# CEN WORKSHOP AGREEMENT

# CWA 18310

December 2025

**ICS** 13.020.40; 25.220.40

English version

# Assessment of ecotoxicity in PVD coatings subjected to accelerated ageing



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**



Ref. No.:CWA 18310:2025 E

# Contents

Page

# Foreword

This CEN Workshop Agreement (CWA 18310:2025) has been developed in accordance with the CEN-CENELEC Guide 29 “CEN/CENELEC Workshop Agreements – A rapid way to standardization” and with the relevant provisions of CEN/CENELEC Internal Regulations - Part 2. It was approved by the Workshop CEN/WS “Evaluating Antimicrobial Coatings: From Air Filtration Efficiency to Antiviral Mechanism and Ecotoxicology”, the secretariat of which is held by UNE (Spanish Association for Standardization) consisting of representatives of interested parties on 2025-07-29, the constitution of which was supported by CEN following the public call for participation made on 2025-06-16. However, this CEN Workshop Agreement does not necessarily include all relevant stakeholders.

The final text of this CEN Workshop Agreement was provided to CEN for publication on 2025-12-15.

Results incorporated in this CWA received funding from the European Union’s Horizon Europe research and innovation programme under grant agreement No 101057597 (NANOBLOC).

The following organizations and individuals developed and approved this CEN Workshop Agreement:

— Politecnico di Torino (POLITO) – Prof. Cristina Balagna (Chair) and Dr. Agelica Luceri

— University of Turin (UNITO) – Prof. Manuela Donalisio, Prof. David Lembo, Mrs. Elisa Feyles and Mrs. Domiziana Porporato

— TEKNIKER – Dr. Rocío Ortiz

— Friedrich Alexander University Erlangen (FAU) – Prof. Aldo R. Boccaccini and Dr. Zoya Hadzhieva and Mrs. Andrada-Ioana Damian

— IRIS Technology Solutions SL. – Dr. Cristina Fernández and Dr. Marina Pellegrino

— AGC Interpane – Dr. Omar Benzine

— SIOEN Industries – Mrs. Sophie Poirier

— GV FILTRI INDUSTRIALI SRL - Mr. Flavio Martina and Mr. Samuele Gentile

— National Technical University of Athens – Prof. Nikolaos Chronis, Dr. Manolis Papazoglou and Dr. Dimitris Barmpakos.

— Spanish Association for Standardization (UNE) – Mr. Javier Idiago (Secretary)

Attention is drawn to the possibility that some elements of this document may be subject to patent rights. CEN-CENELEC policy on patent rights is described in CEN-CENELEC Guide 8 “Guidelines for Implementation of the Common IPR Policy on Patent”. CEN shall not be held responsible for identifying any or all such patent rights.

Although the Workshop parties have made every effort to ensure the reliability and accuracy of technical and non-technical descriptions, the Workshop is not able to guarantee, explicitly or implicitly, the correctness of this document. Anyone who applies this CEN Workshop Agreement shall be aware that neither the Workshop, nor CEN, can be held liable for damages or losses of any kind whatsoever. The use of this CEN Workshop Agreement does not relieve users of their responsibility for their own actions, and they apply this document at their own risk. The CEN Workshop Agreement should not be construed as legal advice authoritatively endorsed by CEN/CENELEC.

# Introduction

The increasing use of functional coatings developed by Physical Vapor Deposition (PVD) technology has led to significant advancements in surface engineering, particularly for high-touch surfaces in public and healthcare environments. These coatings are often engineered to provide not only mechanical and chemical resistance, but also antibacterial and antiviral functionalities through the incorporation of elements such as copper (Cu), silver (Ag), and zinc (Zn).

While such functionalities contribute to improved hygiene and infection control, concerns have emerged regarding the potential environmental impact of these coatings, especially as they age and degrade. Accelerated ageing caused by routine cleaning, moisture, or chemical exposure may lead to the release of these elements into the environment, particularly into wastewater systems. This raises the risk of ecotoxicological effects on aquatic organisms and ecosystems.

