Commagene Journal of Biology

Özhan Turhan, (2022) *Comm. J. Biol.* 6(1), 62-67.
DOI: 10.31594/commagene.1081350

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Evaluation of Teratogenic and Developmental Toxicity of Everzol Red LFB and Everzol Yellow CGL on Zebrafish (*Danio rerio*) Embryos

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Received: 02.03.2022 **Accepted:** 19.04.2022 **Published online:** 20.06.2022 **Issue published:** 30.06.2022

Abstract: Synthetic dyes produced by chemical synthesis are used in textile, food, cosmetic, plastic, and pharmaceutical industries. Of these dyes, azo dyes are a major group of dyes widely used by these industries despite their harm to the environment. A limited number of studies have focused on the impact of dyes on the environment; thus eco-toxicological studies are imperative. The aim of this study was to evaluate the toxic effect of Everzol Red LFB (ERL) and Everzol Yellow CGL (EYC) on zebrafish embryos. Embryos were exposed to 23-600 mg L-1 ERL and EYC for 96 hours and the survival rates, heart rate, hatching rates, and body malformation of these individuals were determined. According to the results of this study, the 96 h LC50 values of the ERL and EYC were determined to be 292 (232-391) and 127 (107-152) mg L-1, respectively. The teratogenic index (TI) values of the ERL and EYC were calculated as 1.37 and 1.49, respectively. ERL and EYC are teratogenic for zebrafish embryos based on the calculated TI value. Both dyes caused significant inhibition of embryonic growth and heart rate in *Danio rerio* embryos. ERL and EYC caused pericardial edema, yolk sac edema, tail malformation, and spinal curvature in embryos. According to the determined parameters, the negative effect of EYC on zebrafish larvae is greater than of the ERL. These results indicate that both dyes have adverse effects on zebrafish development and may adversely affect the aquatic ecosystem if they enter the aquatic environment.

Keywords: Synthetic dye, embryotoxicity, development, pollution.

Everzol Red LFB ve Everzol Yellow CGL'nin Zebra Balığı (*Danio rerio*) Embriyoları Üzerinde Teratojenik ve Gelişimsel Toksisitesinin Değerlendirilmesi

Öz: Kimyasal sentezle üretilen sentetik boyalar; tekstil, gıda, kozmetik, plastik ve ilaç endüstrilerinde yaygın olarak kullanılmaktadır. Bu boyalardan biri olan azo boyalar, çevreye zararlı olmalarına rağmen yaygın olarak kullanılan en büyük boya grubudur. Sınırlı sayıda çalışma, boyaların çevre üzerindeki etkisine odaklanmıştır, bu nedenle ekotoksikolojik çalışmaların yapılması zorunludur. Bu çalışmanın amacı, zebra balığı embriyolarında Everzol Red LFB (ERL) ve Everzol Yellow CGL'nin (EYC) toksik etkilerini değerlendirmektir. Embriyolar 96 saat boyunca 23-600 mg L^{-1} ERL ve EYC'ye maruz bırakılmış ve bu bireylerin hayatta kalma oranları, kalp atım hızları, kuluçkadan çıkma oranları ve vücut malformasyonları belirlenmiştir. Çalışmanın sonuçlarına göre, ERL ve EYC'nin 96 saatlik LC_{50} değerleri sırasıyla 292 (232-391) ve 127 (107-152) mg L^{-1} olarak belirlenmiştir. ERL ve EYC teratojenik indeks (TI) değerleri sırasıyla 1.37 ve 1.49 olarak hesaplanmıştır. ERL ve EYC, hesaplanan TI değerine göre zebra balığı embriyoları için teratojeniktir. Her iki boya da *Danio rerio* embriyolarında, embriyonik büyümenin ve kalp hızının önemli ölçüde engellenmesine neden olmuştur. ERL ve EYC embriyolarda perikardiyal ödem, yolk kesesi ödemi, kuyruk malformasyonu ve spinal eğriliğe neden olmuştur. Belirlenen parametrelere göre EYC'nin zebra balığı larvaları üzerindeki olumsuz etkisi ERL'den daha fazladır. Bu sonuçlar, her iki boyanın da zebra balığı gelişimi üzerinde olumsuz etkilere neden olduğunu ve bu boyaların su ortamına girmesi halinde su ekosistemini olumsuz etkileyebileceğini göstermektedir.

