Available online at BIOMA: Jurnal Ilmiah Biologi
Websites:http://journal.upgris.ac.id/index.php/bioma/index
BIOMA: Jurnal Ilmiah Biologi, 15 (1), April 2026, 26-40
DOI: https://doi.org/10.26877/bioma.v15i1.3390

TESTING OF DEXAMETHASONE TOXICITY TO CARDIOMETABOLIC AND MALFORMATION OF ZEBRAFISH (*Danio rerio* Hamilton, 1882*)* LARVAE

Fatimah Nur Aini, Dewi Puspita Sari*
Biology Education Study Program, Faculty of Teacher Training and Education, Universitas Sebelas Maret
Jl. Ir. Sutami No. 36A, Kentingan, Surakarta 57126, Central Java, Indonesia
**Corresponding author: dewipuspita@staff.uns.ac.id*

ARTICLE INFO

Article history

Submission	2026-03-26
Revision	2026-04-03
Accepted	2026-04-14

Keywords:
Cardiometabolic disease
Dexamethasone
Toxicity
Zebrafish

ABSTRACT

This study aims to evaluate the toxicity effects of dexamethasone on survival, hatching, cardiometabolic function, and the emergence of malformations in zebrafish larvae (Danio rerio), a vertebrate model organism. The study was conducted during the embryonic to early larval phase (0 – 96 hpf) using an in vivo experimental design with complete randomization, following the Zebrafish Embryo Toxicity Test (ZFET) approach, in accordance with OECD 236 guidelines. Zebrafish embryos were exposed to varying concentrations of dexamethasone (0.5,1, 3, and 7 ppm). The parameters observed included survival rate, hatching rate, cardiometabolic function, and changes in larval morphology. Data were analyzed using normality and homogeneity tests, followed by One-Way ANOVA with LSD post hoc tests and Two-Way ANOVA. The results showed that exposure to dexamethasone did not cause lethal toxicity in zebrafish larvae up to 96 hpf (P = 0.949), but significantly inhibited hatching at 48 hpf (P = 0.007) and 72 hpf (P = 0.032). Dexamethasone was also significant in lowering the larvae's heart rate in a dose- and time-dependent manner (P < 0.001). The two-way ANOVA results further indicated that dose, exposure time, and their interaction had significant effects on cardiometabolic function (P < 0.001).

INTRODUCTION

Synthetic glucocorticoids are a group of drugs widely used in modern clinical practice, with dexamethasone (DEX) among the leading agents due to their potent anti-inflammatory and immunosuppressive effects. Dexamethasone is used to treat various clinical conditions such as autoimmune diseases, asthma, allergies, arthritis, and critical conditions such as septic shock and cerebral edema (Hanim et al., 2018). The use of dexamethasone has increased sharply during the COVID pandemic – alongside clinical evidence showing its ability to lower mortality among patients with severe symptoms requiring mechanical ventilation (Horby Peter et al., 2021). The intensification of use has included increased dexamethasone residues excreted into the aquatic environment through hospital and pharmaceutical waste. As active pharmaceutical compounds, dexamethasone residues have the potential to be bioactive and persistent, and should be emerging contaminants that can trigger chronic physiological disturbances in non-target aquatic organisms, even at trace concentrations of nanograms to micrograms per liter (Sayed et al., 2022).

Zebrafish (*Danio rerio*) occupies a strategic position as a model organism in developmental toxicology studies because it has a short life cycle, transparent embryos, and larvae (Indriyanti, 2020). Lestari et al. (2025) reported that zebrafish exhibit high genetic similarity and physiological pathways with other vertebrates, including humans. Zebrafish is internationally recognized as a standard test organism for the Fish Embryo Acute Toxicity Test (ZFET) (Busquet et al., 2014). Several studies have reported that exposure to dexamethasone during the embryonic to larval stages of zebrafish can affect the development of metabolic organs, accelerate the hatching process, alter cardiovascular function, suppress the immune response, and trigger oxidative stress and cell apoptosis with an intensity of effects that depends on the dose and duration of exposure (Di Paola et al., 2022; Sharif et al., 2015; Yin et al., 2017). This study aims to evaluate the toxicity of dexamethasone on survival, hatching, cardiometabolic, and the appearance of malformations in zebrafish larvae, providing a scientific basis for the risk assessment of pharmaceutical residues in the aquatic environment. This research is expected to produce comprehensive quantitative data while enriching the developmental toxicology literature.

MATERIALS AND METHODS

Research Design

The study used an in vivo experimental method, the Zebrafish Embryo Acute Toxicity Test (ZFET), which is based on guidelines (OECD, 2013). The research was conducted from August to October 2025 at the Ecophysiology Laboratory at Universitas Sebelas Maret. The independent variables in this study were dexamethasone concentrations (0.05, 1, 3, and 7 ppm), determined through preliminary LC50 tests. In contrast, the dependent variables included survival rate, hatching rate, cardiometabolic parameters, and larval malformations.

