Zebrafish Embryo Assay for Developmental Toxicity - Summary

**Developmental toxicity**

Potential developmental toxicity (including embryotoxicity and teratogenicity) of a test compound is assessed by examining its effects on the early development or early life stages of zebrafish embryos, which are evaluated for viability and abnormal morphological development.

**Objective & Application**

The zebrafish embryo model is frequently used for studying developmental biology, for the assessment of the effects of environmental pollutants on fish and to elucidate mechanisms of developmental toxicity (Henry *et al.*, 1997; Augustine-Rauch *et al.*, 2010; Brannen *et al.*, 2010; Eimon and Rubinstein, 2009).

The zebrafish (*Danio rerio*) embryo assay is generally used for screening purposes for developmental toxic potential (including embryotoxicity and teratogenicity) of chemicals (Augustine-Rauch *et al.*, 2010; Selderslags *et al.*, 2009).

Alternative test methods for reproductive toxicity are intended to complement existing *in vivo* assays and to reduce or replace experimentation on mammals in future (Brannen *et al.*, 2010; Schenk *et al.*, 2010; Selderslags *et al.*, 2009).

Currently, chemicals are tested for toxic effects on prenatal developmental toxicity with *in vivo* studies according to OECD Test Guidelines (414, 415, 416, 421, 422 and 426; OECD, 2001, 1983, 2001, 1995, 1996 and 2007), EU test methods (B.34, B.35; EC, 2008) and segment 2 studies (teratogenicity and embryotoxicity) according to the ICH guidelines (ICH, 2005).

**Basis of the Method**

Existing scientific literature in the field of aquatic toxicology has shown the successful use of fish systems to study reproductive and developmental toxicity. Since all vertebrates follow a common, basic plan of development, fish embryos offer a simple vertebrate system where the embryo can be manipulated and monitored throughout the entire developmental period. New genetic tools and endpoints have been applied to the model system and increased the knowledge at the molecular level (Augustine-Rauch *et al.*, 2010; Brannen *et al.*, 2010; Hill *et al.*, 2005; den Hertog, 2005).

Zebrafish has gained interest as a model for developmental toxicity studies due to the ease of its breeding, the fact that eggs are fertilised externally, the ease of culture, the rapid development of the embryo-larva and its transparency for several days. Therefore, the morphological development can be studied easily (Augustine-Rauch *et al.*, 2010; Selderslags *et al.*, 2009; Yang *et al.*, 2009). The entire organogenesis can be observed in culture within 3 days after fertilisation (Augustine-Rauch *et al.*, 2010). Moreover, zebrafish embryos are able to absorb compounds through the water (Rubinstein, 2006).

Herrmann of the University of Köln has originally developed an assay using zebrafish embryos. In this assay, retardation of development and malformations have been used as quantitative parameters for the assessment of the effectiveness of each agent (Herrmann 1993 and 1995).

Several laboratories have furthermore proposed to assess embryolethality and malformations on early life stages of Zebrafish (96 h or 7-day exposure periods) in order to predict human toxicity (Selderslags *et al.*, 2009). Several new molecular endpoints have been developed using different techniques (Huuskonen, 2005).

In the meantime, a number of zebrafish developmental toxicity assays have been described, and complete test systems are commercially available (Augustine-Rauch *et al.*, 2010). These tests share the following principle: early stage zebrafish embryos are placed in culture plates with the test compound and culture media, these plates are then incubated for a defined time period and are assessed for viability and morphological development afterwards (Augustine-Rauch *et al.*, 2010; Brannen *et al.*, 2010; Fraysse *et al.*, 2006; Yang *et al.*, 2009).
Experimental Description

Biological and Endpoint Measurement:

According to the protocols used the following endpoints may be assessed:

VIABILITY: survival number; viability assessment of the zebrafish embryos can be conducted based on the absence of heartbeat, body degradation, severe growth retardation and/or dysmorphology

DEVELOPMENTAL STAGE: evaluations are conducted largely similar to foetal examinations in other developmental toxicity studies. Functional observations can be recorded as normal or abnormal only or numerical scores (0-5 (normal)) can be assigned

GENE EXPRESSION: mRNA, protein expression profiles

MORPHOLOGY: examination of abnormalities; morphological scoring is based on larval length, locomotion, cardiovascular function etc

