Received: 15 Jan, 2023 | Revised: 28 February 2024 | Accepted: 4 March 2024

MANUSCRIPT ID: CoAS_SP1_11

REVIEW

Chronicle of Aquatic Science

# Swimming Through Science: Unveiling the Secrets of Endocrine Disruptors with Zebrafish Models

Tejal Bhapkar[1] | Swati Umap*[1] | A. P. Somkuwar[1] | Alka Sawarkar[1]
Gauri Deshmukh[1] | M. M. Purankar[1]

[1]Department of Veterinary Pharmacology and Toxicology, Nagpur Veterinary College, Nagpur.

**Correspondence**
Swati Umap, Department of Veterinary Pharmacology and Toxicology, Nagpur Veterinary College, Nagpur.
Email: swatiumap@mafsu.in

**Publisher's Note**

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**Conflict of Interest**

The authors declare that the manuscript was formulated in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Authors Contribution**

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

## Abstract

The utilization of zebrafish (*Danio rerio*) as a model organism in studying endocrine disruptor chemicals (EDCs) has emerged as a powerful and versatile approach in toxicology research. This review highlights the numerous advantages that make zebrafish an ideal model for assessing the impact of EDCs on endocrine systems. Zebrafish, with their high fecundity, transparent embryos and rapid development, offer a cost-effective and time-efficient platform for observing the effects of EDC exposure on various endocrine-related pathways. Furthermore, the zebrafish's amenability to genetic manipulation has revolutionized the study of EDCs, allowing for precise control and modification of specific genes associated with endocrine function. Genetic application, such as transgenesis and CRISPR/Cas9 technology, enable researchers to create targeted mutants, providing valuable insights into the molecular mechanisms underlying endocrine disruption. This review underscores the pivotal role of zebrafish in advancing our understanding of EDCs, emphasizing the synergy between its inherent advantages and genetic tools, thus positioning it as a premier model for unraveling the complexities of endocrine disruption and facilitating the development of targeted interventions

## KEYWORDS

Zebrafish, Endocrine Disruptor Chemicals, Endocrine Disruption, Toxicology model, EDC effects

This is an open access article under the terms of the https://creativecommons.org/licenses/by/4.0/ License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2024 *Chronicle of Aquatic Science*.

## INTRODUCTION

The Cyprinidae family of freshwater fish includes the zebrafish (Danio rerio). It has a cylindrical body with alternate light and dark longitudinal stripes along the length of the fish (Spence et al., 2008). These are used in drug development, pre-clinical development and studies of biological processes for human or animal health. Francis Hamilton, in the 1900s first described the Danio rerio. Nevertheless, it was only in 1980s that the transgenic Zebrafish technology was developed. They have good regenerative capability and have been modified by researchers to produce transgenic traits. According to Howe et al., (2013), 70% genes of humans are present in Danio rerio, the zebrafish that was used to sequence its genome. Therefore, these vertebrates are preferred for human studies.

Endocrine disruption is upsetting or disturbance in endocrine system of the organism. Endocrine disruption leads to any interference or dysfunction in hormone production, regulation or action thereby leading to a wide variety of health issues. Any chemical, be it synthetic or natural in origin and have abnormal effect on normal functioning of endocrine system are collectively known as Endocrine disruptors. United States Environment Protection Agency (EPA) defines endocrine disrupting chemicals as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood-borne hormones those are present within the body and are liable for homeostasis, reproduction and development process.” Exposure of the same may lead to a cascade of health issues ranging from reproductive disorders, developmental abnormalities, etc. EDCs interfere with endocrine system by forming complex with hormone receptors and are usually highly potent (La Merrill et al., 2020). European Commission proposed that for a chemical to be regarded as an EDC, it should demonstrate endocrine activity, have detrimental or pathologic endocrine mediated activity and have a cause effect relationship between substance and endocrine activity in exposed substance (ESFA 2013). They remain in body and environment unharmed as they are stable and relatively non-biodegradable. They are lipophilic because of which they get stored in fats and adipose tissue of organism. A thorough research on EDCs helps educate regulatory agencies about the potential risks associated with these chemicals. Detailed research on EDCs will help in development of guidelines and regulations to limit expose of such chemicals to humans, animals and environment. Extensive research on this topic also aids in increasing awareness amongst people. Some EDCs which are heavily incorporated in day-to-day life are bisphenol-A(BPA), phthalates, parabens, pesticides, etc. Others include synthetic hormones like contraceptives, natural hormones, phytoestrogens, chlorinated pesticides like DDT, polychlorinated biphenyls and industrial chemicals like alkylphenols (Segnur 2009).

