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The effect of sunset yellow food coloring (E110) on the zebrafish (*Danio rerio*) embryos development

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ABSTRACT

Synthetic food coloring sunset yellow (E110) is widely used due to its color stability and low production cost; however, it may pose toxic effects during the early developmental stages of aquatic organisms. This study aimed to evaluate the embryotoxicity of sunset yellow in zebrafish (*Danio rerio*) embryos, determine the median lethal concentration (LC_{50}) as an indicator of acute toxicity, and analyze its effects on embryonic development, with a particular focus on somite formation. The study employed a completely randomized design (CRD) using the fish embryo toxicity test, with one control group and three treatment groups exposed to sunset yellow at 200 ppm, 400 ppm, and 800 ppm. Observed parameters included survival rate, hatching rate, heart rate, somite development, and morphological alterations in the embryo. Data were analyzed using One-Way ANOVA and Two-Way ANOVA with IBM SPSS software at a significance level of $\alpha = 0.05$. The results showed that the LC_{50} value of Sunset yellow for zebrafish embryos was 416.67 ppm. Exposure to sunset yellow significantly decreased survival rate and hatching rate ($p = 0.004$ and $p = 0.033$, respectively) and significantly affected embryonic heart rate based on concentration and exposure duration ($p < 0.001$). Disruption of somite development was primarily observed during early embryogenesis, characterized by indistinct somite boundaries at higher concentrations. Additionally, concentration- dependent morphological abnormalities were observed, including shortened body length, delayed hatching, and increased body curvature. These findings suggest that Sunset yellow exhibits embryotoxic effects in zebrafish and may pose an ecological risk to aquatic organisms.

Keywords: *Embryotoxicity; LC50; somite development; sunset yellow; zebrafish*

INTRODUCTION

Synthetic dyes among the most widely used food additives in the food, pharmaceutical, and cosmetic Synthetic dyes are among the most widely used food additives in the food, pharmaceutical, and cosmetic industries. These compounds are used for sunset yellow due to their high stability, visual appeal, and relatively low cost (Sunu, 2018). One of the most frequently used synthetic dyes in food products is sunset yellow FCF (E110), a member of the azo dye group (Jiang et al., 2020). This substance imparts a bright orange color to various products, such as soft drinks, candy, cakes, and fast food (Sunu, 2018). Various studies have shown that excessive consumption of unset yellow can have negative health impacts, ranging from allergic reactions and hyperactivity in children to indications of genotoxic and toxic effects on specific organs (Rarassari et al., 2023).

In toxicology, attention is focused not only on the effects of a substance on adult organisms but also on its effects on the early stages of development, namely the embryo. The embryonic phase is a critical period that is highly vulnerable to environmental influences and chemicals (Khosim et al., 2023). Exposure to hazardous substances at this stage can cause embryotoxicity, which is characterized by embryonic death, developmental delays, malformations, and impaired function of basic organs such as the heart (Jiang et al., 2020). Therefore, testing for toxic effects in the embryonic phase is important as part of the safety evaluation of any chemical substance, including synthetic food coloring.

Zebrafish (*Danio rerio*) are widely used as a model organism in developmental toxicology studies due to several advantages, including transparent embryos that facilitate developmental observation, rapid development, and high genetic homology with humans (Khosim et al., 2023). Furthermore, zebrafish have been recommended as a standard test organism in the Fish Embryo Toxicity (FET) Test by the Organization for Economic Co-operation and Development (OECD) to assess the toxicity of various chemical compounds (Scholz et al., 2008). Several studies have reported that exposure to Sunset yellow in zebrafish embryos can cause various developmental disorders, including pericardial and yolk sac edema, spinal curvature, decreased body size, and increased embryonic mortality.

The reported LC_{50} and EC_{50} values indicate that this dye has toxic potential at specific concentrations and has even shown a teratogenic index, suggesting its ability to disrupt vertebrate embryonic development (Barciela et al., 2023).