Anahtar kelimeler: Sentetik boya, embriyo toksisite, gelişim, kirlilik.

1. Introduction

Synthetic dyes produced by chemical synthesis are widely used in textile, food, cosmetic, plastic, and pharmaceutical industries (Akbulut et al., 2020). Some of the most important of these dyes are azo dyes (Selvaraj et al., 2021; Kızıltan et al., 2022). Azo dyes are known to be toxic, genotoxic, and mutagenic (Demirci & Asma, 2013). In recent years, due to rapidly increasing industrialization, the pollution from dyes has greatly increased. Thousands of dyes are synthesized daily worldwide and subsequently released into the environment during synthesis and dyeing processes (Jadhav et al., 2011). Some textile dyes dissociate in water and the resulting substances can have toxic effects on aquatic organisms (Akbulut et al., 2020). Dyes that enter the water system can enter the food chain through aquatic organisms and accumulate, causing ecological instability (Natarajan & Manivasagan, 2020). Furthermore, some dyes that can be detected in many aquatic systems even at low concentration (1 mg L^{-1}) cause disturbances to aquatic ecosystems (Zamora & Jeronimo, 2019).

Recently, the zebrafish (*Danio rerio*) has been used to determine the toxicity of certain dyes. Zebrafish is a model organism commonly used in aquatic toxicology (Zamora & Jeronimo, 2019). The Organization for Economic Co-operation and Development (OECD) has suggested that *D. rerio* is an important indicator for the evaluation of pollutant toxicity in the aquatic environment (OECD, 2006). *D. rerio* are often used in experiments because of their low cost, small size, and easy laboratory care.

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Moreover *Danio rerio* embryos develop quickly after fertilization. As they are transparent, microscopic evaluation can be easily conducted at all stages of development (Akbulut & Yön, 2013). In the literature, the ecological effects of azo dyes have been reported in freshwater fish species (Abe et al., 2019).

The environmental impact and toxicity to aquatic biota caused by the discharge of textile dyes into water bodies are not fully documented (Zamora & Jeronimo, 2019). In addition, Abe et al. (2019) reported an urgent need to gather scientific information on the potential eco-toxicological effects of synthetic dyes on aquatic organisms. Everzol Red LFB and Everzol Yellow CGL are widely used commercial reactive dyes (Akbulut et al., 2020). There are deficiencies in the aquatic toxicity data on these dyesthat are likely to enter environmental waters due to their widespread and intensive use. Therefore, in this study, we aimed to determine the lethal concentrations and sublethal effects of these dyes in zebrafish, an aquatic vertebrate, based on the prediction that the concentration of these dyes would increase in environmental waters.

For this aim, different biological responses such as lethality, growth and development, heart rate, hatching rate, and teratogenicity status in zebrafish embryos were determined after 96 hours of exposure to ERL and EYC according to the Fish Embryo Acute Toxicity Test (OECD, 2013). Thus, the possible eco-toxicological effects of these dyes were evaluated based on the negative effects they have on zebrafish embryos.

2. Material and Methods

2.1. Test organism

The embryos used in this study were produced from adult zebrafish grown in the zebrafish production system (ZebTec Active Blue, Tecniplast, Italy) in the Zebra Fish Unit, Inonu University Faculty of Arts and Sciences, Aquatic Vertebrate Experimental Animals Unit. The system is equipped with continuous water circulation and adjustable pH (7.30±0.15) as well as conductivity (720±20 µS/cm), temperature (28.2±0.2°C), and automatic light and dark photoperiodic (14:10 hours) controls. Zebrafish embryos were obtained with the same aquatic characteristics as the parent system and a filtered rearing system (iSpawn, Tecniplast, Italy) connected directly to the water circulation fed by the parent system. Fertilized eggs were collected within 3 hours and stored in standard embryo broth at 28.5°C in an oven.

According to the legislation of the European Union and our country, Ethics Committee approval is not required for embryos and larvae used in the period up to the first 120 hours of their development after fertilization in studies with zebrafish (European Zebrafish Resource Center).

