Developing an Integrated Approach to Testing and Assessment for Acute Fish Toxicity

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Introduction

One of the most frequently used aquatic toxicity tests is the **Acute Fish Toxicity Test** (OECD TG 203). Since lethality is the endpoint, this test represents a very significant animal-welfare concern.

As knowledge on the scientific limitations (e.g. limited reproducibility and relevance to the environment) of individual fish tests grows, methods that replace, reduce, and refine the use of fish in AFTs have become available:

- The **OECD QSAR Toolbox** and other sources propose numerous models to predict acute fish toxicity.
- The **RTgill-W1 fish cell line test to predict acute fish toxicity** (ISO/DIS 21115)[1,2] was included in the OECD TG work plan in 2019.
- The **Fish Embryo Acute Toxicity Test** using zebrafish (OECD TG 236) represents a refinement.
- Applying the **Threshold Approach for Acute Fish Toxicity** (OECD GD 126) can substantially reduce the number of fish used. An initial fish test is conducted at the threshold concentration (**TC**) derived from test responses in daphnids and algae, and continued testing is triggered only if mortality is observed at the TC.

Therefore, advanced **IATAs** for acute fish toxicity are of high scientific, societal, and environmental interest. IATAs structure and guide the combination of existing information, computational tools, mechanistic information from the molecular and cellular levels, information from *in vitro* assays and species of lower trophic levels, and refinement methods such as using fish at life stages when they are less susceptible to pain and suffering than in adulthood, e.g. fish embryos.

Through the OECD Test Guidelines Programme, the Austrian WNT delegation and the International Council on Animal Protection in OECD Programmes are working with leading experts and the OECD VMG-eco to develop such IATAs. In particular, this OECD project aims to enrich the Threshold Approach for Acute Fish Toxicity with new toxicological approaches.

This poster outlines one potential IATA for acute fish toxicity. The IATA also aims to estimate the lowest EC/LC_{50} from the three trophic levels, represented by algae, daphnids, and fish as a surrogate for all aquatic organisms, according to EU Regulation 1272/2008 on classification, labelling, and packaging.[3]

Proposal for an IATA for Acute Fish Toxicity

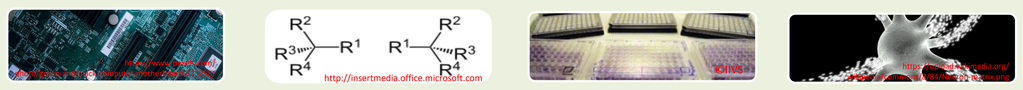
Collect existing reliable, relevant information; apply *in silico* approaches; and conduct weight-of-evidence analysis.

Each data element is characterised for its quality, relevance, and associated uncertainty. The TC is derived.

Internationally Standardised Information Sources

ISO/DIS 21115: RTgill-W1 fish cell line test to predict acute fish toxicity
OECD TG 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test
OECD TG 202: *Daphnia* sp. Acute Immobilisation Test
OECD TG 236: Fish Embryo Acute Toxicity Test

