# Ecotoxicity of Tartrazine Yellow Dye in *Danio rerio* Embryos and Larvae After Multigenerational Exposure

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**Abstract** The toxicological aspects of tartrazine yellow dye for human health are relatively well studied, but for aquatic environments, little is known. In order to understand whether the minimum and maximum safe concentrations for human ingestion (Codex Committee on Food Additives – CCFA) are also safe for aquatic biota, tests were carried out with *Danio rerio*, exposed to tartrazine yellow analytical standard 100% (AS) (50 mg. $L^{-1}$ and 500 mg. $L^{-1}$) and commercial standard 86% (CS) (50 mg. $L^{-1}$) in multigenerational assays. Adults of *D. rerio* (F0 generation) were exposed (21 days) to the above concentrations, followed by mating and evaluation of the number of viable eggs laid (F1 generation). The remaining animals were exposed for 11 months. In both F1 and F2 generations, there was a surplus of eggs laid in the treatments in relation to the controls. However, embryos malformations and larval mortality rates were higher across the treatments. The multigenerational effect of the tartrazine yellow dye on *D. rerio* was confirmed by the pairwise ANCOVAS that always showed much smaller numbers of embryos at 96 hpf for the F2 generation. The results showed that concentrations considered safe for human ingestion represent risks to the aquatic biota.

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## 1 Introduction

Many commercial food dyes and their by-products were proved to be toxic, with chronic effects on human health, and for this reason, the food industry is currently considered as one of the main sources of environmental contamination (Nascimento et al., 2020). The production of dyes and pigments is estimated to be around 800.000 t/year, with 10 to 20% of this total being discarded in effluents worldwide (Katheresan et al., 2018), which makes the excessive use, the inadequate management, and the difficulty to treat the effluents containing these highly soluble pollutants of global concern (Januário, et al, 2021).

The tartrazine yellow dye is used in several industrial segments such as textile, pharmaceutical, and alimentary, especially because of its color and high stability. It is produced by diazotizing aminobenzene 4 acids with nitric acid and sodium nitrite, and the main components found in this dye are sodium chloride and/or sodium sulfate, calcium, potassium salts, and small amounts of potentially toxic metals such as mercury ($\leq 1$ mg $kg^{-1}$), arsenic ($\leq 3$ mg $kg^{-1}$), and lead ($\leq 10$ mg $kg^{-1}$) (FDA 1985; European Commission Directive, 2008/128/EC).

The consumption of dyes is common in different age groups, with greater exposure in children

compared to adults (Bradman, et al., 2022) because of their incorporation in food, medicines, and vitamins (Lehmkuhler et al., 2020; Thilakaratne et al., 2022). Studies on Wistar rats with doses equivalent to that permitted for human consumption evidenced that the tartrazine yellow dye acted as an endocrine disruptor, suggesting that its consumption could be among the causes of the increasingly precocious human puberty (Mindang et al., 2022). Histopathological assays showed that this dye produced lesions in the kidney, spleen, and liver of rodents (Golli et al., 2016). In the liver, it generated products with carcinogenic properties, such as aromatic amines (Moutinho et al., 2007; Zhang & Ma, 2013), and caused significant adverse effects on rat ileum and colon tissue (El-Desoky et al., 2017), kidneys (Erdemli et al., 2021), brain (Gao et al., 2011), and testes (Visweswaran & Krishnamoorthy, 2012). Molecular research also indicated the induction of systemic toxicity (Amin et al., 2010); effects on metabolism and allergies (Axon et al., 2012); interaction with hormone receptors (Kashanian & Zeidali, 2011); destruction of deoxyribonucleic acid (DNA) (Matsuo et al., 2013, Merinas-Amo et al., 2019); anxiety; headaches; blurred vision; and sleep disturbances (Saxena & Sharma, 2015).

A number of studies also aimed to evaluate the use of other chemical substances that can minimize the harmful effects of tartrazine yellow. Essawy et al., (2023) evaluated the function of the neurohormone melatonin to eliminate free radicals derived from tartrazine-induced neurotoxicity in the cerebral cortex and cerebellum of adult male rats, using oral doses permitted in human-consumed products. Altinoz et al., (2021) evaluated the therapeutic effects of crocin (50 mg/kg/day) against oxidative damages induced by tartrazine (500 mg/kg/day) in tissues of the ileum and colon of rats. Although these substances were promising to reverse some toxic parameters for human health, studies addressing the effects of tartrazine yellow discarded in industrial effluents to aquatic organisms are scarce.

The solubility of tartrazine in water is related to the presence of chromophores, carboxylic, nitro, hydroxyl, and amino groups, which can increase the absorption intensity of the dye (Del Giovine & Bocca, 2003). It is classified as an azo dye, that are described in the literature as being genotoxic due to the interactions with blood molecules (Basu & Kuma, 2016; Golli et al., 2016), causing behavioral

changes and developmental alterations in aquatic organisms (Gupta et al., 2019; Jiang et al., 2020; Joshi & Katti, 2017; Linskens, 2018). Furthermore, depending on the amount released into water bodies, changes in water color can potentially imply in reduced photosynthesis and cascading trophic effects (Przystas et al., 2012).

No acute lethal ecotoxicological effects or morphologically visible deformities were found for the fish *D. rerio* exposed to concentrations of $0 - 26.7$ mg. $L^{-1}$ at the embryonic stage (Joshi & Katti, 2017). However, at concentrations of $53 - 80$ mg. $L^{-1}$, it caused cardiac deformities; at concentrations above 80 mg. $L^{-1}$, it caused bradycardia, impaired heart rate, tail distortion, and yolk sac edema, and at concentrations of $133 - 267$ mg. $L^{-1}$, it increased lethality (Joshi & Katti, 2017). Using concentrations of 1.200 mg. $L^{-1}$, Motta et al., (2019) observed embryonic deformations in developing *D. rerio*; increased germination but reduced sprouting rate in *Cucumis sativus*; as well as increased mortality and reduced mobility in *Aartemia salina*. Gupta et al., (2019) studied the embryonic stages of *D. rerio* exposed to 0.1% tartrazine in 100 μL with the enzyme superoxide dismutase and concluded that the dye induced oxidative stress and significant mortality at 96 h post-fertilization. Jiang et al., (2020) exposed *D. rerio* to concentrations from 5 to 50 mM. (2.67 to 26.71 mg. $L^{-1}$) of the dye and observed hatching difficulties and abnormalities in their development (26.71 mg. $L^{-1}$).

The zebrafish *D. rerio* (Hamilton, 1822) is a small freshwater representative (5 cm) of the family Cyprinidae (Eschmeyer & Fricke, 2015) which has gained a notable importance in scientific research as a test organism in recent years. It has been used as an alternative to other animals, such as rodents, in several areas of knowledge (Silveira et al., 2012) because it presents high reproductive rate, fast development with the eggs evolving into larvae between 48 and 72 h after fertilization, and they become adults at only 3 months of age, with transparent embryos and 70% of genetic homology with humans (Howe et al., 2013). Furthermore, as a vertebrate model, *D. rerio* shares many anatomical characteristics with mammals (kidneys, brain, liver, intestine, heart, spine, eyes, mouth, ears, etc.), showing similar functions and physiology in most organs (Macrae & Peterson, 2015).

Considering that, in terms of ecotoxicological knowledge of the yellow tartrazine dye in aquatic environments, the literary base is scarce and that results are based only on acute tests, this research intends to improve the knowledge about the potentially harmful effects of this dye through multigenerational chronic tests for the *D. rerio* exposed to concentrations of an analytical standard and a commercial standard of this dye. Specifically, we investigated the fecundity, as well as the morphology of embryos and larvae over three generations of the test organism exposed to concentrations currently considered safe for human daily ingestion, and we inferred whether these same concentrations, when present in water, could also be safe for aquatic organisms.

## 2 Material and Methods

### 2.1 Ecotoxicological Tests

Adults of *D. rerio* were placed in 2-L aquariums with chlorine-free reconstituted water treated with activated carbon filters. The variables hardness and pH were adjusted to 44 mg CaCO3 $L^{-1}$ and $7.5 \pm 0.5$, respectively; the temperature was maintained at $26 \pm 1$ °C, and the photoperiod was 12-h light: 12-h dark (OECD 2013). For the males, six aquariums containing three individuals each were used per treatment (18 males per treatment). Three aquariums were used per treatment for the females, containing three individuals each (nine females per treatment). For the tests, solutions of 500 and 50 mg. $L^{-1}$ of the tartrazine yellow analytical standard 100% (AS) Dynatech® dye were prepared, and a solution of 50 mg. $L^{-1}$ was prepared for the commercial standard 86% (CS), Sigma Aldrich ®. During the tests, the adult organisms were fed Tetramin® fish food (97% crude protein) twice a day, and from the 15th day of exposure (1 week before mating), *Artemia salina* nauplii were introduced as a food supplement, once a day "Ad libitum."