**TABLE 1. COMMON EDCS AND THEIR HISTORY, USES, SOURCE AND EFFECTS.** *Gore et al., (2015)

| SL. No. | EDCs | Group of chemicals | Introduction year | Route of exposures | Sources | Half-life of chemical | Effects/ animal model notes |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1. | BPA | Bisphenol | 1960s | Oral, inhaling, topical | Plastic wares, resins, food can lining | 4 to 5 hrs | Effects on the nervous system, reproduction, development, obesity & estrogenic hormones |
| 2. | DDT | Organo-chlorine | 1940s | Oral, breathing in, topical | Contaminated fish, crops and water | 6 to 10 yrs | reproductive & hormonal effects, carcinogenic, nervous, renal & hepatic effects |
| 3. | DES | Non-steroidal synthetic estrogen | 1941-1947 | oral, breathing in, intra-vaginally | Human & livestock pharmaceutical | 2 to 3 days | Via placental carcinogenic, teratogenic |
| 4. | Phthalates | Plasticizer | 1920s | Oral, inhaling, topical | Food that has been contaminated, PVC flooring & plastics, personal hygiene items, odors & medical supplies | ~ 12hrs | Carcinogenic, hepatic damage, reproductive and teratogenic consequences, asthma, obesogenic, potential neuroendocrine disruptor, and anti-androgenic activity |
| 5. | TCDD | Dicarb-oximide fungicide | 1981 | Oral, inhaling, topically | Food, job related | 28 days in aerobic soil, 20hrs in plasma | Anti-androgenic action, male reproduction related & neurobiological impact, transgenerational reproduction related impact, carcinogenic |

BPA: Bisphenol A; DDT: Dichloro-diphenyl-trichloroethane; DES: Diethyl-stilbesterol; TCDD: 2,3,7,8-tetrachlorodibenzodioxin

## ENDOCRINE SYSTEM OF ZEBRAFISH

The endocrine system of zebrafish is surprisingly very similar to that of humans. It has a Hypothalamus-Pituitary-Endocrine gland axis like humans. This connects the Central Nervous System to the endocrine system.

Depending on the glands, this axis can be further divided into the Hypothalamus-Pituitary-Gonadal axis (HPG), Hypothalamus-Pituitary-Thyroid axis (HPT), Hypothalamus-Pituitary-Adrenal Axis (HPA) (EFSA, 2013). The functioning of the endocrine system of these fishes is much the same as that of vertebrates, wherein the hypothalamus secretes releasing hormones or release-inhibiting hormones. These hormones are secreted either directly or indirectly into the circulation, which in turn commands other glands to secrete hormones. Such physiological characteristics, along with many technical animal husbandry criteria, have made zebrafish an ideal and exciting animal model for studying EDCs. In female fish, the liver is a functioning endocrine organ, while this is not always true for male fish (Lonare and Sole, 2018).

## PORTALS OF EDCs EXPOSURE IN ZEBRAFISH

Several factors are considered when a route of drug administration is to be chosen, like the physicochemical properties of the drug, target site, animal-related factors, etc. In an immersion bath, drugs are dissolved in water in which zebrafish are housed. The fish absorbs the drug through their skin and gills (Peterson et al., 2000). Drugs can also be given in the form of injectables. They can be directly injected into different regions of Zebrafish, such as the yolk sac, pericardial cavity, muscle tissue, etc. For precise delivery of small volume of drug, microinjections are often used (Lawson and Weinstein 2002). Oral administration of drugs is also a critical route wherein the drug is administered through the mouth, either by adding them to the fish food or by directly delivering the drug into the oral cavity (De Esch et al., 2012). Micro-bead loaded drugs are soaked in water with zebrafish embryos. Zebrafish absorb the drug released from beads (Chakraborty et al., 2009). There is a specialized technique accessible where drugs are injected directly into the bloodstream using a fine needle but this way is less shared due to technical challenges (MacRae et al., 2015). The concentration used in experiments varies according to the individual pilot studies conducted. Mostly, trial and error methods are used to determine the lethal dose of every EDC studied. In several studies conducted on the effect of EDCs on Zebrafish, toxicity was produced at concentrations ranging from 1 to 200 mg/L. The period of study is usually up to a month, depending on the EDC in question.

## ZEBRAFISH: A MODEL ORGANISM

It is anticipated that a model organism will provide physiological and management-related benefits for researching key biological processes. The small animal model should also be representative of a larger group or population (Segnur, 2009). Zebrafish comes fit for both these criteria. The small size of zebrafish and its tolerance to maintenance conditions, along with high fecundity, has made it an intriguing animal model amongst scientists. They are easier to house, require less space, and are cost-efficient. They are completely transparent, so it is easier to see the impact of any drug treatment or genetic mutation without invasion. This is also beneficial for genome sequencing studies and behavioral studies during early growth (Tao et al., 2022). They can produce up to 200 to 300 offspring per week, which shows rapid development. Since Zebrafish have extracorporeal fertilization, all stages of development are within reach. Zebrafish embryos have the ability to absorb chemicals readily. Hence, the induction of genetic mutation is suitable for them. It has been successfully used in different biomedical research fields for vaccine production, cancer, COVID-19, toxicological studies, cardiovascular disorders, neurological disorders, metabolic diseases, etc.