Furthermore, the reported LC_{50} and EC_{50} values indicate that this dye has toxic potential at specific

concentrations. Other studies have also shown that Sunset yellow has a teratogenic index, indicating its ability to disrupt vertebrate embryonic development (Jiang et al., 2020). Somitogenesis is an important parameter used in developmental toxicity tests on zebrafish embryos. This process is the stage of somite formation, namely, the formation of mesodermal segments that will later differentiate into muscle, spine, and connective tissue. Because it occurs during a specific, and highly regulated period, somitogenesis is often used as a sensitive indicator of developmental disorders resulting from chemical exposure (Kimmel et al., 1995). Therefore, observing somitogenesis can be a sensitive indicator in assessing the embryotoxic effects of a compound.

Several studies have reported toxic effects of Sunset yellow on zebrafish embryos; however, studies specifically focusing on its effects on somitogenesis remain limited. The novelty of this study lies in its focus on analyzing zebrafish embryo development disorders directly linked to OECD FET parameters and in detailed observations of somite formation. This study aims to evaluate the embryotoxicity of sunset yellow (E110) on zebrafish (*Danio rerio*) embryos, determine the LC_{50}/EC_{50} values as indicators of acute toxicity, and analyze its effects on embryo somitogenesis.

METHODS

Tools and Materials

The tools used in this study included a spawning aquarium, a broodstock maintenance aquarium, an aerator, a digital thermometer, a pH meter, a dropper, a micropipette, a petri dish, a stereo microscope, a stopwatch, and a 24- well plate. The materials used included: zebrafish (*Danio rerio*) broodstock, E3 media solution, sunset yellow food coloring, aerated and filtered well water, and artemia as a food source for broodstock maintenance.

Instruments

The instruments used in this study included a stereomicroscope to observe the morphological development of zebrafish (*Danio rerio*) embryos, including embryo coagulation, somite formation, tail detachment, and other morphological abnormalities. The stereomicroscope was also used to observe embryonic heart rate during the early larval stage. Heart rate was measured directly by counting heartbeats per minute with a stopwatch, with each embryo observed individually to obtain accurate heart rate values. Embryo exposure was conducted using a 24-well plate system to ensure uniform exposure conditions and minimize variability among embryos. Mortality data and embryonic developmental parameters were quantitatively analyzed to determine the LC_{50} and EC_{50} values as indicators of the toxicity level of Sunset yellow (E110). Statistical analyses were performed in Jamovi (R-based) using appropriate tests at a significance level of 5% ($\alpha = 0.05$).

Experimental Procedures

This study began with the spawning of zebrafish (*Danio rerio*) broodstock to obtain fertilized embryos. Normal embryos at early developmental stages were selected and cleaned, then randomly distributed into treatment containers. The embryos were then exposed to sunset yellow (E110) at various concentrations, while the control group was maintained in a medium without the test substance. All embryos were incubated at temperatures and lighting conditions suitable for normal zebrafish development. Observations were carried out at regular intervals throughout the developmental time of zebrafish embryos, from the segmentation phase through hatching. Toxicity parameters observed included embryo coagulation, somite formation, tail release, hatching, and embryonic heart rate. Mortality and developmental abnormalities data were recorded to calculate the survival rate. Heart rate frequency was measured in the early larval phase using a stereo microscope by counting beats per minute. The data obtained were analyzed quantitatively to determine the LC_{50} and EC_{50} values of sunset yellow in zebrafish embryos. The analysis was carried out based on the relationship between the test substance concentration and the observed toxic response. The analysis results were further interpreted to assess the level of sunset yellow embryotoxicity.

RESULT AND DISCUSSION

Survival Rate

Exposure of zebrafish (*Danio rerio*) embryos to sunset yellow (E110) induced a toxic response that depended on both concentration and exposure time. Based on preliminary toxicity tests, the LC_{50} value of sunset yellow in zebrafish embryos was determined to be 416.67 ppm. This value was used as a reference for selecting the test. Concentrations in the main experiment. Accordingly, concentrations of 200 ppm and 400 ppm were used to represent sublethal exposure levels, while 800 ppm was included to evaluate the effects of higher, potentially lethal exposure on embryo development.

Table 1. Descriptive statistics of survival rate parameters.

