TERATOTOX ASSAY

Biobide is a biotechnology company offering drug discovery services to Pharma, Biotech, Chemical, Cosmetic and Nutraceutical companies. Our services are based on the **zebrafish model** and the capacity to offer highly efficient tailor made assays.

In order to understand the potential of a drug to induce birth defects in offspring, teratogenic studies must be performed. Reproductive toxicity is a major concern and different guidelines, such as the ICH S5(R2), state the need to assess chemicals safety during the Drug Discovery and Development Process or before drug commercialization.

The zebrafish embryo is an emerging model due to its inherent properties (ease of manipulation, external fertilization and transparency) together with the need to apply the 3Rs. This model has a high genetic homology with humans (over 85%) as well as important parallels in organogenesis and functional mechanisms.

The assays in zebrafish have the benefit of being rapid and cost-effective and results are highly transferable to other vertebrates and humans.

The assay is performed under Good Laboratory Practice (GLP) environment.

METHOD DESCRIPTION

Experimental model: zebrafish (Dario rerio) embryos strain expressing a green fluorescent protein in the heart obtained from crossing adult zebrafish are used under strict environmental conditions of temperature, humidity and photoperiod.

Methodology: embryos at 2-4 hpf (hours post fertilization) will be placed in 24 well plates (5 per well) and treated with the test item. 5 concentrations will be assayed per compound and 10 embryos will be treated per experimental condition. A control group of vehicle treated embryos will also be included. Plates will be incubated at 26-28.5 °C and embryos will be analyzed at 2 and 4 dpf (days post fertilization) under the stereoscope. The minimum concentration at which lethality is induced is calculated.
Different organs and processes are analyzed under a dissecting stereoscope, including teratogenic and toxic endpoints (Table 1).
EC50, LC50 , Teratogenic Index (TI) (ratio between LC50 and EC50) and NOAEL (Non observable adverse event level) values are calculated.

Further analysis can be performed, such as: bioavailability by HPLC MS/MS to investigate further false negative results, histopathology, gene expression, etc.

Table 1. List of endpoints of teratogenicity

		2dpf	4dpf
Malformation of the head	Jaw morphology		X
	Microcephaly or abnormal head shape	X	X
	Microphthalmia/Cyclopia	X	X
	Edema	X	X
Malformation of the otoliths			X
Malformation of the heart	Edema/irregular shape	X	X
	Abnormal heartbeat	X	X
Deformed body shape	Length	X	X
	Curved/curled	X	X
	Notochord morphology	X	X
	Somite morphology	X	X
Malformation of the tail (including tail fins)		X	X
Yolk deformation	Edema	X	X
	Yolk opacity	X	X
Other		X	X

VALIDATION RESULTS

Drugs with different human therapeutic indications have been tested (Table 2) at 1-100 µM for the validation study:

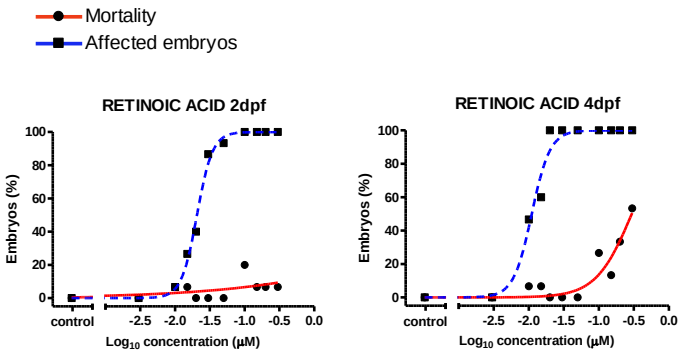
Table 2. Pharmaceuticals and Chemicals tested