**TABLE 2: FACTORS TO TAKE INTO ACCOUNT FOR USING ANIMAL MODELS IN EDC STUDIES** *Patisaul et al., 2018

| |
|---|
| • Take into consideration potential variations in metabolism and metabolite production that are physiologically active among species. |
| • Efforts should be made to select species that is most sensitive. |
| • Verify that a pertinent target of a comparable mechanism of action is present. |
| • Make sure the results are applicable to human illness rather than being specific to that species (certain malignancies in rats are not found in people). |
| • Include animals of both sexes, except if only a particular sex has relevance like in case of prostate cancer. |
| • Ensure that dosing is done in proper critical period for that species. |
| • Pay attention that the latency period for EDCs may extend into advanced adulthood and be prolonged between exposure and impact. |
| • Make sure you utilize a sufficient number of individuals to achieve adequate statistical power. The litter, not the individual pup, should serve as the statistical unit in mammalian developmental toxicity investigations. |
| • ARRIVE standards or something similar should be used in methodological reporting to guarantee thorough and honest reporting. (https://www.nc3rs.org.uk/arrive-guidelines) |

## BIOMARKERS AND ENDPOINTS IN EDCS STUDIES:

Currently, Fish Short-Term Reproduction Assay (FSTRA) is used, which provides only limited information like effects on egg numbers, vitellogenin level, etc., without the accurate mechanism of action. Till now, Zebrafish lifecycle data has been published about estrogen active EDCs only.

In most EDCs investigations, growth, gender distinction, sex ratio, female egg production, successful fertilization, vitellogenin (VTG), and other endpoints are taken into account. Histological examination of gonads for alterations in structure and cell types can be done. Due to genomic data available for zebrafish with extended working knowledge in toxicity testing, it is comparatively easy to establish molecular endpoints for EDC effects assessment. There are some commonly studied behavioral, molecular and cellular changes indicating exposure of EDCs in Zebrafish. Some molecular changes include Vitellogenin (VTG) induction and gene expression alterations. VTG is a well-known biomarker for estrogenic effects. It is seen that Zebrafish males exposed to estrogenic EDCs exhibit upregulated expression of VTG (Zhang et al., 2018). VTG is a yolk precursor protein of vitellin traditionally associated with female reproduction. In a study, VTG concentrations were seen to have prominently increased after 25dph to 15.4 ± 1.4 ng EE2/L as compared to a control concentration of less than 0.05ng EE2/L. The highest concentration of the same was seen during the embryonic stage. The metabolism of VTG occurs by a first-order kinetic process with a half-life of 2.4 days. Key hormone levels like estrogen, testosterone, thyroid hormone, etc., and changes in hormone levels indicate disruption. EDCs exposure induces changes in the expression of genes related to sex differentiation and steroidogenesis (Chen et al., 2017). Behavior modifications seen are mostly in reproductive and locomotor activities. EDCs exposed to zebrafish

appeared to show altered mating preferences or courtship behavior, indicating disruptions in reproductive behavior (Schiller et al., 2013). Studies have reported changes in zebrafish locomotor activity patterns following exposure to EDCs suggesting neurobehavioral effects (Wang et al., 2015). Gonadal development and histological aberrations show cellular responses, EDCs exposed zebrafish often exhibit abnormalities in gonadal development and histology, providing insights into the impact on reproductive organs (Wang et al., 2016). Reproductive parameters are also altered or influenced by water temperature and estrogen exposure. Disturbances in the sex ratio were also observed. Cellular stress responses, like oxidative stress and apoptosis, have been observed in zebrafish exposed to certain EDC, indicating potential cellular damage (Wang et al., 2016).

## EFFECTS OF EDCs ON ZEBRAFISH

Five kinds of EDCs with mild estrogenic action and concomitant anti-androgenic activity were described by the US Environmental Protection Agency (EPA): (1) pharmaceuticals or artificial estrogens (e.g., 17β estradiol, diethylstilbesterol); (2) phytoestrogens (e.g., isoflavonoids, carbamates, lignans, stilbens); (3) pesticides (e.g., organophosphates, carbamates, organochlorines, synthetic pyrethroids); (4) plasticizers and chemicals made from partially burning polyvinyl chloride (PVC), paper, and putrescible materials (e.g., dioxins); (5) industrial substances and their byproducts (e.g., phenols, dioxins, heavy metals, perfluorooctanoic acid, flame retardants). (Harding et al., 2006; Browne et al., 2017)

It was shown that adult zebrafish exposed to 5ng EE2/L for 40 days did not exhibit any changes in their ability to reproduce. Conversely, lifelong exposure to the same concentration of filial generation decreased fecundity and fertility by 56% and 100%, respectively (Nash et al., 2004). Interestingly, once zebrafish were brought back to clean water post-exposure to chronic estrogen exposure, a reversal in loss of reproductive performance was not fully seen. However, the limit of reversal or recovery of reproductive functions appears to depend on the exposure concentration. Varied anatomical alterations were seen in zebrafish exposed to 15.4±1.4 ng EE2/L for varied time frames, i.e., fertilization to 60 days post-hatching (dph). Changes included the occurrence of ovotestis that contained primary oocytes in close proximation to testicular tissues until 30dph to 40dph. In group of 20 to 60dph, all individuals were observed to have developed into females with fewer oocytes, thereby proving that EE2 has a prominent feminizing effect on gonads.

