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Chronic life-cycle exposure to atrazine at an environmentally relevant concentration induces redox imbalance, mortality, and developmental malformations in zebrafish (Danio rerio)

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Abstract

Atrazine (ATZ) is a widely used herbicide and is frequently detected in water bodies, potentially affecting non-target organisms such as fish. This study investigated the effects of chronic exposure to ATZ, at an environmentally relevant concentration (1 µg/L), on behavioral and biochemical parameters of *Danio rerio*. The fish were exposed from the embryonic stage to adulthood. Heart rate (HR), spontaneous movement (SM), and malformations in larvae were evaluated. After 96h of exposure biomarkers of oxidative damage: thiobarbituric acid reactive substances (TBARS) and reactive oxygen species (ROS) were evaluated. In addition, the activity of the enzymes acetylcholinesterase (AChE), and some antioxidant defense parameters also were evaluated: superoxide dismutase (SOD), catalase (CAT), non-protein thiols (NPSH) and total antioxidant capacity against peroxyl radicals (ACAP). No significant changes were observed in spontaneous movement (SM). However, heart rate (HR) was significantly reduced in the atrazine-exposed group. However, a high mortality rate (87.5%) was shown, and malformations were observed after 72 hours post-fertilization in atrazine exposed group. TBARS and SOD levels were not altered by ATZ exposure. In contrast, a significant increase in ROS was observed, along with a reduction in ACAP and in the activity of the enzymes CAT and AChE. NPSH levels were also reduced by chronic exposure to atrazine. The sum of results indicated oxidative damage and impairment of both enzymatic and non-enzymatic antioxidant defenses. In addition, the results concerning AChE activity suggest possible neuromuscular effects. The results also highlight the issue of environmental contamination, because an environmentally relevant concentration of ATZ was used.

1. Introduction

With the growth of the global population, the use of pesticides has become essential to meet the increasing demand for food. Used since antiquity pesticides have evolved from natural substances such as sulfur and ash to synthetic compounds, which are more widely used currently. Although their use remains the basis of global large-scale food production models, long-term exposure to pesticides represents a silent threat to human health and ecosystems. Among the pesticides of current use, that presented toxic potential to non-target species atrazine (ATZ) (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is an herbicide in use since the 1950s. Currently, this compound has an annual use of 70,000 to 90,000 tons worldwide. In the Brazilian context, ATZ ranked seventh among the most used pesticides in 2024 (Zhang et al., 2018; de Albuquerque et al., 2020; IBAMA 2025). Atrazine is a triazine herbicide that exerts its phytotoxic action through inhibition of photosystem II (PSII), blocking electron transport and, consequently, the synthesis of ATP and NADPH, processes essential for photosynthesis. In addition, this compound presents high environmental persistence, being frequently detected in surface waters, groundwater, and even in remote regions. Although its half-life in aquatic environments may vary from days to months depending on environmental conditions, studies demonstrate that atrazine and its metabolites may persist in the environment for more than 90 days, being detected even after more than 20 years since the last application, which highlights its high environmental stability (Jablonowski et al., 2011). Its uncontrolled use has raised concerns regarding its potential adverse effects on non-target organisms. Brazilian legislation establishes legal limits for atrazine in water at 2.0

µg/L, the maximum value for drinking water (Ministry of Health Brazil, 2021). Despite this, ATZ concentrations of up to 4.0 µg/L were detected in the Guarapiranga Reservoir, in São Paulo, and 3.0 µg/L and 3.2 µg/L in the São Francisco River Basin, in the state of Paraná (Zanella et al., 2002; Montagner & Jardim (2011); Júnior et al., 2009). Previous studies have already demonstrated that atrazine can cause oxidative stress in zebrafish, even at sublethal concentrations (Blahová et al., 2013). In addition, the study by Marins et al. (2018) showed that ATZ can trigger damage to proteins and lipids and bioaccumulate in the muscle tissue of carp even at the concentration allowed by the National Council for the Environment (CONAMA), which is 2.0 µg/L. Given the gaps regarding the effects of chronic exposure to ATZ and its toxicity at concentrations below those established by legislation, it becomes essential to investigate these effects in organisms that are indirectly affected by the herbicide, such as fish. In this context, the zebrafish has become established as a model in research, especially in toxicological studies. Its application gained strength mainly after the mapping of its genome, which demonstrated approximately 70% homology with human genes (Howe et al., 2013; Adhish & Manjubala, 2023). Its advantages include external reproduction, accelerated embryonic development, low maintenance cost, and the transparency of embryos and larvae, which allows detailed analysis of physiological, phenotypic, and behavioral characteristics (Zha et al., 2024).