The fish from the first trial (F0 generation), remained under the influence of the dye concentrations for 21 days, while the control fish remained in reconstituted water only. The test solution was renewed every 48 h, with the organisms transferred to a new solution. On the 21st day, 18 males and nine females from each treatment were used for the analyses of reproductive aspects. Each mating tank contained three females and six males, that were exposed in triplicate in breeding tanks that were set up following ABNT NBR 15499 (2016). On the 22nd day, viable eggs from each of the mating tanks were exposed to a washing process in leaching solution (250 μL NaClO $L^{-1}$) to avoid fungal contamination, and they were counted at 0 and 24 h after fertilization. Larvae hatched 48 h after fertilization, and they started to receive compound food diluted in water with biological yeast when they were 7 days old. They were exposed to the potential contaminants since egg laying and their developments were monitored until adulthood. When they were 11 months old, this F1 generation underwent mating tests as described for the F0 generation.

For qualitative morphological analyses, eggs and larvae of the F1 generation (the F2 generation) were placed into wells of cell culture plates (24 wells 2.0-cm deep × 1.5 cm in diameter, each containing 2 mL of each solution or reconstituted water). They were analyzed 3, 24, 48, 72, and 96 h post-fertilization (hpf). Five eggs (3 hpf) were allocated in each well; embryos were observed 24 hpf, and larvae were observed 48, 72, and 96 hpf. In total, 120 eggs for each concentration and control, and 120 embryos for each concentration and control were analyzed. However for the larvae at the highest concentration (500 mg. $L^{-1}$), only 75 survived until 48 hpf; six until 72 hpf, and no larvae survived until 96 hpf. At the 50 mg. $L^{-1}$ AS concentration, at 96 hpf, only 42 larvae remained. Observations were made under an optical microscope (Leica Application Suite—DM 3000 LED) at 500- and 1000-μm magnification, and the deformities found in eggs, embryos, and larvae were photographed and described in detail. Morphological abnormalities in larvae were described according to OECD, 236 (2013); Nagel (2002); Scholz et al., (2008), and Beekhuijzen et al., (2015); and chemical parameters including pH, hardness, and conductivity (OECD 236) were monitored for each well during the time of the experiment (Fig. 1).

### 2.2 Statistical Analyses

We addressed whether the relationships between hpf and the number of viable embryos/larvae differed between treatments using analysis of covariance (ANCOVA). To address whether the slopes of linear regressions have differed, we tested the significance
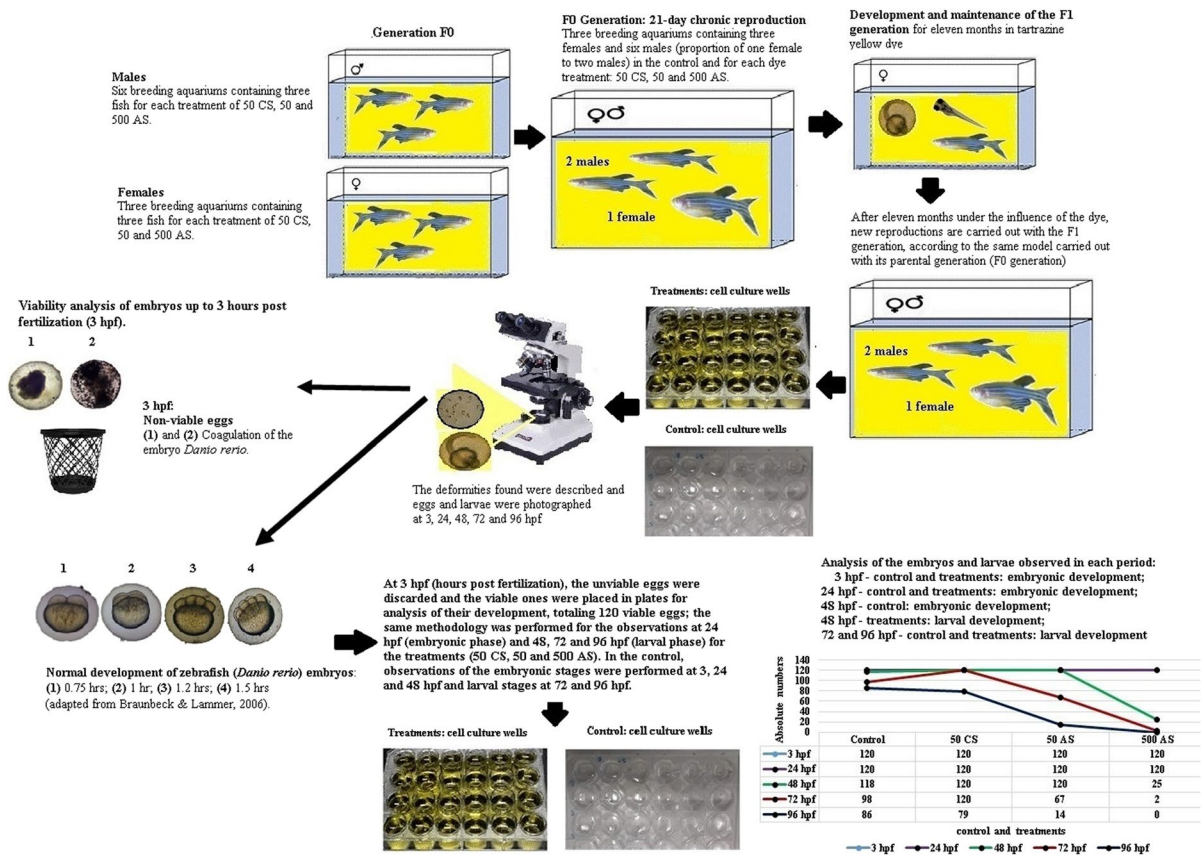
**Fig. 1** Experimental design used for investigating the multigeneration effects of tartrazine yellow dye in different developmental stages of *Danio rerio e*xposed to concentrations of 50 mg. L$^{-1}$ CS e AS (commercial standard and absolute standard) and 500 mg. L$^{-1}$ AS (absolute standard) attachment

of the interaction between treatment (50CS, 50AS, 500AS) and the predictive variable (hpf) (number ~ treatment + hpf + treatment:hpf); and to address whether the intercepts have-differed, we tested the significance of the independent variable "treatment," holding the slopes equal (number ~ treatment + hpf). This approach was used for F1 and F2 generations separately, and we also used ANCOVAS for comparing the relationships between hpf and number of viable embryos/larvae between F1 and F2 generations, for each treatment separately (control, 50CS, 50AS, and 500AS). All of the ANCOVA analyses were performed using the R package "car.''.

To compare statistically the numbers of morphological deformities found in the embryos/larvae of F2 generation between treatments, we counted the numbers of deformities found in samples with a maximum of 120 individuals that were chosen aleatorily in the respective aquariums at 3, 24, 48, 72, and 96 hpf, with the health individuals returned to the aquariums. For this comparison, all of the types of deformities were pooled together, and when an embryo or larvae presented more than one type alteration, they were all counted. Because the numbers of available individuals varied with time due to mortality, we used the proportions of the total number of deformities subdivided by the number of available embryos/larvae of each treatment/hpf for statistical comparisons, with the non-parametric Kruskal–Wallis test, followed by Dunn post-test, using the approaches implemented in the software Past 4.03 free.

## 3 Results

The slopes and the intercepts of the relationships between hpf and the number of viable embryos/larvae among the different treatments have differed
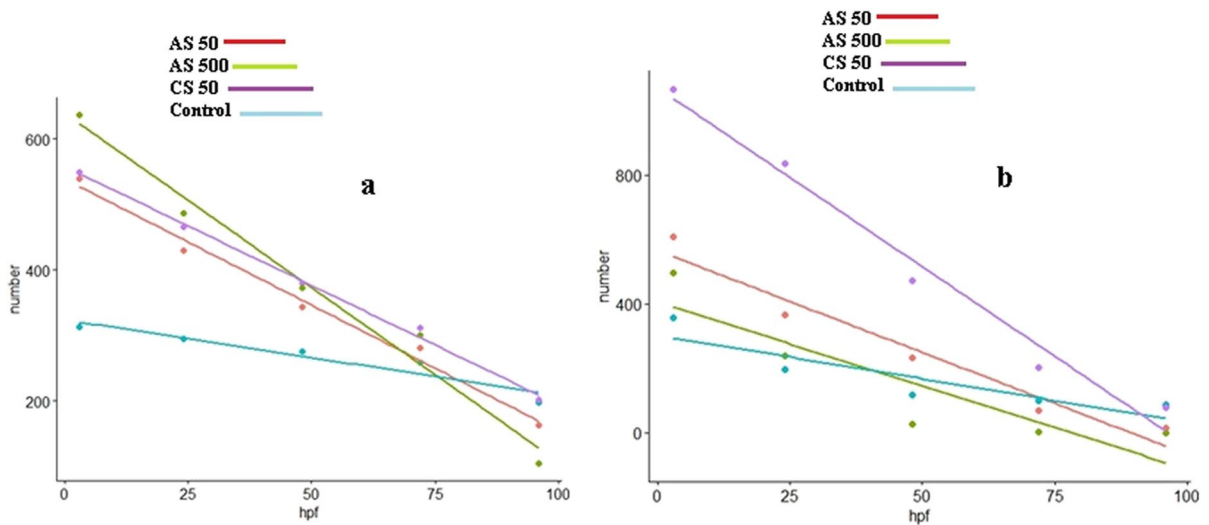
**Fig. 2** Comparison of the relationship between hpf and numbers of eggs/embryos/larvae across treatments for F1 (**a**) and F2 (**b**)

significantly in both F1 (slope: $F = 36.56$, $P < 0.0001$; intercept: $F = 3.92$, $P = 0.03$) and F2 generations (slope: $F = 10.33$, $P = 0.001$; intercept: $F = 3.92$, $P = 0.03$) (Fig. 2).

