LRI ECO70: EXPANDING THE APPLICABILITY DOMAIN OF THE OECD 309 TEST GUIDELINE FOR BIODEGRADATION SIMULATION TESTING TO ADDRESS DIFFICULT-TO-TEST PROPERTIES

Background

The potential persistence of non-readily biodegradable test substances is often assessed through higher-tier simulation tests, specifically, the OECD Test Guidelines (TG) 307, 308 and 309. The OECD TG 309 ,the focus of this RfP, was originally developed for plant protection products (PPP) run-off to receiving water bodies. The above test guidelines were adopted for substances with low volatility and measurable water solubility; such that a clear applicability domain in line with validity criteria was not defined.

The assessment of substances with difficult-to-test properties, such as those being poorly soluble, volatile, or with a high sorption potential, routinely requires test method adaptations, leading to potential study design changes, beyond any available recommendation from the TG.

The lack of recommendations within the TG introduces potential uncertainty related to the acceptability and validity of these tests. Variation in tests on difficult substances undermines the goal of standardized testing protocols that enable comparison against regulatory criteria across chemicals and regulatory regimes. In a 2021 survey requesting recommendations for improvements to the environmental OECD TGs, the German Environmental Agency (UBA) received requests to accommodate difficult to test substances in biodegradation testing1.

To address this issue, a need for further guidance addressing biodegradation testing of difficult-to-test substances was identified at the ECHA PBT Expert Group2. This topic is also the subject of a recently awarded framework contract, where Fraunhofer Gesellschaft and Technical University of Denmark will work on identifying methodologies for biodegradation tests of difficult-to-test substances that can be used for regulatory decision-making, with the goal of developing guidance for the degradation testing of difficult-to-test substances.

Some methods for addressing difficult-to-test substances in aquatic systems already exist and are discussed in the OECD guidance document 234. A similar OECD guidance document for degradation testing of difficult-to-test substances would be beneficial. This should include both dosing mechanisms for difficult-to-test substances as well as property-appropriate test system configurations that would allow accurate data generation for the test substance without affecting the integrity and validity of the test , ensuring regulatory acceptance of the data produced.

Several works have investigated ways to improve the feasibility of these TGs, e.g. *Birch et al.* published a review of dosing methods for difficult substances and mixtures for persistence testing in water. However, those methods, such as passive dosing and micro-volume spiking with co-solvent, have been developed with non-radiolabelled test material and have not been validated by tracking the mass-balance in the system. The

lack of validations for use in the OECD simulation tests has resulted in them currently being considered solely as supporting information rather than key data for regulatory assessments. Furthermore, as these methods have been developed relatively recently, few commercial laboratories have experience using them, limiting their use in practice.

Objectives

We propose here to compile, validate, and (where needed) improve upon the existing dosing and test system configurations to allow testing of difficult substances within the current OECD TG 309. The goal would be to provide input to any further biodegradation guidance document development.

The project's objectives are to:

1. Define a set of metrics (e.g., mass balance calculations, amount of test item in the water phase, etc.) against which the standard set up and any modifications of the OECD 309 test should be evaluated. These metrics should set the foundation to assess the efficacy of any alternative dosing or test system configuration identified later in this work.
2. Re-evaluate and establish data-driven boundaries for the physico-chemical applicability domain (volatility, solubility and hydrophobicity) in which the current guidelines are suitable (compared to the metrics from objective 1), where no modifications to the dosing or study design would be required. Combinations of physico-chemical properties should also be evaluated (e.g., volatile and hydrophobic substances). Partitioning models could be used in establishing applicability domain.
3. Based on the gaps found in the applicability domain, identify suitable methods for dosing difficult-to-test substances and needed adjustments to the test system configuration when compared to the metrics from objective 1. Methods beyond those mentioned in the OECD GD 23 and Birch *et al.* 2023 may exist in literature. Such information may come from the ongoing work at Fraunhofer Gesellschaft and Technical University of Denmark. However, depending on the outcome of that work, a more detailed literature review may still be needed. Different dosing methods may be applicable to substances with different properties. The identified

methods should be prioritized for effectiveness and practicality in an OECD TG309 test set up.