Endpoint Value:

EC50: effect concentration; concentration or dose that produces an alteration of an examined effect to 50% compared to the control value

LC50: lethal concentration; concentration which produces 50% or 25% dead embryos

LOEL/LOAEL/LOAEC: Lowest (adverse) observed effect level/concentration: Lowest dose or concentration administered leading to significant adverse effects

MATC: the maximal acceptable toxicant concentration is calculated as the geometric mean of the NOEL and the LOEL

MC50: morbid concentration; concentration which produces 50% malformed embryos

NOEL/NOAEL/NOAEC: No (adverse) observed effect level/concentration: Highest dose or concentration administered not leading to significant adverse effects

Experimental system:

Fertilised eggs are collected at 4-24 hours post fertilisation (hpf), sometimes even at the 2-cell stage (Augustine-Rauch et al., 2010; Brannen et al., 2010; Eimon and Rubinstein, 2009). The fertilised eggs may be dechorionated (that is removal of the outer shell, the chorion, of the fertilised egg) for better delivery of the test compound to the embryo (Augustine-Rauch et al., 2010; Brannen et al., 2010).

Chemical treatment:

For chemical exposure, the fish embryos are transferred to a multi-well culture plate (each into a different well), to dishes or vials with media and chemical to be tested. Control embryos remain unexposed under the same conditions. The embryos are exposed for 1-6 days (Brannen et al., 2010; Selderslaghs et al., 2009; Yang et al., 2009).

An alternative way of test substance exposure is the microinjection into the yolk ball of zebrafish embryos, especially useful for compounds with low water solubility (Brannen et al., 2008).

Data collection:

At selected time points during exposure or afterwards, larvae are assessed for viability (for the determination of LC25 or LC50, see "endpoint values") and are scored for morphology and functional development (Augustine-Rauch et al., 2010; Brannen et al., 2010; Chapin et al., 2008; Eimon and Rubinstein, 2009; Selderslaghs et al., 2009). This investigation can be done immediately after exposure or after different time periods in chemical-free conditions.

For observation of internal organs via a microscope, larvae can be immobilised in a gel (Redfern et al., 2008).

Viability assessment of the zebrafish embryos can be conducted based on the absence of heartbeat, body degradation, severe growth retardation and/or dysmorphology (Brannen et al., 2010). Morphological scoring is based on larval length, locomotion, cardiovascular function etc. (Brannen et al., 2010; Fraysse et al., 2006; Selderslaghs et al., 2009; Teraoka et al., 2006). Evaluations are conducted largely similar to fetal examinations in other developmental toxicity studies (Brannen et al., 2010). Functional observations can be recorded as normal or abnormal only or numerical scores (0-5 (normal)) can be
assigned (Brannen et al., 2010; Fraysse et al., 2006; Selderslaghs et al., 2009).

The NOAEL (see “endpoint values”) is determined with the presence or absence of defects and with the identification of a dose-response increase of abnormalities. The LOAEL (see “endpoint values”) is identified with the lowest tested chemical concentration producing adverse effects; the NOAEL is defined as the next lowest tested concentration (Brannen et al., 2010).

Basic procedures of Zebrafish culturing and its developmental stages can be found in The Zebrafish book at www.zfin.org (Spargue et al., 2001). The website is a resource for genetic, genomic and developmental research for zebrafish researchers.

**Data Analysis/Prediction Model**

Mortality of the exposed fish is expressed as the lethal concentration parameters LC50 or LC25 (Augustine-Rauch et al., 2010). The NOECs or NOAECs (see “endpoint values”) are estimated based on the developmental and teratogenic responses, for example larval length, pigmentation, hatching success etc., see also “endpoints” and “basic procedure”. The NOEC or NOAEC represents the highest chemical concentration used in a test which did not produce significant effects (Hallare et al., 2006).

For teratogenicity assays, typically a ratio of the LC50 and LOAEC or NOAEC is established as a threshold value that is supposed to separate teratogens from non teratogens (Augustine-Rauch et al., 2010). The NOEC or NOAEC represents the highest chemical concentration used in a test which did not produce significant effects (Hallare et al., 2006).

**Test Compounds**

Various industrial chemicals, pharmaceuticals, agrochemicals, environmental contaminants and complex mixtures have been tested.