When we compared F1 and F2 generations, the regressions of the controls have not differed in the slope ($F = 3.45$, $P = 0.11$), but they have differed in the intercept ($F = 9.07$, $P = 0.02$) (Fig. 3a); the CS50 treatments have differed in the slope ($F = 55.23$, $P = 0.0003$), but not in the intercept ($F = 2.29$, $P = 0.17$) (Fig. 3b); the AS50 treatments have differed in the slope ($F = 7.82$, $P = 0.03$), but not in the intercept ($F = 5.18$, $P = 0.06$) (Fig. 3c), and the 500AS treatments have not differed in the slope ($F = 0.006$, $P = 0.94$), but they have differed in the intercept ($F = 20.89$, $P = 0.003$) (Fig. 3d).

### 3.1 Morphological Observations of D. rerio Larvae

The proportions of the total numbers of deformities subdivided by the number of embryos/larvae analyzed across hpf (Table 1) have differed significantly among treatments from the F2 generation (KW = 10.7, $P = 0.01$). Dunn-post test revealed that all of the treatments (50CS, 50AS, and 500AS) have differed significantly in relation to the control ($P = 0.03$, 0.03, and 0.001, respectively), but they have not differed among them.

Normal larvae and embryos, as well as the different types of deformations observed in F2 generation

exposed to the different treatment conditions, are described and explained in details in Figs. 4, 5, 6, 7, 8, and 9. At 24 hpf, the main deformities observed in the embryos exposed to the concentrations were embryos without movement; yolk sac deformity; and tail curvature (Fig. 5); at 48 hpf: body malformation, impairing tail movement; diffuse pigmentation of the body and tail; head malformation and edema formation (Fig. 6); at 72 hpf: head malformation; tail sprain; edema formation in the pericardial edema with irregular heartbeat; and leakage of fluid from the heart (Fig. 7); at 96 hpf: edema formation in the yolk sac; formation of pericardial edema; and malformation on the body, without pigmentation (Fig. 8).

## 4 Discussion

In the present research, the multigenerational toxicity of tartrazine yellow dye on *Danio rerio* was evidenced for both commercial and analytical products at different concentrations, and these results were supported by survival and morphological biomarkers of embryos and larvae. The higher numbers of eggs laid by parents exposed to the dyes when compared to the control in both F1 and F2 generations suggested that these dyes have stimulated spraws. It was an unexpected result, and possible explanations could be the stress caused by changes in the color of the water,
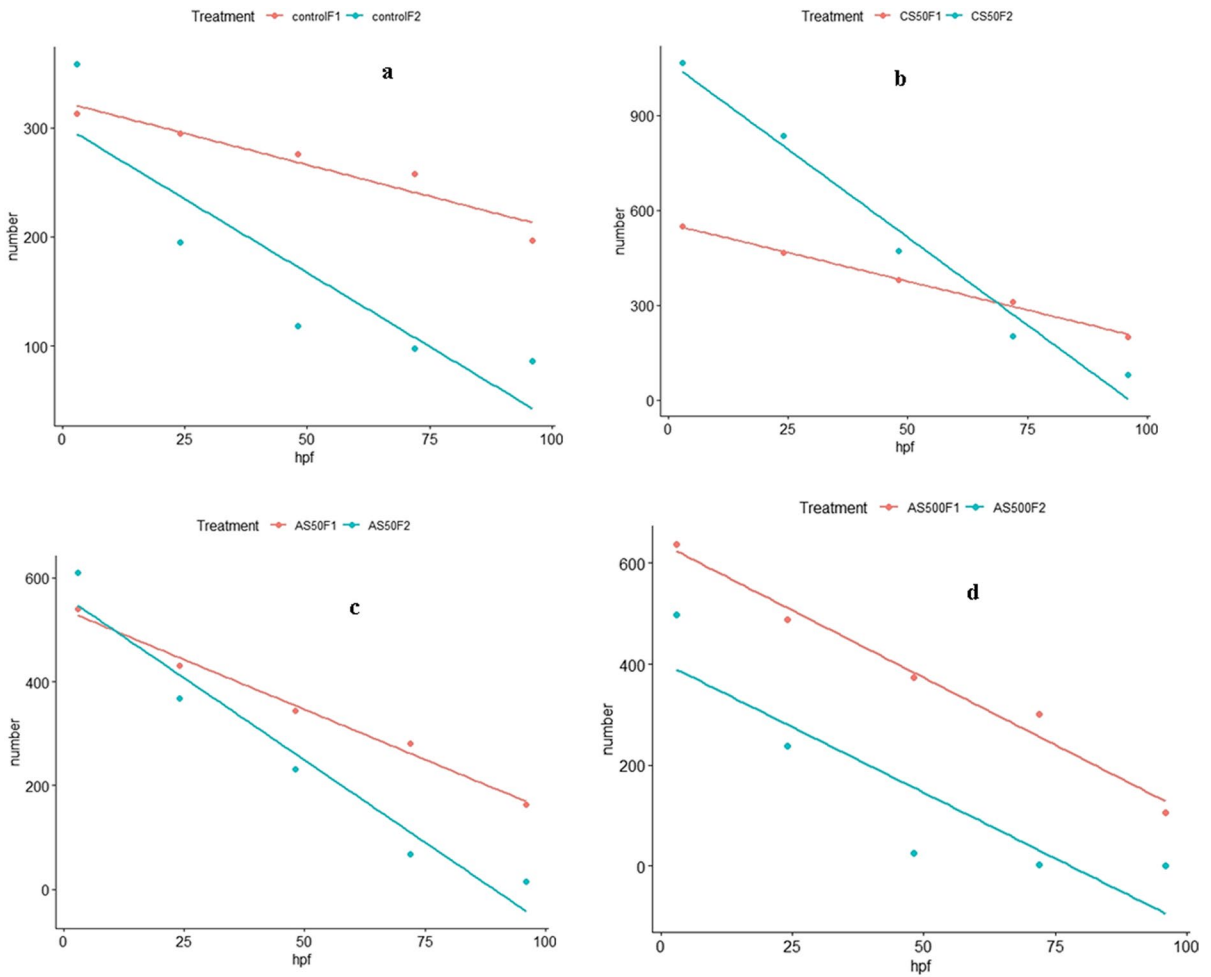
**Fig. 3** Comparisons of tehe relationship between hpf and numbers of eggs/embryos/larvae for each treatment between F1 and F2 generation. **a** (control). **b** (50CS). **c** (50AS) **d** (500AS)

**Table 1** Numbers of morphological changes observed in eggs (3 hpf), embryos (24 to 48 hpf), and larvae (72 to 96 hpf) from F2 generation allocated into wells of cell plates for qualitative-quantitative analyses (Control; 50 mg. L$^{-1}$ SC; 50 mg. L$^{-1}$AS; 500 mg. L$^{-1}$AS)