EDCs have been shown to have transgenerational effects through the use of epigenetic imprinting in mammals. DNA methylation is important for epigenetic reprogramming. This reprogramming undergoes a significant alteration during zebrafish embryo development (Mhanni and McGowan, 2004). A study was conducted to see the effects on ovum development, sex hormones, reproduction related histology and transcriptional patterns along the HPG axis. Results suggested a reduced reproductive capacity, disrupted endocrine system and compromised craniofacial skeletons and vertebrae development in the progeny. It has come in attention that EDCs have a significant role in decreasing both male and female fertility, testicular hypertrophy and polycystic ovarian syndrome (PCOS) (Schug et al., 2011, De Coster and Larebeke 2012). Some other related observations are onset of testicular dysgenesis syndrome, impaired spermatogenesis, testicular cancer, etc. Testicular Dysgenesis Syndrome (TDS) is observed due to an interference in

physiological embryonal programming and Gonadal development during fetal life (Shakkebaek et al., 2001, Virtanen et al., 2005). Transcription of genes related to immune response, namely, IFNγ, IL1β, IL10, Mx, TNFα, CC-chemokine and CXCL-clc showed significant rise in their level on exposure to EDCs. On exposure of Zebrafish embryos to EDCs, there was a noticeable increase in the quantities of the pro-inflammatory mediator nitric oxide, along with an increase in the activity of nitric oxide synthase (NOS) and up-regulation of inducible NOS gene expression (Xu et al., 2013).

Fetal life is a critical time in development because it experiences rapid structural and functional changes. The process of development involves more than just the inheritance of a genetic program, cell commitment to particular lineages and morphological and functional differentiation in the corresponding organs and tissues. Environmental and genomic (epigenetic and genetic) influences can affect an animal's developmental plasticity, which can result in significant modifications to the fetus's developmental path for adaptive responses (Bateson et al., 2004; Gluckman et al., 2009; Tseet al., 2013). Some major teratogenic effects seen post-exposure were cardiac edema, spinal malformation, pigment reduction, cranial hemorrhage and yolk sac deformity.

In a study conducted by Patil and Sole (2019) following the exposure of zebrafish to varying drug doses, behavioral alterations were noted in each group both immediately after the exposure and every day for 21 days prior to and following an hour-long meal.

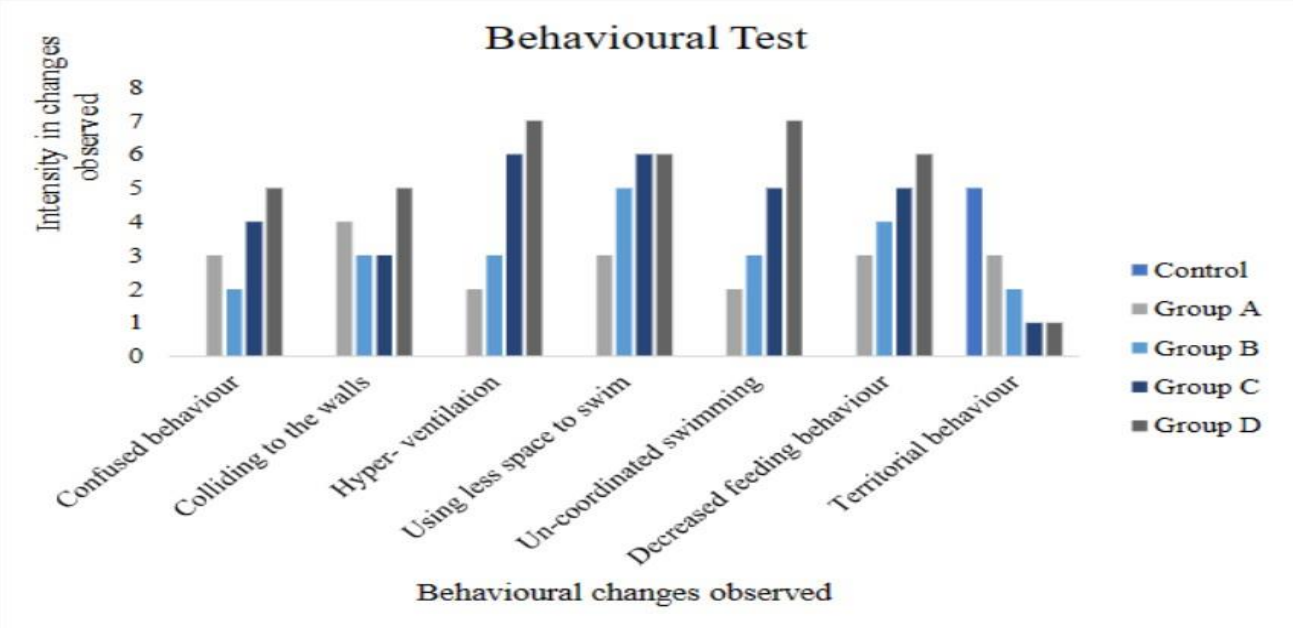


Figure 1. Behavioral Changes Observed in Zebrafish

Control: Unexposed males, Group A, B, C & D of test drug Fluralaner in ascending concentration i.e., Group A < Group B < Group C < Group D *Adapted from Patil and Sole 2021

