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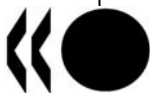
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**Series on Testing and Assessment
No. 126**

SHORT GUIDANCE ON THE THRESHOLD APPROACH FOR ACUTE FISH TOXICITY

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TOXICITY**

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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Environment Directorate
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FOREWORD

This document presents a short guidance on the Threshold Approach for Acute Fish Toxicity.

The project proposal was submitted by the European Commission to the OECD in 2006. The draft guidance document was circulated to the Working Group of the National Coordinators of the Test Guidelines Programme several times for comments in 2008 and 2009. The document was finally approved by the Working Group of the National Coordinators of the Test Guidelines Programme at its meeting held on 23-25 March 2010. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 5 May 2010.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

THE THRESHOLD APPROACH FOR ACUTE FISH TOXICITY TESTING

General considerations

1. In the interest of animal welfare and efficient use of resources, it is important to avoid the unnecessary use of animals whenever possible. In the field of aquatic toxicology, this especially applies to the acute toxicity testing of fish according to OECD TG 203. The threshold approach described hereafter addresses fish toxicity by initially using a single-concentration test (limit test) requiring less fish compared to the full acute fish toxicity study. The selection of a single concentration is based on the derivation of a *threshold concentration (TC)* from reliable algae and acute invertebrate (e.g. daphnia) toxicity data. Fish toxicity is then tested at the TC to consider if fish are more or less sensitive than groups/species for which an E/LC₅₀ is available. If no mortality occurs in the limit test using the TC, the TC might be used as a surrogate of the LC₅₀ value in the further hazard or risk assessment.

2. The threshold approach proposes good practice for *in vivo* testing for acute fish toxicity. The threshold approach is based on the observation that fish is not always the most sensitive test species (1, 2). The concept initially described for pharmaceuticals (2) was further developed for chemical substances at the European Commission's Joint Research Centre (3) taking into consideration the requirements of the limit test in OECD TG 203 (4, 5)¹. In addition, several publications confirm the potential of the threshold approach in reducing the number of fish for acute toxicity testing (6, 7), also when applied to other substances than chemicals.

3. The threshold approach provides a concentration-response relationship allowing a precise LC₅₀ derivation if fish is the most sensitive group of test organism after short-term exposure (see 7).

Description of the threshold approach

4. When acute fish toxicity data need to be generated, this guidance document recommends that the threshold approach should be applied whenever possible. The whole approach might include the performance of tests in a step-wise manner according to the following OECD Guidelines:

- TG 201 – Freshwater Alga and Cyanobacteria, Growth Inhibition Test
- TG 202 – Daphnia sp. Acute Immobilisation Test
- TG 203 – Fish, Acute Toxicity Test (Limit test, paragraph 20)

¹ Incorporated into the “Guidance on information requirements and chemical safety assessment” for REACH. See: ECHA (2008). Guidance on information requirements and chemical safety assessment. Chapter R.7B – Endpoint specific guidance (p. 41 ff, Chapter 7.8)

- TG 203 – Fish, Acute Toxicity Test.

5. It is recommended that the following step-wise procedure should be utilized (Figure):

6. Derivation of the threshold concentration (Step 1 - 3): The lowest EC₅₀ value of existing and reliable algae or acute invertebrate (e.g. daphnia) toxicity data is set as threshold concentration (TC). If these data are not available they need to be determined according to OECD TG 201 and OECD TG 202 or any other standard test method generating reliable data.

7. Assessment of acute fish toxicity (limit test) at the TC (Step 4): An acute fish test is performed according to the limit test (OECD TG 203, paragraph 20) at the TC. If the TC is >100 mg/l, the test substance concentration should be 100 mg/l in the limit test. The absence of mortality at a TC<100mg/L indicates that the fish is not the most sensitive group of test organism after short-term exposure and that, with at least 99% of confidence, the LC₅₀ is greater than the TC. If sublethal effects are observed, these should be recorded. The test should be terminated when one or more fish from the test group die, since this finding requires a full study (step 5). In compliance with the OECD Guidance Document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (8), the remaining fish should be humanely killed.

8. Performance of a full OECD TG 203 (Step 5): If mortality is observed in the limit test using the TC, a full OECD TG 203 study should be conducted.

FIGURE

The Threshold Approach for Acute Fish Toxicity Testing

<u>Activity</u>	<u>Finding</u>	<u>Conclusion</u>
1 Evaluation of existing EC ₅₀ values from algae and invertebrates (e.g. daphnids) tests	Reliable EC ₅₀ values from both algae and invertebrates (e.g. daphnids) are available	proceed to step 3
↓ <i>Reliable EC₅₀ values from algae and/or invertebrates (e.g. daphnids) tests are not available</i> ↓		
2 Generate the missing EC ₅₀ value(s) e.g. according to OECD TG 201 and OECD TG 202	EC ₅₀ algae and invertebrates available	proceed to step 3
↓		
3 Derivation of threshold concentration (TC) using lowest EC ₅₀ of invertebrates / algae tests	lowest EC ₅₀ = TC	proceed to step 4
↓		
4 Limit test according to OECD TG 203, paragraph 20, at TC or 100mg/L (whichever is lowest)	no mortality sublethal effects One or more mortality(ies) in test group	LC ₅₀ > TC or 100 mg/L; no further testing observation recorded terminate test and proceed to step 5; humanely kill remaining fish
↓		
5 Performance of full study according to OECD TG 203	dose-response curve	LC ₅₀ fish

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