Preparation of Test and Maintenance Organisms

Adult zebrafish (*Danio rerio*) were maintained in aquariums with continuous filtration and aeration systems for 24 hours under a natural light cycle (Nurfakhrurajab et al., 2025). Spawning was carried out at a 2:1 male-to-female ratio using a likely meant mating chamber (Khusna, 2025). The spawned eggs were then selected using an IRIS OptiLab microscope with egg criteria, namely transparent membranes and a developmental stage of 2 hours post–fertilization (hpf), for use as samples.

Dexamethasone Exposure Procedure

A Dexamethasone stock solution is prepared by dissolving 0.0050 g of dexamethasone (Sigma Aldrich, USA) drug powder into absolute ethanol until it reaches a concentration of 1000 ppm (Permatasari et al., 2021). The test solution is then vortexed until dexamethasone dissolves completely. The test solution is then diluted with absolute alcohol to the treatment concentration. 20 embryos per treatment, with three replicates, were placed into 24-well plates. Observation times were conducted at 24, 48, 72, and 96 hpf to evaluate toxicity endpoints.

Measurement of Toxicity Parameters

Based on the measurement (OECD, 2013), the survival rate is calculated based on the percentage of embryos that live in the 96 hpf observation period, while the hatching rate is calculated based on the percentage of embryos that have successfully released from the chorion in the observation period of 48 hpf and 72 hpf. The heartbeat rate, commonly referred to as heart rate in studies of zebrafish embryos and larvae, was

measured using an IRIS OptiLab microscope for 30 seconds. Data is converted to beats per minute (bpm). Morphological observations include pericardial edema (PE), yolk sac edema (YSE), scoliosis (SC), and blood circulation disorders (BC).

Data Analysis

The data from the study were analyzed using IBM SPSS Statistics 22 with normality and homogeneity tests, followed by One-Way ANOVA and LSD post hoc tests at a 95 % confidence level ($\alpha = 0.05$). The LC_{50} value was determined using SPSS probit analysis.

RESULTS AND DISCUSSION

Effects of Dexamethasone Exposure on Survival Rate and Hatching Rate

Table 1. Survival Rate and Hatching Rate of *Danio rerio* Larvae at Various Concentrations of Dexamethasone

Parameter	(Mean ± SD)					P value
	Control	0.05 ppm	1 ppm	3 ppm	7 ppm	
Survival Rate (%)	90 ± 5	85 ± 10	88.3 ± 10.4	88.3 ± 10.4	90 ± 5	0.949
Hatching Rate 48 hpf (%)	93.3 ± 7.63	86.6 ± 7.63	78.3 ± 7.63	76.6 ± 1.52	65 ± 5	0.007
Hatching Rate 72 hpf (%)	95 ± 5	91.6 ± 7.63	85 ± 13.2	83.3 ± 7.63	70 ± 5	0.032

Based on the toxicity tests carried out, the data in Table 1 show that dexamethasone exposure has no significant effect on zebrafish larvae survival rate (P=0.949). The survival rate in each treatment group remained between 85% and 90%, indicating that exposure to dexamethasone at doses of 0.05-7 ppm did not reach the acute lethality threshold at 96 hpf. Meanwhile, observations on hatching rates showed a significant effect (P<0.05). The embryo hatching rate at 48 hours post fertilization (hpf) decreased significantly (P=0.007) in line with the increase in the concentration dose of dexamethasone exposure, which was at the highest dose (7 ppm), showing a relatively low hatching rate compared to the control group (93.3%), which decreased to 65% at 7 ppm. The consistent decrease in hatching rate up to 72 hpf (P=0.032) indicates dose-dependent impairment of embryonic development.

The declining hatching rate, without an increase in larval mortality, indicates that exposure to dexamethasone did not cause acute embryonic death and is therefore non-lethal within the tested dose range. (Di Paola et al., 2022). These results are in line with

previous developmental toxicology research in *Danio rerio,* which found that exposure to synthetic glucocorticoid compounds at sublethal concentrations tends to affect differentiation and maturation in embryos without causing significant embryo mortality (C. Wang et al., 2023). The delayed hatching process indicates a disturbance in the biochemical and mechanical mechanisms of the embryo that break down the chorion (McNeil et al., 2016). Physiologically, the hatching gland, under endocrine regulation, produces the enzyme chorionase, which regulates the hatching process (Nagasawa et al., 2016). Dexamethasone, a glucocorticoid receptor agonist, modulated the hypothalamic-pituitary-interrenal (HPI) axis in fish, playing an important role in stress regulation and embryonic development (Nesan & Vijayan, 2016). Overactive glucocorticoid receptors suppress the expression of genes involved in proteolytic enzyme synthesis and release, thereby inhibiting chorion softening and delaying hatching (Gerber et al., 2021).