The chronic effects of the use of these compounds are still uncertain, reinforcing the need for investigations that are indispensable for understanding their effects. Given the gaps presented and the scarcity of results on the chronic effects of ATZ, the effects of exposure to an environmentally relevant concentration (1 µg/L) were investigated across all stages of zebrafish (*D. rerio*) development, from the first embryonic hours (3 hpf) to sexual maturity (90 days).

2. Material and Methods

2.1 Chemicals and reagents

2,4-dinitrophenylhydrazine (DNPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), and malondialdehyde (MDA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The pure compound atrazine ($C_8H_{14}ClN_5$; molecular weight: 215.68 g/mol; purity ≥ 97) was used, obtained from the Laboratory for Analysis of Pesticide Residues (LARP).

2.2 Breeding Stock (fish)

Sixty adult short-fin zebrafish (*Danio rerio*), six months old, were acquired from a specialized supplier in the region of Santa Maria, RS, Brazil. After the acclimation period, the fish were transferred to the zebrafish facility of the Department of Biochemistry and Molecular Biology, Federal University of Santa Maria. The animals were housed in six tanks with a capacity of 12 liters each, in strict accordance with the standards established by the National Council for Animal Experimentation (CONCEA), according to Normative Resolution No. 37, February 15, 2018. The experimental arrangement followed a 2:1 ratio (males:females) per tank, totaling 12 individuals per experimental unit, according to the reproductive management guidelines described in the ZFIN Protocol (https://zfin.org/zf_info/zfbook/zfbk.html).

Maintenance conditions were strictly controlled: dechlorinated water with a constant aeration system, a photoperiod of 14 hours light/10 hours dark, and a temperature of 28 ± 1°C (thermostat) (Ames et al., 2023). The physicochemical parameters of the water (ammonia, oxygen, and nitrite concentrations) were monitored daily using colorimetric tests, maintaining non-detectable levels. Throughout the experimental period, the fish were fed daily with commercial feed OVOVIT (Tropical®) supplemented with live brine shrimp nauplii (*Artemia* sp.). Portions were calculated for complete consumption within no more than five minutes, in order to avoid residue accumulation and maintain water quality.

The reproductive protocol consisted of reducing the water volume to approximately one-third of the aquarium on the day prior to embryo collection. Simultaneously, a mesh divider was installed at the base of each aquarium, thus preventing adult fish from accessing the eggs after spawning. The environment received environmental enrichment through the introduction of artificial structures, including plants and rocky substrates, simulating natural ecological conditions, while the water temperature remained stabilized at 28°C. The combined stimuli (environmental and behavioral) proved effective in inducing the reproductive process, with spawning occurring predominantly during the first hours of light on the following day, as described by Wafer et al. (2016) and Ames et al. (2023). This study was approved by the Ethics Committee for the Use of Animals of UFSM (N°: 4344290925).

2.3 Exposure and maintenance of organisms

Zebrafish (*Danio rerio*) embryos were collected 60 min after the onset of mating. A total of 240 fertilized embryos was obtained and divided into two experimental groups: control group (CT) and atrazine-exposed group (ATZ) at a concentration of 1 µg/L, totaling 120 embryos per group. The eggs were collected and washed in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, and 0.00001% w/v methylene blue). Subsequently, the embryos were transferred to Petri dishes and subjected to random screening under a Vastar Mega Pixels microscope (500×) for the selection of viable embryos. Healthy embryos were distributed into 24-well plates, with five embryos per well containing 2 mL of solution. Embryos in the control group received 2 mL of dechlorinated water, while embryos in the experimental group received 2 mL of solution containing 1 µg/L of ATZ. The plates were maintained in an incubator with controlled photoperiod and temperature of 28°C.