Currently, no standardized methodology exists to evaluate the ecotoxicity of aged PVD coatings under realistic usage conditions. Existing guidelines and regulations, such as the OECD 23 (2018) "Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals", provide important foundations for aquatic toxicity assessment, but are not specifically tailored to solid surface coatings undergoing environmental degradation. In parallel, the EN ISO 11998 standard for wet scrub resistance offers a relevant framework for simulating cleaning activities on coated surfaces [1]. The EU Ecolabel Regulation (EC) No 66/2010 also underlines the importance of assessing environmental performance, including chemical emissions, as part of the product lifecycle, highlighting the need for a harmonized testing methodology applicable across coatings and use cases. [2]

This CEN Workshop Agreement (CWA) addresses that gap by defining a reproducible, practical procedure to assess the ecotoxicity of PVD coatings when exposed to two representative ageing scenarios: (1) daily cleaning cycles and (2) prolonged exposure to humid or wet conditions. It supports the Safe and Sustainable by Design (SSbD) framework and contributes to compliance with environmental and chemical safety legislation in the EU, including REACH and circular economy goals outlined in the European Green Deal.

This document is intended for use by researchers, manufacturers, environmental testing laboratories, and regulatory bodies involved in the development, evaluation, and approval of functional surface coatings.

This document has been based on the knowledge generated in the EU-funded research project NANOBLOC, which received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101057597.

# 1 Scope

This CWA defines a methodology for assessing the potential ecotoxicity of coatings developed using Physical Vapor Deposition (PVD) technology when applied to metallic substrates and subjected to accelerated ageing conditions.

The procedure is intended to evaluate the release of functional elements, such as copper (Cu), silver (Ag), and zinc (Zn) from PVD coatings under conditions simulating two common environmental exposure scenarios:

a) Repeated surface cleaning using chemical and mechanical actions;

b) Prolonged exposure to humid or wet environments, such as those found in sanitary facilities.

This methodology enables the collection of leachates and their subsequent ecotoxicological evaluation using aquatic toxicity assays. It provides guidance on sample preparation, ageing protocols, leachate collection, and reference to existing toxicological test methods.

The procedure applies to the screening and development of PVD coatings intended for high-touch surfaces, particularly in public, medical, and sanitary environments. It supports compliance with EU environmental and product safety frameworks and aligns Safe and Sustainable by Design (SSbD) principles.

This CWA does not cover the mechanical or antimicrobial performance assessment of PVD coatings, nor does it replace existing ecotoxicity testing standards for soluble chemicals or effluents.

# 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document.

EN ISO 11348-2:2008, *Water Quality —Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (luminescent bacteria test) — Part 2: Method using liquid dried bacteria (ISO 11348-2:2007)*

ISO 22196:2011, *Measurement of antibacterial activity on plastics and other non-porous surfaces*

# 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp/

— IEC Electropedia: available at http://www.electropedia.org/

**3.1**
**ecotoxicity**
capability of a compound or any chemical or physical agent to show a harmful effect on both the environment and organisms

**3.2**
**PVD**
physical vapor deposition (PVD) technologies are processes that involve depositing a source material (which can be either from a solid, liquid, or gas) onto the surface of the component

**3.3**
**leachate**
chemicals extracted from the water immersion ageing tests

**3.4**
**lixiviates**
soluble substances extracted from the coated metallic samples after ageing tests

**3.5**
**$EC_{50}$**
Effective Concentration when the test substance in water is estimated to result in a 50 percent reduction in reproduction of the organism tested within a particular period of exposure

**3.6**
**correction factor ($F_{kt}$)**
measurement of the changes in the intensity of the control samples during exposure time in a LUMIStox test

**3.7**
**ICP assay**
Inductively Coupled Plasma (ICP) assay is a type of mass spectrometry that uses an inductively coupled plasma to ionize the sample and identify and quantify elements in the sample

# 4 Methodological framework

## 4.1 Overview of the procedure

The antibacterial activity of coated metallic samples shall be evaluated before and after the accelerated ageing tests, which simulate daily cleaning procedures and humid environmental exposure, using a quantitative test based on ISO 22196:2011 guideline for non-porous surfaces and textile materials. The general scheme for this standard is shown in Figure 1. The ageing tests consist in water immersion tests designed ad hoc, where coated samples are placed in corrosion test cells and subjected to elevated temperatures (typically 60 °C) for defined durations (e.g., 72, 168, 240, and 336 hours). The resulting leachates are analysed for metal-release by ICP for ecotoxicity assessment and are also evaluated for antibacterial activity.