## SPECIALITY OF ZEBRAFISH OVER OTHER ANIMAL MODEL FOR ENDOCRINE DISRUPTIONSTUDIES:

To accurately replicate the human experience, including pertinent dose and exposure windows, and to guarantee translational value and reproducibility, the selection of a suitable animal model is crucial. In contrast to classical toxicology, which primarily uses inbred rats and mice and concentrates on apical endpoints like tumor formation or birth defects, researchers at EDC have successfully modeled more intricate but important outcomes like changes in pubertal timing, mammary gland development and social behaviors by utilizing a wider variety of species. EDC researchers now have greater access to a broader range of animal models with developments in genetics, neuro-imaging, and many other technologies. For example, due to housing challenges, use of sheep and quail for EDC studies has been denied. Scientists have preferred Zebrafish as a model for EDC studies. They share a considerable number of genetic similarities with humans, including a similar endocrine system. This makes them a relevant model for studying the effects of EDCs on endocrine function (Hill et al., 2005). The embryos of Zebrafish are transparent, allowing direct observation of developmental processes. Their external fertilization also simplifies exposure assessments and facilitates the monitoring of early developmental stages (McGrath and Li 2008). They also have a rapid development and reproductive rates. The embryos develop rapidly and they have a high reproductive rate. This allows for efficient screening of chemicals over multiple generations and the assessment of long-term effects (Westerfield et al., 2000). Management-related and animal husbandry factors have been major attractions for preference as a model. They are relatively easy and cost-effective to maintain in the laboratory compared to some other vertebrate models, making them accessible to a wide range of researchers (Lieschke et al., 2007).

Zebrafish, though being a preferred animal model, is only partially ideal. There are certain limitations to their use. The most obvious is that, in contrast to what human encounter that is exposure via ingestion or inhalation, EDC exposure is usually obtained by absorbing chemical from water in the tank. This absorption occurs dermally in early stage and after the gills have matured, it occurs through circulation. This means that chemical absorption and metabolism might differ significantly. Even though they share genetic similarities with humans, there are also significant evolutionary differences, particularly in the complexity of their endocrine system. Some responses to EDCs may differ between Zebrafish and humans (Brion et al., 2004). Zebrafish metabolize and biotransform chemicals differently than mammals which can impact the interpretation of toxicity data (Sipes et al., 2011). They have also been shown to have a limited tissue complexity. Zebrafish lack some of the tissue complexities as compared to mammals, which may limit the ability to study certain aspects of EDC effects (Peterson et al., 2012). However, the frequent incidence of two infectious illnesses, mycobacteriosis and microsporidiosis, which are challenging to treat and remove from culture facilities, is a persistent issue in zebrafish laboratory colonies (Spitsbergen and Kent, 2003).

There is hesitation to use of some lower-order species like the fruit fly (Drosophila melanogaster) and the nematode (Caenorhabditis elegans), for toxicology related testing due to issues regarding translation of data to humans, despite the fact that some branches especially genetics and neurosciences have contributed significant discoveries with them (Patisaul et al., 2018). The usage of transgenic rodents for the same has certain limitations. There are significant possibilities of incomplete knockout genes, off-the-target expression,

physiological compensation, and imperfect replication of the unexpected disease phenotype. Later, it was mainly observed in Alzheimer's mice (Patisaul et al., 2018).

**TABLE 3: STRENGTHS AND WEAKNESSES OF SOME COMMON ANIMAL MODELS USED IN EDCS RESEARCH** *Patisaul et al., 2018

| Sr. No. | Animal | Strain | Characteristics |
|---|---|---|---|
| 1. | Mice | CD1 | Huge litters, outstanding maternal conduct, sensitivity to estrogens, strong historical data on the prevalence of control diseases, an outbred strain that increases experiment variability |
| | | C57BL/6J | Especially immune-challenged inbred strain that makes an excellent embryo donor & a transplant recipient |
| | | BTBR | Absence of a corpus callosum and has significantly diminished hippocampal commissure, making it an imperfect model for autism. |
| | | B6C3F1 | litters of an acceptable size, a hybrid between two inbred strains, a sizable control dataset, and lifespan |
| | | Collaborative Cross | Genetic diversity along with reproducibility |
| | | Diversity outbred | Maximal genetic diversity with optimal QTL mapping precision and power |
| 2. | Rats | Wistar Han | Outstanding maternal conduct, less occurrence of spontaneous malignancies |
| | | Long Evans | More ethologically significant traits are spontaneously displayed by outbred strains rather than albinos. |
| | | Sprague Dawley | Vendor strains are inconsistent; the best sources are Taconic or Harlan. Cardiomyopathy can have a background incidence of 100%. CRL shows a high risk of breast galactocoeles, poor lifespan, and little sensitivity to estrogen. |
| | | F344/N | Prolactin sensitivity causes a high tumor rate in the mammary gland and testes. |
| 3. | Voles (Microtus) | | Certain strains exhibit pro-social characteristics like as social monogamy, allopaternal care, parental care, and other qualities; the biological foundation for these behaviors is well established. |
| 4. | Deer Mice (Peromyscus) | | Some strains have been utilized to describe "real world" exposures from contaminated locations |

| | | | |
|---|---|---|---|
| | | | because they exhibit pro-social features such as paternal care and social monogamy. |
| 5. | Zebrafish | | Transparent; rather simple and affordable to breed, house, and keep; development happens quickly |
| 6. | Guinea Pig | | Human-related placental anatomy |
| 7. | Sheep | | Human-related placental anatomy |

