Fish embryo test for acute toxicity testing of chemicals

Commonly used acronym: *FET-test, OECD TG 236*

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Organisation

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Department Health
Country Belgium
Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Environment
The Method is situated in	Regulatory use - Routine production
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

Fish embryo
Fertilised zebrafish eggs
Somite formation
Fertilisation rate
Reproducibility
Lethality
Coagulation
LC50Fish embryo
Fertilised zebrafish eggs
LC50

Scientific area keywords

Chemical testing
Ecotoxicity Acute toxicity
In vitro toxicologyChemical testing
EcotoxicityAcute toxicity
in vitro toxicology

Method description

The method has been fully described in OECD TG 236 and is intended to determine the acute or lethal toxicity of chemicals on embryonic stages of fish (*Danio rerio*). Newly fertilized zebrafish eggs are exposed to the test chemical for a period of 96 hrs. Every 24 hrs. Twenty embryos (one embryo per well) are exposed to the chemical tested at each concentration level. The test includes five increasing concentrations of the chemical tested and a control. Every 24 hours, four apical observations are recorded as indicators of lethality: (i) coagulation of fertilised eggs, (ii) lack of somite formation, (iii) lack of detachment of the tail-bud from the yolk sac, and (iv) lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC50 is calculated. The test report also includes a number of other important information elements related to the conduct of the test, in particular: the concentration of dissolved oxygen, pH, total hardness, temperature and conductivity of solutions, measured concentrations of the chemical tested, and whether the validity criteria of the test were met.

Lab equipment

- Fish tanks made of chemically inert material and of a suitable capacity;
- Inverted microscope or binocular with a capacity of at least 80-fold magnification;
- Test chambers (glass or polystyrene);
- Incubator or air-conditioned room with controlled temperature;
- Spawn trap.

Method status

Published in peer reviewed journal
Validated by an external party (e.g. OECD, EURL ECVAM,…)

PROS, CONS & FUTURE POTENTIAL

Advantages

- Fast and cost-effective;
- The FET-test has been successfully applied to a wide range of substances exhibiting diverse modes of action, solubility, volatility, and hydrophobicity.

Challenges

- If the method is used for the testing of a mixture, its composition should, as far as possible, be characterized. Before use for regulatory testing of a mixture, it should be considered whether it will provide acceptable results for the intended regulatory purpose.
- The metabolic capabilities of fish embryos are not always similar to that of juvenile or adult fish. For instance, the protoxicant allylalcohol has been missed in the FET.
- For substances with a molecular weight ? 3kDa, a very bulky molecular structure, and substances causing delayed hatch which might preclude or reduce the post-hatch exposure, embryos are not expected to be sensitive because of limited bioavailability of the substance, and other toxicity tests might be more appropriate.
- The use of solvents should be avoided, but may be required in some cases in order to produce a suitably concentrated stock solution. Where a solvent is used to assist in stock solution preparation, its final concentration should not exceed 100 µl/L and should be the same in all test vessels. When a solvent is used, an additional solvent control is required.

For more information see OECD TG nr. 236

Future & Other applications

The test can also be used for the selection and ranking of candidate chemicals during the development of new chemicals and products and in toxicology research.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Busquet F, Strecker R, Rawlings JM, Belanger SE, Braunbeck T, Carr GJ, Cenijn P, Fochtman P, Gourmelon A, Hübler N, Kleensang A, Knöbel M, Kussatz C, Legler J, Lillicrap A, Martínez-Jerónimo F, Polleichtner C, Rzodeczko H, Salinas E, Schneider KE, Scholz S, van den Brandhof EJ, van der Ven LT, Walter-Rohde S, Weigt S, Witters H, Halder M. (2014). OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Regul Toxicol Pharmacol. 69 (3), 496-511

Associated documents

Busquet Witters et al 2014_Regul Tox Pharmacol.pdf
OECD Test No. 236.pdf

Links

OECD guideline n°236

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