

## CEFIC Long-range Research Initiative Request for Proposals (RfP)

### ***Title and Code Number:***

Assessing the Impact of Sample Collection on Microbial Population and Validity Criteria in the OECD 309 Surface Water Mineralisation Test – LRI ECO55

### ***Background***

The OECD 309 is often demanded by ECHA as the initial higher tier biodegradation simulation test. Together with the OECD 307 and OECD 308 guidelines, the OECD 309 test should yield results to directly assess a substances persistence with the criteria laid down in ANNEX XIII of the REACH regulation, or, with the potential to elucidate the degradation pathway and identify relevant metabolites. With the last update of the ECHA REACH Chapter R11 Guidance Document (June, 2017), the OECD 309 test is mostly required at a test temperature of 12°C for the derivation of degradation kinetics unless metabolite formation is in focus which would require a test temperature of 20°C.

Several factors (e.g. microbial viability in the test matrix, test temperature, test setup and geometry) can have significant influence on the results of biodegradation simulation tests (i.e. OECD 309 as well as OECD 308 and OECD 307). The impact of these factors is, for the major part, not well understood. Besides these factors (see ***Objectives***), the shift in the test temperature, from in-situ temperature to 12°C, creates uncertainty regarding the relevance of the test's outcome and the subsequent assessment of the results including the validity criteria for the reference substance kinetics. Although broad experience with the OECD 309 exists, several of these recurring questions and unknowns need to be elucidated and assessed in order to perform valid experiments and to robustly interpret the results.

### ***Objectives***

The project's objectives are to elucidate the following issues:

1. The impact of sample collection, storage and treatment prior to use: In its current state, the OECD 309 test guideline (TG) stipulates that the OECD 309 study should be performed at between 20 – 25°C, or, at the actual temperature that the surface water was sampled. The revised ECHA guidance document suggests that the test should be performed at 12°C. Therefore, the surface water collected should ideally be at an ambient temperature of 12°C to be in-line with the TG. The TG further suggests that if the surface water sample is not to be used in the definitive study within 2 to 3 hours of its collection, then the sample should be stored at 4 °C. If the available technical guidance is followed, this could lead to major and rapid temperature changes which could lead to a significant alteration of the diversity and abundance of the natural microbial community. The surface-water mineralisation test may also be performed with suspended solids amended up to 1 g/L of sediment collected from the same sampling site. Similar to the water, it is

also important for the sediments to maintain consistent temperatures to prevent major shift in the microbial community as they are likely an additional source of microbes to the test system.

A technical guidance document on sample collection, storage and treatment shall be issued to avoid effects related to significant temperature differences between the collection site and the requested laboratory test temperature. This should include a scientifically derived maximum range between source and lab temperature. Furthermore, the guidance should include a tangible microbial viability assessment with special emphasis on temperature as an influencing factor. Other factors e.g. storage time should be assessed too. Efforts should be taken to set up metrics which help to robustly assess the microbial activity of an inoculum and potential changes between sampling and test initiation.

2. Reference Substances: The OECD 309 TG suggests the use of Sodium Benzoate and Aniline as appropriate reference substances to demonstrate the validity of the study. The pass criteria for test validity is stipulated, as follows *“If the reference substance is not degraded within the expected time interval (for aniline and sodium benzoate, usually less than two weeks), the validity of the test is suspected and must be further verified”*, leaving room for interpretation. It has also been suggested from biodegradation screening studies, that the mineralisation of Sodium Benzoate and Aniline will take place even in test systems with low microbial diversity and abundance and that their complete biodegradation may not be a realistic representation of the microbial viability of a test system. To this end, it will be necessary to assess the suitability and limitations of the existing reference substances within the OECD 309 test system, and, identify reasonable new reference substances which are realistic of test system performance and which require the presence of microbial communities in order to undergo significant mineralisation. These are required to provide a robust guidance on the test validity criteria with special emphasis on the reference substances mineralisation at different temperature regimes and to develop applicable biodegradation rate constants at 12 °C.
3. Other influencing factors: Identify and assess other relevant influencing factors (e.g. test system geometry and setup like flow-through vs. closed system, use of suspended solids in OECD 309, sub-sampling, aeration etc.) and give guidance on best practices and how they can be accounted for in the interpretation of the test results.
4. Applicability domain: Refine the applicability domain of the OECD 309 method with regard to substance properties. If possible, a comparison on the robustness of OECD 309 tests at 20°C and 12°C should be derived.
5. Any new approach should be validated with reasonable reference substances for biodegradation but also for no occurring biodegradation (=persistence). In this regard, positive controls for detecting biodegradation should only follow this fate path which means a viable inoculum is required and should not have other properties prone to yield artefacts.

## **Scope**

Enhance the applicability of the OECD 309 methodology with regard to the adapted temperature requirement and elucidate and assess the relevant influencing factors to robustly assess the results of biodegradation simulation tests.

## **Deliverables**

- A tangible guidance document on the microbial activity of the surface water, and, where appropriate, sediment samples shall be issued containing chapters on sample collection, storage and pre-treatment as well as techniques to qualitatively assess the microbial viability. Preferably, these techniques should have a broad applicability to samples also from other environmental compartments.
- Identification of reasonable new reference substances which are realistic of test system performance and which require the presence of microbial communities in order to undergo significant mineralisation, and develop applicable biodegradation rate constants at 12 °C. Develop a detailed test protocol and organise and administer an inter-laboratory validation.
- Report findings and present to international test guideline authorities (e.g. OECD, ISO) proposing updates to existing Test Guideline text.

The final report shall contain an executive summary (2 pages max), a main part (max. 50 pages) and a detailed bibliography. 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).

## **Cost and Timing**

Start in Q1 2021

Duration: 3 years

Budget in the order of 500.000 Euro

## **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.

## **References**

OECD Guidelines for testing of Chemicals. Test Guideline 309: Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test. Adopted November 2004.

OECD Guidelines for testing of Chemicals. Test Guideline 308: Aerobic and anaerobic transformation in aquatic sediment systems. Adopted April 2004.

ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment, version 3, June 2017.

([https://echa.europa.eu/documents/10162/13632/information\\_requirements\\_r11\\_en.pdf](https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf))

Mark Honti and Kathrin Fenner. Deriving persistence indicators from regulatory water-sediment studies – Opportunities and limitations in OECD 308 data. *Environ Sci Technol.* 2015 May 19;49(10):5879-86.

Mark Honti, Stefan Hahn, Dieter Hennecke, Thomas Junker, Prasit Shrestha, Kathrin Fenner. Bridging across OECD 308 and 309 Data in Search of a Robust Biotransformation Indicator. *Environ Sci Technol.* 2016 Jul 5;50(13):6865-72.

Prasit Shrestha, Thomas Junker, Kathrin Fenner, Stefan Hahn, Mark Honti, Rani Bakkour, Cecilia Diaz, Dieter Hennecke. Simulation Studies to Explore Biodegradation in Water-Sediment Systems: From OECD 308 to OECD 309. *Environ Sci Technol.* 2016 Jul 5;50(13):6856-64.

Delina Y. Lyon, David Saunders, Sarah Hughes, Erin Maloney, Charles Meyer. Inoculum criteria for ready biodegradability tests. Poster, presented at SETAC North America Annual Meeting, 2018.

**DEADLINE FOR SUBMISSIONS: August 31, 2020**

Please see [www.cefic-lri.org/funding-opportunities/apply-for-a-grant/](http://www.cefic-lri.org/funding-opportunities/apply-for-a-grant/) for general LRI objectives information, project proposal form and further guidance for grant applications.