METHODS FOR PREDICTING ACUTE FISH TOXICITY

Acute fish toxicity is any toxicity caused in a short-term test on fish. The concentration to kill 50% of the fish (LC_{50}) is determined. The OECD test guidelines below can be used to assess a substance's potential to cause acute fish toxicity.

METHOD	TEST PRINCIPLE	APPLICABILITY DOMAIN	DATA INTERPRETATION
OECD TG 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay[1] Adopted in 2021	RTgill-W1 cells from rainbow trout are exposed to 6 test concentrations of the test chemical in 24-well cell culture plates in serum-free medium. Three fluorescent indicator dyes are used simultaneously to measure cell viability based on metabolic activity, and membrane and lysosomal integrity. Cell viability is expressed as a percentage of cell viability in the control group. EC_{50} (half maximal effective concentration) values are estimated for each of the three endpoints, and the lowest EC_{50} value is used as an acute fish toxicity estimate.	OECD TG 249 predicts acute fish toxicity well overall;[2-4] its applicability domain includes narcotics, volatile and hydrophobic fragrances with a variety of different structures (e.g., alcohols, esters, aromatic, aliphatic; log K_{ow} at least up to 6.7),[3,5] pesticides/agrochemicals,[6,7] nanomaterials,[8] whole-effluent toxicity (WET) testing including metals,[9] and negatively charged compounds including cationic surfactants.[10-11] A broad spectrum of *in vitro* enzymatic activity in salmonid models, including RTgill-W1, has been demonstrated.[12]	OECD TG 249 may be accepted as an element in a weight-of-evidence approach for acute fish toxicity under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation. According to the European Union Biocidal Products Regulation (BPR), short-term testing on fish, OECD TG 203, is not required if "sufficient weight of evidence including the use of other data such as the Fish Embryo Acute Toxicity (FET, OECD TG 236) and/or results obtained from non-animal methods is available for this data requirement."[13]

Data from multiple sources can be considered together in integrated approaches:

- Applying the Threshold Approach for Acute Fish Toxicity set out in OECD Guidance Document (GD) 126 can substantially reduce the number of fish used to assess acute fish toxicity.[14] In this approach, an initial limit fish test is conducted at the threshold concentration (the lowest EC_{50} from algae [OECD TG 201] and daphnid acute testing [OECD TG 202]), and a full TG 203 test is triggered only if death is observed at this concentration. For toxicity assessment in the context of a persistent, bioaccumulative and toxic (PBT) assessment, an acute fish toxicity limit test may be performed to assess whether acute toxicity is <0.1 mg/L, i.e., screens as toxic.[15] If derivation of Predicted No-Effect Concentrations (PNECs) using fish is required under REACH, the threshold approach may be applied. The threshold approach is accepted under the BPR.[16-17]
  In a comparison of acute toxicity data on 165 chemicals, algae and daphnids were more sensitive than fish around 80% of the time.[18] According to an analysis of 805 chemicals in the EnviroTox database, the acute fish toxicity data requirement can be addressed by TGs 249 and 236, computational approaches, and available daphnid acute toxicity data and algae toxicity data.[19]
- A Defined Approach has been developed by Macmillan et al.[20] It integrates three *in silico* predictions from freely available (Q)SAR models along with OECD TG 249 data. The Defined Approach provides a categorical output aligned with the UN GHS Classification and Labelling framework (Acute Categories 1, 2, or 3, or Not Classified) with an overall accuracy of 80%. The Defined Approach is applicable to a wide range of chemical substances with diverse modes of action.
- An Integrated Testing Strategy using QSARs, as well as OECD TGs 249 and 236 has been proposed.[21] LC_{50} values were determined using a preference-dependent strategy, a sequential testing strategy, and a sensitivity-dependent strategy. All the strategies demonstrated a predictive power of at least 73%.

Note: OECD TG 236 on the fish embryo acute toxicity (FET) test uses live animals and is therefore considered a refinement. Some regulatory agencies may require reduction and refinement approaches, while complete replacement methods are undergoing development and validation.

+49 (0) 711-860-591-0
ThePSCI.eu - Info@thepsci.eu
PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