After complete hatching (96 hpf), the larvae were transferred to 2 L beakers previously adapted with small perforations (~ 1 mm) on the sides and base, to facilitated circulation and renewal of the aquatic medium. The containers were maintained in a water bath at 28°C, with continuous aeration, until 7 days post-fertilization (dpf). Total water changes (100%) were performed at 7-day intervals throughout the experiment to ensure water quality and maintain ATZ concentration. Physicochemical parameters, including pH (7.2), conductivity (400 mS), dissolved oxygen (4 mg/L), and temperature (28°C), as well as the absence of nitrite and ammonia, were monitored daily as described by Ames et al. (2023). Larval feeding was initiated from 120 hpf with paramecium concentrate (*Paramecium* spp.) obtained by simple filtration. At 7 dpf, gradual introduction of brine shrimp nauplii began along with paramecia. Between 10 and 15 dpf, the diet consisted exclusively of brine shrimp nauplii. From 15 dpf onwards, gradual adaptation to dry feed OVOVIT (Tropical®) was initiated, maintaining the supply of *Artemia* as a dietary

supplement. At 7 dpf, the larvae were transferred to 6 L aquaria, where they remained under the same management conditions until 45 dpf. Subsequently, each group was transferred to 20 L aquaria, where the fish were maintained until 90 days post-fertilization. To ensure maintenance of ATZ concentration, the solutions were completely renewed every 7 days. Measurements of ATZ concentration in the water showed a 10% reduction in ATZ concentration every 7 days.

2.4 Survival and hatching rate

Survival was evaluated daily from 3 hpf exposure until 90 dpf. Embryonic viability was analyzed by a blinded observer who was unaware of the identity of the animals, through the presence of egg coagulation and the absence of heartbeats in embryos and larvae; in adults, the absence of opercular movement, body movements or swimming, and the absence of response to stimuli were evaluated. The hatching rate was analyzed by a blinded observer, who counted the hatched animals at 24, 48, 72, and 96 hpf. All parameters described here were evaluated according to previously published protocols (OECD, 2013; Westerfield, 2000; Severo et al., 2020; Ames et al., 2023). For the analysis of these parameters, two hundred and forty animals were used.

2.5 Heart rate

Heart rate was evaluated at 48 hours post-fertilization (hpf) as described by Ames et al. (2023). The larvae were filmed under a stereoscopic microscope. Heartbeats were quantified manually; 15 embryos from each group were filmed for 1 minute by a blinded observer, and the number of heartbeats observed during this time interval was counted with the aid of ImageJ/Fiji software.

2.6 Embryo/larval malformations

A total of 50 larvae at 72 hpf were randomly selected for the analysis of morphological abnormalities. The number of individuals with and without malformations was recorded. The evaluated alterations included: pericardial edema (PE), yolk sac edema (YSE), body malformations (BM), encompassing tail deformities, shortened tail and cranial anomalies, and spinal curvature (SC). The larvae were photographically documented using a professional digital microscope with 300× zoom, coupled to a Vastar Mega Pixels 500× microscope, and with the aid of the rear camera of an iPhone 12 (dual 12 MP: wide-angle f/1.6 and ultra-wide f/2.4), which enabled the acquisition of high-resolution images.

2.7 Spontaneous movement (SM)

Embryos at 28 hpf were randomly selected for the analysis of spontaneous movement. Fifteen embryos per group were observed, and the number of spontaneous movements was counted manually over a period of 60 seconds. Observations were performed using a Vastar Mega Pixels stereoscopic microscope at 40×, according to a methodology adapted from Ames et al. (2023). This analysis allowed the evaluation of the initial motor pattern of the larvae, considered an important indicator of neurofunctional development.