## INTEGRATION OF EDC STUDIES WITH MOLECULAR AND GENETIC TOOLS

Wang et al., in 2015, have come up with a genetically modified zebrafish-based high-throughput screening method. Due to this, the Zebrafish have “reporter” genes, which glow when a gene is active. It is possible to determine the effects of an administered chemical by interpreting the intensity of the fluorescence. CRISPR/Cas9 i.e., Clustered Regularly Interspaced Short Palindromic Repeats, technology has revolutionized the field of molecular biology and genetic engineering. In the context of studying specific gene targets in EDC research, CRISPR/Cas9 can be applied to investigate the effects of these compounds on the endocrine system at a molecular level. The first step is to identify specific genes associated with the endocrine system that may be affected by EDCs. This can include genes involved in hormone synthesis, receptor signaling, or other critical regulatory elements. Once these targets have been identified, researchers design CRISPR/Cas9 constructs to target and modify those genes specifically. This involves creating guide RNAs that guide the Cas9 enzyme to the desired DNA sequence. The designed CRISPR/Cas9 constructs are introduced into relevant cell lines or tissues. This allows researchers to study the effects of gene modifications in a controlled environment. Researchers can then analyse the functional consequences of gene modifications induced by CRISPR/Cas9. This may involve assessing changes in gene expression, protein levels, or cellular phenotypes (Zhao et al., 2023).

## TRANSLATION OF DATA TO HUMAN HEALTH

For appropriate relation between chemical consequences across species, knowledge of the effects of pathway disruption is necessary. A framework known as adverse outcome pathways is used to arrange the mechanistic and predictive linkages that exist between pathways, bad phenotypic outcomes that are pertinent to hazard assessment, and early chemical biological interactions, also known as Molecular Initiating Events or MIEs. An AOP framework informs the extrapolation of chemical impacts across species, hence enabling the use of alternative models. It is evident that HTS experiments with an emphasis on humans or mammals that target conserved molecular starting events can be helpful in forecasting possible higher-level impacts in other species. Additionally, pathway-based data can frequently be extended considerably beyond the phylogenetic group from which it was created when orthologous pathways arise (Perkins et al., 2013). There are still definite benefits even though fish and mammals may have slightly different biotransformation capacities, and hence, caution should be used when extrapolating results to humans. Zebrafish have been suggested to be a nearly perfect in vivo model with the ability to do high throughput screening.

## FUTURE DIRECTIONS

The field of Endocrine Disrupting Chemicals has been continuously evolving and researchers are actively working on refining the use of zebrafish models. Incorporation of omics technologies like genomics, transcriptomics, proteomics and metabolomics can deliver a comprehensive understanding of the molecular mechanisms underlying EDC effects (Filby and Tyler 2017). High-throughput omics approaches allow researchers to identify critical pathways and biomarkers associated with EDC exposure in zebrafish. This integrative approach can enhance the precision and depth of EDC research. Continued development and validation of behavioral assays that capture a broad range of endpoints are also essential. Automated and standardized assays can improve the reproducibility of behavioral studies, allowing for more accurate assessments of EDCs effects on zebrafish behavior (Kalueff et al., 2014). Extended exposure studies can be conducted to investigate the long-term effects of EDCs on zebrafish health (AnvariFar et al., 2020). This may involve studying transgenerational effects and assessing the persistence of EDCs induced changes across multiple generations. The biomarkers for EDCs exposure can be identified and validated. This can enhance the sensitivity and specificity of assessments. This includes the development of molecular, biochemical and physiological markers that are specifically responsive to EDCs in zebrafish (Schiller et al., 2013). The ecological relevance of zebrafish models can be improved by considering interactions between EDCs and other environmental factors. Some assays like cell-based models, organoids and other systems, can be developed and validated to reduce reliance on whole animal testing (Antczak and Meister 2017). Open comparison to datasets and the establishment of standardized protocols can facilitate cross-study comparisons and enhance the robustness of findings (Howe et al., 2013).

## CONCLUSION

The utilization of the zebrafish model for studying endocrine disruptor chemicals has emerged as a powerful and versatile tool in the field of aquatic toxicology. This review has given a comprehensive overview of the strengths and limitations of the zebrafish model, shedding light on its potential to revolutionize our understanding of endocrine disruption. The zebrafish model's ability to mimic human endocrine systems, cost-effectiveness, and high throughput capabilities make it an attractive choice for researchers investigating its impact. Looking ahead, the future of research in this area holds great promise. Further exploration of zebrafish models can deepen our understanding of specific pathways affected by endocrine disruptors, elucidate dose-response relationships, and identify novel chemicals with endocrine-disrupting potential. Additionally, advancements in genomic and molecular techniques can enhance the precision and reliability of zebrafish studies.

The implications of zebrafish-based research extend beyond the laboratory, particularly in the context of regulatory practices in India. The adoption of zebrafish models in toxicity assessments can provide more relevant and reliable data for regulatory decision making. This tactic aligns with the global inclination in the direction of incorporating alternative approaches in toxicity testing to ensure chemical safety. In light of these advancements, it is foreseeable that the Zebrafish model will play a pivotal role in shaping regulatory frameworks for endocrine disruptors in India. As researchers continue to unveil the intricacies of endocrine disruption using this model, regulatory agencies can leverage this knowledge to establish more effective

guidelines and policies for the assessment and management of EDCs. The integration of zebrafish-based data into regulatory practices holds the potential to enhance public health protection and environmental conservation, setting a benchmark for evidence-based decision-making in the realm of chemical safety.