*HPF*, hours post-fertilization

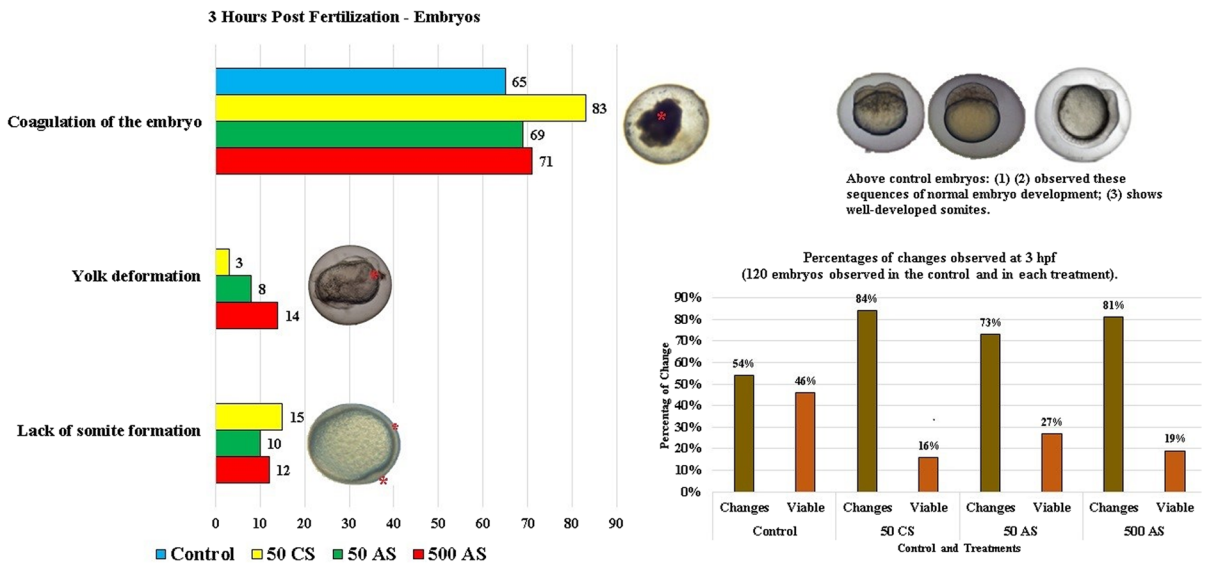| Treatments (mg. L$^{-1}$) | Number of changes/number of organisms | | | |
|---|---|---|---|---|
| | Control | 50 CS | 50 AS | 500 AS |
| HPF | F2 | F2 | F2 | F2 |
| 3 | 65/120 = 0.54 | 101/120 = 0.84 | 87/120 = 0.72 | 97/120 = 0.80 |
| 24 | 64/120 = 0.53 | 68/120 = 0.56 | 100/120 = 0.83 | 105/120 = 0.87 |
| 48 | 5/118 = 0.04 | 81/120 = 0.67 | 47/120 = 0.39 | 20/25 = 0.80 |
| 72 | 5/98 = 0.05 | 66/120 = 0.55 | 53/67 = 0.79 | 2/2 = 1 |
| 96 | 2/86 = 0.02 | 74/79 = 0.93 | 14/14 = 1 | 0 |

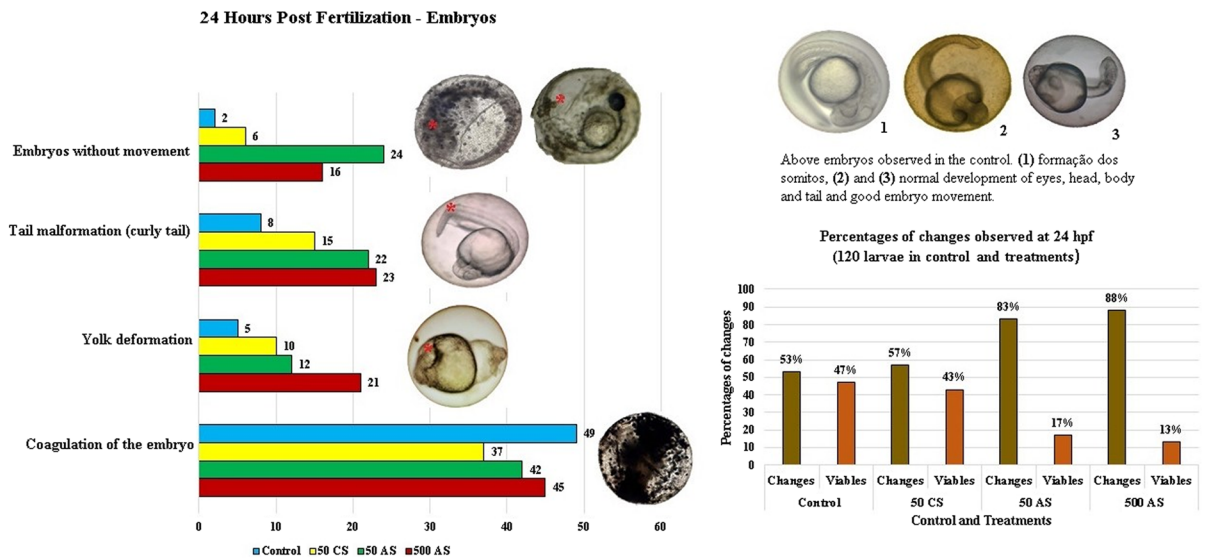**Fig. 4** Photos and graphs describing the types of deformities observed and the number of embryos/larvae with and without alterations in the offspring of the F2 generation of *D. rerio* exposed to different concentrations of the tartrazine yellow dye (50 mg. L$^{-1}$ CS, 50 mg. L$^{-1}$ AS, and 500 mg. L$^{-1}$ AS) at 3 hpf



**Fig. 5** Photos and graphs describing the types of deformities observed and the numbers of embryos with and without alterations in the F2 generation of *D. rerio* exposed to different concentrations of the tartrazine yellow dye (50 mg. L$^{-1}$ CS, 50 mg. L$^{-1}$ AS, and 500 mg. L$^{-1}$ AS) at 24 hpf

stimulating egg laying. This effect was previously reported by Fontana et al. (2021), who exposed fishes to different classes of pollutants, or the dyes can act as endocrine disruptors capable of inducing egg laying, but these effects are still to be investigated. However, it did not reduce the harmful effects of these

dyes once the numbers of viable larvae and embryos declined much faster within the treatments than in the control, in such a way that at 96 hpf, the numbers of viable embryos and larvae became higher in the control. The idea of a multigenerational effect of the tartrazine yellow dye on *D. rerio* was confirmed by
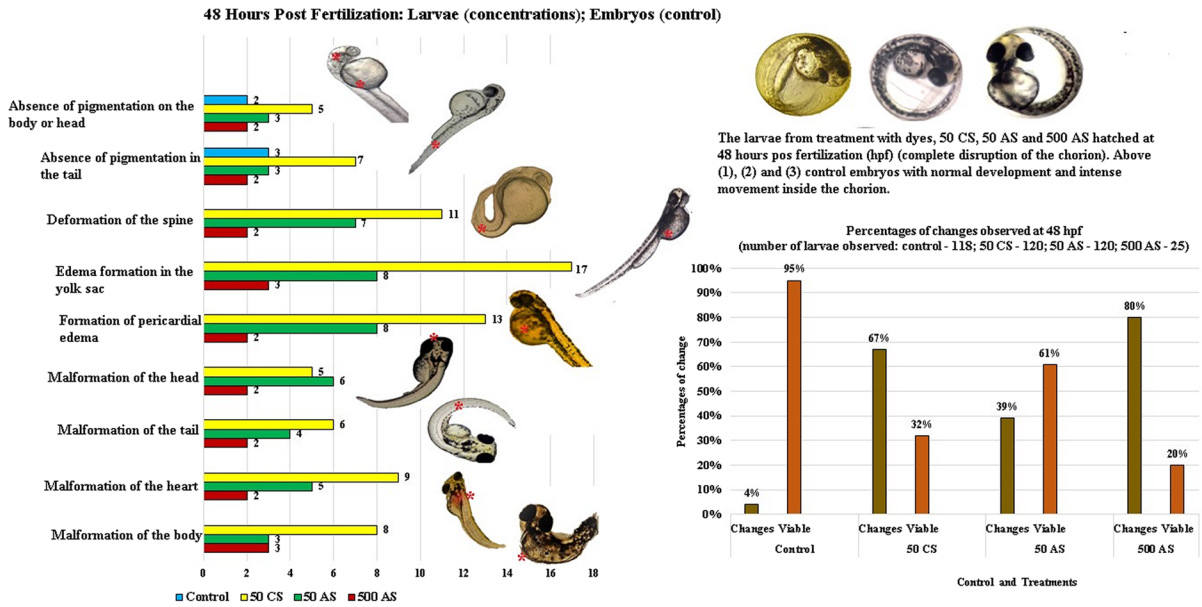
**Fig. 6** Photos and graphs describing the types of deformities observed and the number of embryos/larvae with and without alterations in the F2 generation of *D. rerio* exposed to different concentrations of the tartrazine yellow dye (50 mg. L$^{-1}$ CS, 50 mg. L$^{-1}$ AS, and 500 mg. L$^{-1}$ AS) at 48 hpf
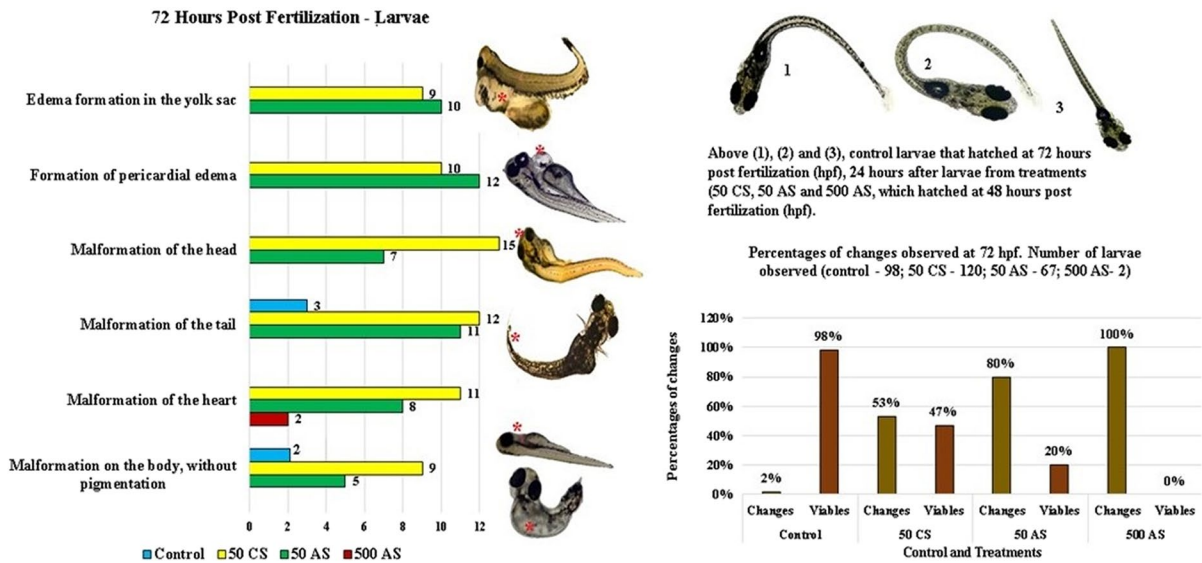