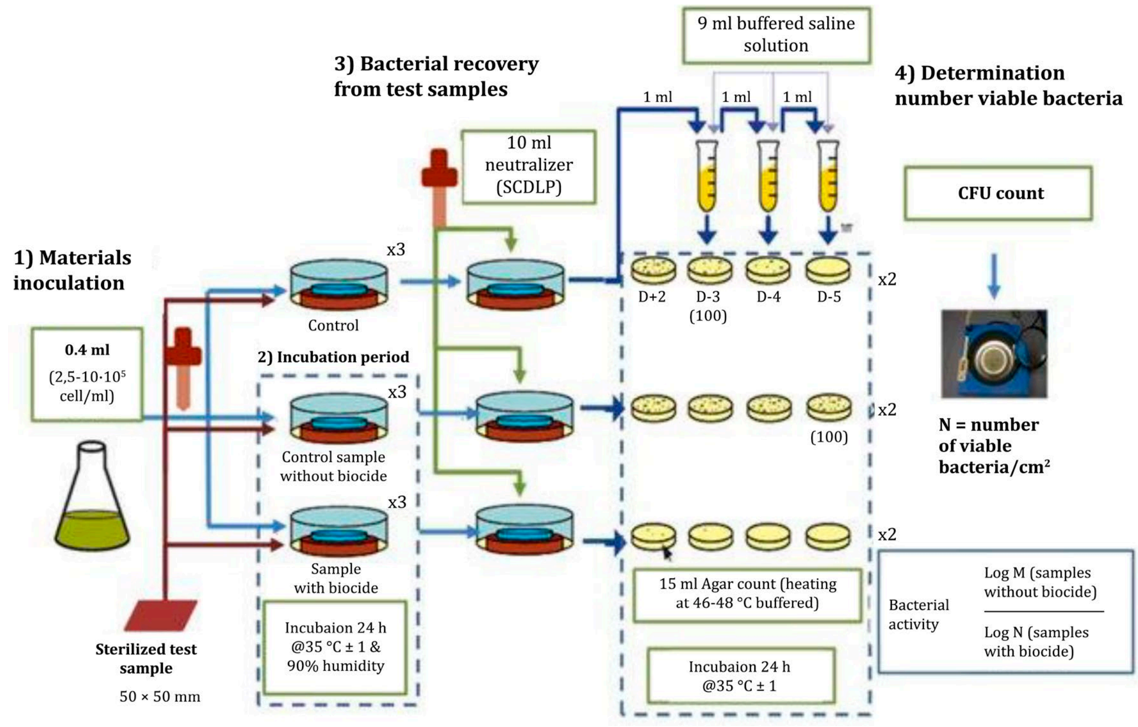


**Figure 1 — Scheme of antibacterial activity test**

### 4.2 Applicability and limitations

The application of this procedure considers PVD-coated flat metallic substrates.

The ecotoxicity assay procedure described herein can be applied using the bacteria strain included in the OECD 23 guideline [3], such as Vibrio fischeri" (NRRL B-11177), or using the test organism specified in OECD guidelines 201 [4], 202 [5] and 203 [6].

## 5 Preparation of test samples

### 5.1 Substrate selection and coating description

Metallic samples are considered substrates. Coatings deposited by PVD technologies and containing antimicrobial elements such as copper (Cu), silver (Ag), and zinc (Zn) can be evaluated.

