Expert Meeting on OECD TG 309: Surface Water Composition and Relevant Conditions

17 May 2026, Maastricht

EXPERT MEETING REPORT

Background and Objectives

The Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 309 "Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test", which assesses aerobic primary degradation and mineralisation in surface water, is a cornerstone method for deriving degradation half-lives used in chemical hazard and risk assessments, including Persistence assessment (P/vP) under European regulatory frameworks (e.g., CLP, REACH etc.).

ECETOC held a workshop in January 2025, "Overcoming challenges and advancing (bio)degradation guidelines: OECD TG309 revisited", during which the OECD TG 309 was extensively discussed (ECETOC, 2025). Inherent shortcomings related to the regulatory purpose of the test, as well as technical challenges, were highlighted. Several longer-term proposals for improving the robustness of the test were put forward, which tackle topics falling outside of the specifications of the current TG and requirements of regulatory frameworks.

An ECETOC Expert Meeting held on 17th May 2026 brought together twenty experts specialising in the OECD TG 309 and its application within regulatory frameworks. Participants represented a range of perspectives from industry, academia, and regulatory agencies.

Building on the key outcomes of the 2025 workshop, the objectives of the meeting were to:

(i) Develop recommendations that could be applied in the shorter-term within the framework of the current TG 309 and regulatory frameworks;

(ii) Make recommendations for associated longer-term research priorities to improve the robustness of TG 309 tests.

These recommendations aim to support chemical registrants and contract research organisations (CROs) in improving current practices, while also assisting regulatory experts in the assessment of such studies. They are intended to contribute to ongoing scientific efforts to advance the understanding, application, and further development of OECD TG 309, including through future research initiatives supported by organisations such as ECETOC.

Introductory remarks and General views on OECD TG 309

The meeting co-chairs highlighted the fundamentals of the OECD TG 309 study, i.e. a laboratory test incubating environmentally-relevant concentrations of chemicals with natural pelagic inocula, and its application in chemicals regulation.

Recent research reporting the potential over-stringency of these tests relative to environmental conditions, as well as the high variability in resulting biodegradation half-lives and limited robustness due to sensitivity to test system parameters, was also mentioned (e.g. Honti et al. (2018), Seller et al. (2020), Seller et al. (2021), Hakvåg et al. (2025)).

There was broad agreement among meeting participants that the OECD TG 309 pelagic test is stringent, with outcomes (i.e. degradation half-lives) being sensitive to multiple variables, including experimental conditions and the inoculum. Several participants highlighted considerable variability, with reported half-lives for the same substance differing by several orders of magnitude among independent pelagic and water-sediment studies employing distinct inocula.

It was highlighted that the OECD TG 309 aims to have a sufficiently low concentration of test substance so that it is a secondary carbon substrate, i.e. the test substance is degraded via co-metabolism, which is defined as the degradation of a compound by enzymes targeting other substrates used as microbial energy sources.

It was noted by some participants that the long test duration may allow the microbial community to drift from its original composition and activity which, however, is a general problem that is inevitable in every laboratory test system. In addition, depletion of nutrients may lead to starvation, altering microbial metabolism and reducing the environmental relevance of the test conditions. As a result, the derived half-life may increasingly reflect changes in the microbial community, including adaptation to the test substance, where growth-linked processes described by Monod kinetics become more important than co-metabolic degradation processes that are more representative of natural surface waters.

There was some discussion around use of biodegradability potential (yes/no) rather than numerical biodegradation half-lives, but participants did not come to a consensus on this point. Overall, it was suggested that the OECD TG 309 test may provide qualitative insight into biodegradability but has limitations in terms of reliably delivering robust half-life values.

Divergent opinions were expressed regarding the regulatory protection goal. Some participants argued that the goal is to represent pristine environments with low microbial abundance as required for the identification of Substances of Very High Concern, thereby justifying the stringency of the test. Others countered that most chemicals are not directly released into pristine environments but rather first pass through wastewater systems and journey through anthropogenically impacted streams and rivers.