**Modifications**

To assess developmental toxicity of test substance metabolites, a zebrafish teratogenicity test with metabolic activation (mDarT) has been developed (Busquet et al., 2008; Weigt et al., 2010). The test protocol is similar to those described before, but with the additional exposure to a metabolic activation system composed of rat liver microsomes and NADPH, able to biotransform chemicals (Busquet et al., 2008; Weigt et al., 2010).

For research purposes, other fish systems have been utilized for developmental toxicity studies as well: Japanese medaka, African and Silver catfish, salmon and Fundulus heteroclitus (Benaduce et al., 2008; Farwell et al., 2006; Osman et al., 2007; Oxendine et al., 2006a; Wassenberg et al., 2005; Hammock et al., 2003).

Various exposure protocols are possible with fish embryos including static and non-static flow systems and embryo microinjection (Wanget al., 2005). The endpoints assessed and the exposure periods vary according to the protocols (Augustine-Rauch et al., 2010).

The Japanese medaka is also emerging as an alternative test system to mammals (Wang et al., 2006). The medaka fish is described as a small oviparous fish which is easily obtained, cultivated and bred. Elaine Faustman at the University of Washington has developed a method using Medaka embryos in early organogenesis (stage 20), which are exposed for 2 h to the test compound and assessed daily for viability and gross malformations until hatching using a morphological scoring system (Shi and Faustman, 1989). Fertilised Japanese medaka eggs are collected at the 4-, 8- or 16-cell stage (stages 6 and 7) and are reared in Petri dishes in isotonic rearing media at 22°C. Treatment is initiated at stage 20 (period of early organogenesis). After 2 h of exposure to the dark, embryos are rinsed and transferred to fresh embryo rearing medium. Using a dissecting microscope, embryos are assessed daily for viability and malformations up to 28 days post-fertilisation. Other broadly similar protocols have been published by Oxendine et al. (2006), Farwell et al. (2006) and Hanno et al. (2010).

Multi-generation or life-cycle studies with zebrafish or medaka allow for the completion of one or two reproductive cycles (Patyna et al., 2006; Seki et al., 2005; van der Ven et al., 2006). Kuiper et al. (2007) published a zebrafish partial life cycle test with the exposure of adult zebrafish and their offspring. Individuals are examined for survival, development (length, weight), sex ratio, fecundity (number of...
produced eggs) and behavioural changes; histopathological and phenotypical evaluations can be conducted as well (Patyna et al., 2006). The offspring is analysed for hatching numbers, mortality, phenotype and malformations (Kuiper et al., 2007).

Artificially fertilised African catfish were immediately transferred to 24-well multiwell plates with 1 egg/well in 2 ml test solution (Nguyen and Janssen, 2002). Temperature was 27±1°C and photoperiod of 0 hours light and 24 hours dark. In another protocol by Osman et al. (2007), fertilised eggs were exposed in aquaria.

Zebrafish as a neurotoxicological model could be a useful tool in investigating neurotoxicants (Linney et al., 2004). New molecular markers in developmental toxicity are reviewed by Huuskonen (2005). New and more sensitive endpoints which monitor the function of different processes in the individual instead of measuring the morphological appearance have been tested (Caravan III et al., 2004; Bilotta et al., 2004). Furthermore, the zebrafish model has been used to study geno-, hepato- and cardiotoxicity (Kari et al., 2007; McGrath and Li, 2008; Rubinstein, 2006) and vertebrate hematopoiesis and hematological disorders, respectively (Trompouki and Zon, 2010).

Zebrafish studies using toxicogenomics techniques are under development as well (Yang et al., 2009). Changes in gene expression after exposure to toxicants can be monitored for example with the microarray technique, compared to effects in mammals and the specific gene expression profiles can be used for mechanistic evaluations of the mode of action of toxicants, due to the sequenced genome of the zebrafish (Aleström et al., 2006; Shi et al., 2008; Yang et al., 2009).

**Discussion**

Fish exhibit patterns of embryotoxic development similar to those of mammals and offer numerous advantages over mammalian systems for the screening of teratogens: low cost, ease of culturing large numbers of embryos, the ability to directly examine the effect of a chemical, and an expanded period of development which can be observed *in vitro* (Augustine-Rauch et al., 2010; Brannen et al., 2008 and 2010).

