background information on the specifications given in the REACH guidance on the test procedure of OECD TG 309.

1990: Graduated with a degree in ecotrophology at the University of Applied Sciences at campus Niederrhein, Mönchengladbach
1991 to 2007: section IV 2.3 Biodegradation and Bioaccumulation at the Federal Environment Agency, specialising in the evaluation of test protocols for chemicals, but also for plant protection products or pharmaceuticals.
Since 2007: section IV 2.3 Chemicals, evaluating of or preparing dossiers as part of the REACH processes, e.g. SVHC identification. In charge of the degradation team, member of UBA’s coordinating group on degradation and exposition and supporting expert for the German member of MSC and the Agency’s expert in ECHA’s PBT expert group. Initiation and supervision of scientific research projects, e.g. on NER.

PILLAR 1: ROBUSTNESS: Enhancing Standardization and Reproducibility in OECD 309 Biotransformation Studies for Chemical Persistence Assessment **Dr. Carolin Seller, Eawag – Swiss Federal Institute for Aquatic Science and Technology**

For chemical persistence assessment, regulatory frameworks following the REACH regulation rely on chemical half-lives (DT₅₀) derived from OECD biotransformation simulation studies, including the OED 309 guideline, which aims to assess biodegradation in surface waters. REACH pursues a cautious approach, meaning that the longest DT₅₀ available from at least two different OECD 309 tests is compared against persistence cut-off criteria to depict a “worst-case” scenario. This implies the assumption that variable DT₅₀ values, to large extents, represent variability of a chemical’s biotransformation behavior in natural surface water bodies. Unfortunately, differing OECD 309 DT₅₀ values also stem from laboratory artefacts resulting from insufficiently standardized conditions allowing for differences in test setups that can significantly impact study outcomes. As just a few examples, the OECD 309 guideline allows for (i) batch or semi-continuous operation, (ii) systems with or without suspended sediment, (iii) a range of initial chemical concentrations, (iv) largely undefined inoculum sources, (v) various test temperatures and many more, making the comparison between study outcomes highly uncertain. Our research suggests that proper standardization of those test conditions, e.g., addition of suspended sediment and reduction of chemical concentrations, holds the potential to significantly reduce laboratory artifacts and therewith increase reproducibility of test results. Finally, the OECD 309 guideline does not require any analysis and reporting of the composition or diversity of the microbial community present in the test systems, even though both parameters

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strongly influence a compound's biotransformation behavior in aquatic environments and should be included to allow for a proper data analysis.

Carolyn's research is focused on exploring biotransformation behavior of anthropogenic chemicals, i.e., pharmaceuticals, pesticides or industrial chemicals, in aquatic environments. The main objective is to understand which chemical properties in combination with which environmental parameters would favor a rather rapid substance degradation and avoid its release and widespread transport in the environment. Thereby, she has been gathering and working with field and laboratory data describing biotransformation in water, water-sediment, or activated sludge systems.

PILLAR 2: IMPLEMENTATION

Dr. Dieter Hennecke, Fraunhofer Institute

From theory to testing practice: to conduct practical e-fate testing under GLP for regulatory purposes is in general a challenging task. It might become a nightmare if the testing guideline is unspecific in relevant sections or if requests by a respective regulatory framework are unclear (e.g. suspended sediment amount in OECD 309 pelagic test for REACH). Though on the other hand some flexibility is necessary to be able to test chemicals with a certain range of Phys-chem properties within the frame given by a guideline.

Examples will be given of challenges, questions and experiences in practical OECD 309 standard tests conducted at a GLP testing facility under the required standardized conditions with typical substances. It will demonstrate that a guideline cannot have an answer to all – foreseeable and unforeseen – issues that may arise during a real GLP-test, and that the line between a standard test and a research project can be very thin. This is important to understand for registrants and regulators as expectations to executing laboratories for a “standard test” are usually quite different.

Dieter Hennecke is a chemist with more than 30 years of experience in applied research on the fate of chemicals in the environment. In his department at Fraunhofer IME, guideline-driven standard studies are carried out for regulatory purposes under GLP, mostly with substances that are difficult to test. He is further active in the development of new and improvement of existing guidelines to support chemical regulation. His particular expertise lies in ¹⁴C-radiolabeling technology and its application in novel research fields (e.g. fate of polymers).

PILLAR 3: RELEVANCE: Environmental relevance of the OECD 309 test

Dr. Michael McLachlan, University of Stockholm

Recent research exploring the application of OECD 309 type tests to study the biodegradation rates of organic contaminants in rivers will be presented. The underlying premise of the work is that the initial biodegradation rate in the test is the best measure of the biodegradation rate in the environment; all changes in biodegradation rate during the test reflect departures of the test conditions from environmental conditions. The research shows that almost all of the chemicals studied did not degrade in a pelagic test. Large quantities of sediment (50 g/L) were required in the incubation to increase the microbial community density to a level sufficient to biodegrade most test chemicals to a measurable extent. While the repeatability of the test for a specific water/sediment mixture was good, when the test was conducted with water/sediment mixtures from different rivers then the

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biodegradation rate constant for a given chemical varied by up to several orders of magnitude. These results will be discussed in the context of the environmental relevance of the OECD 309 test and the information that the test can provide in chemical regulation.

* *Michael is a professor of environmental chemistry at the Department of Environmental Science at Stockholm University. Michael's research interest is the fate of organic contaminants in the environment. During the past years his work has focused on biodegradation in aquatic systems, and he is particularly interested in quantifying biodegradation rates in the natural environment. He has led research investigating the use of OECD 309 for this purpose.*

Organising Committee

Michael	McLachlan	Stockholm University
Dieter	Hennecke	Fraunhofer Institute
Louise	Camenzuli	Exxonmobil
Chris	Hughes	Embark Chemical Consulting
Delina	Lyon	Concawe
Katherine	Santizo	Cefic
Christian	Schlechtriem	Fraunhofer Institute
Blanca	Serrano	ECETOC
Megan	D'souza	ECETOC
Andrea	Salvadori	ECETOC