### 5.2 Sample conditioning

Before testing, both sides of the metallic samples and cover films shall be sterilized by UV radiation in a laminar airflow chamber for 10 minutes.

### 5.3 Replicates and controls

Six uncoated samples shall be used as controls, and six coated samples are required for each coating condition.

# 6 Accelerated ageing protocols

## 6.1 Simulation of daily cleaning procedures

### 6.1.1 General

This section describes the procedure used to simulate the physical and chemical ageing of PVD coatings under typical cleaning conditions, based on the methodology adapted from EN ISO 11998:2006.

### 6.1.2 Objective

To simulate the effects of routine surface cleaning on PVD-coated metallic substrates in order to assess the potential release of functional elements and the resulting ecotoxicity.

### 6.1.3 Test equipment and parameters

Unless otherwise specified, use the following equipment and parameters.

A wet abrasion scrub tester (Figure 2) that simulates repeated washing by moving a brush or abrasive pad pack and forth across the coated surface.

Test parameters shall be as follows:

- Cycle frequency: 37 ± 2 cycles/min;
- Estimated contact pressure: 1.6 kPa (achieved with ≈500 g weight);
- Cleaning medium (chemical cleaning agent): 1% aqueous solution of Probio Total (non-ecotoxic commercial cleaner);
- Cleaning cloth (physical cleaning agent): Blue microfibre;
- Number of cycles: 3,650 (equivalent to ∼1 year of daily cleaning);
- Motion: Linear horizontal back-and-forth scrubbing;
- Sample mounting: Coated metallic substrates fixed securely to a flat support.

### 6.1.4 Procedure

The following steps shall be followed.

1) Prepare the cleaning solution by diluting Probio Total to 1 % in distilled water.
2) Mount each coated sample on the scrub testing device, ensuring flat and uniform exposure.
3) Attach a microfibre cloth to the scrub arm and apply a load of 500 g to achieve an approximate pressure of 1,6 kPa (see Figure 3).
4) Apply the cleaning solution uniformly to the cloth or the sample surface.
5) Run the test for 3,650 cycles at 37 ± 2 cycles/min.
6) Upon completion of the test, collect any visible residue and rinse the surface with deionized water.
7) Collect the rinsing solution (and optionally, extract residual elements) for subsequent ecotoxicity or chemical analysis.

The test set-up with real samples is shown in Figure 4.