References

1. OECD. Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay. OECD Guidelines for the Testing of Chemicals. 2021. https://doi.org/10.1787/c66d5190-en
2. Fischer M, Belanger SE, Berckmans P, et al. Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. *Toxicol Sci*. 2019;169(2):353-364. https://doi.org/10.1093/toxsci/kfz057
3. Natsch A, Laue H, Haupt T, et al. Accurate prediction of acute fish toxicity of fragrance chemicals with the RTgill-W1 cell assay. *Environ Toxicol Chem*. 2018;37(3):931-941. https://doi.org/10.1002/etc.4027
4. Tanneberger K, Knöbel M, Busser FJ, et al. Predicting fish acute toxicity using a fish gill cell line-based toxicity assay. *Environ Sci Technol*. 2013;47(2):1110-9. https://doi.org/10.1021/es303505z
5. Laue H, Hostettler L, Sanders G, et al. PeBiToSens™: A platform for PBT screening of fragrance ingredients without animal testing. *Chimia (Aarau)*. 2020;74(3):168-175. https://doi.org/10.2533/chimia.2020.168
6. Emberley-Korkmaz S, Mittal K, Temlock N, et al. Cytotoxicity of 19 pesticides in rainbow trout gill, liver, and intestinal cell lines. *Environ Toxicol Chem*. 2025;44(9):2443-2454. https://doi.org/10.1002/etc.5808
7. Lange et al. Prediction of the in vivo acute toxicity of agrochemicals to fish with the in vitro RTgill-W1 cell line assay. 2024. *SETAC Europe 34th Annual Meeting*.
8. Kühnel D, Busch W, Meissner T, et al. Agglomeration of tungsten carbide nanoparticles in exposure medium does not prevent uptake and toxicity toward a rainbow trout gill cell line. *Aquat Toxicol*. 2009;93(2-3):91-99. https://doi.org/10.1016/j.aquatox.2009.04.003
9. Scott J, Belden JB, Minghetti M. Applications of the RTgill-W1 cell line for acute whole-effluent toxicity testing: in vitro-in vivo correlation and optimization of exposure conditions. *Environ Toxicol Chem*. 2021;40(4):1050-1061. https://doi.org/10.1002/etc.4947
10. Balk F, Hollender J, Schirmer K. Investigating the bioaccumulation potential of anionic organic compounds using a permanent rainbow trout liver cell line. *Environ Int*. 2023;174:107798. https://doi.org/10.1016/j.envint.2023.107798. Erratum in: *Environ Int*. 2024;186:108624. https://doi.org/10.1016/j.envint.2024.108624
11. Balk F, Hüsser B, Hollender J, et al. Bioconcentration assessment of three cationic surfactants in permanent fish cell lines. *Environ Sci Technol*. 2024;58(3):1452-1461. https://doi.org/10.1021/acs.est.3c05360
12. Martin BPM, Franco ME, Schirmer K. Comparative characterization of organ-specific phase I and II biotransformation enzyme kinetics in salmonid S9 sub-cellular fractions and cell lines. *Cell Biol Toxicol*. 2025;41(1):37. https://doi.org/10.1007/s10565-025-09992-8
13. European Union. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Annex II, 9.1.1 Short term testing on fish. https://eur-lex.europa.eu/eli/reg/2012/528/oj/eng
14. OECD. No. 126: Short Guidance on the Threshold Approach for Acute Fish Toxicity. OECD Guidelines for the Testing of Chemicals. 2010. https://doi.org/10.1787/3f8559d8-en.
15. European Chemicals Agency. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Section R.11.4.1.3.6 Use of non-testing data. 2023. https://www.echa.europa.eu/documents/10162/17224/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f
16. European Union. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Annex II, 9.1.1 short term testing on fish. 2012. https://eur-lex.europa.eu/eli/reg/2012/528/oj/eng
17. European Chemicals Agency. Guidance on the Biocidal Products Regulation. Volume IV. Part A: Information Requirements. 2022. https://echa.europa.eu/documents/10162/2324906/bpr_guidance_vol_iv_part_a_en.pdf/4a70aa9e-7491-7fc5-0734-6777ade10b02?t=1648525444087
18. Rawlings JM, Belanger SE, Connors KA, et al. Fish embryo tests and acute fish toxicity tests are interchangeable in the application of the threshold approach. *Environ Toxicol Chem*. 2019;38(3):671-681. https://doi.org/10.1002/etc.4351
19. Schür C, Paparella M, Faßbender C, et al. Daphnids can safeguard the use of alternative bioassays to the acute fish toxicity test: a focus on neurotoxicity. *Environ Toxicol Chem*. 2025;44(9):2635-2647. https://doi.org/10.1093/etojnl/vgaf014
20. Macmillan DS, Ambure P, Aranda V, et al. Addressing the challenges of acute fish toxicity hazard classification using a non-animal defined approach. *Environ Toxicol Chem*. 2025;44(9):2659-2672. https://doi.org/10.1093/etojnl/vgaf046
21. Lee YL, Chen ZY, Li TN, et al. A novel integrated testing strategy (ITS) for evaluating acute fish toxicity with new approach methodologies (NAMs). *Environ Int*. 2024;193:109112. https://doi.org/10.1016/j.envint.2024.109112

+49 (0) 711-860-591-0
ThePSCI.eu - Info@thepsci.eu
PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.