Delayed hatching is not only caused by disturbances in enzymatic mechanisms, but is also related to the embryo's low motor activity (Wisenden et al., 2022). Previous research has shown that exposure to dexamethasone during the early phase of embryogenesis can interfere with neuromuscular development, characterized by decreased spontaneous movements and tail wagging activity in embryos within the chorion (Luo et al., 2023). Motor activity exerts mechanical pressure on the chorionic wall to facilitate the hatching (Shafei et al., 2017). Decreased motor activity in the chorion traps the embryo for longer, with a profound impact on the physiological status of the larvae, as the delay in hatching prolongs exposure to the micro-hypoxic environment in the chorion (Duan et al., 2020). Continuous exposure to hypoxia can trigger oxidative stress, leading to impaired cardiovascular system development, including heart rate regulation and energy metabolism after hatching (W. Wang et al., 2024). These findings suggest that dexamethasone residues in aquatic environments may pose a risk to early vertebrate development by disrupting hatching processes and physiological functions, even at sublethal concentrations.

Effects of Dexamethasone Exposure on Survival Rate and Hatching Rate

Data on the heart rate of *Danio rerio* larvae at 48 hpf and 72 hpf are presented in Table 2. Exposure to dexamethasone at various doses showed a significant effect on cardiometabolic function through the measurement of the heart rate of *Danio rerio* larvae. Based on Table 2, heart rate decreased consistently with increasing dexamethasone

concentration at both 48 and 72 hpf (P<0.001). During the 48 hpf observation period, the control group's heart rate of 175.6 ± 1.52 bpm decreased to 161.6 ± 2.08b bpm at a 7 ppm exposure dose. At 72 hpf, a more pronounced decrease was observed at the highest exposure dose (7 ppm), with an average heart rate of 137.3 ± 5.6 bpm.

Table 2. Average Heart Rate (mean ± SD) of *Danio rerio* Larvae at 48 hpf and 72 hpf After Exposure to Various Concentrations of Dexamethasone.

Parameter	(Mean ± SD)					P value
	Control	0,05 ppm	1 ppm	3 ppm	7 ppm	
Heart Rate 48 hpf	175.6 ± 1.52[a]	173.6 ± 2.08 [a]	168 ± 3.6 [ab]	165 ± 3.6 [b]	161.6 ± 2.08 [b]	0.000
Heart Rate 72 hpf	173.6 ± 2.51[a]	146.6 ± 4.04[b]	143.3 ± 2.51[b]	137.3 ± 3.05 [b]	137.3 ± 5.6 [b]	0.000

Note: Superscripts with different letters in columns and rows indicate significant differences in the Bonferroni post hoc test (P < 0.05).

A decreased heart rate, commonly referred to as bradycardia, is closely associated with delayed hatching, as discussed in the previous section. A decreased heart rate is an early indicator of metabolic disorders and the emergence of homeostatic circulatory failures due to chemical stress (Sarmah & Marrs, 2016). Dexamethasone is a synthetic glucocorticoid that can penetrate the chorion and bind to glucocorticoid receptors (GR) in developing heart tissue. Excessive GR activation can impair calcium (Ca2+) signaling in cardiomyocytes, a critical mechanism underlying the regulation of cardiac contractility and heart rate. (Wilson et al., 2015)

Gans & Coffman (2021) align with the view that exposure to glucocorticoids during embryogenesis can trigger reprogramming of the cardiovascular system, often resulting in a decrease in heart rate. In addition, bradycardia at 72 hpf, compared with 48 hpf, indicates the accumulation of toxic effects, consistent with the longer exposure duration (Wu et al., 2023). These results support previous studies showing that although larvae can survive (high survival rate), their cardiometabolic function can undergo significant degradation (Meador, 2021).

The relationship between bradycardia and low hatching rates can also be explained by the energy supply theory, which holds that decreased blood flow may disrupt the distribution of oxygen and nutrients throughout the larval body, including the hatching glands. The result is that the larvae have limited energy available to perform mechanical activities to break down the chorion (Ping et al., 2022). These findings suggest that dexamethasone residues in aquatic environments may disrupt early cardiovascular

development in fish, potentially affecting larval fitness and survival at later developmental stages.

Table 3. ANOVA Two-Way Test Results of Dexamethasone Dosage and Exposure Time on Heart Rate of *Danio rerio* Larvae

Variasi	Sum of Squares	df	Mean Square	F	P value
Dosage	0.018	4	0.005	50.4	0.000
Time	0.026	1	0.026	292.4	0.000
Dosage*Time	0.006	4	0.001	15.61	0.000

Based on the results of the Two-Way ANOVA test presented in Table 3, the dosage factor, dexamethasone exposure time, and interaction between dose and exposure time had a significant effect on the heart rate of *Danio rerio* larvae ($P<0.001$). High F-values were observed in the variables of time ($F=292.4$) and dose ($F=50.4$), indicating that these two factors are independently the main determinants in the physiological changes of the larval heart.

A significant effect of dose ($P<0.001$) indicates a dose-dependent toxic effect, i.e., a condition in which an increase in dexamethasone concentration was directly proportional to a decrease in cardiometabolic function (Ryu et al., 2021). On the other hand, a significant time variable ($P<0.001$) showed that the duration of dexamethasone exposure exacerbated bradycardia, making the glucocorticoid effect cumulative during the cardiac organogenesis period (Saettele et al., 2022).