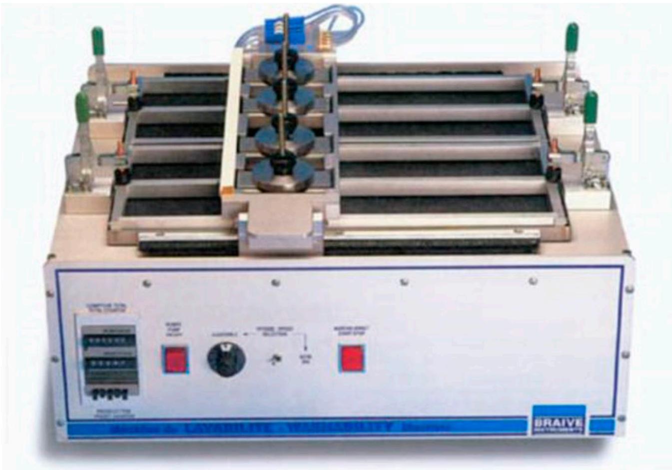


**Figure 2 — Wet-scrub tester-washability tester (Neurtek)**

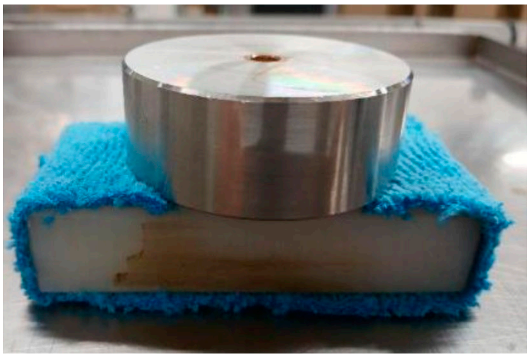

**Figure 3 — Cleaning cloth fixed to the support.**

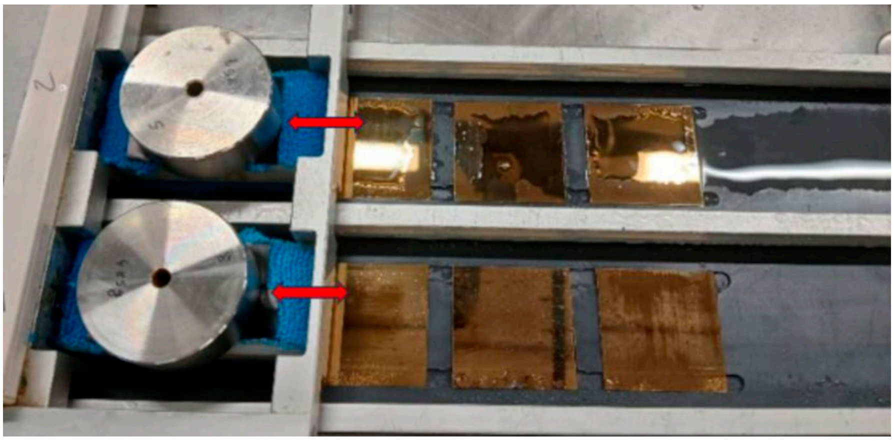

**Figure 4 — Set-up of the test with coated metallic samples.**

### 6.1.5 Notes

This test simulates one year of routine cleaning exposure in public or sanitary settings. The use of a non-ecotoxic cleaning product ensures that any observed ecotoxicity in the leachates originates from the coating itself.

## 6.2 Simulation of humid environmental exposure

### 6.2.1 General

This section describes the immersion ageing procedure developed ad hoc to simulate the prolonged exposure of PVD-coated metallic surfaces to humid environments, such as those found in sanitary facilities (e.g., faucets, drains), and to evaluate the release of functional elements under these conditions.

### 6.2.2 Objective

To assess the potential release of functional elements (e.g., Cu, Ag, Zn) from PVD coatings during prolonged exposure to elevated temperatures in aqueous environments, simulating accelerated ageing.

### 6.2.3 Test equipment and parameters

Unless otherwise specified, use the following equipment and parameters.

A static immersion test using 100 mL of distilled water is carried out in individual cells, which are placed in a WEISS C340/70 climatic chamber (Figure 5).

Test parameters shall be as follows:

- Temperature: 60 °C;
- Duration: 72 h, 168 h, 240 h, and 336 h (four separate test intervals);
- Test cell: Corrosion test cells (custom-designed);
- Medium: Deionized water;
- Volume/sample ratio: Sufficient to allow full immersion of each specimen (recommendation: 20 mL/cm$^2$).

### 6.2.4 Procedure

The following steps shall be followed:

1) Each coated metallic sample shall be placed in an individual corrosion test cell.
2) A measured volume of deionized water shall be added to each cell to ensure complete immersion of the sample. The upper side of each cell shall be sealed to minimize evaporation of the contained water.
3) The test cells shall be placed in a temperature-controlled environment maintained at 60 °C.
4) At each specified exposure time (72, 168, 240, and 336 hours), the leachate from each cell shall be collected.
5) The collected leachates shall be filtered and stored in clean containers for subsequent analysis (e.g., quantification of copper release, aquatic toxicity assays).
6) The leachates obtained from the immersion test shall be analyzed by inductively coupled plasma (ICP) to determine the release of ions.
7) (Optional): The antibacterial performance of the exposed samples may be assessed following immersion to evaluate potential functional degradation.