2.8 Mortality

Mortality was monitored every 24 hours until the end of the experiment. For its determination, criteria such as egg coagulation and absence of heartbeat in embryos and larvae were considered, following established protocols (OECD, 2013; Westerfield, 2000; Severo et al., 2020).

3. Biochemical analyses

At the end of the experimental period (90 days post-fertilization), zebrafish were euthanized by immersion in ice (0°C) for 10 min, according to Normative resolution N° 37 from National Council for Science and Animal Experimentation (CONCEA). The whole body was homogenized for biochemical analysis (n=8) per group. The fish were homogenized in 2 mL of buffer (50 mM Tris-HCl, pH 7.4) and then centrifuged at 1000 x G for 10 min at 4°C. The supernatant obtained was transferred to microtubes and stored at −80 °C for biochemical analysis

The biochemical analysis was:

3.1 Total proteins: Proteins were quantified according to a colorimetric method, following the methodology of Bradford (1976) and using bovine albumin as standard. The obtained values were expressed in (mg/mL).

3.2 Thiobarbituric Acid Reactive Substances (TBARS): Lipid peroxidation was analyzed by the TBARS technique, using the methodology described by Buege J.A & Aust S.D (1978). The results were expressed as nmol MDA/mg protein.

3.3 Acetylcholinesterase activity (AChE): AChE activity was evaluated by the method of Ellman (1961), with adaptations. The results were expressed as µmol/min/mg protein.

3.4 Total Antioxidant Capacity Against Peroxyl Radicals (ACAP): ACAP was evaluated by measuring the amount of ROS production induced by ABAP, according to the methodology described by Amado et al. (2009). Fluorescence was measured at 545/570 nm (excitation/emission), using DCF (dichlorofluorescein) as standard. The results were expressed as relative area of ROS.

3.5 Reactive Oxygen Species (ROS): ROS quantification was performed using the methodology described by Ali et al. (1992). The results were expressed as nmol DCF/mg protein.

3.6 Superoxide dismutase enzyme (SOD): SOD activity was evaluated by the method described by Dieterich et al. (2000). The results were expressed in units (U) of SOD/mg protein.

3.7 Catalase enzyme (CAT): Catalase activity was evaluated following the methodology described by Hadwan and Abed (2016). Activity was expressed in micromoles of substrate degraded per minute per milligram of protein (µmol min/mg protein).

3.8 Non-protein thiols (NPSH): NPSH quantification was performed by the colorimetric method of Ellman (1961). The results were expressed as micromoles of sulfhydryl groups per g of tissue (µmol

SH/g tissue).

4. Statistical analysis

Statistical analyses were performed using GraphPad Prism software. Data normality was evaluated using the Shapiro–Wilk test. For normally distributed data, comparisons between groups were performed using the unpaired t-test with Welch's correction when necessary. For data that did not meet normality assumptions, the Mann–Whitney test was used. Results were considered statistically significant when $p < 0.05$.

5. Results

5.1 Morphological, behavioral changes and mortality

Statistical results between the CT and ATZ groups (Fig. 1a–d) showed no significant difference in the outcomes of spontaneous movement (SM) and hatching (HC). For spontaneous movement, no difference was observed between the groups ($p = 0.8674$). The hatching rate (HC) was analyzed descriptively over time (24, 48, 72, and 96 hpf). Up to 48 hpf, no hatching was observed in either group. At 72 hpf, all embryos in the control group had hatched, while in the atrazine-exposed group hatching was partial. At 96 hpf, an increase in the hatching rate was observed in the ATZ group, with approximately 35% of embryos hatching during this period. Heart rate (HR) was significantly reduced in the ATZ group compared to the control group (Mann–Whitney test, $p = 0.0441$). Median values decreased from 180.5 bpm in the control group to 164.0 bpm in the atrazine-exposed group. On the other hand, survival/mortality analysis (Fig. 1d) revealed a marked difference between the groups, demonstrating that exposure to atrazine significantly increased embryo mortality. The mortality rate was substantially higher in the atrazine-exposed group (87.5%) compared to the control group (12.5%). The calculated relative risk was 0.1429 (95% CI: 0.08789 to 0.2253), indicating that animals in the control group had an approximately 7-fold lower risk of death compared to those exposed to atrazine.