The Zebra fish is easy to grow, spawning is triggered by light; about 100 translucent eggs can be obtained per day per female, and it develops rapidly (McGrath and Li, 2008). Development of embryos is highly synchronous so that the developmental stages could be accurately determined. Early developmental stages can be easily and accurately determined (Brannen et al., 2008; Rubinstein, 2006). The scoring method allows to yield response curves that are useful for risk assessment.

As a first approach, the effects in zebrafish has been quantified by the observed retardation of development (Herrmann, 1993), later the quantification of malformations in zebrafish has also been proposed (Herrmann, 1995). According to the author, scoring provides important information for a risk evaluation of substances and it enables a further analysis of the mechanism of the molecular action of the drugs.

It has been mentioned that it is a great advantage of the translucent zebrafish embryo that the formation of internal organs can be monitored without interfering with development; usually, the investigation of brain development is only possible by histological methods, but in zebrafish, it could be detected easily by use of a dissecting microscope. According to the author, the advantages of zebrafish development and the scoring method presented are that, in addition to the order of effectiveness, it is possible to analyse similarities and differences in detail and to get clues as to the underlying molecular mechanisms and action of drugs. Further, it appears that the substances tested so far defined distinct 'effect classes' with distinct properties. New substances tested can be evaluated by determining their effects as more 'retinoid' or 'valproic'-like by the following properties: the range of the effective concentrations, the slope of the dose-response curves, the type of malformations generated by the classes of substances and the type of malformations generated by some substances only (Herrmann, 1995).

Protocols using early life stages zebrafish embryos are usually used in ecotoxicology but several authors proposed their use as a method for predicting human toxicity (Groth et al. 1994, Van Leeuwen et al. 1990). According to Van Leeuwen et al. (1990), early life stage (ELS) tests are an essential element in environmental hazard assessment for chemicals and they may also have a predictive value in human teratology for direct-acting teratogens. According to the authors, the method deserves further optimization and standardization (Augustine-Rauch et al., 2010)

The zebrafish genome is almost known and different molecular approaches can be used to study the gene expression and its effects (Huuskinen, 2005). Transgenic models with fluorescent markers can easily be monitored to study gene activation (Kari et al., 2007).
Despite all the similarities in the developmental processes to humans, one should keep in mind that there are also differences (Brannen et al., 2008). For instance, sex determination can, in some fish species, be determined by ambient water temperature.

The zebrafish embryo as a model for predicting the developmental toxicity of chemicals is advantageous for its cost-effectiveness, ease of use and rapid development with similarities to mammalian development (Augustine-Rauch et al., 2010; Brannen et al., 2010; Selderslaghs et al., 2009; Yang et al., 2009). Brannen et al. (2010) developed an experimental protocol and a prediction model that was challenged with 31 compounds (tested in vivo). 87% of the compounds were successfully categorised as being teratogenic or not. Therefore, this assay is found to be promising for screening compounds for their teratogenic potential (Brannen et al., 2010; Rubinstein, 2006; Augustine-Rauch et al., 2010; Selderslaghs et al., 2009).

Some unanswered questions concerning the broad use of zebrafish for developmental toxicity predictions remain. Little is known about pharmacokinetics and metabolism of waterborne chemicals in zebrafish embryos (Brannen et al., 2010; Selderslaghs et al., 2009). Furthermore, chorion removal or not is an area of controversy (Brannen et al., 2010; Selderslaghs et al., 2009). Variations between zebrafish strains remain to be explored (Brannen et al., 2010). Extrapolation of zebrafish data to humans remains an issue, as it does for many alternative methods (Selderslaghs et al., 2009). The reliability of the zebrafish model for predicting toxicity in general needs to be challenged by further systematic assessments (McGrath and Li, 2008; Yang et al., 2009).

Despite these issues, the zebrafish embryo model is gaining popularity in safety assessment research and has become a commonly used model (Brannen et al., 2008; Brannen et al., 2010; McGrath and Li, 2008; Selderslaghs et al., 2009). A large amount of research papers using this species is available (Redfern et al., 2008).

Many publications are available on developmental toxicity studies using zebrafish. Other fish have been used as well and are valuable for comparative studies, as sensitivity differences can appear between fish species (Wanget al., 2006; Wassenberg et al., 2005).