**Fig. 7** Photos and graphs describing the types of deformities observed and the number of larvae with and without alterations in the F2 generation of *D. rerio* exposed to different concentrations of the tartrazine yellow dye (50 mg. L$^{-1}$ CS, 50 mg. L$^{-1}$ AS, and 500 mg. L$^{-1}$ AS) at 72 hpf

the pairwise ANCOVAS, that always showed much smaller numbers of embryos at 96 hpf for the F2 generation.

In fish, the chorion (a cellular membrane that surrounds the embryo until the moment of its hatching) is obtained by the secretion of the enzyme choriolysin
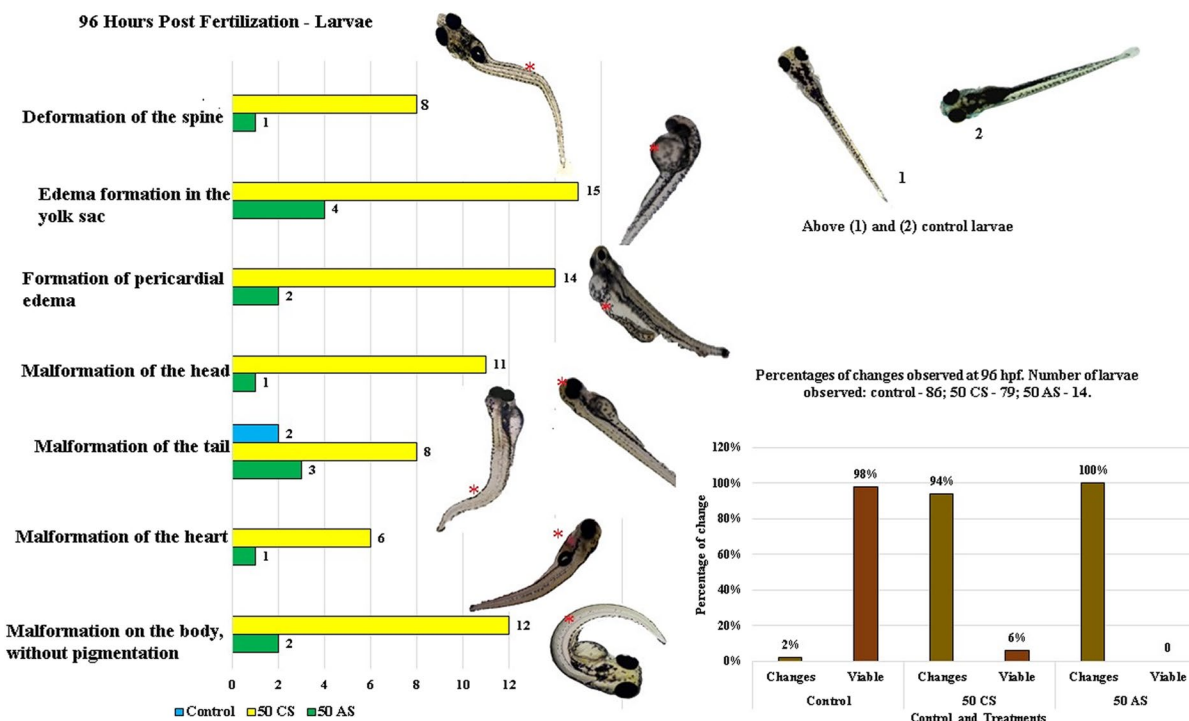
**Fig. 8** Photos and graphs describing the types of deformities observed and the number of larvae with and without alterations in the F2 generation of *D. rerio* exposed to different concentrations of the tartrazine yellow dye (50 mg. L$^{-1}$ CS, 50 mg.L$^{-1}$ AS, and 500 mg. L$^{-1}$ AS) at 96 hpf

in the glandular cells (HGCs) of the perivitelline space (PVS), together with spontaneous movements of the developing embryo (Baran et al., 2021). The difficulty of hatching could be explained by neuromuscular deficiencies that cause the weakening of spontaneous muscle movements, triggered by the presence of external chemical substances (De la Paz et al., 2017). Some authors have argued that the chorion could provide physical protection against pollutants of high molecular weight (Chen et al., 2016), but this structure has pores between 0.5 and 0.7 mm in diameter and, therefore, only partially isolates the embryo from the environment (Medeiros et al., 2017). In the case of azo dyes, with high solubility, they can easily break this barrier and change hatching rates.

The decrease in hatching rate in *D. rerio* exposed to tartrazine yellow dye was also observed by Joshi and Katti (2017), who exposed embryos to dye dilutions of 0, 0.1, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 75, and 100 mM, and analyzed their developments from the gastrulation stage (5.25 hpf) to the seventh day. At concentrations of 20 mM (53 mg.L$^{-1}$) and 30 mM

(80 mg.L$^{-1}$), the dye caused anomalies such as cardiac deformities, and embryos exposed to concentrations greater than 30 mM (80 mg.L$^{-1}$) had heartbeat mismatch and tail distortion followed by yolk sac edema. At the highest concentrations (50–100 mM equivalent to 133 mg. L$^{-1}$ and 267 mg. L$^{-1}$), embryo development stopped, causing mortality. The authors concluded that the tartrazine yellow dye was teratogenic or embryotoxic for *D. rerio* at the mentioned concentrations, corroborating the effects observed in the present study at similar concentrations, however, over a longer period of exposure. Kiziltan et al. (2022) exposed *Danio rerio* to the azocarmoisine dye, in doses of 4 to 2000 ppm for 96 h and detected, among other embryonic alterations, the reduced capacity for movement and hatching, attributed to the difficulty in breaking the chorion.