Discussions on surface water composition, including inoculum, and environmental relevance

The meeting co-chairs highlighted the influence of the test inoculum on test outcome, and that test inoculum can vary in terms of biological composition (specific bacteria, enzymes expressed, competition etc.) and this composition may be influenced by environmental factors (temperature, season etc.) (e.g. Wang (2026), Tian et al. (2024a), Tian et al. (2024b)). However, so far, robust proxies that can reliably show the metabolic activity and functional relevance of inocula have not been identified.

The meeting co-chairs also shared some initial thoughts on relevance of the test system set-up relating to representativeness of environmental conditions (including inoculum characteristics).

There was broad agreement amongst meeting participants that inoculum can be highly variable and dependent on location of sampling, season of sampling etc, with key aspects being both concentration/cell density as well as community composition. It was further highlighted that temperature may influence both microbial activity and community composition.

Use of standard inoculum

It was briefly discussed whether use of a standardised inoculum could be a potential solution to minimise the TG 309 variability. This was eventually deemed impractical due to technical limitations and the risk of reducing microbial diversity and activity through procedures that shall standard inocula.

Increasing test water volume

Increasing test water volume was proposed by some participants to enhance the probability of including competent degraders, hence increasing the robustness of the test. However, practical constraints were acknowledged, as incubating volumes exceeding one litre may be technically challenging. Use of tangential flow to concentrate the inoculum was proposed as an alternative, to achieve the objective of including more microorganisms while avoiding unpractically large volumes.

Test concentrations

There was broad agreement amongst participants that testing below 1 µg/L (c.f. less than 1 µg/L to 100 µg/L recommended in OECD TG 309, with maximum test concentrations <10 µg/L or lower preferred) will likely better reflect co-metabolic processes, whereas higher concentrations (e.g. 100 µg/L) may provide sufficient carbon for direct microbial utilisation and associated less environmentally relevant growth-linked kinetics as well as shifts in microbial composition.

Reference substances

It was briefly discussed whether the reference substances, used in OECD TG 309 tests to ensure that an active microbial population is present, were fit for purpose. OECD TG 309 provides both sodium benzoate and aniline as

examples of appropriate reference substances. Sodium benzoate is very easily degraded and may not be sufficiently discriminatory, whilst aniline more frequently fails to pass the OECD TG 309 reference substance validity criteria (degradation in less than two weeks) and in some cases may form NER if the amount of suspended solids is high, whilst in others maybe lost by air partitioning.

In terms of aniline as a reference substance, some participants expressed scepticism due to it often failing to meet the validity criteria whilst others felt that this is rather an illustration of the high stringency of the OECD TG 309 pelagic test.

Testing multiple inocula

Participants considered whether testing more than one test water might be appropriate. Such an approach would be more in line with the OECD TG 308 (sediment-water simulation) and OECD TG 307 (soil simulation) tests where more than one sediment-water system and soil are tested in a single experiment.

Testing multiple test waters/inocula in a single experiment (e.g., sampling along a river gradient) and deriving an average or median half-life was suggested. One participant mentioned that they are currently conducting research to determine the optimal number of sampling sites needed to approximate a representative median value in Europe.

It was noted, for context, that CLP and the REACH guidance R.11 (PBT) recommend using the longest half-life in regulatory persistence assessments when multiple equivalent studies are available. Provided that specific conditions are fulfilled, a median can be used according to the REACH guidance.

Sampling and storage temperature

As temperature can influence both microbial activity and community composition, it was discussed whether sampling and testing at the same temperature might be appropriate, so as better reflect environmental relevance. This approach may also reduce the need to apply Arrhenius corrections to extrapolate half-lives between temperatures (e.g. to the 12°C often used in regulatory assessments), which accounts for temperature-dependent reaction rates but not for differences in microbial communities resulting from sampling at different temperatures.