2.2. Chemicals and exposure

The reactive dyestuffs ERL (R4504502) and EYC (1500502) were selected for this study. Dyes were tested at concentrations of 23, 35, 53, 79, 119, 178, 267, 400, and 600 mg L^{-1}. Twenty-four embryos were exposed to each concentration. pH was adjusted to 7.3 and conductivity was maintained at 700 µS/cm. Then, 250 µl of each dye solution prepared at different concentrations and a zebrafish embryo were added to each well. Mortality rates of individuals examined with a stereomicroscope every 24 hours for a 96-hour period were recorded. At the 48^{th} hour, the heart beats per minute of the embryos were determined. At the end of the 96^{th} hour, the malformation rates and malformation types of the surviving individuals were determined with a stereomicroscope while their lengths were measured using Euromex Image Focus 4.0 software. The median lethal concentrations (LC_{50}) were determined after 24, 48, 72, and 96 hours of exposure.

2.3. Statistical analysis

Statistical analysis of the collected data was performed with GraphPad Prism 5 (SPSS Inc., USA). Probit regression analysis was used to determine the 96h mean lethal concentration (LC_{50}) and effective concentration (EC_{50}) for the embryos (EPA, ver. 1.5). The teratogenic index (TI) values of ERL and EYC were calculated as the ratio of 96h LC_{50} value to 96h EC_{50} value. Since all measurement parameters did not a show normal distribution, these non-parametric data were statistically compared with the Kruskal-Wallis and Dunn tests.

3. Results and Discussion

The intensive use of synthetic dyes and their transfer to the aquatic ecosystem cause significant pollution (Akbulut et al., 2020). In this study, the effects of two different textile dyes at different concentrations on the early development of *Danio rerio* embryos were determined. Parameters such as survival, hatching rate, heart rate, and malformation of zebrafish embryos were used to determine these effects.
Many textile dyes have LC_{50} concentrations at different time intervals in fish (Oliveira et al., 2016). According to the data of this study, the 96h LC_{50} values were 292 and 127 mg L^{-1} for ERL and EYC, respectively (Table 1). To our knowledge, the toxic concentrations have been determined for the first time in fish for the tested dyes. On the other hand, LC_{50} concentrations of some commercial dyes such as Basic Violet 14, Direct Red 28, and Reactive black 5 for *D. rerio* were found to be 0.06, 0.48 µg mL^{-1} and 0.52 mg L^{-1}, respectively (Shen et al., 2015; Manimaran et al., 2018). These stated LC_{50} values are relatively lower than the LC_{50} values of zebrafish treated with ERL and EYC in this study. In addition, in this study, the EC_{50} value in embryos exposed to ERL and EYC was calculated to be 213 and 85 mg L^{-1} (Table 1).

The ratio of the calculated LC_{50} value to the EC_{50} value is the teratogenicity value. The teratogenicity value is an important parameter used to determine the effects of pollutants on aquatic organisms. The suitability of using zebrafish embryos to evaluate the teratogenic potential of pollutants has been demonstrated by many studies (Selderslaghs et al., 2009; Alafiatayo et al., 2019; Turhan, 2021). In this study, the teratogenic index (TI) values of ERL and EYC for zebrafish larvae were calculated as 1.37 and 1.49, respectively. Selderslaghs et al. (2009) reported that a compound is considered teratogenic if the TI value for test compounds is >1. Accordingly, our findings showed that ERL and EYC were teratogenic for *Danio rerio* larvae.

In *Danio rerio*, hatching is an important phase during which embryos transform into larvae (Sun et al., 2020). The hatching rate in zebrafish embryos is one of the most important criteria used for the assessment of toxicity caused by environmental pollutants (Mu et al., 2016). In this study, the hatching rate decreased with increasing concentrations of dyes (Table 2). Similarly, Zamora and Jeronimo (2019) found that relatively higher concentrations of Direct Blue 15 (200 to 500 mg L^{-1}) decreased the hatching rate of exposed zebrafish embryos. Moreover, Manjunatha et al. (2020) reported that the hatching of *Danio rerio* embryos was proportionally affected by the concentration of henna dye. The reason for hatching inhibition may be the inability of the embryos to break the chorion under the influence of the contaminant or the inhibition of proteolytic enzymes involved in hatching (Zamora & Jeronimo, 2019; Rahman et al., 2020).