## REFERENCES

Antczak, P., & Meister, A. (2017). A versatile new tool to quantify ciliate ingestion by meiofauna using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Molecular Ecology Resources, 17(6), 1293-1305.

AnvariFar, H., Ahmadi, F., & Adel, M. (2020). Long-term exposure to environmentally relevant concentrations of estrogen induces metamorphosis and alters sex steroid levels in zebrafish (Danio rerio). Environmental Science and Pollution Research, 27(21), 26313-26323.

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R. A., & Sultan, S. E. (2004). Developmental plasticity and human health. Nature, 430(6998), 419-421.

Brion F, Tyler CR, Palazzi X, Laillet B, Porcher JM, Garric J, Flammarion P. (2004) Impacts of 17β-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (Danio rerio). Aquat. Toxicol. 68(3), 193-217

Browne, P., Noyes, P. D., Casey, W. M., & Dix, D. J. (2017). Application of adverse outcome pathways to US EPA's endocrine disruptor screening program. Environmental health perspectives, 125(9), 096001.

Chakraborty, C., Hsu, C. H., Wen, Z. H., & Lin, C. S. (2009). Aggregation of Zebrafish Embryos for High-throughput Drug Toxicity Screening. Journal of Visualized Experiments, (25), e1422

Chen, Q. L., Yu, T., Zhou, J., Huang, H. W., & Wang, P. (2017). Proteomic and metabolomic responses of different zebrafish tissues to BDE-47 and TBBPA exposure. Science of the Total Environment, 574, 1147-1155.

De Coster S, van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. J Environ Public Health. (2012) 2012:713696. doi 10.1155/2012/713696

De Esch, C., van der Linde, H., Slieker, R., Willemsen, R., & Hermsen, T. (2012). Zebrafish as a model to study the blood-brain barrier in disease and treatment. Drug discovery today: technologies, 9(4), e227-e233

EFSA J. (2013). Committee ES. Scientific Opinion on the hazard assessment of endocrine disruptors: scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. 11:3132. doi: 10.2903/j.efsa.2013.3132

Filby, A. L., & Tyler, C. R. (2017). Appropriate 'housekeeping' genes for use in expression profiling the effects of environmental estrogens in fish. BMC Molecular Biology, 18(1), 1-11.

Gluckman P. D., Hanson M. A., Bateson P., Beedle A. S., Law C. M., Bhutta Z. A., Anokhin K. V., Bougnères P., Chandak G. R., Dasgupta P.et al. (2009). Towards a new developmental synthesis: adaptive developmental plasticity and human disease. Lancet 373, 1654–1657.

Gore, A. C. et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 36, E1-E150, doi:10.1210/er.2015-1010 (2015)

Harding, A. K., Daston, G. P., Boyd, G. R., Lucier, G. W., Safe, S. H., Stewart, J.,.... & Van Der Kraak, G. (2006). Endocrine disrupting chemicals research program of the US Environmental Protection Agency: summary of a peer-review report. Environmental Health Perspectives, 114(8), 1276-1282.

Hill AJ, Teraoka H, Heideman W, Peterson RE. (2005) Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol. Sci. 86(1), 6-19

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., ....& Teucke, M. (2013). The zebrafish reference genome sequence and its relationship to the human genome. Nature, 496(7446), 498-503.

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. Trends in Pharmacological Sciences, 35(2), 63-75.

Lawson, N. D., & Weinstein, B. M. (2002). In vivo imaging of embryonic vascular development using transgenic zebrafish. Developmental biology, 248(2), 307-318

Lieschke GJ, Currie PD. (2007) Animal models of human disease: zebrafish swim into view. Nat. Rev. Genet. 8(5), 353-367

Lonare S. M., Sole S. S. (2018) Assessment of Ractopamine for Endocrine Dysfunction & Teratogenic Effects in Zebrafish.M.V.Sc. Thesis.Maharshtra Animal & Fishery Sciences University, 27

La Merrill, M. A., Vandenberg, L. N., Smith, M. T., Goodson, W., Browne, P., Patisaul, H. B.,.... & Zoeller, R. T. (2020). Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nature Reviews Endocrinology, 16(1), 45-57.

MacRae, C. A., & Peterson, R. T. (2015). Zebrafish as tools for drug discovery. Nature Reviews Drug Discovery, 14(10), 721-731

McGrath P, Li C-Q. (2008) Zebrafish: a predictive model for assessing drug-induced toxicity. Drug Discovery Today 13(9-10), 394-401

Mhanni, A. A., & McGowan, R. A. (2004). Global changes in genomic methylation levels during early development of the zebrafish embryo. Development genes and evolution, 214, 412-417.

Nash, J. P., Kime, D. E., Van der Ven, L. T., Wester, P. W., Brion, F., Maack, G., ....& Tyler, C. R. (2004). Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environmental health perspectives, 112(17), 1725-1733.

