

# Zebrafish Embryo Developmental Toxicity Assay

*Commonly used acronym: ZEDTA*

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Regulatory use - Routine production, Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Animal derived cells / tissues / organs
<b>Species from which cells/tissues/organs are derived</b>	Zebrafish
<b>Type of cells/tissues/organs</b>	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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Pype, C., et al., Antioxidants reduce reactive oxygen species but not embryotoxicity in the metabolic *Danio rerio* test (mDarT). *Reprod Toxicol*, 2017. 72: p. 62-73

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Saad, M., et al., *In vitro* CYP-mediated drug metabolism in the zebrafish (embryo) using human reference compounds. *Toxicol In Vitro*, 2017. 42: p. 329-336

Verbueken, E., et al., *In Vitro* Biotransformation of Two Human CYP3A Probe Substrates and Their Inhibition during Early Zebrafish Development. *Int J Mol Sci*, 2017. 18(1)

Verbueken, E., et al., From mRNA Expression of Drug Disposition Genes to *In Vivo* Assessment of CYP-Mediated Biotransformation during Zebrafish Embryonic and Larval Development. *Int J Mol Sci*, 2018. 19(12)

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Saad, M., et al., *In vitro* CYP-mediated drug metabolism in the zebrafish (embryo) using human reference compounds. *Toxicol In Vitro*, 2017. 42: p. 329-336

## Associated documents

## Links

[PhD thesis Moayad Saad](#)

[PhD thesis Casper Pype](#)

## PARTNERS AND COLLABORATIONS

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