Treatment	N	Mean ± SD
Control	3	66.7 ± 10.4
200 ppm	3	80.0 ± 13.2
400 ppm	3	65.0 ± 5.00
800 ppm	3	18.3 ± 7.64

Based on Table 1, descriptive statistical analysis showed that the survival rate of zebrafish embryos varied among treatment groups. The control group exhibited a survival rate of 66.7 ± 10.4%, whereas exposure to 200 ppm sunset yellow resulted in a higher survival rate of 80.0 ± 13.2%. In contrast, the survival rate decreased to 65.0 ± 5.0% at 400 ppm. A marked reduction was observed at 800 ppm, with survival declining sharply to 18.3 ± 7.64%, indicating a pronounced toxic effect at higher concentrations.

Table 2. Results of statistical analysis of zebrafish embryo survival rate.

Treatment	F	df1	df2	P value
Survival rate	24.2	3	4.19	0.004

One-way ANOVA results based on Table 2 indicated that the treatments had a significant effect on the survival rate of zebrafish embryos ($F = 24.2$; $p = 0.004$). Therefore, post hoc tests were subsequently performed to identify significant differences among treatment groups.

Table 3. Tukey's p-value of the zebrafish embryo survival rate.

Treatment	Control	200 ppm	400 ppm	800 ppm
Control	-	0.381	0.996	0.001*
200 ppm	0.381	-	0.293	<0.01*
400 ppm	0.996	0.293	-	0.002*
800 ppm	0.001*	<0.01*	0.002*	-

(*) indicates a significant difference

Tukey's post hoc test results presented in Table 3 indicated that the 800 ppm treatment differed significantly from the control ($p = 0.001$), 200 ppm ($p < 0.001$), and 400 ppm ($p = 0.002$). In contrast, no significant differences were observed between the control and 200 ppm ($p = 0.381$), the control and 400 ppm ($p = 0.996$), or between the 200 ppm and 400 ppm treatments ($p = 0.293$). These findings indicate that the decrease in zebrafish embryo survival rate occurred primarily at the 800 ppm concentration, whereas low to moderate concentrations did not result in statistically significant differences compared to the control.

In addition to Sunset yellow exposure, environmental conditions during incubation also play an important role in determining embryo survival. Previous studies have shown that incubation temperatures outside the optimal range can significantly increase embryo mortality by disrupting physiological processes during development (Al- Zghoul & El-Bahr, 2019). Moreover, thermal stress from excessively high or low temperatures can disrupt embryonic physiological homeostasis and increase the risk of mortality (Lockwood et al., 2017).

Statistical analysis using one-way ANOVA demonstrated that sunset yellow treatment had a significant effect on zebrafish embryo survival ($p = 0.004$). The high mortality observed at elevated concentrations suggests that sunset yellow exerts acute toxic effects on embryos, particularly during later stages of development when vital organ systems become functionally active. This finding is consistent with previous studies reporting that zebrafish embryos are highly susceptible to exposure to synthetic chemical compounds during the organogenesis period (Oetelaar et al., 2025).

Hatching Rate

Table 4. Descriptive statistical results of hatching rate parameters.

Treatment	N	Mean ± SD
Control	3	56.7 ± 20.2
200 ppm	3	64.3 ± 9.02
400 ppm	3	60.0 ± 5.00
800 ppm	3	33.3 ± 7.64

In addition to affecting survival, sunset yellow exposure also influenced the hatching rate of zebrafish embryos, as presented in Table 4. The control group exhibited a hatching rate of 56.7 ± 20.2%, whereas embryos exposed to 200 ppm showed a higher hatching rate of 64.3 ± 9.02%. At 400 ppm, the hatching rate was recorded at 60.0 ± 5.00%. In contrast, a marked reduction was observed at 800 ppm, with the hatching rate decreasing significantly to 33.3 ± 7.64%, indicating disruption of the hatching process at high concentrations.

Table 5. Results of statistical analysis of zebrafish embryo hatching rate.

Treatment	F	df1	df2	P value
Hatching rate	7.88	3	4.29	0.033

Statistical analysis based on Table 5 indicated that exposure to sunset yellow at different concentrations had a significant effect on the hatching rate of zebrafish embryos. One-way ANOVA yielded an F value of 7.88 ($df_1 = 3$, $df_2 = 4.29$) with a p value of 0.033 ($p < 0.05$), demonstrating significant differences among the control, 200 ppm, 400 ppm, and 800 ppm treatment groups.