There were differing views on the appropriate storage temperature prior to testing. While aeration was widely agreed upon, debate remained on whether to store samples at 4°C (as recommended in the guideline) or at test temperature. Some participants argued that if kept for 24 hours or less, they should be kept at the temperature of the test (i.e. 12°C) to avoid thermal shock.

TOC

Recent research (Tian et al., 2024a) found that waters with higher TOC tend to show shorter degradation half-lives in TG 309 studies. This could potentially be due to increased number of microorganisms present and/or increased availability of nutrients.

Some meeting participants highlighted that a biofilm is often formed during OECD TG 309 studies which can, in some instances, be a sign of microbial stress possibly due to limitation of nutrients.

It was noted that characterising the composition of TOC in tested waters may provide more useful information than measuring TOC alone, as both its bioavailable fraction and its sorption capacity can vary considerably. BOD was mentioned as a possible additional or alternative metric.

It was highlighted that hydrophobic substances can sorb strongly to organic matter and form non-extractable residues (NER), thereby complicating the interpretation of degradation data and the distinction between true biodegradation and reduced extractability of the parent substance, with some NER fractions being considered as non-degraded. Some participants highlighted the technical challenges to quantify NERs on the very small amount of suspended solids present in a pelagic test.

Removing protozoa

It was considered whether filtering out protozoa, who may feed on the bacterial microorganisms, could support maintenance of the inoculum. It was suggested to remove them as soon as possible to avoid any selective removal of specific strains and hence reduction of diversity.

Inoculum activity characterisation

Inoculum characterisation was raised as a potentially useful add-on to the OECD TG 309, beyond use of reference substances. This could be testing for types of enzymes present, or, more simply, a measure of microbial concentration (e.g. colony-forming units). BOD_5 testing could also be applied. However, such characterisation should ideally provide results within one day, as the collected test water (inoculum) should be used promptly after collection, with OECD TG 309 recommending preferably starting the test within one day after sample collection.

Semi-continuous or flow-through systems

The use of a semi-continuous systems (as recommended in OECD TG 309 for prolonged incubations) or flow-through systems, rather than the batch systems of up to 60-days that are standard in OECD TG 309, was considered as a means to better preserve inoculum composition over the course of the study. Similarly, the adaptation of OECD TG 314-type studies (simulation tests for degradation in wastewater systems) using surface water inocula was proposed.

Addition of sediment

It was noted that the use of a more standardised and environmentally relevant quantity and type of sediment (e.g. particle size distribution) could help to further characterise and harmonise the test system. Depending on the characteristics and depth of the simulated surface water body, sediment concentration of up to 2 to 50 g/L may be required to achieve laboratory degradation rates comparable to those observed in rivers (c.f. 0.01 – 1 g dw/L recommended for the OECD TG 309 'suspended sediment test').

Some participants considered that the pelagic OECD TG 309 test alone provides a limited representation of environmental conditions. It was highlighted that presence of sediment in the test system has been shown to increases biomass, reduce inter-replicate variability and improve robustness of OECD TG 309 studies.

Testing of mixtures and Benchmarking

The testing of several test chemicals simultaneously in the same experiment was proposed by some participants for consideration. It was hypothesised that if each chemical is present at a low concentration (e.g. 1 µg/L) then each chemical is unlikely to influence the biodegradation of the others, e.g. by competing for microbial enzymes, inhibition of microorganisms or co-metabolism effects. It was emphasised that this hypothesis would need to be tested and confirmed before applying such an approach.

Co-testing multiple chemicals could improve the comparability of biodegradation data by exposing all compounds to the same inoculum and environmental conditions. This could also facilitate benchmarking approaches where the chemical of interest is tested in the same system as benchmark chemicals whose degradation behaviour is already well characterised.

Shorter-term recommendations that could be applied within the framework of the current TG and regulatory frameworks

Building on the above discussion points, complemented with specific proposals in Wang (2026), the following were identified as short-term recommendations to increase the environmental relevance and robustness of the OECD TG tests.

