Zebrafish Embryo Developmental Toxicity Assay

Commonly used acronym: *ZEDTA*

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Organisation

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Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production, Translational - Applied Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Zebrafish

Type of cells/tissues/organs	Embryo

DESCRIPTION

Method keywords

development

toxicity

teratogen

screening

regulatory toxicology

malformations

drug development

chemicals

automated analysis

zebrafish

embryo

Scientific area keywords

toxicity testing

drug screening

drug development

preclinical

teratogenicity

Method description

In view of safety of pregnant women, a promising *in vitro* zebrafish embryo developmental toxicity assay has been developed to test pharmaceutical and chemical compounds for their teratogenic potential. The protocol deals with exposing zebrafish embryos to a range of compound concentrations at 28°C throughout organogenesis, i.e. from the gastrulation stage (5.25 hours post-fertilization [hpf]) up to 120 hpf. Morphological development is monitored at 5, 12, 24, 48, 72, 96 and 120 hpf. Larvae are evaluated for lethality in order to identify an LC25 (the compound concentration in which 25% lethality is observed) and morphological anomalies using a numerical scoring system to identify the NOAEL (no observed adverse effect level). These values are used to calculate the teratogenic index (LC25/NOAEL ratio) of each compound. If the teratogenic index is equal to or greater than 10 then the compound is classified as a teratogen, and if the ratio is less than 10 then the compound is classified as a non-teratogen. Currently the assay is optimized by including several skeletal endpoints after skeletal staining at 120 hpf and exogenous metabolic activation systems are developed to encompass the limited biotransformation capacity of the zebrafish embryos. Automation of the morphological scoring is also explored.

Lab equipment

Stereomicroscope ;

Aquaria ;

Incubator.

Method status

Still in development

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Fast results ;

Medium-throughput ;

Cost effective ;

Limited compound requirements ;

Longitudinal follow-up.

Challenges

Compound uptake (internal concentrations) ;

Limited biotransformation ;

Less morphological endpoints compared to the mammalian *in vivo* tests.

Modifications

Skeletal staining methods and exogenous metabolic activation systems are currently developed to increase the sensitivity of the assay. The main focus is to reduce the number of false negative results.

Future & Other applications

The main goal is to optimize and use the assay for (regulatory) developmental toxicity testing, but the assay could potentially also be used for chronic toxicity testing in the future.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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