The optical clarity of the medaka fish eggs offers additional advantages for screening assays. The medaka test system has been widely used for developmental studies and its normal developmental stage has been characterized. The medaka fish seems to be highly adaptive to a wide range of temperatures and osmotic pressures (Hanno et al., 2010). It remains to be investigated further (Hanno et al., 2010). The malformations observed in tests with medaka fish are similar to those of other test species, like zebrafish and the fathead minnow (Farwell et al., 2006). A disadvantage of using medaka embryo-larval assays is the high natural variability in the hatching time (8–35 days), increasing duration and cost of such tests (Farwell et al., 2006). The medaka has been used for assessing developmental effects of chemicals, for evaluating endocrine active chemicals and to investigate chemical effects on reproduction (Hall et al., 2007; Hano et al., 2009; Kang et al., 2008; Nassef et al., 2010; Urushitani et al., 2007).

According to the authors, the ability of medaka embryos to display varied dysmorphogenic teratogenic responses, which include those observed in rodent embryos, suggests that medaka embryo culture systems may be useful models for screening potential mammalian teratogens (Solomon and Faustman 1987). To achieve more precise and systematic evaluations of embryonic development, Shi and Faustman (1989) have developed a quantitative morphological scoring system for medaka embryo; this system is similar to that developed for use with rodents by Brown and Fabro (1981). According to the authors, this scoring system allows to distinguish developmental delays from specific teratogenicity.

Fewer studies were carried out catfish embryos and larvae. These fishes may be valuable models for further studies as they form a staple diet for many people for example in Africa and America (Benaduce et al., 2008; Osman et al., 2007). Studies using the mummichog fish (Fundulus heteroclitus), the java medaka and the fathead minnow are available as well (Ankley et al., 2005; Imai et al., 2007; Peters et al., 2007; Peters et al., 2010; Timme-Laragy et al., 2006). Also cultured embryos of the giant freshwater prawn have been proposed as a simple and low-cost model for developmental toxicity testing in an invertebrate model (Porntrai and Damrongphol, 2008).

In general, the relevance of non-mammalian test species for predicting human health effects is still under discussion (Bremer et al., 2007).

**Status**

**In Development:**
A consortium for sharing results and experiences and for developing a harmonised assay with a defined protocol is currently underway (Augustine-Rauch et al., 2010). The final assay will be tested thoroughly
with a large compound set and several participating laboratories (Augustine-Rauch et al., 2010; Adler et al., 2011).

So far, no publications about studies regarding within-laboratory and between-laboratory variability or about formal validation studies are available in the international literature. This method is not mentioned in any official regulations, guidelines or recommendations on reproduction toxicity testing.

**Known Laboratory Use:**
A questionnaire survey was performed among members of EPAA (The European Partnership for Alternative Approaches to Animal Testing, a collaboration between the European Commission, European trade associations, and companies from seven industry sectors) in November 2010 to investigate the real use of each method among the industries.

The survey showed that this method is used in the laboratories of 3 (out of 11 companies responding) major international pharmaceutical and chemical companies for the assessment of developmental toxicity (incl. embryotoxicity/teratogenicity) for substance-related mechanistic studies, screening or priority testing, further internal test development or for regulatory purposes (i.e. in reaction to prevailing regulatory requirements). It is used as part of a test battery or as a stand-alone test. Two companies started using this method 2007 and 2008 and use it 5-15 times per year to date. One company states that it is still in the phase of internal test development. Altogether, about 3 to 15 substances have been tested with this method per company so far. The companies reported good, promising or unknown predictivity. One company is part of the consortium that is driving a validation effort for this assay. This company states that Zebrafish and FETAX tests are faster and cheaper than e.g. WEC and EST. Two companies state that this method is useful for mechanistic studies, one company uses this method for prioritisation when a developmental effect is suspected or in case of negative *in vivo* results.