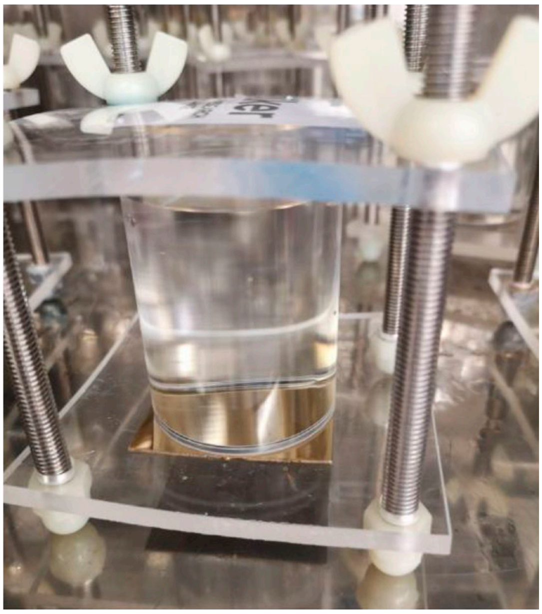

**Figure 5 — Static individual sample immersion test inside a climatic chamber**

### 6.2.5 Notes

The procedure described has been specifically developed to simulate realistic environmental exposure scenarios relevant to high-humidity applications.

A temperature of 60 °C shall be employed to accelerate leaching phenomena, while ensuring that the value remains below the degradation thresholds applicable to most coating systems.

The leachates obtained shall be suitable for direct use in ecotoxicological testing or elemental analysis.

# 7 Ecotoxicity testing

## 7.1 Selection of test organisms

The toxicity of coated samples shall be assesed using luminescent bacteria “Vibrio fischeri” (NRRL B-11177) as the test organism (Figure 6). This species produces bioluminescence as a byproduct of cellular respiration, a process essential to its metabolic activity and overall viability. Due to the direct correlation between bioluminescence and metabolic function, any inhibition or reduction in light emission shall be interpreted as an indicator of toxicity in the test medium.

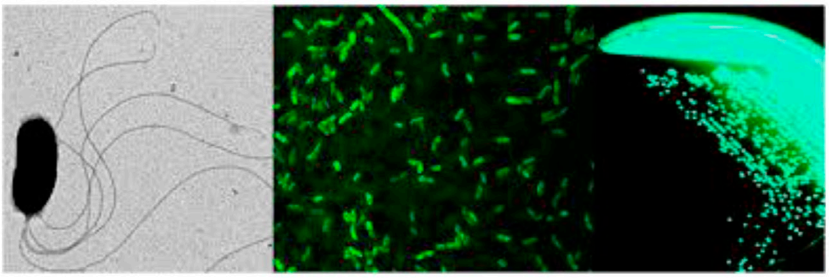

**Figure 6 — Image of Vibrio fischeri bacteria**

### 7.2 Test equipment and conditions

In order to assess the toxicity of the lixiviates obtained from the ageing process of various coatings, the LUMISTox test shall be performed in accordance with EN ISO 11348-2, using dehydrated *Vibrio fischeri* (NRRL B-11177) as the test organism (see Figure 7). This photobacterial assay is based on evaluating the effect of a series of sample dilutions on the bioluminescence of *Vibrio fischeri*.

Test conditions shall be as follows:

- Equal volumes of test suspension and water sample or diluted sample shall be mixed. As a result, dilution levels within the series shall be D ≥ 2.
- Sodium chloride shall be added to the test samples at a concentration of 20 g/L to ensure appropriate salinity for the marine bacteria. The final solutions shall contain 2 % NaCl.
- A geometric dilution series of the sample shall be prepared.
- In parallel, a test-ready concentration of luminescent bacteria shall be reconstituted, and the initial (baseline) luminescence of the bacterial suspension shall be recorded.
- The sample dilutions shall then be mixed with the bacterial suspension, and after 15 minutes of incubation, the final luminescence shall be measured.
- Incubation shall be conducted at a temperature of 15 °C.
- The concentration of the sample that causes a 50 % reduction in luminescence shall be determined and reported.