The interaction between dose and time ($P<0.001$; $F=15.61$) showed that the effect of dexamethasone dose on heart rate was not static but varied with larval development stage. The results of this study are consistent with previous research, which found that hormonal signaling pathways during the critical period of embryonic development produce increasingly pronounced toxic effects (amplification) as exposure time increases (Hong et al., 2024). Research has also shown that synthetic glucocorticoid exposure will interfere with the regulation of ion channels in the heart pacemaker cells of *Danio rerio* larvae, leading to a decrease in heart rate and an increase in the duration of dexamethasone exposure (Dinarello et al., 2020). In addition, the condition of bradycardia that decreases during the increased exposure time, which is up to 72 hpf, supports the theory of the vulnerability of the organogenesis phase to the accumulation of xenobiotic substances, which is evidenced by the existence of a significant relationship between dose factors and time (Chahardehi et al., 2020).

Overall, these results demonstrate that dexamethasone-induced cardiotoxicity in zebrafish larvae is influenced by both exposure concentration and duration, highlighting the importance of considering temporal dynamics in ecotoxicological risk assessments of pharmaceutical contaminants.

Morphological Malformations

The results of the morphological observation of *Danio rerio* larvae showed that exposure to Dexamethasone was able to cause the appearance of various types of malformations, including swelling of the pericardial area, impaired blood accumulation, swelling of the yolk sac, and abnormal curvature of the spine of *Danio rerio* larvae. The distribution of the number of malformations across the exposure dose groups is presented in Figure 1. Descriptively, there is a pattern of increasing the number of larvae with malformations with increasing exposure dose to dexamethasone. In the control group, the median malformation value was 0%, indicating no larval malformations at that dose, whereas the highest- exposure dose group (7 ppm) had the highest median value of 15%.

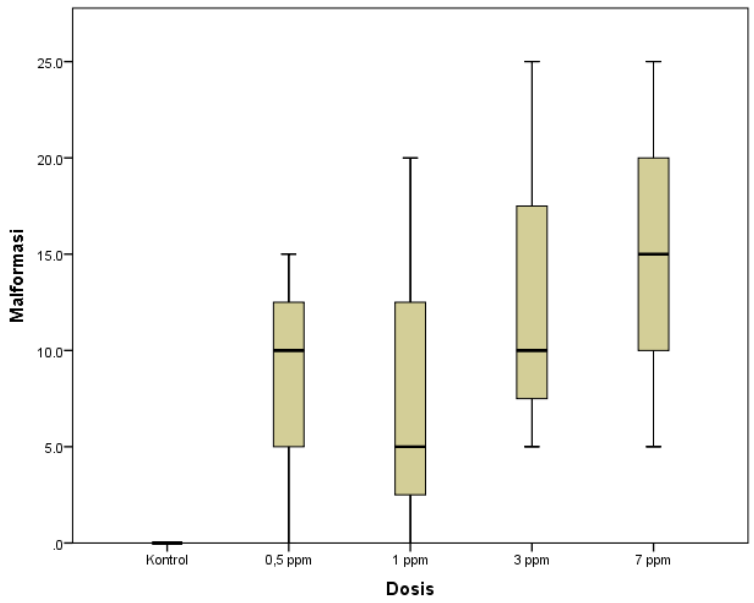

Figure 1. Boxplot distribution of the number of malformations in larvae of various doses of Dexamethasone. The center line indicates the median (%), the box indicates the interquartile range, and the whiskers indicate the distribution of the data. Descriptively, there is a tendency to increase malformations at higher doses, but the results of the ANOVA test showed no significant difference between groups ($p>0.05$).

The boxplot showed a tendency for malformations to increase with higher doses and greater exposure. However, the ANOVA results showed that the difference in

malformations between the treatment groups was not statistically significant ($P > 0.05$). Although dexamethasone exposure affects the physical development of *Danio rerio* larvae, variation in individual responses within each dose group is considerable. Exposure to dexamethasone does not uniformly cause defects in all larvae; its toxic effects persist at the cellular and systemic levels that underlie the organism's cardiometabolic function (Saettele et al., 2022). The results of this study showed that in the dose range of 0.05 ppm to 7 ppm, exposure to dexamethasone is more functionally toxic, affecting heart rate and hatching rate than teratogenetic – lethal, i.e., conditions in which a uniform massive physical record occurs (Heigwer et al., 2024).

The study results showing an increase in malformations include pericardial edema, which is closely related to the bradycardia data in Table 2. A significant decrease in heart rate reduces the efficiency of blood circulation in larvae. These findings are in line with research by Mitovic et al. (2025), which indicates that hemodynamic disorders in larvae result in decreased cardiac contractility, triggering fluid stasis in the pericardial cavity, which subsequently manifests as edema. In addition, the hatching delays are reported in Table 1. Prolonged larval time in the chorion, under limited space and low oxygen conditions, leads to abnormalities in spinal curvature (Li et al., 2022).

