

Abstract

Beyond Fish Lethality: Shifting from Traditional Ecotoxicology Toward Ecologically Relevant and Humane Alternative Tests in Chemical Assessment [†]

Matilde Moreira-Santos ^{1,*‡}, Laura Guimarães ² and Cristiano V. M. Araújo ²

¹ CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

² Department of Ecology and Coastal Management, Institute of Marine Sciences of Andalusia (ICMAN-CSIC), Puerto Real, 11510 Cádiz, Spain

* Correspondence: matildemsantos@ciimar.up.pt

[†] Presented at the XI Iberian Congress of Ichthyology, Vila Real, Portugal, 23–27 June 2026.

[‡] Presenting author (Oral Presentation).

Abstract

Introduction: The rationale of traditional toxicity assessments, such as fish lethality tests (e.g., OECD TG 203), is to use forced exposure tests to characterise the ecotoxicity of chemicals by deriving concentration–response relationships based on observed physiological effects to estimate environmental risks. This approach assumes that physiological mechanisms, such as detoxification, are the main means that organisms use to minimise contamination effects. However, non-forced exposure approaches, where organisms can freely move along a contamination gradient, show that mobile species like fish can avoid adverse contaminant levels and escape to favourable areas. As populations are exposed to disturbed habitats, direct ecosystem-level effects may occur through population down-sizing, even in the absence of individual suffering. Contaminants may thus act as habitat disturbers, regulating fish dispersion patterns by provoking emigration from contaminated areas at concentrations well below lethal levels. Spatial avoidance responses therefore align with a key priority in environmental risk assessment (ERA): progressing beyond standard tests to gain ecological realism when assessing impacts on biodiversity, habitats, ecological processes and recovery. **Objective:** To increase ecological relevance in ERA while halting animal distress, pain and suffering. This study reviews existing data on fish avoidance tests, with the ultimate goal of discussing their value and fostering their implementation as an ecologically relevant and more humane alternative to fish lethal testing in chemical ERA. **Methodology:** This review analyses results from studies using two main non-forced multi-compartment exposure systems: linear systems and the bi-dimensional HeMHAS (Heterogeneous Multi-Habitat Assay System), compared with traditional forced exposure tests. **Conclusions:** Spatial avoidance is generally triggered after short exposure periods (5 min to 48 h) at concentrations causing no mortality. Fish populations may therefore become locally extinct before any deaths occur, as individuals promptly emigrate without physiological impairment. The simplicity of experimental design provides strong potential for standardisation and routine implementation in ERA. Fish avoidance tests represent a key ecologically relevant tool at ecosystem and landscape levels and support the 3Rs (replacement, reduction and refinement) as well as the new 3Rs (reproducibility, relevance and regulatory applicability), helping reduce uncertainty in chemical assessment, as urged by many EU legislations.



Academic Editor: Alberto Teodorico Correia

Published: 17 June 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

Keywords: fish lethality; fish spatial avoidance/colonisation; ecological relevance; animal testing alternatives; 6Rs principle

Author Contributions: Conceptualisation, M.M.-S. and C.V.M.A.; methodology, M.M.-S. and C.V.M.A.; investigation, M.M.-S., L.G. and C.V.M.A.; resources, L.G.; data curation, M.M.-S.; writing—original draft preparation, M.M.-S.; writing—review and editing, M.M.-S., L.G. and C.V.M.A.; visualisation, M.M.-S., L.G. and C.V.M.A.; supervision, L.G. and C.V.M.A.; project administration, L.G.; funding acquisition, L.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by SIGNAL (COMPETE2030-FEDER-00877500; <https://doi.org/10.54499/2023.16585.ICDT>), supported by the European Regional Development Fund (FEDER) through the Competitiveness and Internationalization Operational Program (COMPETE 2030) and by national funds through the Foundation for Science and Technology (FCT), and also by the European Commission’s Recovery and Resilience Facility, within the scope of UID/04423/2025 (<https://doi.org/10.54499/UID/04423/2025>), UID/PRR/04423/2025 (<https://doi.org/10.54499/UID/PRR/04423/2025>), and LA/P/0101/2020 (<https://doi.org/10.54499/LA/P/0101/2020>).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.