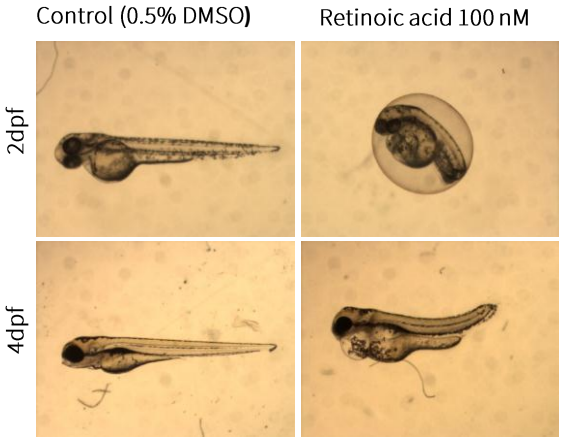
Reference products	Classification	BIOBIDE Classification
Aflatoxin A	Teratogenic in rodents	POSITIVE
Acetaminofen	Moderate risk teratogen	NEGATIVE
Dexamethasone	Teratogenic	POSITIVE
Tetracycline	Teratogenic	POSITIVE
Penicillin G	Non Teratogenic	NEGATIVE
Warfarin	Teratogenic	POSITIVE
Chlorambucil	Teratogenic	POSITIVE
5-Fluorouracil	Teratogenic	NEGATIVE
Thalidomide	Teratogenic	NEGATIVE
Hydroxyurea	Teratogenic	NEGATIVE
Amiodarone	Teratogenic	POSITIVE
Sotalol	Non Teratogenic	NEGATIVE
Acebutolol	Non Teratogenic	NEGATIVE
Carbamazepine	Teratogenic	POSITIVE
Valproic Acid	Teratogenic	POSITIVE
Pilocarpine	Non Teratogenic	NEGATIVE
Tacrine	Teratogenic	POSITIVE
Testosterone	Teratogenic	POSITIVE
Norepinephrine	Teratogenic	POSITIVE
Hydrocortisone	Teratogenic	POSITIVE
Ascorbic acid	Non Teratogenic	NEGATIVE
Retinol	Teratogenic	POSITIVE
N-Acetyl-Cysteine	Non Teratogenic	NEGATIVE
Sucrose	Non Teratogenic	NEGATIVE
Retinoic Acid	Teraratogenic	POSITIVE

Reference products	Classification	BIOBIDE Classification
Difenoconazole	Non teratogenic in animal experiments	NEGATIVE
Epoxiconazole	Teratogenic	POSITIVE
Flusiloazole	Teratogenic	POSITIVE
Cyclopamine	Teratogenic in animal experiments	POSITIVE
Myclobutanil	Teratogenic	POSITIVE
Metconazole	Teratogenic	POSITIVE
Propiconazole	Developmental toxicity in rats	POSITIVE
Ipconazole	Developmental toxicity in rat and rabbit	POSITIVE
Penconazole	Not development toxicity in rat and rabbit	NEGATIVE
Diniconazole	Development toxicity in rats and not in rabbits	POSITIVE
Voriconazole	Teratogenic in rats (not in rabbits)	POSITIVE
Glycolic Acid	Teratogenic	NEGATIVE
Camphor	Non teratogenic	NEGATIVE
Dimethyl phthalate	Non Teratogenic	POSITIVE
Levothyroxine	Non Teratogenic	POSITIVE
Metoclopramide	Non teratogenic	NEGATIVE
Saccharin	Non teratogenic	NEGATIVE
Tetrabromobisphenol A	Non teratogenic	NEGATIVE
Caffeine	Teratogenic	POSITIVE
Ramelton	Teratogenic	POSITIVE

POSITIVE
NEGATIVE
FALSE NEGATIVE
FALSE POSITIVE

Specificity: 86,2%
Sensitivity: 87,5%





- ✓ Biobide's Teratotox assay in zebrafish is a valid tool to evaluate teratogenic properties of potential drugs at an early preclinical phase in a cost-effective manner, as shown in the validation data.
- ✓ The level of teratogenic properties was concentration and drug dependent and a high reproducibility of the results was observed