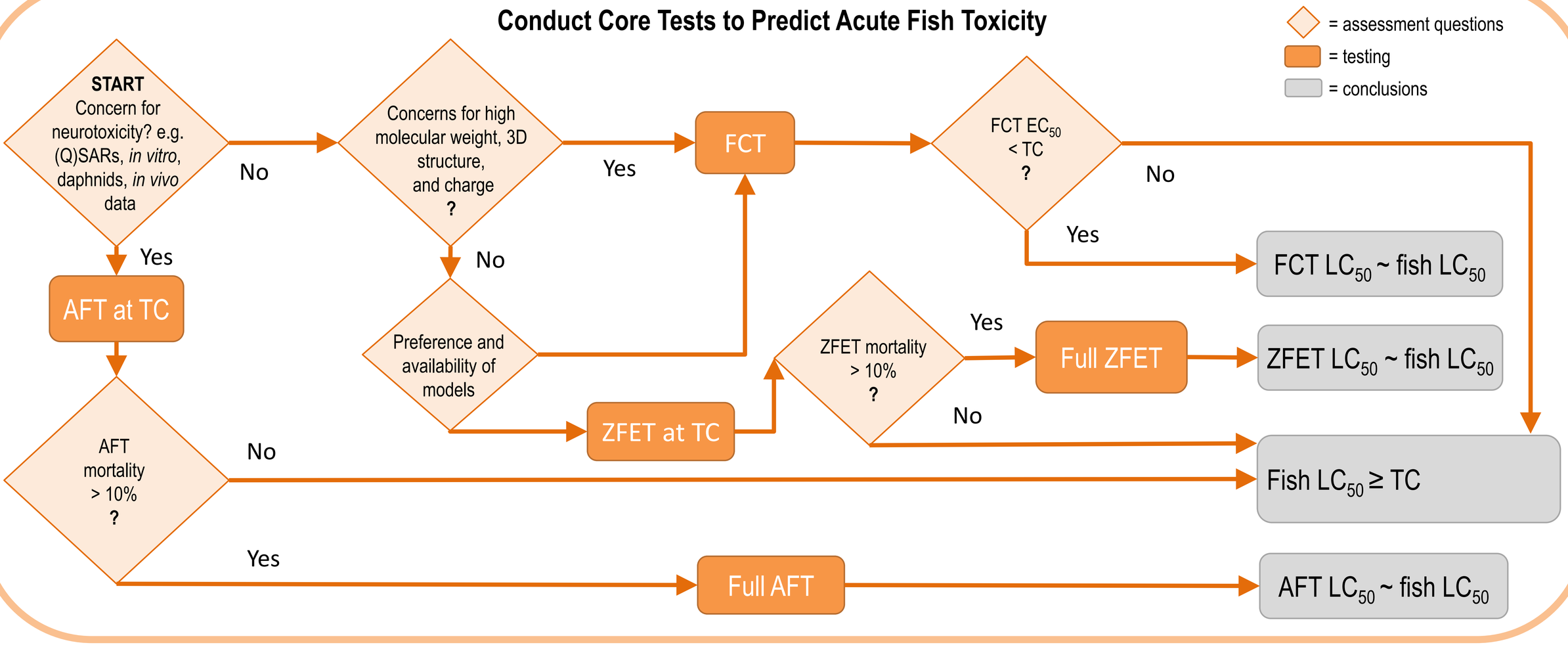
Not Internationally Standardised Information Sources



In silico data, e.g. (Q)SARs, grouping/read-across, bridging
Physical-chemical information, e.g. on molecular weight, bulkiness, charge
Additional *in vitro* and *in vivo* data relevant to acute fish toxicity, e.g. from high-throughput screening and other taxa

WoE inconclusive | **WoE conclusive** → **No further testing**

Conduct Core Tests to Predict Acute Fish Toxicity



Outlook

- Refinement and review by OECD VMG-eco, OECD Fish Drafting Group, and OECD WNT
- Update of OECD GD 126 to include the IATA(s)

The views, conclusions, and recommendations presented here are those of the authors and do not necessarily represent the policies or positions of the organisations to which the authors are affiliated.

Abbreviations and References

AFT	Acute fish toxicity test	(Q)SAR	(Quantitative) structure-activity relationship
EC_{50}	Median effective concentration	TC	Lowest EC_{50} value of algae or acute invertebrate (e.g. daphnid) toxicity data
FCT	RTgill-W1 fish cell line test	TG	Test guideline
GD	Guidance document	VMG-eco	Validation Management Group for Ecotoxicity Testing
IATA	Integrated approach to testing and assessment	WNT	Working Group of National Co-ordinators of the Test Guidelines Programme
LC_{50}	Median lethal concentration	WoE	Weight of evidence
		ZFET	Fish Embryo Acute Toxicity Test

References

[1]Fischer M, *et al*. Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. *Toxicol Sci*. 2019;1-12.
[2]ISO 21115:2019: Water quality – determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1). https://www.iso.org/obp/ui/#iso:std:iso:21115:ed-1:v1:en.
[3]Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.