**Abbreviations & Definitions**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>EC50</td>
<td>half maximal Effect Concentration</td>
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<tr>
<td>EPA</td>
<td>The European Partnership for Alternative Approaches to Animal Testing</td>
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<td>EST</td>
<td>Embryonic Stem Cells test</td>
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<td>EU</td>
<td>European Union</td>
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<td>FETAX</td>
<td>Frog Embryo Assay</td>
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<tr>
<td>hpf</td>
<td>hours post fertilisation</td>
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<td>ICH</td>
<td>International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use</td>
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<td>LC50, LC25</td>
<td>Concentration which produced 50% or 25% dead embryos</td>
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<td>LOEL/LOAEL/LOAEC</td>
<td>Lowest (adverse) Observed Effect Level/Concentration</td>
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<td>MATC</td>
<td>Maximal Acceptable Toxicant Concentration</td>
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<td>MC50</td>
<td>Concentration which produced 50% malformed embryos</td>
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<tr>
<td>mRNA</td>
<td>messenger RiboNucleic Acid</td>
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<tr>
<td>NADPH</td>
<td>reduced form of Nicotinamide Adenine Dinucleotide Phosphate (NADP+)</td>
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<tr>
<td>NOEL/NOAEL/NOAEC</td>
<td>No (adverse) Observed Effect Level/Concentration</td>
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<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
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<td>WEC</td>
<td>Whole Embryo Cultures test</td>
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**Bibliography**

Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (Pimephales promelas)

Toxicological Sciences 86, 300-308
In vitro developmental toxicity assays: A review of the state of the science of rodent and zebrafish whole embryo culture and embryonic stem cell assays

Birth Defects Research. Part C, Embryo Today: Reviews 90, 87-98

Toxicity of cadmium for silver catfish Rhamdia quelen (Heptapteridae) embryos and larvae at different alkalinites

Archives of Environmental Contamination and Toxicology 54, 274-282

Ethanol exposure alters zebrafish development: A novel model of fetal alcohol syndrome

Neurotoxicology and Teratology 26, 737-743


Development of a zebrafish embryo teratogenicity assay and quantitative prediction model

Birth Defects Research. Part B, Developmental and Reproductive Toxicology 89, 66-77

Quantitation of rat embryonic development in vitro: A morphological scoring system.

Teratology 24, 65-78

Development of a new screening assay to identify proteratogenic substances using zebrafish danio rerio embryo combined with an exogenous mammalian metabolic activation system (mDarT)

Toxicological Sciences 104, 177-188

Ethanol effects on the developing zebrafish: neurobehavior and skeletal monrphogenesis

Neurotoxicology and Teratology 26, 757-768

State of the art in developmental toxicity screening methods and a way forward: a meeting report addressing embryonic stem cells, whole embryo culture, and zebrafish

Birth Defects Research. Part B, Developmental and Reproductive Toxicology 83, 446-456

EU (2008)

Official Journal of the European Communities L 142, 141-443

Eimon, P.M.; Rubinstein, A.L. (2009)
The use of in vivo zebrafish assays in drug toxicity screening

Expert Opinion on Drug Metabolism & Toxicology 5, 393-401

Development of a zebrafish 4-day embryo-larval bioassay to assess toxicity of chemicals

Ecotoxicology and Environmental Safety 63, 253-267

Effects of N,N-dimethylformamide and its degradation products in zebrafish embryos.

Toxicology In Vitro 8(3), 401-406

Surflan and oryzalin impair reproduction in the teleost medaka (Oryzias latipes)

Marine Environmental Research 63, 115-131

Comparative embryotoxicity and proteotoxicity of three carrier solvents to zebrafish (Danio rerio) embryos

Ecotoxicology and Environmental Safety 63, 378-388

The effect of humic acid on the uptake of mercury(II), cadmium(II), and zinc(II) by Chinook salmon (Oncorhynchus tshawytscha) eggs
Archives of Environmental Contamination and Toxicology 44, 83-88

Effects of dioxin isomers on induction of AhRs and CYP1A1 in early developmental stage embryos of medaka (Oryzias latipes)
Chemosphere 78, 830-839

In ovo nanoinjection of nonylphenol affects embryonic development of a transgenic see-through medaka (Oryzias latipes), olvas-GFP/STII-YI strain
Chemosphere 77, 1594-1599


Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in zebrafish.
Toxicology and Applied Pharmacology 142, 56-68

Teratogenic effects of retinoic acid and related substances on the early development of the zebrafish (Brachydanio rerio) as assessed by a novel scoring system.
Toxicology In Vitro 9(3), 267-283

- Herrmann K. (1993)
Effects of the anticonvulsant drug valproic acid related substances on the early development of the Zebrafish (Brachydanio rerio).
Toxicology In Vitro 7(1), 41-54