Recommendation	Current TG/guidance	Reasoning
Surface water inoculum sampling		
Consider sampling from large rivers, provided they have not been previously exposed to the test chemical in a way that could cause adaptation.	TG: Surface water that has not been contaminated with the test substance or its structural analogues within the previous four years.	More representative of environmental waters: Large rivers, which tend to have inputs from diverse upstream environments, may better represent the range of microbial communities experienced in receiving waters than small streams or highly localised sites.
Sample at a temperature between 10 and 14°C and maintain temperature during storage.	TG: Store at 4◦C until use. Temperature during transport should not significantly exceed the temperature used in the test.	Support maintenance of microbial community as present at sampling. Reduce inter-replicate variability.

Recommendation	Current TG/guidance	Reasoning
	ECHA guidance R7b: Conduct study at environmentally relevant temperatures: 12°C for freshwater 9°C for marine. Collect from locations where conditions resemble the conditions targeted for the test.	
Use inoculum/test water within 24 hours of sampling.	TG: Preferably use within one day after sample collection.	Support maintenance of microbial community as present at sampling. Reduce inter-replicate variability.
Surface water inoculum characteristics		
Aim for a suspended solids concentration slightly below 20 ppm/0.02 g dw/L.	TG: 0.01 – 1 g dw/L suspended solids for the 'suspended sediment test'. ECHA R7b: 0.01 and 0.02 g dw/L suspended particulate matter. [Note: the 'suspended sediment test' is generally not recommended].	More representative of environmental waters (Bečvář, 2006). Support maintenance of microbial community. Reduce inter-replicate variability.
Aim for a TOC concentration close to 10 mg/L or higher.	No specific recommendations.	More representative of environmental waters (Berggren and Al-Kharusi, 2020). Support maintenance of microbial community. Reduce inter-replicate variability.
Aim for cell concentrations slightly below 10^6 cells/L.	No specific recommendations.	More representative of environmental waters (OECD, 2006). Reduce inter-replicate variability.
Test system setup		
Consider use of aniline as reference substance rather than sodium benzoate.	TG: A substance, which is normally easily degraded under aerobic conditions (e.g. aniline or sodium benzoate).	Sodium benzoate is very easily degraded and may not be sufficiently discriminatory.

Recommendation	Current TG/guidance	Reasoning
[Though note potential issues with NER formation & being blown off from the test system]		
Test concentration 1 µg/L or lower.	TG: less than 1 µg/L to 100 µg/L recommended, with maximum test concentrations <10 µg/L or lower preferred.	Better reflect co-metabolic processes and avoid shifts in microbial composition.

Recommendations on mid- to long-term research priorities to improve the robustness of TG 309

A criterial gap for a mid-term research priority was identified in terms of developing a better understanding of the properties of the inoculum and how they influence degradation. Further research is needed to better characterise the inoculum, perhaps defining quality criteria in terms of biomass and diversity. This could include the characterisation of its initial activity and readily accessible energy content (e.g. via BOD_5 testing), or its diversity in terms of taxonomy and/or functionality. Improved understanding of test conditions that support maintenance of a relevant inoculum is also key.

Other recommendations that could be considered on the longer term included:

- Investigate whether using multiple water inocula from different sampling points and of different types in a test (urban vs rural, pond vs fast flowing river) – either in same or parallel vessels - could increase the robustness of the pelagic study
- Identify more fit-for-purpose reference substances
- Consider whether higher suspended solids concentrations >20 ppm could be more representative of environmental waters
- Investigate application of semi-continuous or flow-through test systems to better maintain microbial community
- Develop a fast-screening method for inoculum activity/quality, e.g. via BOD testing
- Consider relevance of increasing vessel size or using tangential flow to concentrate inocula
- Further investigate testing of several test chemicals simultaneously and benchmarking options to improve the comparability of biodegradation data.

It was broadly recognised that improving the environmental relevance and robustness of OECD TG 309 should go hand-in-hand with strengthening the science-policy interface. This includes defining clear protection goals to ensure

data generation is fit-for-purpose, as well as developing improved approaches for the evaluation and interpretation of degradation data.

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