4. Based on the findings in the first 3 objectives, optimise (as needed) and assess a range of dosing methods and test system configurations; the range of test substances will depend on the breadth of the targeted applicability domain expansion. If desired, the selected dosing methods/test system configurations can be shown to be transferable by testing in at least 1 other laboratory.

Scope

The work should provide options for dosing in the OECD TG 309 of Poorly or sparingly water-soluble; Hydrophobic; Volatile; MCS/UVCB, which are among the properties identified as difficult-to-test in the list from the OECD 23 Guidance).

Out of scope

Difficult-to-test substances that are out of scope:

Toxic at low concentrations; Photo-degradable; Complexing; Colloids; Polymers; Ionisable; Surfactant

Deliverables

Key deliverables include:

1. A set of metrics to compare different dosing and test system configurations of the OECD TG 309 test.
2. Cut-off ranges for a variety of physico-chemical properties that would define the applicability domain of OECD TG 309 for the aforementioned properties.
3. Overview of different dosing methods and test system configurations, paired with the targeted physico-chemical properties and demonstration of how these alternative dosing methods and test system configurations expand the applicability domain.
4. Optimisation (as needed) and evaluation against the metrics established in Deliverable 1 for a range of dosing methods/test system configurations suitable for the OECD TG 309 , with concise method descriptions and justification for the expansion of the

applicability domain to be included in potential upcoming guidance documents.

A final report containing an executive summary (2 pages max), a main part (max. 50 pages) and a detailed bibliography, with the search criteria that were used and any laboratories which were consulted. It is expected that the findings will be developed into at least one peer reviewed publication, following poster(s) and presentation(s) at suitable scientific conference(s).

It is also expected that the findings would be presented to the relevant regulatory bodies allowing for an update on the relevant TG or guidance document.

Cost and Timing

This project would expect to start in Q4 2025 with a duration 2 years and a total budget of 300-400k €. Applicants are requested to break down the costs and timing according to deliverable. This project is cofunded with Concawe.

Partnering / Co-funding

Applicants should provide an indication of additional partners and funding opportunities that can be appropriately leveraged as part of their proposal. Partners can include, but are not limited to industry, government/regulatory organizations, research institutes, etc. Statements from potential partners should be included in the proposal package.

Fit with LRI objectives / Possible regulatory and policy impact involvements / Dissemination

Applicants should provide information on the fit of their proposal with LRI objectives and an indication on how and where they could play a role in the regulatory and policy areas. Dissemination plans should also be laid down.

DEADLINE FOR SUBMISSIONS: June 27th, 2025 at 11:59 PM (Central European Time).

Please see www.cefic-lri.org/funding-opportunities/apply-for-a-grant/ for general LRI objectives information, project proposal form and further guidance for grant applications. Please note that, if awarded the project, the institute must be able to accept Belgian Law (or an equivalent European legal framework) in the contract. If there are questions, please email lri@cefic.be.

Related links

- Review of the OECD Test Guidelines relevant to environmental assessment with regard to the state of the art in science and technology (umweltbundesamt.de)
- SUMMARY REPORT OF THE 34th PBT EXPERT GROUP MEETING 11268044-c161-5ace-705a-5d3078bf18e1 (europa.eu)
- News - ECHA (https://echa.europa.eu/view-article/-/journal_content/title/echa-weekly-23-april-2025)
- OECD (2019), *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures*, OECD Series on Testing and Assessment, OECD Publishing, Paris, https://doi.org/10.1787/0ed2f88e-en.
- Birch H, Hammershøj R, Møller MT, Mayer P. Technical guidance on biodegradation testing of difficult substances and mixtures in surface water. MethodsX. 2023 Mar 20;10:102138

Timing: Q4 2025, duration 2 years

LRI funding: 300-400k €

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chemical safety

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