Jiang et al., (2020) studying the toxicity of azo dyes tartrazine, sunset yellow, amaranth, and Allura red in *D. rerio* embryos, in 96 h, verified the teratogenic potential in the development of embryos in concentrations of 5 to 50 mM. At
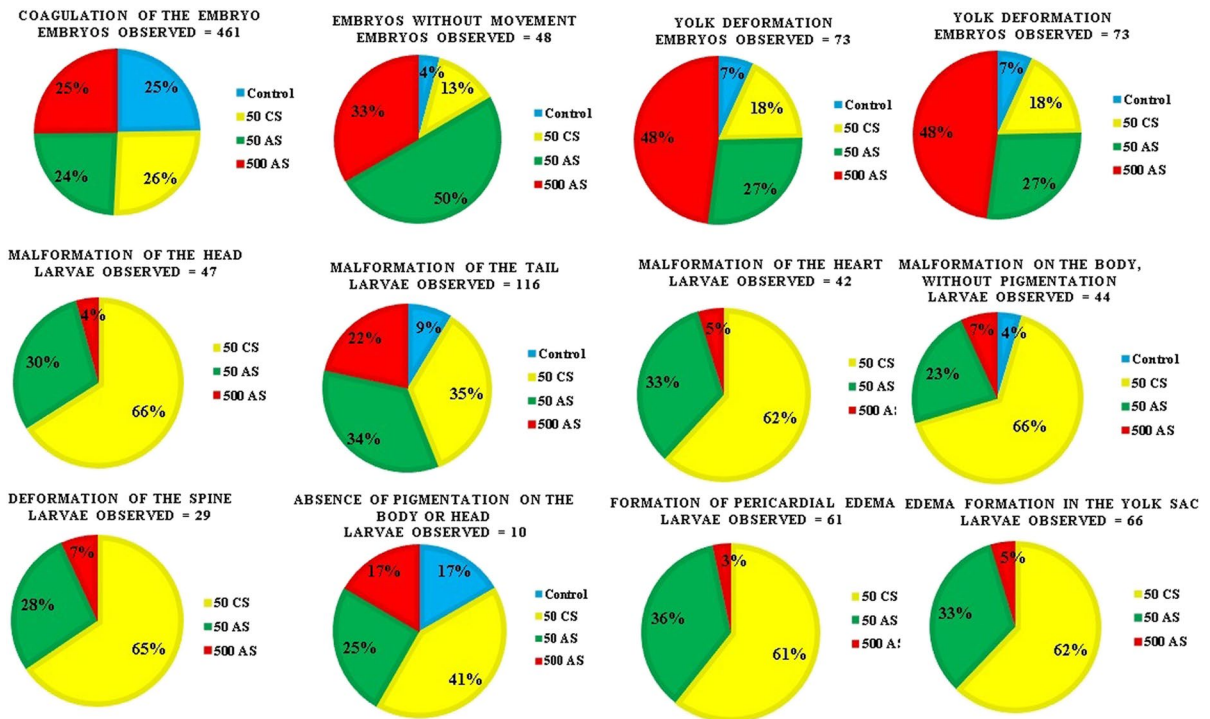
**Fig. 9** Changes found in *Danio rerio* embryos and larvae at different observation times in control and at concentrations of 50 mg. L$^{-1}$ CS; 50 mg. L$^{-1}$ AS, and 500 mg. L$^{-1}$ AS

concentrations of 10 to 50 mM (5.34–26.7 mg. L$^{-1}$), the dyes induced difficulties in hatching and abnormal developments, such as cardiac edema, slow heart rate, yolk sac edema, and in spinal development, including spinal curvature and tail distortion. At 100 mM (53.4 mg. L$^{-1}$), there were few survivors and the azodye group was lethal to embryos. In the present research, expressive lethality was observed in the F2 generation, from the concentration of 50 mg.L$^{-1}$, and the same morphological alterations described by the above authors were detected.

In embryo development, important factors to be considered are the yolk deformities, which can selectively aggregate lipophilic xenobiotics from the surrounding aquatic environment in fish. Some studies have quantified the bioaccumulation of xenobiotics specifically in the yolk, such as aqueous exposure to estradiol (Paige Souder & Gorelick, 2017), selenium (Dolgova et al., 2016), graphene oxide (Chen et al., 2015), and hydrogen sulfide (Choi et al., 2016). The main content of the yolk is vitellogenin, and due to its chemical

composition (lipoglycoprotein), it can associate with toxic substances from maternal exposure and modify its configuration, compromising the reabsorption capacity of fish embryos (Ulhaq et al., 2015). *D. rerio* is purely lecithotrophic, dependent on a finite supply of yolk. The, if yolk absorption transporters are inhibited by pollutants, yolk utilization will be impaired, resulting in malnutrition (De la Paz et al., 2017). In the present study, deformations were detected in the yolk of embryos at all dye concentrations in the F2 generation, which may have influenced the significant reduction of viable eggs in relation to the initial number of eggs.

Also noteworthy is the fact that the high solubility of azo dyes in water can interrupt the osmotic balance in the yolk sac in *D. rerio* (Sant & Timme-Laragy, 2018). This causes excessive water intake forming edema (Jiang et al., 2020; Motta et al., 2019). It can explain the yolk sac edemas observed in our study with tartrazine yellow.

Considering the embryo hatching times, which for *D. rerio* is 72 hpf, there is a consensus in the literature that exposure to tartrazine advances the process

by 24 h, that is, hatching occurs at 48 hpf, which was also detected in the present research for the F1 and F2 generations (after multigenerational exposure), except for the controls. It is possible that the dyes can produce physical and chemical signals capable of activating the chorionase enzyme, which initiates the deterioration of the inner layer of the chorion and stimulates the embryo's movements, culminating in premature hatching. In this sense, Motta et al. (2019) exposed embryos to 1.2 g. $L^{-1}$ of tartrazine yellow after 6 hpf, and at 48 hpf hatching rate was 60% higher than in the control. The authors proposed two hypotheses to account for these findings: first, the dye could have altered the osmotic equilibrium, increasing the pressure inside the chorion envelope; second, the dye could have directly affected the structure or composition of the chorion, reducing its resistance.

In the present study, we observed curvature of the spine in larvae of the F2 generation. Gupta et al., (2019) reported that *D. rerio* embryos exposed to tartrazine and erythrosine dyes at concentrations of 5 mg. $L^{-1}$, showed a delay in bone and muscle development, considering embryos aged between 3.5 and 10 dpf (days after fertilization). Locomotor activity, controlled by the nervous system, is susceptible to different endogenous/exogenous disturbances, and changes in locomotor activities may indicate changes at the neuromuscular junction. Studies revealed that the malformations observed due to developmental toxicity in *D. rerio* larvae exposed to xenobiotics affected locomotor activity, compromising the survival of the species in natural environments (Abe et al., 2017; Sulukan et al., 2021; To et al., 2021).

In this study, another evident alteration in the F2 generation (500 mg. $L^{-1}$) was the accelerated heart rate, evidencing pericardial edemas, and, in some cases, a total disruption of the pericardial region was detected. The solubility of tartrazine yellow dye in water can impair the osmotic gradient, and excessive water absorption can lead to the formation of cardiac edema (Jiang et al., 2020). Heart rate and blood flow rate are valuable parameters used to determine developmental toxicity and regulate heart rate in the environment through branches of the sympathetic and parasympathetic autonomic nervous systems (Li et al., 2017). Corroborating with the results obtained in the present study, Wang et al., (2020) studied the effects of two commonly used food additives, the natural dye cochineal red and brilliant blue, belonging to the class of triphenylmethane dyes (usually used together with tartrazine yellow dye to impart a green color to food), and they demonstrated that, by exposing *D. rerio* from 4 to 6 hpf to 0.2‰ of the brilliant blue food additive, the heart rates were 10% higher than in the control group, and in the cochineal red additive, they were 32% higher than in the control group.

The absence of pigmentation in the head, body, and tail found in this study in the F2 generation from 48 hpf onwards may be related to individuals who had deficiencies in somite formation, detected in individuals at 24 hpf. There are no descriptions in the current literature of this type of effect associated with dyes (Stickney et al., 2000).

## 5 Conclusion

The reproductive and morphological biomarkers of *D. rerio*, exposed to commercial and analytical standards of tartrazine yellow, observed in chronic multigenerational assays showed the toxicity of the referred dye, in all generations and suggest that concentrations considered safe for human consumption pose risks to aquatic biota.

Therefore, in order to establish ranges of safe concentrations of exposure to aquatic biota of tartarzine yellow, in addition considering to the ecotoxicological data generated here and in others studies present in the literature, risk analyses with species of different trophic levels and further studies of biological reversibility are still needed.

**Author contribution** Janete da Silva: methodology, investigation, processing data, formal analysis, processing data, writing original draft.

Renata Fracacio Francisco: methodology, project administration, writing review and editing, conceptualization, supervision, funding acquisition.

**Conflict of Interest** The authors declare no competing interests.

# References

Abe, F. R., Mendonça, J. N., Moraes, L. A., Oliveira, G. A., Gravato, C., Soares, A. M., & Oliveira, D. P. (2017). Toxicological and behavioral responses as a tool to assess the effects of natural and synthetic dyes on zebrafish early life. *Chemosphere, 178*, 282–290. https://doi.org/10.1016/j.chemosphere.2017.03.030

ABNT – Brazilian Association of Technical StandardS (2016) NBR 15499: Aquatic ecotoxicology – Short-term chronic toxicity – Test method with fish, 2nd edn. Rio de Janeiro.