5.2 ATZ causes alterations in markers of oxidative damage

Statistical analyses revealed different effects of ATZ on the evaluated biochemical parameters. Among the variables analyzed by the non-parametric Mann–Whitney test (TBARS, ACAP, and ROS), TBARS showed no significant difference between the groups ($p > 0.05$) (Fig. 2a). Reactive oxygen species (ROS) showed a significant increase in the ATZ-exposed group ($p = 0.0002$) (Fig. 2b), while antioxidant capacity against peroxyl radicals (ACAP) showed a significant reduction ($p = 0.0258$) in the atrazine group (Fig. 2c).

For the parameters non-protein thiols (NPSH) (Fig. 2d), CAT (Fig. 2f), and AChE (Fig. 2g), analyzed by Welch's t-test, significant reductions were observed in these parameters in organisms exposed to ATZ ($p < 0.0001$; $p = 0.0007$; and $p < 0.05$, respectively), evidencing toxic effects of the herbicide. Finally, the

enzyme SOD (Fig. 2e), evaluated by standard unpaired t-test, showed no significant difference between the groups ($p = 0.1401$).

5.3 Embryo/larval malformations

We recorded malformations induced by exposure to 1 µg/L of ATZ in zebrafish embryos and larvae (Fig. 3). Malformations such as pericardial edema (PE), yolk sac edema (YSE), body malformations (BM) (including tail malformation, short tail, and head malformation), and spinal curvature (SC) were analyzed in all groups at 24, 48, and 72 hpf. Animals with malformations were observed in the exposed group at 72 hpf.

6. Discussion

In the present study, we performed a chronic exposure to atrazine in zebrafish at an environmentally relevant concentration (1 µg/L), covering the period from 3 hours post-fertilization (3 hpf) to adulthood. To our knowledge, this is the first study to investigate the effects of continuous exposure for 90 days throughout the entire ontogenetic development. Previous studies have evaluated exposures of up to 30 days, generally restricted to juvenile and adult stages, at concentrations ranging from 0.3 to 90 µg/L (Blahová et al., 2013; Destro et al., 2021). Developmental toxicity affecting early life stages, including alterations in swimming behavior, mortality, and morphological abnormalities, has also been reported in zebrafish exposed to pesticide mixtures containing atrazine (Pérez et al., 2013). In addition, the literature reports gene silencing effects mediated by DNA methylation after short exposures (72 h), with persistent alterations observed after long periods under control conditions (Wang et al., 2022).

In our study, we verified that atrazine induced a significant reduction in CAT activity and in NPSH levels. No difference was observed in superoxide dismutase (SOD). On the other hand, we observed an increase in ROS production accompanied by a reduction in ACAP. Interestingly, we did not observe a significant increase in oxidative damage markers (TBARS). Similar effects have been reported in adult zebrafish, with reduced CAT activity accompanied by compensatory increases in other antioxidant enzymes, such as SOD, glutathione peroxidase (GPx), and glutathione reductase (GR) (Blahová et al., 2013). Although not evaluated in this study, other antioxidant enzymes such as glutathione S-transferase (GST) and GPx may have contributed to preventing lipid peroxidation. The reduction of ACAP itself may help to explain the absence of significant differences in parameters indicative of oxidative damage.