New models and molecular markers in evaluation of developmental toxicity
Toxicology and Applied Pharmacology 207, S495-S500

- ICH (2005)
International Conference on Harmonisation

Effects of estrone on full life cycle of Java medaka (Oryzias javanicus), a new marine test fish
Environmental Toxicology and Chemistry (SETAC) 26, 726-731

The effects of methyltestosterone on the sexual development and reproduction of adult medaka (Oryzias latipes)
Aquatic Toxicology 87, 37-46

Zebrafish: an emerging model system for human disease and drug discovery
Clinical Pharmacology and Therapeutics 82, 70-80

Toxicity of tetrabromobisphenol A (TBBPA) in zebrafish (Danio rerio) in a partial life-cycle test
Archives of Toxicology 81, 1-9

Zebrafish as a neurotoxicological model
Neurotoxicology and Teratology 26, 709-718

Strain-dependent effects of developmental ethanol exposure in zebrafish
Neurotoxicology and Teratology 26, 745-755
- McGrath, P.; Li, C.Q. (2008)
  Zebrafish: a predictive model for assessing drug-induced toxicity
  *Drug Discovery Today* 13, 394-401

  In ovo nano-injection of triclosan, diclofenac and carbamazepine affects embryonic development of medaka fish (Oryzias latipes)
  *Chemosphere* 79, 966-973

  Embryo-larval toxicity tests with the African catfish (Clarias gariepinus): comparative sensitivity of endpoints
  *Archives of Environmental Contamination and Toxicology* 42, 256-262

- OECD (2007)
  *OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects*

- OECD (2001)
  *OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects*

- OECD (2001)
  *OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects*

- OECD (1996)

- OECD (1995)

- OECD (1983)
  *OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects*

  Lead induced malformations in embryos of the African catfish Clarias gariepinus (Burchell, 1822)
  *Environmental Toxicology and Chemistry (SETAC)* 22, 375-389

  Adapting the medaka embryo assay to a high-throughput approach for developmental toxicity testing
  *Neurotoxicology* 27, 840-845

  Vulnerable windows for developmental ethanol toxicity in the Japanese medaka fish (Oryzias latipes)
  *Aquatic Toxicology* 80, 396-404

  Hazard evaluation of diisononyl phthalate and diisodecyl phthalate in a Japanese medaka multigenerational assay
  *Ecotoxicology and Environmental Safety* 65, 36-47

  Effects on reproductive potential and endocrine status in the mummichog (Fundulus heteroclitus) after exposure to 17α-ethynylestradiol in a short-term reproductive bioassay
  *Aquatic Toxicology* 85, 154-166

  Effects of 17α-ethynylestradiol on early-life development, sex differentiation and vitellogenin induction in mummichog (Fundulus heteroclitus)
  *Marine Environmental Research* 69, 178-186
Fish embryos as teratogenicity screens: a comparison of embryotoxicity between fish and birds.
Ecotoxicology and Environmental Safety 20, 42-52

Inhibition of embryonic development by microcystin-LR in zebrafish, Danio rerio
Toxicon 45, 303-308

Japanese medaka (Oryzias latipes): developmental model for the study of alcohol teratology
Birth Defects Research. Part B, Developmental and Reproductive Toxicology 77, 29-39

Effects of the polycyclic aromatic hydrocarbon heterocycles, carbazole and dibenzothiophene, on in vivo and in vitro CYP1A activity and polycyclic aromatic hydrocarbon-derived embryonic deformities
Environmental Toxicology and Chemistry (SETAC) 24, 2526-2532

Zebrafish teratogenicity test with metabolic activation (mDarT): Effects of phase I activation of acetaminophen on zebrafish Danio rerio embryos
Toxicology 275, 36-49

Zebrafish embryos as models for embryotoxic and teratological effects of chemicals
Reproductive Toxicology 28, 245-253

Chemical genetics: drug screens in zebrafish
Bioscience Reports 25, 289-297

Dithiocarbamates are teratogenic to developing zebrafish through inhibition of lysyl oxidase activity
Toxicology and Applied Pharmacology 244, 156-161

Effects of the antithyroid agent propylthiouracil in a partial life cycle assay with zebrafish
Environmental Science & Technology 40, 74-81

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