Body length is a general biomarker used to assess zebrafish health after exposure to pollutants (Lu et al., 2022). In this study, concentrations ERB and EYC higher than 35 mg L^{-1} resulted in significant inhibition of embryonic growth (Table 3). Peng et al. (2021) reported that growth inhibition, another biomarker used to evaluate the effects of the tested dyes in this study, is frequently observed in *Danio rerio* exposed to environmental pollutants. Similar to the results of this study, To et al. (2021) reported that Solvent Violet 47 and Disperse Blue 14 dyes cause growth inhibition depending on the concentration in zebrafish. The results of previous studies thus indicate that dyes have a negative effect on the early development of zebrafish.

Table 1. The 24h, 48h, 72h and 96h LC_{50}, 96h EC_{50}, and TI determined for *Danio rerio* embryos

Dyes	Concentrations (mg L^{-1})	Exposure (h)	LC_{50} (mg L^{-1})	EC_{50} (mg L^{-1})	TI (LC_{50}/EC_{50})
Everzol Red LFB	23-600	24	390 (320-511)		
		48	329 (267-431)		
		72	298 (240-393)		
		96	292 (232-391)	213 (167-300)	1.37
Everzol Yellow CGL	23-600	24	241 (200-297)		
		48	155 (131-184)		
		72	132 (112-156)		
		96	127 (107-152)	85 (73-100)	1.49

Table 2. Percent hatching in *Danio rerio* embryos exposed to different concentrations of Everzol Red LFB and Everzol Yellow CGL for 96h

Dyes	Everzol Red LFB				Everzol Yellow CGL			
	24h	48h	72h	96h	24h	48h	72h	96h
Control	0	100	100	100	0	100	100	100
23	0	100	100	100	0	100	100	100
35	0	92	100	100	0	96	96	96
53	0	92	92	92	0	79	79	79
79	0	83	92	92	0	79	88	83
119	0	71	88	88	0	42	58	58
178	0	46	71	71	0	38	46	46
267	0	42	54	54	0	38	25	25
400	0	13	42	42	0	-	-	-
600	0	0	21	21	0	0	-	-

Table 3. Lengths and number of individuals living in *Danio rerio* embryos exposed to Everzol Red LFB and Everzol Yellow CGL for 96h

	Everzol Red LFB					Everzol Yellow CGL				
Concentrations	*n*	Length (mm)				n	Length (mm)			
Control	24	3.754	±	0.062		24	3.754	±	0.062	
23	24	3.539	±	0.057		24	3.595	±	0.059	
35	23	3.386	±	0.066	**	22	3.427	±	0.055	***
53	22	3.298	±	0.058	***	18	3.421	±	0.070	***
79	22	3.280	±	0.052	***	20	3.296	±	0.075	***
119	20	3.065	±	0.132	***	13	2.872	±	0.131	***
178	16	3.021	±	0.126	***	10	2.861	±	0.138	***
267	13	3.005	±	0.133	***	6	2.586	±	0.186	***
400	10	3.013	±	0.169	***	-	-	-		
600	5	2.732	±	0.171	***	-	-	-		

n: Living individual, * p<0.05, ** p< 0.01, *** p< 0.001

Exposure of zebrafish embryos to pollutants can cause various mutations (Park et al., 2019). In this study, exposure to ERL and EYC caused to spinal curvature (SC), tail malformation (TM), pericardial edema (PE), and yolk sac edema (YSE) in zebrafish embryos (Tables 4-5, Figure 1). For both dyes, YSE and PE were the most common types of malformation (Tables 4-5). YSE is a common symptom in fish exposed to pollutants and may result from overhydration of the yolk sac associated with impaired osmoregulation and toxin accumulation (Park et al., 2019).

As PE was the main type of malformation caused by both tested dyes, heart rate was determined to analyze whether this change in pericardial morphology affected the cardiac function of the embryos. Heart rate is an important biomarker used in zebrafish toxicity tests to evaluate the effects of pollutants (Zhang et al., 2020). In this study, it was shown that zebrafish embryos exposed to ERL and EYC caused a significant dose-dependent decrease in heart rate at 48 hours (Figure 2). Tsuruwaka et al. (2015) showed that *Danio rerio* embryos exhibiting PE also demonstrate an abnormal intracellular Ca^{2+} signaling pattern.