The P value > 0.05 in the malformation data of Figure 1, and the cardiometabolic significance of $P < 0.001$ indicate that the cardiac physiological system of *Danio rerio* larvae is much more sensitive to exposure to glucocorticoids compared to the morphological structure of the larvae outside (McNeil et al., 2016). These findings are in line with Santos et al. (2014), the Sub-lethal stress theory, as reported in research conducted by the University of Wisconsin, which states that disruptions in hormonal signaling pathways often impair internal functions (such as heart rate) long before the damage manifests as permanent physical disability. The variability shown by the boxplot, particularly the long whisker, indicates differences in the level of resilience or sensitivity of glucocorticoid receptors among embryonic individuals exposed to xenobiotic substances.

The results of microscopic observations of *Danio rerio* larvae in Figure 2 show specific manifestations of malformations induced by dexamethasone exposure. The control group showed normal development (ND) with a straight-looking spine axis (Normal Spine Axis / NSA) and a healthy heart structure. On the other hand, in the

treatment group, the most dominant malformations included swelling of the heart cavity area (Pericardial Edema/PE), edema in the yolk sac (Yolk Sac Edema/YSE), abnormalities in the curvature of the spine (Scoliosis/SC), and disturbances in blood circulation (Blood Circulation Disorder/BC).

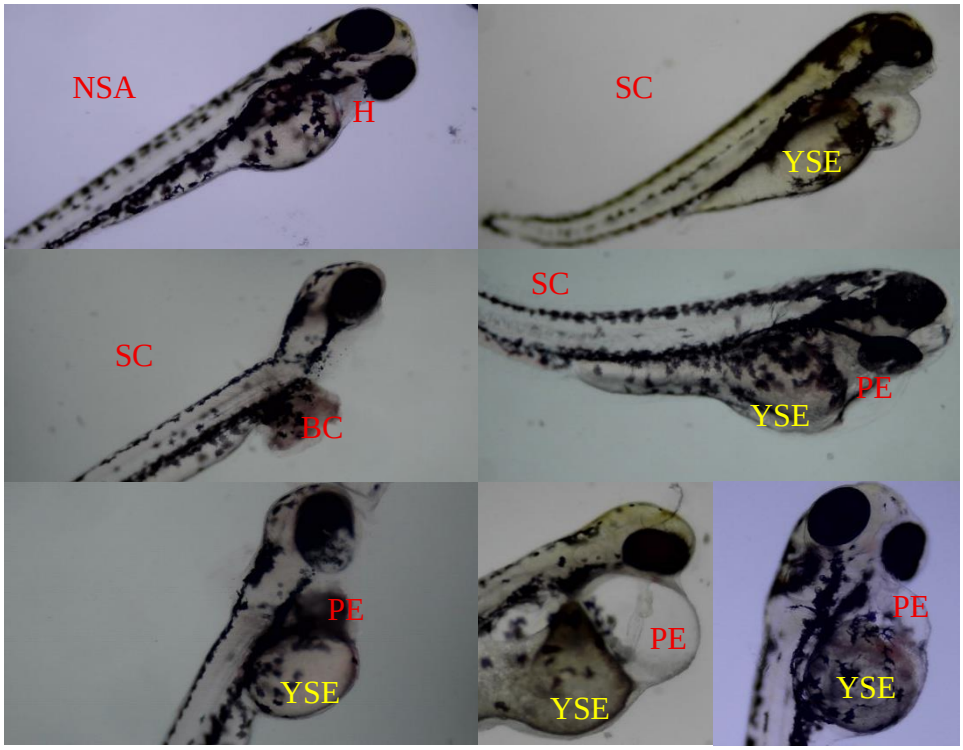

Figure 2. The results of microscopy observations of Zebrafish larvae (*Danio rerio*) at 96 hpf showed the presence of malformations of *Danio rerio* larvae at various dose levels of Dexamethasone exposure. (A) Control, (B-D) 3 ppm, (E-G) 7 ppm. PEP – Pericardial Edema, YSE – Yolk Sac Edema, SC – Scoliosis, H – Heart, ND – Normal Development, NSA – Normal Spine Axis, and BC – Blood Circulation Disorder.

The findings of morphological malformations in larvae are related to the previously discussed cardiometabolic dysfunction. The appearance of Pericardial Edema (PE) and Yolk Sac Edema (YSE) indicates the clinical manifestation of extreme bradycardia, as observed in Table 2, where the heart rate decreases drastically to 137.3 ± 5.6b at the highest dose of 7 ppm during the observation period of 72 hpf. Decreased heart pump function causes hemodynamic failure and accumulation of interstitial fluid in the body cavities of *Danio rerio* larvae (Gu et al., 2017). The results of this study are in line with previous research by Chen et al. (2015), which found that blood circulation disorders (BC) occur due to exposure to xenobiotic substances, leading to edema and an inability of the heart to maintain stable osmotic pressure.

Scoliosis (SC) is most likely due to an obstacle to hatching rate (Table 1). Based on the data, the larvae exposed to a dexamethasone dose of 7 ppm had a hatching rate of 65% at 48 hpf and 70% at 72 hpf. The condition of larvae confined in the chorion for a longer period, with limited space and exposed to continuous chronic hypoxia, can interfere with skeletal ossification and trigger bending of the spinal axis (Yang et al., 2011). Research indicates that mechanical stress and limited oxygen available to the embryo during the critical period of spinal development may be the main risk factors for the development of permanent scoliosis in larvae (Li et al., 2022). These morphological observations further confirm that dexamethasone exposure primarily disrupts physiological processes related to cardiovascular function and embryonic development, leading to secondary structural abnormalities in zebrafish larvae.