Patil P. N., Sole S. S. (2021). “Evaluation of Fluralaner for Endocrine Disruption & Embryonic Toxicity in Zebrafish Model”. M.V.Sc. Thesis. Maharshtra Animal& Fishery Sciences University, 48-50

Patisaul, H. B., Fenton, S. E., & Aylor, D. (2018). Animal models of endocrine disruption. Best Practice & Research Clinical Endocrinology & Metabolism, 32(3), 283-297.

Perkins, E. J., Ankley, G. T., Crofton, K. M., Garcia-Reyero, N., LaLone, C. A., Johnson, M. S., ... & Villeneuve, D. L. (2013). Current perspectives on the use of alternative species in human health and ecological hazard assessments. Environmental health perspectives, 121(9), 1002-1010.

Peterson, R T., Link, B. A., & Dowling, J. E. (2000). Zebrafish as a model for the study of retinal and optic nerve regeneration: implications for the treatment of glaucoma. Investigative ophthalmology & visual science, 41(10), 2678-2686

Peterson RT, Macrae CA. (2012) Systematic approaches to toxicology in the zebrafish. Annu. Rev. Pharmacol. Toxicol. 52, 433–453

Schiller, V., Wichmann, A., Kriehuber, R., &Muth-Köhne, E. (2013). Influence of water temperature and estrogen exposure on endocrine and reproductive parameters in male zebrafish. Environmental Toxicology and Chemistry, 32(7), 1606-1617.

Schiller, V., Wichmann, A., Kriehuber, R., Muth-Köhne, E., Giesy, J. P., Hecker, M., & Fenske, M. (2013). Effects of long-term UV filters (4-MBC, OMC, BP-3, BZ-12) on fish, crustaceans, algae and mollusks in a lotic ecosystem. Science of the Total Environment, 463, 45-55.

Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol. (2011) 127:204–15. doi: 10.1016/j.jsbmb.2011.08.007

Segnur, H. (2009). Zebrafish (Danio rerio) as a model organism for investigating endocrine disruption. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 149(2), 187-195

Sipes NS, Padilla S, Knudsen TB. (2011) Zebrafish: as an integrative model for twenty-first century toxicity testing. Birth Defects Res. Part C Embryo Today Rev. 93(3), 256–267

Skakkebaek, N. E., De Meyts, E. R., & Main, K. M. (2001). Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Apmis, 109(S103), S22-S30.

Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, Danio rerio. Biological reviews, 83(1), 13-34.

Spitsbergen, J. M., & Kent, M. L. (2003). The state of the art of the zebrafish model for toxicology and toxicologic pathology research—advantages and current limitations. Toxicologic pathology, 31(1_suppl), 62-87.

Tse, W. K., Yeung, B. H., Wan, H. T., & Wong, C. K. (2013). Early embryogenesis in zebrafish is affected by bisphenol A exposure. Biology open, 2(5), 466-471.

Virtanen, H. E., Rajpert-De Meyts, E., Main, K. M., Skakkebaek, N. E., & Toppari, J. (2005). Testicular dysgenesis syndrome and the development and occurrence of male reproductive disorders. Toxicology and applied pharmacology, 207(2), 501-505.

Wang, G., Rajpurohit, S. K., Delaspre, F., Walker, S. L., White, D. T., Ceasrine, A., ... & Mumm, J. S. (2015). First quantitative high-throughput screen in zebrafish identifies novel pathways for increasing pancreatic β-cell mass. Elife, 4, e08261.

Wang, Q., Liu, S., Jiang, Y., Zhao, Y., Liu, H., Liu, Z., & Wang, C. (2015). Estrogenic endocrine-disrupting effects of organophosphorus flame retardants and related mechanisms in early life stages of zebrafish. Environmental Science & Technology, 49(21), 13370-13379

Wang, Q., Wang, X., Niu, C., & Li, Y. (2016). Endocrine disruption and reproductive impairment in zebrafish by exposure to 8:2 fluorotelomer alcohol. Aquatic Toxicology, 175, 109-118

Westerfield M. (2000) The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio). University of Oregon Press

Xu, H., Yang, M., Qiu, W., Pan, C., & Wu, M. (2013). The impact of endocrine-disrupting chemicals on oxidative stress and innate immune response in zebrafish embryos. Environmental Toxicology and Chemistry, 32(8), 1793-1799.

Tao, Y., Li, Z., Yang, Y., Jiao, Y., Qu, J., Wang, Y., & Zhang, Y. (2022). Effects of common environmental endocrine-disrupting chemicals on zebrafish behavior. Water Research, 208, 117826.

Zhang, Y., Luo, H., Zhang, T., Yang, M., Yu, Y., Shi, W., & Zhu, K. (2018). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 208, 18-25.

Zhao, F., Ding, X., Liu, Z., Yan, X., Chen, Y., Jiang, Y., ....& Zheng, J. (2023). Application of CRISPR/Cas9-based genome editing in ecotoxicology. Environmental Pollution, 122458.

**How to cite this article:** Bhapkar T, Umap S, Somkuwar AP, Sawarkar A, Deshmukh G and Purankar MM. Swimming Through Science: Unveiling the Secrets of Endocrine Disruptors with Zebrafish Models. *Chron Aquat Sci*. 2024;1(10):115-129