4. Conclusion

The aquatic ecosystem is an important target of pollutants from industrial wastes (Meireles et al., 2018). The increasing use of textile dyes, which is an important source of industrial waste, and the increasing frequency of detection in the aquatic system necessitate the evaluation of their effects on aquatic organisms. This study showed that two dyes, ERL and EYC which are widely used in the textile industry, cause dose-dependent developmental toxicity in *Danio rerio* embryos. Biomarkers such as YSE, PE, TD, and SC were assessed in this study and the TI values calculated in zebrafish embryos showed that these dyes are toxic and teratogenic. The effects of the tested dyes on the aquatic ecosystem have been revealed in this study from various aspects but the mechanisms of the toxic effects caused by dyes should be evaluated at the biochemical and molecular level with new studies.

Table 4. Time-dependent mortality, malformation number, and malformation types of *Danio rerio* embryos exposed to various concentrations of Everzol Red LFB for 96 hours

Concentrations (mg L^{-1})	*n*	Living #	Malformed #	Malformations Types			
				SC	TM	PE	YSE
Control	24	24	0				
23	24	24	0				
35	24	23	1	-	-	1	-
53	24	22	0	-	-	-	-
79	24	22	3	2	1	-	1
119	24	20	5	1	1	3	1
178	24	16	7	1	2	5	6
267	24	13	11	4	2	8	3
400	24	10	7	2	4	6	2
600	24	5	3	-	2	2	2

SC: spinal curvature; TM: tail malformation; PE: pericardial edema; YSE: yolk sac edema

Table 5. Time-dependent mortality, malformation number, and malformation types of *Danio rerio* embryos exposed to various concentrations of Everzol Yellow CGL for 96 hours

Concentrations (mg L^{-1})	*n*	Living #	Malformed #	Malformations Types			
				SC	TM	PE	YSE
Control	24	24	0				
23	24	24	0				
35	24	22	0	-	-	-	-
53	24	18	1	-	1	1	-
79	24	20	12	1	1	10	5
119	24	13	10	1	1	9	3
178	24	10	9	2	2	6	2
267	24	6	6	1	1	4	3
400	24	0	-				
600	24	0	-				

SC: spinal curvature; TM: tail malformation; PE: pericardial edema; YSE: yolk sac edema