**Figure 7 — LUMIStox test equipment**

## 8 Data analysis and reporting

### 8.1 Data validation

The validity of the test results shall be confirmed based on the following criteria:

- The method shall be considered valid when an inhibition of the initial luminescence between 40 and 60 % is observed.
- The pH value of the sample shall be within the range of 6,0 to 8,5.
- Sample coloration: Losses of luminescence caused by absorption or scattering of light may occur in the case of strongly colored or cloudy samples.
- The $f_{kt}$ value after 30 minutes of incubation shall be between 0,6 and 1,8. If the optical density (OD) measured in the LUMIStox system exceeds 1,800 E, the sample shall be diluted accordingly. Hardness

or salt concentration: Sodium chloride concentrations below 15 g/L or above 50 g/L may result in osmolarity changes that inhibit luminescence.Concentration of oxygen ($O_2$) dissolved in the sample: Samples with rapid oxygen consumption may inhibit luminescence. A minimum dissolved oxygen concentration of 0,5 mg/L shall be maintained. Determinations carried out in duplicate shall not deviated from their mean values more than 3 %. This requirement applies to both control samples and values more used to determine $EC_{50}$ values.

## 8.2 Calculation of ecotoxicity indicators

The inhibition of luminescence produced by Vibrio fischeri cultures shall be determined using a batch assay. When this type of bacteria comes into contact with the toxic product, the light emission decreases in proportion to the toxicity of the sample. When the luminescence is reduced to 50 % of its initial value, the corresponding concentration shall be referred to as the Effective Concentration ($EC_{50}$). The inhibitory effect of the sample shall be determined by calculating $EC_{50}$ values after 15 minutes and/or 30 minutes of exposure, based on a series of sample dilutions. An $EC_{50}$ value below the 50 % threshold shall be classified as the lowest level of ecotoxicity corresponding to a category considered relatively harmless (Table 1).

**Table 1 — $EC_{50}$ criteria for evaluation of ecotoxicity**

| Category | % P | Photobacterium (mg/L)<br>Photobacterium<br>(mg/L) |
|---|---|---|
| Relatively harmless | 50 | > 500 000 |
| Virtually non-toxic | > 5 - 50 | > 50 000 – 500 000 |
| Slightly toxic | > 0,5 - 5 | > 5 000 – 50 000 |
| Moderately toxic | > 0,05 - 0,5 | > 500 - 5 000 |
| Highly toxic | < 0,05 | < 500 |

The evaluation of the test shall be based on on the reduction in luminescence measured after contact times of15 min and 30 min A correction factor ($f_{kt}$), shall be applied to account for changes in the luminescence intensity of the control samples during the exposure period. The correction factor shall be calculated from the measured luminescence intensity using equation (1):

$$f_{kt} = I_{kt}/I_0 \quad (1)$$

Where $I_0$ shall be the measured luminescence intensity at time 0, and $I_{kt}$ shall be the measured luminescence intensity at contact times of 5 min, 15 min, and 30 min. This value serves to correct the initial $I_0$ values of all test samples, before they can be used as reference values for the determination of the decrease in luminescence caused by water.

## 8.3 Reporting template

The test report shall include the following information:

**Table 2 — Results from the ecotoxicity test**

| Aged, coated sample | $EC_{50}$ at 15 min (%) | $EC_{50}$ at 30 min (%) |
|---|---|---|
| | | |
| | | |

# Bibliography

[1] EN ISO 11998:2006, *Paints and varnishes — Determination of wet-scrub resistance and cleanability of coatings (ISO 11998:2006)*

[2] Regulation (EC) No 66/2010 of the European Parliament and of the Council of 25 November 2009 on the EU Ecolabel

[3] OECD. Nº 23 (2018), Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals (OECD Series on Testing and Assessment No. 23, Second Edition)

[4] OECD Nº 201 (2011), Guidelines for the testing of chemicals: Freshwater Alga and Cyanobacteria, Growth Inhibition Test

[5] OECD Nº 202 (2004), Guidelines for the testing of chemicals: Daphnia sp., Acute Immobilisation Test

[6] OECD Nº 203 (2025), Guidelines for the testing of chemicals: Fish, Acute Toxicity Testing

[7] ISO 11885:2007, *Water quality — Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES)*