

# OECD GUIDELINE FOR TESTING OF CHEMICALS

## DRAFT REVISED GUIDELINE 203:

### Fish, Acute Toxicity Test

#### **INTRODUCTION**

1. OECD Guidelines for Testing of Chemicals are periodically reviewed to ensure that they reflect the best available science. In the revision of this Guideline (originally adopted in 1981, updated in 1984 and 1992), special attention was given to possible improvements in relation to animal welfare concerns in order to avoid unnecessary testing in laboratory animals.
2. According to this updated version of Guideline 203, prior to undertaking the described fish acute toxicity test it should be evaluated whether the Threshold Approach provided as Annex 2 to this guideline is applicable. The Threshold Approach takes into consideration EC<sub>50</sub> values from relevant algae and acute invertebrate (e.g. daphnia) tests.

(...)

#### **LIMIT TEST**

20. Using the procedures described in this Guideline, a limit test may be performed at 100 mg(active ingredient)/l or at the Threshold Concentration (derived as described in Annex 2 to this guideline) in order to demonstrate that the LC<sub>50</sub> is greater than this concentration. The limit test should be performed using a minimum of 7 fish, with the same number in the control(s). (Binomial theory dictates that when 10 fish are used with zero mortality, there is a 99.9 % confidence that the LC<sub>50</sub> is greater than 100 mg/l. With 7, 8 or 9 fish, the absence of mortality provides at least 99% confidence that the LC<sub>50</sub> is greater than the concentration used in the limit test.) If any mortalities occur, a full study should be conducted. If sublethal effects are observed, these should be recorded.

## ANNEX 2

### The Threshold Approach for Acute Fish Toxicity Testing

#### GENERAL CONSIDERATIONS

1. It should be carefully considered whether the respective legislation requires the description of the dose-response-relationship and the derivation of a LC<sub>50</sub>. In this case, the threshold approach is not applicable.
2. The threshold approach represents 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, 4) taking into consideration existing OECD guidelines<sup>1</sup> (5). 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. 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 203. The threshold approach described hereafter addresses fish toxicity by using a single-concentration test (limit test) requiring less fish compared to the full acute fish toxicity study. It is based on the derivation of a *threshold concentration (TC)* from relevant algae and acute invertebrate (e.g. daphnia) tests at which fish toxicity is tested. If no mortality occurs in the limit test using the TC, the TC might be used as a surrogate LC<sub>50</sub> value in the further hazard or risk assessment.

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<sup>1</sup> 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)

## **DESCRIPTION OF THE THRESHOLD APPROACH**

1. When acute fish toxicity data need to be generated the threshold approach should be applied whenever possible. It might include the performance of tests 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.

The following procedure should be utilized (see Figure):

2. Derivation of the threshold concentration (Step 1 - 3): The lowest EC50 value of existing relevant algae or acute invertebrate (e.g. daphnia) tests is set as threshold concentration (TC). If these data are not available they can be determined according to TG201 and TG202.
3. Assessment of acute fish toxicity (limit test) at the TC (Step 4): An acute fish test is performed according to the limit test (paragraph 20) at the TC. If the TC is >100 mg/l, the test substance concentration should be 100mg/l in the limit test. The absence of mortality indicates that the fish is not the most sensitive test species 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, 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 (ENV/JM/MONO(2000)7), the remaining fish should be humanely killed.
4. Performance of a full OECD 203 (Step 5): If any mortality occurs, a full study should be conducted.

## **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.
- (2) Hutchinson, T.H., Barrett, S., Buzby, M., Constable, D., Hartmann, A., Hayes, E., Huggett, D., Länge, R., Lillicrap, A.D., Straub, J.O., Thompson, R.S., 2003. A strategy to reduce the numbers of fish used in acute ecotoxicity testing of pharmaceuticals. *Environ. Toxicol. Chem.* 22, 3031-3036.
- (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) 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.
- (5) 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.
- (6) 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.

**Figure**

**The Threshold Approach for Acute Fish Toxicity Testing**