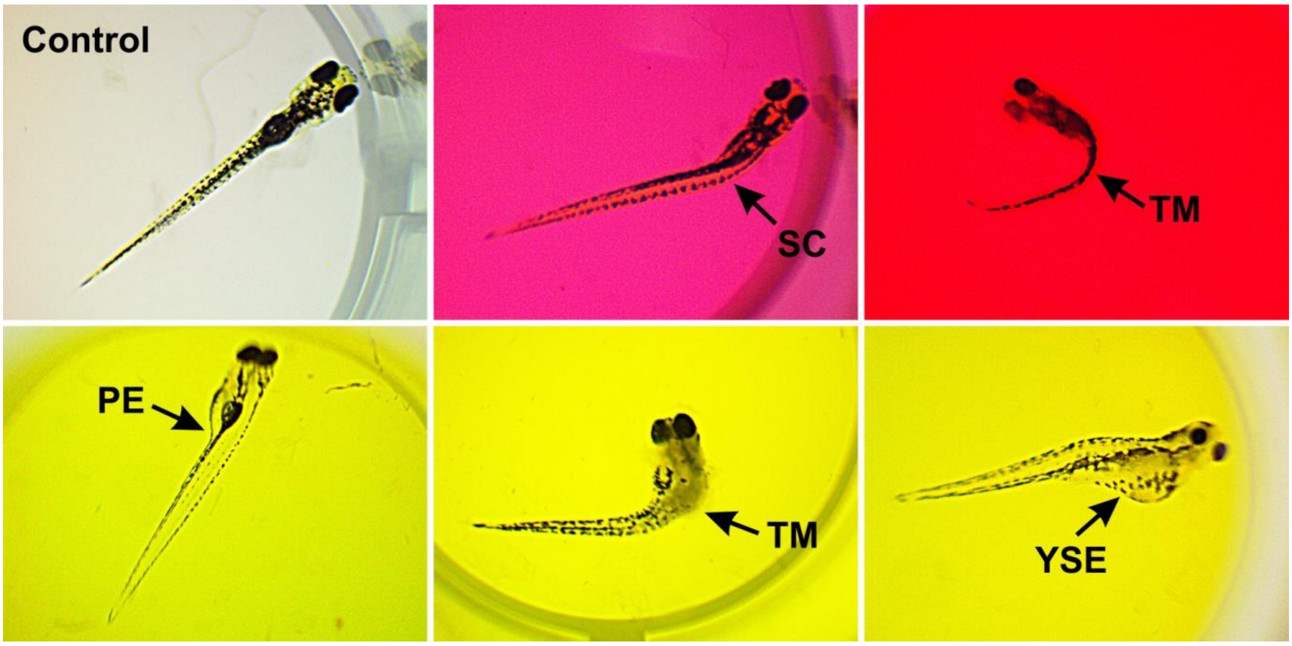

Figure 1. Microscopic images of embryos. Control embryos and Everzol Red LFB and Everzol Yellow CGL treatment groups 96h (SC: spinal curvature; TM: tail malformation; PE: pericardial edema; YSE: yolk sac edema)

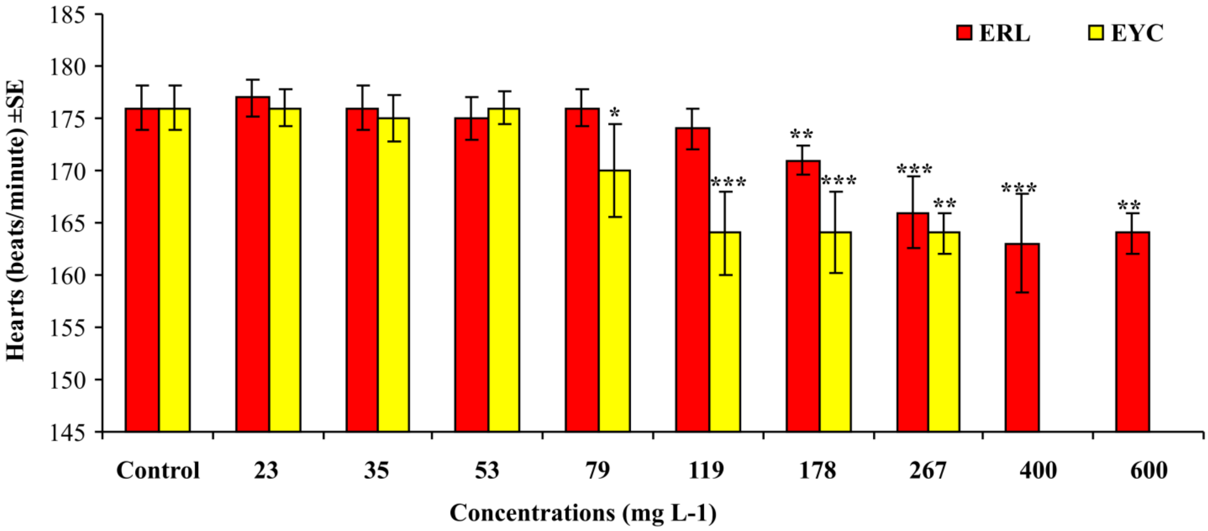

Figure 2. Heart rate per minute at 48h in *Danio rerio* embryos exposed to different concentrations of Everzol Red LFB and Everzol Yellow CGL

Acknowledgments: The author would like to thank Prof. Dr. Murat ÖZMEN for the laboratory facilities provided, Prof. Dr. Abbas GÜNGÖRDÜ for their support at every stage of the study and article writing, and Nazan BATALOĞLU for the technical support in the laboratory studies.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

References

Abe, F.R, Machado, A.L., Soares, A.M.V.M., Oliveira, D.P., & Pestana, J.L.T. (2019). Life history and behavior effects of synthetic and natural dyes on *Daphnia magna*. *Chemosphere*, 236, 124390. https://doi.org/10.1016/j.chemosphere.2019.124390

Akbulut, G.B.A., Turhan, D.Ö., & Yiğit, E. (2020). Alleviation of Everzol Red LFB Toxicity in Duckweed (*Lemna minor* L.) by Exogenous Salicylic Acid. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 23(4), 876-884. https://doi.10.18016/ksutarimdoga.vi.683962

Akbulut, C., &Yön, N.D. (2013). Bisfenol a'nın zebra balıklarında (*Danio rerio*) teratolojik etkileri. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 17, 105-111.

