DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment Category 4: Other approached

Toolbox component: Lab toxicity studies

Fact Sheet 4.3: Zebrafish embryo acute toxicity test (FET)

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What is it?

Ecotoxicological test to determine acute toxicity of chemicals on fish embryos, using zebrafish as test species (*Danio rerio*). The test has been applied to various substances and is described in OECD guideline 236 (2013) "Guideline for the Testing of Chemicals Test No. 236: Fish Embryo Acute Toxicity (FET) Test". The test was developed in order to reduce the numbers of animal testing for risk assessment (Braunbeck et al. 2005).

What does it tell you?

The FET is used to determine the acute toxicity of chemicals on newly fertilized zebrafish eggs over 96 hours. By observation of lethal as well as sublethal endpoints after every 24 hours a so called median lethal concentration (LC_{50}) and median effective concentration (EC_{50}) can be calculated.

Type of Indicator (tick box)

☐ non-specific stress indicator
☒ specific for groups of contaminants incl. CWA or explosives
☐ CWA-specific indicator
☒ specific for substances related to explosives (e.g. TNT)

How to measure it?

Newly fertilized zebrafish eggs are used for the test. In general, every soluble substance can be tested. The procedure of the test is defined in OECD guideline 236 (2013). Prior to the test start, a solution of the test medium is prepared according to OECD 203, Annex 2 (1992). The solution is aerated overnight, until oxygen saturation.

Test species: Newly fertilized zebrafish eggs.

Equipment: Incubator (26° C) with photoperiodical illumination, stereomicroscope (> 25 x), glass or plastic jar with mesh (< 1000 µm) for egg collection, pipettes, glass beakers or 96-well plates, parafilm.

Additional notes:

Depending on the stability of the chemical, a semi-static exposure, with renewal of the test solution, should be considered.

If plastic well-plates are used for incubation, the plastic surface should be saturated with test solution 12 hours prior to the test star. Alternatively, glass vessels can be used for incubation.

If necessary, the exposure time of embryos can be prolonged up to 120 hours after fertilization as they are not subject to animal testing until then (Strähle et al. 2012).

How to analyse and assess the data?

The observed cumulative percentage of mortality and effects is plotted against the logarithmic exposure concentrations. A nonlinear regression is then fitted to the data to derive threshold concentrations such as LC_{50}. Detailed information on how to apply the appropriate statistical method to the test data is given in OECD Document No. 54 (2014).

The test report should include all relevant data of the tested chemical, tested organism, test conditions and procedures.

References

Braunbeck T, Böttcher M, Hollert H, et al (2005) Towards an alternative for the acute fish LC(50) test in chemical assessment: the fish embryo toxicity test goes multi-species - an update. ALTEX 22:87–102

OECD (2013) Guideline for the Testing of Chemicals Test No. 236: Fish Embryo Acute Toxicity (FET) Test. Organ Econ Co-operation Dev Paris

OECD (1992) Guideline for the Testing of Chemicals Test No. 203. Fish, Acute Toxicity Test. Organ Econ Co-operation Dev Paris

OECD (2014) Current Approaches in the Statistical Analysis of Ecotoxicity Data

Strähle U, Scholz S, Geisler R, et al (2012) Zebrafish embryos as an alternative to animal experiments - a commentary on the definition of the onset of protected life stages in animal welfare regulations. Reprod Toxicol 33:128–132