Table 6. Tukey's p-value of the zebrafish embryo hatching rate.

Treatment	Control	200 ppm	400 ppm	800 ppm
Control	-	0.361	0.361	0.190
200 ppm	0.361	-	1.000	0.017*
400 ppm	0.361	1.000	-	0.017*
800 ppm	0.190	0.017*	0.017*	-

(*) indicates a significant difference

Tukey's post hoc test results, presented in Table 6, showed differences in the hatching rate responses of zebrafish embryos among sunset yellow concentration treatments. The comparison between the control and 200 ppm treatments yielded a p-value of 0.361, indicating no statistically significant difference. These results suggest that exposure to sunset yellow at a concentration of 200 ppm did not exert a significant toxic effect on the embryo hatching process.

Comparisons between the 400 ppm and 800 ppm treatments and the control and 200 ppm groups revealed significant differences ($p = 0.017$; $p < 0.05$). In addition, a significant difference was also observed between the 400 ppm and 800 ppm treatments ($p = 0.017$). These results indicate that increasing concentrations of sunset yellow were associated with a progressive decrease in the hatching rate of zebrafish embryos.

The reduction in hatching rate at higher concentrations suggests that sunset yellow has the potential to inhibit the hatching process of zebrafish embryos. Successful hatching depends on the structural integrity of the embryo, the activity of the chorionase enzyme that softens the chorion, and the coordinated function of the nervous and muscular systems during eclosion (Sano et al., 2008). Exposure to toxic compounds at high concentrations may disrupt one or more of these processes, leading to delayed or failed embryo hatching (Huang et al., 2018).

Mechanistically, synthetic azo dyes such as sunset yellow are known to induce oxidative stress and reduce embryonic metabolic activity. Reduced energy metabolism may inhibit chorionase production and activity, thereby impairing optimal chorion softening. Additionally, disrupted development of the embryo's nervous and muscular systems can compromise the contractions required for hatching (Small et al., 2020). These mechanisms likely explain the low hatching rates observed at high sunset yellow concentrations.

At a concentration of 200 ppm, the embryo hatching rate was slightly higher than that of the control group, suggesting that low concentrations of sunset yellow do not exceed the physiological tolerance threshold of zebrafish embryos. At this stage, embryos retain an initial adaptive capacity, allowing for a relatively normal hatching process. However, increasing sunset yellow concentrations led to a reduction in hatching rates, indicating a clear dose- response relationship between exposure and hatching success. This observation is consistent with previous studies reporting that exposure to synthetic food additives can reduce fish embryo hatchability through concentration- dependent toxic mechanisms (Jiang et al., 2020).

Heart Rate

Table 7. Two-way ANOVA test results on zebrafish embryo heart rate.

Treatment	df	F	400 ppm	800 ppm
Concentration	3	8.25	<0.001*	0.540
Time	1	209.44	<0.001*	0.422
Concentration x time	3	1.01	0.416	0.006
Residuals	16			