CONCLUSION

The results showed that exposure to dexamethasone during the embryonic phase and early larval stages of zebrafish does not cause lethal toxicity, but does cause physiological disturbances during early development. The findings indicate that larval survival remained high; however, dexamethasone inhibited the hatching process and decreased cardiometabolic function, as evidenced by bradycardia, in a dose- and time-dependent manner. These findings indicate that the toxic effects of synthetic glucocorticoids are more sub-lethal and functional, primarily through disruption of cardiovascular regulation during the critical period of organogenesis.

REFERENCES

Bauer, B., Mally, A., & Liedtke, D. (2021). Zebrafish embryos and larvae as alternative animal models for toxicity testing. In *International Journal of Molecular Sciences* (Vol. 22, Number 24). https://doi.org/10.3390/ijms222413417

Busquet, F., Strecker, R., Rawlings, J. M., Belanger, S. E., Braunbeck, T., Carr, G. J., Cenijn, P., Fochtman, P., Gourmelon, A., Hübler, N., Kleensang, A., Knöbel, M., Kussatz, C., Legler, J., Lillicrap, A., Martínez-Jerónimo, F., Polleichtner, C., Rzodeczko, H., Salinas, E., … Halder, M. (2014). OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. *Regulatory Toxicology and Pharmacology*, *69*(3), 496–511. https://doi.org/10.1016/j.yrtph.2014.05.018

Chahardehi, A. M., Arsad, H., & Lim, V. (2020). Zebrafish as a successful animal model for screening toxicity of medicinal plants. In *Plants* (Vol. 9, Number 10). https://doi.org/10.3390/plants9101345

Chen, M., Chen, S., Du, M., Tang, S., Chen, M., Wang, W., Yang, H., Chen, Q., & Chen, J. (2015). Toxic effect of palladium on embryonic development of zebrafish. *Aquatic Toxicology*, *159*. https://doi.org/10.1016/j.aquatox.2014.12.015

Di Paola, D., Abbate, J. M., Iaria, C., Cordaro, M., Crupi, R., Siracusa, R., D'amico, R., Fusco, R., Impellizzeri, D., Cuzzocrea, S., Spanò, N., Gugliandolo, E., & Peritore, A. F. (2022). RETRACTED: Environmental Risk Assessment of Dexamethasone Sodium Phosphate and Tocilizumab Mixture in Zebrafish Early Life Stage (*Danio rerio*). In *Toxics* (Vol. 10, Number 6). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/toxics10060279

Dinarello, A., Licciardello, G., Fontana, C. M., Tiso, N., Argenton, F., & Valle, L. D. (2020). Glucocorticoid receptor activities in the zebrafish model: A review. In *Journal of Endocrinology* (Vol. 247, Number 3). https://doi.org/10.1530/JOE-20-0173

Duan, Z., Duan, X., Zhao, S., Wang, X., Wang, J., Liu, Y., Peng, Y., Gong, Z., & Wang, L. (2020). Barrier function of zebrafish embryonic chorions against microplastics and nanoplastics and its impact on embryo development. *Journal of Hazardous Materials*, *395*. https://doi.org/10.1016/j.jhazmat.2020.122621

Gans, I. M., & Coffman, J. A. (2021). Glucocorticoid-Mediated Developmental Programming of Vertebrate Stress Responsivity. In *Frontiers in Physiology* (Vol. 12). https://doi.org/10.3389/fphys.2021.812195

Gerber, A. N., Newton, R., & Sasse, S. K. (2021). Repression of transcription by the glucocorticoid receptor: A parsimonious model for the genomics. *Journal of Biological Chemistry*, *296*. https://doi.org/10.1016/j.jbc.2021.100687

Gu, G., Na, Y., Chung, H., Seok, S. H., & Lee, H. Y. (2017). Zebrafish larvae model of dilated cardiomyopathy induced by terfenadine. *Korean Circulation Journal*, *47*(6). https://doi.org/10.4070/kcj.2017.0080

Hanim, R., Darusman, H. S., & Rahminiwati, M. (2018). Studi Karakteristik Tipe Diabetes pada Tikus (Rattus novergicus) yang Diinduksi Deksametason (Characteristic Study of the Type of Diabetes in Rat (*Rattus Novergicus*) Model Induced by Dexamethasone). *Jurnal Veteriner*, *19*(1). https://doi.org/10.19087/jveteriner.2018.19.1.1

Heigwer, J., Steenbergen, P. J., Gehrig, J., & Westhoff, J. H. (2024). Corticosteroids alter kidney development and increase glomerular filtration rate in larval zebrafish (*Danio rerio*). *Toxicological Sciences*, *201*(2), 216–225. https://doi.org/10.1093/toxsci/kfae085

