OECD DRAFT GUIDANCE DOCUMENT

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. If no mortality occurs in the limit test using the TC, the TC might be used as a surrogate LC_{50} value in the further hazard or risk assessment.

2. The threshold approach proposes both best practice and an ethical benchmark for *in vivo* testing for acute fish toxicity. It 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 is not applicable where a concentration-response relationship and an LC50 derivation are legally required.

DESCRIPTION OF THE THRESHOLD APPROACH

4. When acute fish toxicity data need to be generated, this guidance document recommends that the threshold approach 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)
   - TG 203 – Fish, Acute Toxicity Test.

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

[1] Incorporated into the "Guidance on information requirements and chemcial 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)

5. 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.

6. 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 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 threshold concentration. If sublethal effects are observed, these should be recorded. The test should be terminated when 1 fish of the test group dies or is moribund, 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.

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

Figure

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	Activity	Finding	Conclusion
1	Evaluation of existing LC_{50}/EC_{50} values from algae and invertebrates (e.g. daphnids) tests	Both LC_{50}/EC_{50} values from algae/invertebrates (e.g. daphnids) tests are available and relevant	proceed to step 3
	↓ *Relevant LC_{50}/EC_{50} values from algae and/or invertebrates (e.g. daphnids) tests are not available*		
2	Generate the missing LC_{50}/EC_{50} value(s) e.g. according to OECD TG 201 and OECD TG 202	EC_{50} algae / LC_{50} invertebrates available	proceed to step 3
	↓		
3	Derivation of threshold concentration (TC) using lowest LC_{50}/EC_{50} of invertebrates / algae tests	lowest LC_{50}/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 fish dies 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 fish toxicity

LITERATURE

(1) Weyers, A., Sokull-Klüttgen, B., Baraibar-Fentanes, J., Vollmer, G., 2000. Acute toxicity data: a comprehensive comparison of results of fish, *Daphnia* and algae tests with new substances notified in the EU. Environ. Toxicol. Chem. 19, 1931-1933.

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(3) Jeram, S., Riego Sintes, J.M., Halder, M., Baraibar Fentanes, J., Sokull-Klüttgen, B., Hutchinson, T.H. (2005) A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the European Union. Regulatory Toxicology and Pharmacology 42, 218-224.

(4) ECVAM (2006). Statement of the ECVAM Scientific Advisory Committee on the Scientific validity of the Upper Threshold Concentration (UTC) step-down approach for acute Aquatic Toxicity testing. ECVAM website: http://ecvam.jrc.it/index.htm (Validated Methods) and ATLA 35, 199-208, 2006.

(5) Hoeger et al (2006). Reduction of animal use in acute aquatic toxicity testing: Further development of the threshold approach and its application to existing chemicals and plant protection products. Poster presentation at SETAC Europe 16th Annual Meeting 7-11 May 2006, abstract no MO1/AM/P05.

(6) Hoekzema, C.C., Murk A.J., van de Waart, B.J., van der Hoeven, J.C.M, de Roode D.F. (2006). Alternative approaches can greatly reduce the number of fish used for acute toxicity testing. Environ. Toxicol. Chem., 25, 1322–1325.

(7) Sewell (2008). Reduction in the numbers of fish used in aquatic acute toxicity testing. Poster presentation at SETAC Europe 18th Annual Meeting 25-29 May 2008, abstract no MO 253.

(8) OECD, 2008. Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints. OECD Series on Testing and Assessment, No.19. Organisation for Economic Cooperation and Development. Paris, 39 pp.