(*) indicates a significant difference

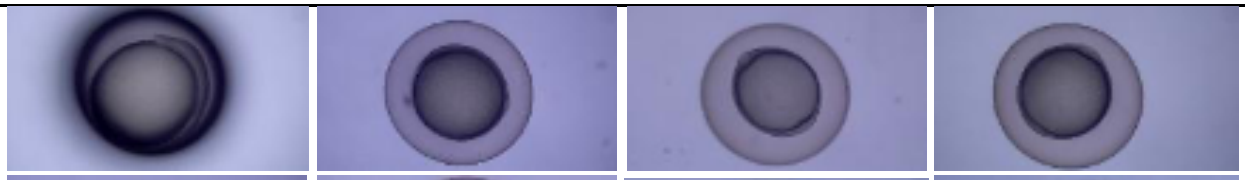
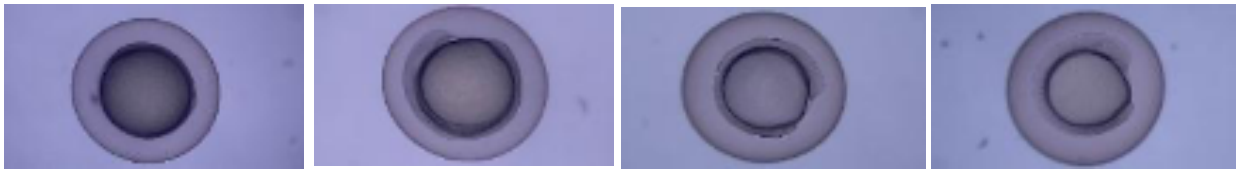
The physiological parameter of heart rate was significantly affected by sunset yellow exposure, as shown in Table 7. Two-way ANOVA results (Table 6) indicated that both the concentration of sunset yellow and exposure time had significant effects on embryonic heart rate, with F values of 89.25 and 209.44, respectively ($p < 0.001$). In contrast, the interaction between concentration and time was not significant ($F = 1.01$; $p = 0.416$), suggesting that the effect of sunset yellow on heart rate was relatively consistent across observation times. At the highest concentration (800 ppm), the observed decrease in heart rate reflects a cardiotoxic effect of sunset yellow, likely due to physiological disturbances and oxidative stress in embryonic tissues.

The decreased heart rate in zebrafish embryos suggests impaired cardiovascular function resulting from sunset yellow exposure. The 48–72 hpf period represents a critical phase in embryonic heart development, during which the heart structure is fully formed and begins to function actively in supporting blood circulation. Disruption during this stage can directly compromise oxygen and nutrient delivery to embryonic tissues, contributing to reduced embryo viability at high concentrations.

Disruption of Ca^{2+} homeostasis can reduce both the frequency and strength of heart contractions, leading to bradycardia in fish embryos. Additionally, the autonomic nervous system, which regulates heart rhythm, is still developing during the embryonic phase, making embryos susceptible to toxic compounds that may impair nerve impulse transmission and heart rate regulation (He et al., 2020). The findings of this study indicate that heart rate is a sensitive physiological parameter for detecting the cardiotoxic effects of sunset yellow in zebrafish embryos. The observed concentration-dependent decrease in heart rate confirms that this synthetic food dye can disrupt embryonic cardiovascular development, particularly at higher concentrations.

Somite Development and Alteration

Table 8. Somite development of *Danio rerio* embryos at various observation times after exposure to sunset yellow.

Hpf	Control	200 ppm	400 ppm	800 ppm
11				
16				
22				

Observations of somite development in Danio rerio embryos at 11, 16, 22 hpf indicate that sunset yellow exposure affects the embryonic segmentation process in a gradual, concentration-dependent manner (Table 8). At 11 hpf, the control group exhibited early somite formation, which was simple but clearly recognizable. In contrast, embryos exposed to sunset yellow at 200, 400, and 800 ppm did not show clearly visiblesomitestructures at this stage. These observations suggest that, during the early phase of segmentation, sunset yellow exposure may delay the initiation of somite formation compared to normal embryonic development. At 11 hpf, zebrafish embryos typically have formed approximately three somites, consistent with the control group observations (Kimmel et al., 1995).

Differences in somite development became increasingly apparent at 16 and 22 hpf. In the control group, somite structures were clearly formed, arranged in an orderly manner, and exhibited a normal segmentation pattern. This observation is consistent with typical zebrafish developmental stages, where approximately 14 somites have formed by 16 hpf and about 26 somites by 22 hpf (Kimmel et al., 1995). In contrast, embryos exposed to sunset yellow at 200, 400, and 800 ppm exhibited delayed somite development. At 16 hpf, the number of somites ranged from 6 to 10 across all concentrations, while at 22 hpf, only 20 to 24 somites were formed, lower than in the control group. These results suggest that sunset yellow exposure slows somitogenesis, with the effect becoming more pronounced at higher concentrations.

Somitogenesis involves the proliferation and differentiation of mesodermal cells, regulated by tightly coordinated molecular mechanisms. Exposure to synthetic food dyes such as sunset yellow may disrupt these processes by inhibiting cell division, altering the expression of segmentation-regulating genes, or increasing oxidative stress. These results are consistent with previous studies reporting that synthetic food dyes can be embryotoxic, interfering with cell proliferation and differentiation during fish embryonic development (Jiang et al., 2020).