Hong, T., Park, J., Park, H., An, G., Lee, H., Song, G., & Lim, W. (2024). Exposure to acifluorfen induces developmental toxicity in the early life stage of zebrafish. *Comparative Biochemistry and Physiology Part - C: Toxicology and*

Pharmacology, *281*. https://doi.org/10.1016/j.cbpc.2024.109909

Horby Peter, Lim Wei Shen, Emberson Jonathan R, Mafham Marion, Bell Jennifer L, Linsell Louise, Staplin Natalie, Brightling Christopher, Ustianowski Andrew, Elmahi Einas, Prudon Benjamin, Green Christopher, Feltob Timothy, Chadwick David, & Rege Kanchan. (2021). Dexamethasone in Hospitalized Patients with Covid-19. *New England Journal of Medicine*, *384*(8), 693–704. https://doi.org/10.1056/NEJMoa2021436

ndriyanti, N. (2020). Zebrafish (*Danio rerio*) Sebagai Model Hewan Coba pada Pengujian Aktivitas Obat. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *11*. https://doi.org/10.25026/mpc.v11i1.398

Khusna, V. D. Z. (2025). Perbandingan Metode Pemijahan Terhadap Fertilitas Embrio Ikan Zebra. *Journal of Comprehensive Science*, *4*(10). https://doi.org/10.59188/jcs.v4i10.3608

Lestari, K. S., Widiati, A. U., & Istiqomah, A. N. (2025). Pengaruh Toksisitas Akut Ekstrak Etanol Rimpang Pacing (*Costus speciosus*) pada Embrio Zebrafish (*Danio rerio*). In *Indonesian Journal of Herbal Science and Innovation*.

Li, X., Chen, C., He, M., Yu, L., Liu, R., Ma, C., Zhang, Y., Jia, J., Li, B., & Li, L. (2022). Lead Exposure Causes Spinal Curvature during Embryonic Development in Zebrafish. *International Journal of Molecular Sciences*, *23*(17). https://doi.org/10.3390/ijms23179571

Luo, M., Yi, Y., Huang, S., Dai, S., Xie, L., Liu, K., Zhang, S., Jiang, T., Wang, T., Yao, B., Wang, H., & Xu, D. (2023). Gestational dexamethasone exposure impacts hippocampal excitatory synaptic transmission and learning and memory function with transgenerational effects. *Acta Pharmaceutica Sinica B*, *13*(9). https://doi.org/10.1016/j.apsb.2023.05.013

McNeil, P. L., Nebot, C., & Sloman, K. A. (2016). Physiological and Behavioral Effects of Exposure to Environmentally Relevant Concentrations of Prednisolone during Zebrafish (Danio rerio) Embryogenesis. *Environmental Science and Technology*, *50*(10). https://doi.org/10.1021/acs.est.6b00276

Meador, J. P. (2021). The fish early-life stage sublethal toxicity syndrome – A high-dose baseline toxicity response. In *Environmental Pollution* (Vol. 291). https://doi.org/10.1016/j.envpol.2021.118201

Mitovic, N., Kovacevic, S., Ostojic, J. N., Puflovic, D., & Stankovic, M. S. (2025). Functional Cardiac Imaging in Zebrafish Embryos Using Standard Microscopy and Video Analysis: Applications in Environmental and Biomedical Research. *Journal of Visualized Experiments*, *2025-October*(224). https://doi.org/10.3791/68941

Nagasawa, T., Kawaguchi, M., Yano, T., Sano, K., Okabe, M., & Yasumasu, S. (2016). Evolutionary changes in the developmental origin of hatching gland cells in basal ray-finned fishes. *Zoological Science*, *33*(3). https://doi.org/10.2108/zs150183

Nesan, D., & Vijayan, M. M. (2016). Maternal cortisol mediates hypothalamus-pituitary-

interrenal axis development in Zebrafish. *Scientific Reports*, *6*. https://doi.org/10.1038/srep22582

Nurfakhrurajab, I., Elfita, L., & Fitriana, N. (2025). Studi Farmakologi Ikan Zebra sebagai Model Obesitas dan Hiperglikemia: Pengembangan Induksi Diet-Induced Obesity. *Pharmaceutical and Biomedical Sciences Journal*, *7*.

OECD. (2013). OECD Guidelines for the Testing of Chemicals nr 236: Fish Embryo Acute Toxicity (FET) Test. In *OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing* (Number July).

Permatasari, D. A. I., Kurniasri, N., & Mahardika, M. P. (2021). Qualitative and Quantitative Analysis of Dexamethasone in Rheumatic Pain Herbal Medicine Using Thin-Layer Chromatography (TLC) – Densitometry. *Journal of Fundamental and Applied Pharmaceutical Science*, *2*(1). https://doi.org/10.18196/jfaps.v2i1.12450

Ping, S., Lin, W., Ming, R., He, Y., Yin, Y., & Ren, Y. (2022). Toxic effects of four cardiovascular drugs on the development and epigenetics of zebrafish (*Danio rerio*). *Science of the Total Environment*, *846*. https://doi.org/10.1016/j.scitotenv.2022.157360