Altinoz, E., Erdemli, M. E., Gül, M., Erdemli, Z., Gül, S., & Turkoz, Y. (2021). Prevention of toxic effects of orally administered tartrazine by crocin in Wistar rats. *Toxicological & Environmental Chemistry, 103*(2), 184–198. https://doi.org/10.1080/02772248.2021.1942472

Amin, K. A., Hameid, A., II., & Elsttar, A. H. A. (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. *Food and Chemical Toxicology, 48*(10), 2994–2999. https://doi.org/10.1016/j.fct.2010.07.039

Axon, A., May, F. E. B., Gaughan, L. E., Williams, F. M., Blain, P. G., Matthew, C., & Wright, M. C. (2012). Tartrazine and sunset yellow are xenoestrogens in a new screening assay to identify modulators of human oestrogen receptor transcriptional activity. *Toxicology, 298*(1–3), 40–51. https://doi.org/10.1016/j.tox.2012.04.014

Baran, A., Yildirim, S., Ghosigharehaghaji, A., Bolat, İ, Sulukan, E., & Ceyhun, S. B. (2021). An approach to evaluating the potential teratogenic and neurotoxic mechanism of BHA based on apoptosis induced by oxidative stress in zebrafish embryo (*Danio rerio*). *Human Experimental Toxiclogy, 40*(3), 425–438. https://doi.org/10.1177/0960327120952140

Basu, A., Kuma, G. S. (2016). Multispectroscopic and calorimetric studies on the binding of the food colorant tartrazine with human hemoglobin. *Journal of Hazardous Materials,* 35468–476. https://doi.org/10.1016/j.jhazmat.2016.07.023

Beekhuijzen, M., De Koning, C., Flores-Guillén, M. E., De Vriesbuitenweg, S., Tobor-Kaplon, M., Van DE Waart, B., Emmeh, H. (2015). From cutting edge to guideline: A first step in harmonization of the zebrafish embryotoxicity test (ZET) by describing the most optimal test conditions and morphology scoring system. *Reproductive Toxicology*, *56*(64–76). https://doi.org/10.1016/j.reprotox.2015.06.050

Bradman, A., Castorina, R., Thilakaratne, R., Gillan, M., Pattabhiraman, T., Nirula, A., Marty, M., & Miller, M. D. (2022). Dietary exposure to United States Food and Drug Administration-approved synthetic food colors in children, pregnant women, and women of childbearing age living in the United States. *International Journal of Environmental Research, 19*, 9661. https://doi.org/10.3390/ijerph1915966

Chen, M., Yin, J., Liang, Y., Uan, S., Wang, F., Song, M., & Wang, H. (2016). Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. *Aquatic Toxicology, 174*, 54–60. https://doi.org/10.1016/j.aquatox.2016.02.015

Chen, Y., Ren, C., Ouyang, S., Hu, X., & Zhou, Q. (2015). Mitigation in multiple effects of graphene oxide toxicity in zebrafish embryogenesis driven by humic acid. *Environmental Science & Technology, 49*(16), 10147–10154. https://doi.org/10.1021/acs.est.5b02220

Choi, S. A., Park, C. S., Kwon, O. S., Giong, H. K., Lee, J. S., Ha, T. H., & Lee, C. S. (2016). Structural effects of naphthalimide-based fluorescent sensor for hydrogen sulfide and imaging in live zebrafish. *Scientific Reports, 18*(6), 26203. https://doi.org/10.1038/srep26203

De la Paz, J. F., Beiza, N., Paredes-Zúñiga, S., Hoare, M. S., & Allende, M. L. (2017). Triazole fungicides inhibit zebrafish hatching by blocking the secretory function of hatching gland cells. *International Journal of Molecular Sciences, 18*(4), 710. https://doi.org/10.3390/ijms18040710

Del Giovine, L., & Bocca, A. P. (2003). Determination of synthetic dyes in ice cream by capillary electrophoresis. *Food Control, 14*, 131–135. https://doi.org/10.1007/s12161-016-0645-9

DIRECTIVE2008/128/EC *European Parliament* 314. Available www.europarl.eu. Accessed: 2023, mai, 27.

Dolgova, N. V., Hackett, M. J., MacDonald, T. C., Nehzati, S., James, A. K., Krone, P. H., George, G. N., & Pickering, I. J. (2016). Distribution of selenium in zebrafish larvae after exposure to organic and inorganic selenium forms. *Metallomics, 8*(3), 305–312. https://doi.org/10.1039/c5mt00279f

El-Desoky, G. E., Abdel-Ghaffar, A., Al-Othman, Z. A., Habila, M. A., Al-Sheikh, Y. A., Ghneim, H. K., Giesy, J. P., & Aboul-Soud, M. A. (2017). Curcumin protects against tartrazine-mediated oxidative stress and hepatotoxicity in male rats. *European Review for Medical and Pharmacological Sciences, 21*(3), 635–645.

Erdemli, Z., Altinoz, E., Erdemli, M. E., Gul, M., Bag, H. G., & Gul, S. (2021). Ameliorative effects of crocin on tartrazine dye-induced pancreatic adverse effects: A biochemical and histological study. *Environmental Science and Pollution Research International, 28*(2), 2209–2218. https://doi.org/10.1007/s11356-020-10578-6

Eschmeyer, W.N., & Fricke, R. (Eds.) (2015). Catalog of fishes. Updated internet version of 3 December 2015. Retrieved May, 12, 2022. https://www.catacademy.org/scientists/projects/eschmeyers-catalog-of-fishes

Essawy, A. E., Mohamed, A. I., Ali, R. G., Ali, A. M., & Abdou, H. M. (2023). Analysis of melatonin-modulating effects against tartrazine-induced neurotoxicity in male rats: Biochemical, pathological and immunohistochemical markers. *Neurochemical Research, 48*(1), 131–141. https://doi.org/10.1007/s11064-022-03723-9

Fontana, B. D., Alnassar, N., & Parker, M. O. (2021). The impact of water changes on stress and subject variation in a zebrafish (*Danio rerio*) anxiety-related task. *Journal of Neuroscience Methods, 363*, 109–347. https://doi.org/10.1016/j.jneumeth.2021.109347

FoodAnd Drug Administration – FDA (1985). FD&C Yellow No. 5 final rule, removal of stay. *Federal Register, 50*, 35774-35783.

Gao, Y., Li, C., Shen, J., Yin, H., An, X., & Jin, H. (2011). Effect of food azo dye tartrazine on learning and memory

functions in mice and rats, and the possible mechanisms involved. *Journal of Food Science, 76*(6), T125–T129. https://doi.org/10.1111/j.1750-3841.2011.02267.x

Golli, E. N., Bini-Dhouib, I., Jrad, A., Boudali, I., Basma, B., Belhadjhmida, N., & El Fazaa, S. (2016). Toxicity induced after subchronic administration of the synthetic food dye tartrazine in adult rats, role of oxidative stress. *Recent Advances in Biology and Medicine, 2*, 20–28. https://doi.org/10.18639/RABM.2016.02.284474

Gupta, R., Ranjan, S., Yadav, A., Verma, B., Malhotra, K., Madan, M., Chopra, O., Jain, S., Gupta, S., Joshi, A., Bhasin, C., & Mudgal, P. (2019). Toxic effects of food colorants erythrosine and tartrazine on zebrafish embryo development. *Current Research in Nutrition and Food Science, 7*(3), 876–885. https://doi.org/10.12944/CRNFSJ.7.3.26

Hamilton (1822). Catalogue *Danio rerio* (Hamilton, 1822) in GBIF Secretariat (2022). GBIF *Backbone Taxonomy*. Checklist dataset https://doi.org/10.15468/39omei. accessed via GBIF.org on 2023-07-23.

Howe, K., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496*(7446), 498–503. https://doi.org/10.1038/Nature12111

Januário, E. F. D., Vidovix, T. B., Beluci, N. C. L., Paixão, R. M., Silva, L. H. B. R., Homem, N. C., Bergamasco, R., & Vieira, M. A. S. (2021). Advanced graphene oxide-based membranes as a potential alternative for dyes removal: A review. *Science of the Total Environment, 789*, 147957. https://doi.org/10.1016/j.scitotenv.2021.147957

Jiang, L. L., Li, K., Yan, D.-L., Yan, M.-F., Ma, L., & Xie, L.-Z. (2020). Toxicity assessment of 4 azo dyes in zebrafish embryos. *International Journal of Toxicology, 39*(2), 115–123. https://doi.org/10.1177/1091581819898396

Joshi, V., & Katti, P. (2017). Developmental toxicity assay for food additive tartrazine using zebrafish (*Danio rerio*) embryo cultures. *International Journal of Toxicology, 37*(1), 38–44. https://doi.org/10.1177/1091581817735227

Kashanian, S., & Zeidali, S. H. (2011). Binding studies of tartrazine food additive. *DNA and Cell Biology, 30*(7), 499–505. https://doi.org/10.1089/dna.2010.1181

Katheresan, V., Kansedo, J., & Lau, S. Y. (2018). Efficiency of various recent wastewater dye removal methods: A review. *Journal of Environmental Chemical Engineering, 6*(4), 4676–4697. https://doi.org/10.1016/j.jece.2018.06.060