Morphological observations at 11, 16, and 22 hpf indicate that somitogenesis is a highly sensitive stage of zebrafish embryonic development to sunset yellow exposure. Delays in somite formation, particularly at 400 and 800 ppm, suggest disruption of paraxial mesoderm segmentation. Embryonic development at all stages was documented using a folding tool (Yesudhason et al., 2020). Additionally, exposure to certain chemical compounds can induce oxidative stress, which may affect the distribution and activity of melanocytes in zebrafish embryos (Hahn et al., 2014).

During the advanced developmental phase (60–96 hpf), zebrafish embryos have developed into larvae with more complete body morphology, including the head, tail, and major organs. At this stage, somites have differentiated into skeletal muscle tissue and are no longer visible as separate segmentation units. Nevertheless, general morphological differences can still be detected. Larvae exposed to high concentrations of sunset yellow exhibited more intense body pigmentation and signs of developmental delay compared to control larvae. These observations are consistent with previous reports indicating that high concentrations of sunset yellow can induce developmental disorders in aquatic organisms (Kimmel et al., 1995).

Based on Table 9, somites at 11 hpf were observed only in the control group, with an average of 1.80 ± 1.55. In contrast, embryos exposed to 200, 400, and 800 ppm

Table 9. Descriptive statistical results of the parameter number of somites 11 hpf.

Treatment	Number of somites (mean ± SD)
Control	1.80 ± 1.55
200 ppm	0.00 ± 0.00
400 ppm	0.00 ± 0.00
800 ppm	0.00 ± 0.00

of sunset yellow showed no somite formation (0.00 ± 0.00). These results suggest that, during the early phase of embryonic development, somitogenesis had not yet initiated in treated embryos, whereas untreated embryos had begun the process of somite formation.

Table 10. Descriptive statistical results of the parameter number of somites 16 hpf.

Treatment	Number of somites (mean ± SD)
Control	12 ± 2.83
200 ppm	6.30 ± 1.25
400 ppm	6.80 ± 0.919
800 ppm	7.30 ± 0.675

Based on Table 10, at 16 hpf, the number of somites increased in all groups. The control group exhibited the highest somite count, with 12.00 ± 2.83. In embryos exposed to sunset yellow, somite numbers were lower, with 6.30 ± 1.25 at 200 ppm, 6.80 ± 0.92 at 400 ppm, and 7.30 ± 0.68 at 800 ppm. These results suggest that Sunset Yellow exposure delays somite formation, with treated embryos consistently exhibiting fewer somites than controls.

Table 11. Descriptive statistical results of the parameter umber of somites 22 hpf.

Treatment	Number of somites (mean ± SD)
Control	25.4 ± 0.966
200 ppm	23.1 ± 1.91
400 ppm	22.4 ± 2.07
800 ppm	21.6 ± 1.26

Based on Table 11, at 22 hpf, somite numbers increased in all groups. The control group exhibited the highest count, with 25.4 ± 0.97 somites. In embryos exposed to sunset yellow, somite numbers were lower: 23.1 ± 1.91 at 200 ppm, 22.4 ± 2.07 at 400 ppm, and 21.6 ± 1.26 at 800 ppm. These results suggest that increasing concentrations of sunset yellow are associated with a reduction in somite formation during the later stages of embryonic development.

During the advanced developmental phase (60–96 hpf), zebrafish embryos have developed into larvae with more complete body morphology, including the head, tail, and major organs. At this stage, somites have differentiated into skeletal muscle tissue and are no longer visible as separate segmentation units. Nevertheless, general morphological differences can still be detected. Larvae exposed to high concentrations of sunset yellow exhibited more intense body pigmentation and signs of developmental delay compared to control larvae. These observations are consistent with previous reports indicating that high concentrations of sunset yellow can induce developmental disorders in aquatic organisms (Kimmel et al., 1995).