Ryu, B., Je, J. G., Jeon, Y. J., & Yang, H. W. (2021). Zebrafish model for studying dexamethasone-induced muscle atrophy and preventive effect of maca (*Lepidium meyenii*). *Cells*, *10*(11). https://doi.org/10.3390/cells10112879

Saettele, A. L., Wong, H. tung C., Kindt, K. S., Warchol, M. E., & Sheets, L. (2022). Prolonged Dexamethasone Exposure Enhances Zebrafish Lateral-Line Regeneration But Disrupts Mitochondrial Homeostasis and Hair Cell Function. *JARO - Journal of the Association for Research in Otolaryngology*, *23*(6). https://doi.org/10.1007/s10162-022-00875-x

Santos, D., Matos, M., & Coimbra, A. M. (2014). Developmental toxicity of endocrine disruptors in early life stages of zebrafish, a genetic and embryogenesis study. *Neurotoxicology and Teratology*, *46*. https://doi.org/10.1016/j.ntt.2014.08.002

Sarmah, S., & Marrs, J. A. (2016). Zebrafish as a vertebrate model system to evaluate effects of environmental toxicants on cardiac development and function. In *International Journal of Molecular Sciences* (Vol. 17, Number 12). https://doi.org/10.3390/ijms17122123

Sayed, A. E. D. H., Taher, H., Soliman, H. A. M., & Salah El-Din, A. E. D. (2022). Immunological and hemato-biochemical effects on catfish (*Clarias gariepinus*) exposed to dexamethasone. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.1018795

Shafei, R. A., Syrkasheva, A. G., Romanov, A. Y., Makarova, N. P., Dolgushina, N. V., & Semenova, M. L. (2017). Blastocyst hatching in humans. *Russian Journal of Developmental Biology*, *48*(1). https://doi.org/10.1134/S1062360417010106

Sharif, F., Steenbergen, P. J., Metz, J. R., & Champagne, D. L. (2015). Long-lasting

effects of dexamethasone on immune cells and wound healing in the zebrafish. *Wound Repair and Regeneration*, *23*(6). https://doi.org/10.1111/wrr.12366

Strähle, U., Scholz, S., Geisler, R., Greiner, P., Hollert, H., Rastegar, S., Schumacher, A., Selderslaghs, I., Weiss, C., Witters, H., & Braunbeck, T. (2012). Zebrafish embryos as an alternative to animal experiments-A commentary on the definition of the onset of protected life stages in animal welfare regulations. *ReproductiveToxicology*, *33*(2). https://doi.org/10.1016/j.reprotox.2011.06.121

Wang, C., Li, M., Gui, W., Shi, H., Wang, P., Chen, J., Fent, K., Zhang, K., Dai, J., Li, X., & Zhao, Y. (2023). Prednisolone Accelerates Embryonic Development of Zebrafish via Glucocorticoid Receptor Signaling at Low Concentrations. *Environmental Science and Technology*, *57*(42). https://doi.org/10.1021/acs.est.3c02658

Wang, W., Ye, Y., Liu, Y., Sun, H., Gao, C., Fu, X., & Li, T. (2024). Induction of oxidative stress and cardiac developmental toxicity in zebrafish embryos by arsenate at environmentally relevant concentrations. *Ecotoxicology and Environmental Safety*, *280*. https://doi.org/10.1016/j.ecoenv.2024.116529

Wilson, K. S., Baily, J., Tucker, C. S., Matrone, G., Vass, S., Moran, C., Chapman, K. E., Mullins, J. J., Kenyon, C., Hadoke, P. W. F., & Denvir, M. A. (2015). Early-life perturbations in glucocorticoid activity impacts on the structure, function and molecular composition of the adult zebrafish (*Danio rerio*) heart. *Molecular and Cellular Endocrinology*, *414*. https://doi.org/10.1016/j.mce.2015.07.025

Wisenden, B. D., Paulson, D. C., & Orr, M. (2022). Zebrafish embryos hatch early in response to chemical and mechanical indicators of predation risk, resulting in underdeveloped swimming ability of hatchling larvae. *Biology Open*, *11*(12). https://doi.org/10.1242/bio.059229

Wu, Y., Ye, X., Jiang, L., Wang, A., Wang, J., Yao, W., Qin, Y., & Wang, B. (2023). Developmental toxicity induced by brodifacoum in zebrafish (*Danio rerio*) early life stages. *Birth Defects Research*, *115*(3). https://doi.org/10.1002/bdr2.2118

Yang, D. C., Tsai, C. C., Liao, Y. F., Fu, H. C., Tsay, H. J., Huang, T. F., Chen, Y. H., & Hung, S. C. (2011). Twist controls skeletal development and dorsoventral patterning by regulating runx2 in zebrafish. *PLoS ONE*, *6*(11). https://doi.org/10.1371/journal.pone.0027324

Yin, G., Cao, L., Du, J., Jia, R., Kitazawa, T., Kubota, A., & Teraoka, H. (2017). Dexamethasone-induced hepatomegaly and steatosis in larval zebrafish. In *Journal of Toxicological Sciences* (Vol. 42, Number 4). https://doi.org/10.2131/jts.42.455