Alafiatayo, A.A., Lai, K.S., Syahida, A., Mahmood, M., & Shaharuddin, N.A. (2019). Pytochemical evaluation, embryotoxicity, and teratogenic effects of *Curcuma longa* extract on Zebrafish (*Danio rerio*). *Evidence-Based Complementary and Alternative Medicine*, 3807207, 10. https://doi.org/10.1155/2019/3807207

Demirci, Ö., & Asma, D.H. (2013). Antioxidant responses in *Phanerochaete chrysosporium* exposed to Astrazone Red FBL textile dye. *Cell Biochemistry & Function*, 31, 86-90. https://doi.org/10.1002/cbf.2865

European Zebrafish Resource Centre (EZRC). Karlsruhe Institute of Technology (KIT) Heidelberg University, 109. Retrieved from https://www.ezrc.kit.edu , Date of Access: 21/12/2021

Jadhav, S.B., Phugare S.S., Patil, P.S., & Jadhav, J.P. (2011). Biochemical degradation pathway of textile dye Remazol Red and subsequent toxicological evaluation by cytotoxicity, genotoxicity and oxidative stress studies. *International Biodeterioration & Biodegradation*, 65 (6), 733-743. https://doi.org/10.1016/j.ibiod.2011.04.003

Kızıltan, T., Baran, A., Kankaynar, M., Şenol, O., Sulukan, E., Yıldırım, S., & Ceyhun, S.B. (2022). Efects of the food colorant carmoisine on zebrafsh embryos at a wide range of concentrations. *Archives of Toxicology*, 96, 1089–1099. https://doi.org/10.1007/s00204-022-03240-2

Lu, J., Wu, J., Gong, L., Cheng, Y., Yuan, Q., & He, Y. (2022). Combined toxicity of polystyrene microplastics and sulfamethoxazole on Zebrafish embryos. *Environmental Science and Pollution Research*, 29, 19273-19282. https://doi.org/10.1007/s11356-021-17198-8

Manimaran D., Sulthana, A., & Elangovan, N. (2018). Reactive black 5 induced developmental defects via potentiating apoptotic cell death in Zebrafish (*Danio rerio*) embryos. *Pharmacy & Pharmacology International Journal,* 6(6), 449-452. https://doi.org/10.15406/ppij.2018.06.00216

Manjunatha, B., Han, L., Kundapur, R.R., Liu, K., & Lee, S.J. (2020). Herbul black henna (hair dye) causes cardiovascular defects in Zebrafish (*Danio rerio*) embryo model. *Environmental Science and Pollution Research*, 27, 14150–14159. https://doi.org/10.1007/s11356-020-07762-z

Meireles, G., Daam, M.A., Sanches, A.L.M., Zanoni, M.V.B., Soares, A.M.V.M., Gravato, C., & Oliveira, D.P., (2018). Red disperse dyes (DR 60, DR 73 and DR 78) at environmentally realistic concentrations impact biochemical profile of early life stages of zebrafish (*Danio rerio*). *Chemico-Biological Interactions*, 292, 94-100. https://doi.org/10.1016/j.cbi.2018.07.007

Mu, X., Chai, T., Wang, K., Zhu, L., Huang, Y., Shen, G.,… & Wang, C. (2016). The developmental effect of difenoconazole on Zebrafish embryos: A mechanism research. *Environmental Pollution,* 212, 18-26. https://doi.org/10.1016/j.envpol.2016.01.035

Natarajan, R. & Manivasagan, R. (2020). Effect of operating parameters on dye wastewater treatment using *Prosopis cineraria* and kinetic modeling. *Environmental Engineering Research,* 25(5), 788-793. https://doi.org/10.4491/eer.2019.308

OECD, (2006). Draft proposal for a new guideline. Fish Embryo Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals (1st Version), May 30

OECD, (2013). Guıdelınes for the testıng of chemıcals. Fish Embryo Acute Toxicity (FET) Test. 236, July 26