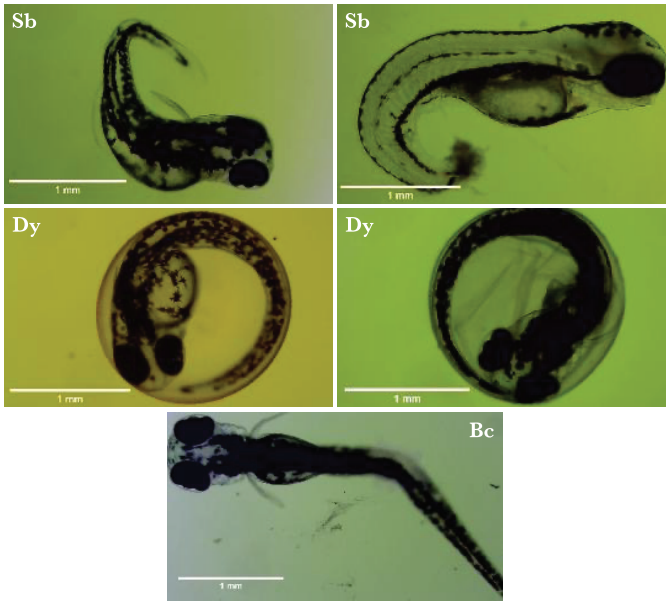


Figure 1. Morphology of zebrafish embryo abnormalities after exposure to sunset yellow (Sb) Short body, (Dy) Delay, (Bc) Body curvature.

In addition to developmental delays, exposure to sunset yellow induced various morphological alterations, including reduced body length, delayed hatching, and body curvature, as shown in figure 1. These abnormalities were absent in the control group and were more pronounced at higher concentrations of the test substance. Such morphological defect reflect disruptions in normal embryogenesis, particularly affecting the development of the musculoskeletal and nervous systems.

Altered body form, such as reduced body length (dwarfism), indicates a disruption in the growth and differentiation of embryonic tissues. This condition is generally associated with impaired somite formation and reduced elongation of the anterior-posterior axis during early embryogenesis. Exposure to sunset yellow is thought to inhibit cell proliferation and differentiation by increasing oxidative stress, thereby impairing musculoskeletal development. These findings are consistent with previous reports that synthetic food dyes can inhibit fish embryo growth by interfering with the regulation of cell and tissue development (Kimmel et al., 1995).

Delayed hatching reflects physiological disturbances in embryos during the final phase of development

(Rousseaux, 2005). The hatching process in fish embryos depends on chorionase enzyme activity and the embryo's ability to perform body movements to rupture the chorion. Exposure to sunset yellow is thought to reduce embryonic metabolic activity and inhibit hatching enzyme function, thereby delaying eclosion. This observation is consistent with reports that delayed hatching is a common response of zebrafish embryos to toxic compounds that induce oxidative stress and disrupt energy metabolism (Koca et al., 2015).

Body curvature in embryos indicates impaired musculoskeletal and nervous system development (Roy et al., 2016). Axial deformities are often linked to defective somite formation, uncoordinated muscle contractions, and disruptions in the central nervous system. Exposure to sunset yellow is thought to interfere with tissue differentiation and cellular ionic balance, both of which are essential for normal body structure formation. These findings are consistent with previous reports suggesting that toxic compounds can induce axial deformities in fish embryos by impairing mesodermal tissue development (OECD, 2018).

CONCLUSION

This study demonstrates that the synthetic food coloring sunset yellow (E110) exerts embryotoxic effects on zebrafish (*Danio rerio*) embryos. Exposure to sunset yellow caused a significant, concentration-dependent decrease in survival and hatching rates, as indicated by one-way ANOVA results ($p = 0.004$ for survival rate and $p = 0.033$ for hatching rate), with the most pronounced effects observed at higher concentrations. Embryonic heart rate was also significantly affected by both concentration and exposure time (Two-Way ANOVA, $p < 0.001$), although the interaction between these factors was not significant. Furthermore, sunset yellow disrupted somitogenesis during early embryogenesis and induced various morphological abnormalities, including reduced body length (dwarfism), delayed hatching, and body curvature. These effects reflect disturbances in the development of embryonic tissues and organs. Overall, the findings suggest that sunset yellow has the potential to pose ecological risks to non-target aquatic organisms if it accumulates in aquatic environments.

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