Kiziltan, T., Baran, A., Kankaynar, M., Şenol, O., Sulukan, E., Yildirim, S., & Ceyhun, S. B. (2022). Effects of the food colorant carmoisine on zebrafish embryos at a wide range of concentrations. *Archives of Toxicology, 96*(4), 1089–1099. https://doi.org/10.1007/s00204-022-03240-2

Lehmkuhler, A. L., Miller, M. D., Bradman, A., Castroina, R., & Mitchell, A. E. (2020). Certified food dyes in over the counter medicines and supplements marketed for children and pregnant women. *Food and Chemical Toxicology, 143*, 111499. https://doi.org/10.1016/j.fct.2020.111499

Li, K., Wu, J. Q., Jiang, L. L., Shen, L. Z., Li, J. Y., He, Z. H., Wei, P., Lv, Z., & He, M. F. (2017). Developmental toxicity of 2,4-dichlorophenoxyacetic acid in zebrafish embryos. *Chemosphere, 171*, 40–48. https://doi.org/10.1016/j.chemosphere.2016.12.032

Linskens, A. (2018). The long term effects of tartrazine (FD&C Yellow No. 5) on learning, cognitive flexibility, and memory of zebrafish (Danio rerio) embryos into adulthood. Seymour Community High School, Seymour, WI, 54165. Retrieved July 30, 2022, from https://W2.wpmucdn.com/sites.uwn.edu/dist/8/202/files2018/06/Linskens_paperLMSeymour_2018--1lxiur8.pdf.

Macrae, C. A., & Peterson, R. T. (2015). Zebrafish as tools for drug discovery. *Nature Reviews Drug Discovery, 14*(10), 721. https://doi.org/10.1038/nrd4627

Matsuo, H., Yokooji, T., Morite, H., Ooi, M., Urata, K., Ishii, K., Takahagi, S., Yanase, Y., Hiragun, T., Mihara, S., & Hide, M. (2013). Aspirin augments IgE-mediated histamine release from human peripheral basophils via Syk kinase activation. *Allergology International, 62*, 503–511. https://doi.org/10.2332/allergolint.13-OA-0536

Medeiros, A. M. Z., Silva, G. H., Castro, V. L. S. S., Monteiro, R. T. R., & Martinez, D. S. T. (2017). Nanoecotoxicity of graphene oxide: Influence of the chorion of zebrafish (Danio rerio) embryos and co-exposure with humic acid. In: IX Workshop on Nanotechnology Applied to Agribusiness. São Carlos: Embrapa - Brazilian Agricultural Research Company, São Carlos/SP, November, 21 to 22, 2017. Retrieved September 30, 2022. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/171384/1/2017AA57.pdf

Merinas-Amo, R., Martinez-Jurado, M., Jurado-Gueto, S., Alonso-Moraga, A., & Merinas-Amo, T. (2019). Biological effects of food coloring in in vivo and in vitro model systems. *Foods, 8*, 176. https://doi.org/10.3390/foods8050176

Mindang, E. L. N., Awounfack, C. F., Ndinteh, D. T., Krause, R. W. M., & Njamen, D. (2022). Effects of tartrazine on some sexual maturation parameters in immature female wistar rats. *International Journal of Environmental Research and Public Health, 19*, 10410. https://doi.org/10.3390/ijerph191610410

Motta, C. M., Simoniello, P., Arena, C., Capriello, T., Panzuto, R., Vitale, E., Agnisola, C., Tizzano, M., Avallone, B., & Ferrandino, I. (2019). Effects of four food dyes on development of three model species, *Cucumis sativus*, *Artemia salina* and *Danio rerio*: Assessment of potential risk for the environment. *Environmental Pollution, 253*, 1126–1135. https://doi.org/10.1016/j.envpol.2019.06.018

Moutinho, I. L. D., Bertges, L. C., & Assis, R. V. C. (2007). Prolonged use of the food dye tartrazine (FD&C yellow no. 5) and its effects on the gastric mucosa of Wistar rats. *Brazilian Journal of Biology, 67*, 141–145. https://doi.org/10.1590/S1519-69842007000100019

Nagel, R. (2002). DarT: The embryo test with the zebrafish *Danio rerio* – a general model in ecotoxicology and toxicology. *Biology, Medicine Altex, 19*(Suppl 1), 38–48.

Nascimento, G. E., Cavalcanti, V. O. M., Santana, R. M. R., Sales, D. C. S., Rodriguez-Diaz, J. M., Napoleão, D. C., & Duarte, M. M. M. B. (2020). Degradation of a sunset yellow and tartrazine dye mixture: Optimization using statistical design and empirical mathematical modeling. *Water, Air, & Soil Pollution, 231*, 254. https://doi.org/10.1007/s11270-020-04547-5

OECD - Organisation for Economic Cooperation and Development (2013). Guidance document on the threshold

approach for acute fish toxicity 1 testing. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. Paris: OECD.

Paige Souder, J., Gorelick, D. A. (2017).Quantification of estradiol uptake in zebrafish embryos and larvae. *Toxicological Sciences: An Official Journal of the Society of Toxicology,* 2017. https://doi.org/10.1093/toxsci/kfx107

Przystas, W., Zablocka-Godlewska, E., & Grabinoka-Sota, E. (2012). Biological removal of azo and triphenylmethane dyes and toxicity of process by – products. *Water, Air, & Soil Pollution, 223*(4), 1581–1592. https://doi.org/10.1007/s11270-011-0966-7

Sant, K. E., & Timme-Laragy, A. R. (2018). Zebrafish as a model for toxicological perturbation of yolk and nutrition in the early embryo. *Current Environmental Health Reports, 5*(1), 125–133. https://doi.org/10.1007/s40572-018-0183-2

Saxena, B., & Sharma, S. (2015). Food color induced hepatotoxicity in Swiss albino rats Rattus norvegicus. *Toxicology International, 22*(1), 152–157. https://doi.org/10.4103/0971-6580.172286

Scholz, S., Fischer, S., Gundel, U. E., Kuster, E. T., Luckenbach, T., & Voelker, D. (2008). The zebrafish embryo model in environmental risk assessment -applications beyond acute toxicity testing. *Environmental Science and Pollution Research, 15*, 394–404. https://doi.org/10.1007/s11356-008-0018-z

Silveira, T. R. D., Schneider, A. C., & Hammes, T. O. (2012). Zebrafish: An established model for studies of human diseases. *Science and Culture, 64*(2), 4–5.

Stickney, H.L., Barresi, M.J.F., Devoto, S.H. (2000). Somite development in zebrafish. *Developmental Dynamics, 219*, 287–303. https://doi.org/10.1002/1097-0177(2000)9999:9999<::AID-DVDY1065>3.0.CO;2-A

Sulukan, E., Ghosigharehagaji, A., Baran, A., Yildirim, S., Bolat, İ, & Ceyhun, S. B. A. (2021). Versatile toxicity evaluation of ethyl carbamate (urethane) on zebrafish embryos: Morphological, physiological, histopathological, immunohistochemical, transcriptional and behavioral approaches. *Toxicology Letters, 2021*(353), 71–78. https://doi.org/10.1016/j.toxlet.2021.09.012

Thilakaratne, R., Castorina, R., Gillan M., Han, D., Pattabhiraman, T., Nirula, A., Miller, M. D., Marty, M., Lehmkuhler, A., Mitchell, A., Bradman, A. (2022). Exposures to FD&C synthetic color additives from over-the-counter medications and vitamins in United States children and pregnant women. *Journal of Exposure Science Environmental Epidemiology*, 1. https://doi.org/10.1038/s41370-022-00418-9

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

Ulhaq, M., Sundström, M., Larsson, P., Gabrielsson, J., Bergman, Å., Norrgren, L., & Örn, S. (2015). Tissue uptake, distribution and elimination of (14)C-PFOA in zebrafish (*Danio rerio*). *Aquatic Toxicology, 163*, 148–57. https://doi.org/10.1016/j.aquatox.2015.04.003

Visweswaran, B., & Krishnamoorthy, G. (2012). Oxidative stress by tartrazine in the testis of Wistar rats. *IOSR Journal of Pharmacy and Biological Sciences, 2*, 44–49. https://doi.org/10.9790/3008-0234447

Wang, Y. F., Chen, I. W., Subendran, S., Chun-Wei, K., Bivas, P., Tzu-Fun, F., & Chia-Yuan, C. (2020). Edible additive effects on zebrafish cardiovascular functionality with hydrodynamic assessment. *Scientific Reports, 10*, 16243. https://doi.org/10.1038/s41598-020-73455-9

Zhang, G., & Ma, Y. (2013). Mechanistic and conformational studies on the interaction of food dye amaranth with human serum albumin by multispectroscopic methods. *Food Chemistry, 136*, 442–449. https://doi.org/10.1016/j.foodchem.2012.09.026