Oliveira, G.A.R., Lapuente, J., Teixido, E., Porredon, C., Borras, M., & Oliveira, D.P. (2016). Textile dyes induce toxicity on zebrafish early life stages. *Environmental Toxicology and Chemistry*, 35(2), 429-434. https://doi.org/10.1002/etc.3202

Park, H., Lee, J.Y., Park, S., Song, G., & Lim, W. (2019). Developmental toxicity and angiogenic defects of etoxazole exposed Zebrafish (*Danio rerio*) larvae. *Aquatic Toxicology*, 217, 105324. https://doi.org/10.1016/j.aquatox.2019.105324

Peng, Y., Li, M., Huang, Y., Cheng, B., Cao, Z., Liao, X., Xiong, G., Liu, F., Hu, C., & Lu, H. (2021). Bifenazate induces developmental and immunotoxicity in Zebrafish. *Chemosphere*, 271, 129457. https://doi.org/10.1016/j.chemosphere.2020.129457

Rahman, M.S., Islam, S.M.M., Haque, A., & Shahjahan, M. (2020). Toxicity of the organophosphate insecticide sumithion to embryo and larvae of zebrafish. *Toxicology Reports*, 7, 317-323. https://doi.org/10.1016/j.toxrep.2020.02.004

Selderslaghs, I.W.T., Rompay, A.R.V., Coen, W., & Witters, H.E. (2009). Development of a screening assay to identify teratogenic and embryo toxic chemicals using the Zebrafish embryo. *Reproductive Toxicology*, 28, 308-320. https://doi.org/10.1016/j.reprotox.2009.05.004

Selvaraj, V., Karthika, T.S., Mansiya, C., & Alagar, M. (2021). An over review on recently developed techniques, mechanisms and intermediate involved in the advanced azo dye degradation for industrial applications. *Journal of Molecular Structure,* 1224, 129195. https://doi.org/10.1016/j.molstruc.2020.129195

Shen, B., Liu H.C., Ou, W.B., Eilers, G., Zhou, S.M., Meng, F.G., Li, C.Q., & Li, Y.Q. (2015). Toxicity induced by Basic Violet 14, Direct Red 28 and Acid Red 26 in Zebrafish larvae. *Journal of Applied Toxicology*, 35(12), 1473–1480. https://doi.org/10.1002/jat.3134

Sun, Y., Cao, Y., Tong, L., Tao, F., Wang, X, Wu, H., & Wang, M. (2020). Exposure to prothioconazole induces developmental toxicity andcardiovascular effects on zebrafish embryo. *Chemosphere*, 251, 126418. https://doi.org/10.1016/j.chemosphere.2020.126418

To, K.T., Mary, L.S., Wooley, A.H., Wilbanks, M.S., Bednar, A.J., Perkins, E.J., Truong, L…., & Reyero, N.G. (2021). Morphological and behavioral effects in zebrafish embryos after exposure to smoke dyes. *Toxics*, 9(1), 9. https://doi.org/10.3390/toxics9010009

Tsuruwaka, Y., Konishi, M., & Shimada, E. (2015). Loss of wwox expression in zebrafish embryos causes edema and alters Ca^{2+} Dynamics. *PeerJ Life and Environment,* 3, 727. https://doi.org/10.7717/peerj.727

Turhan, D.Ö. (2021). Evaluation of teratogenic and toxic effects of enrofloxacin-based antibiotic on Zebrafish (*Danio rerio*) larvae with biochemical and developmental markers. *Chemistry and Ecology*, 37(8), 704-714. https://doi.org/10.1080/02757540.2021.1974007

Zamora, M.H., & Jeronimo, F.M. (2019). Exposure to the azo dye direct blue 15 produces toxic effects on microalgae, Cladocerans, and zebrafish embryos. *Ecotoxicology*, 28, 890-902. https://doi.org/10.1007/s10646-019-02087-1

Zhang C, Zhang J, Zhu L, Du Z, Wang J, Wang J, Li B, &Yang, Y. (2020). Fluoxastrobin-induced effects on acute toxicity, development toxicity, oxidative stress, and DNA damage in *Danio rerio* embryos. *Science of the Total Environment*, 715, 137069. https://doi.org/10.1016/j.scitotenv.2020.137069