Considering the central role of non-protein thiols, especially reduced glutathione, in maintaining cellular homeostasis, their decrease indicates excessive consumption in response to atrazine-induced oxidative stress. This interpretation is supported by previous evidence demonstrating the ability of atrazine to negatively affect the antioxidant system in aquatic organisms. Blahová et al. (2013), similarly reported reduced catalase activity and absence of SOD response in zebrafish exposed to atrazine (0, 0.3, 30, and 90 µg/L) for 28 days. As mentioned earlier, Wang et al. (2022) conducted atrazine exposures (0, 0.3, 3, or 30 µg/L) in zebrafish embryos. After 9 months, they observed silencing of pathways crucial for

antioxidant defense, such as the Sirtuin 1 (SIRT1) signaling pathway. SIRT1 directly regulates the nuclear factor erythroid 2–related factor 2 (Nrf2), responsible for promoting the transcription of antioxidant enzymes (Singh et al., 2018). Notably, Wang et al. (2022) also observed mitochondrial dysfunction, which is consistent with our results, evidenced by the high ROS production. Mitochondrial alterations were also observed in neutrophils in different proteins of the cytochrome family (CYP). In addition, ROS production is involved in pro-inflammatory signaling of tumor necrosis factor alpha (TNF-α) and in the activation of apoptosis involving caspase signaling pathways and the Bcl-2/BAX ratio (Wang et al., 2019). Therefore, early-life exposure increases organism susceptibility to contaminants, mainly through oxidative stress and cell death pathways, with chronic effects extending into adulthood.

Among the biomarkers sensitive to pesticide toxicity, acetylcholinesterase (AChE) is widely recognized as a key indicator. In our study, we observed a significant reduction in AChE activity after atrazine exposure. Inhibition of this enzyme may lead to acetylcholine accumulation in synapses, impairing cholinergic transmission and affecting physiological and behavioral functions. For example, we observed pericardial edema and yolk sac edema during the embryonic-larval stage. We also identified morphological abnormalities such as head and tail malformations and shortened tail, which was reported previously after atrazine exposure in fish embryos (Chromcova et al., 2013). Taken together, the association between increased ROS levels and reduced AChE activity suggests that atrazine-induced neurotoxic mechanisms impair neuromuscular transmission, development, and locomotion in these organisms. Reduced AChE activity in the brains of adult zebrafish has been associated with pronounced alterations in social behavior and exploratory patterns. Additionally, zebrafish larvae (72 hpf) exposed to atrazine (0.3, 3, and 30 ppb) showed persistent effects, with impairment of locomotor function and significant modulation of genes involved in neurodevelopment and neurotransmission, even after 9 months (Schmidel et al., 2014; Horzmann et al., 2021). In addition to neurobehavioral effects, atrazine exposure has also been consistently associated with reproductive dysfunction. Specifically, exposure to the herbicide (0, 0.3, 3, and 30 ppb) resulted in downregulation of genes related to kisspeptin (kiss1, kiss2), as well as genes involved in gonadotropin synthesis (fshb), leading to reduced levels of reproductive hormones (Stradtman et al., 2024). Taken together, these results reinforce the notion that atrazine exerts toxicity at multiple levels, especially neurological during the embryonic-larval phase, leading to long-term behavioral and reproductive impairments.

Embryos that survived ATZ exposure (12.5%) showed some compensatory mechanisms and were thus able to control some oxidative damage, such as lipid peroxidation. Despite the absence of significant changes in spontaneous movement, atrazine exposure significantly reduced heart rate in zebrafish embryos. This result suggests that atrazine may interfere with early cardiovascular physiology during development, even at environmentally relevant concentrations. This contrast between molecular responses and the absence of detectable physiological effects indicates that atrazine toxicity, at the tested concentration, may initially manifest at more sensitive levels of biological organization, without necessarily translating into functional alterations in the short term. When comparing this mortality result with the literature, an important divergence emerges, as previous studies often report no significant mortality in chronic atrazine exposures, even at similar or higher concentrations (Corvi et al., 2012).

However, these differences may be explained by differences in experimental design. In the present study, exposure was initiated early, from 3 hours post-fertilization (3 hpf), covering the entire development of the organism. In contrast, studies such as Corvi et al. (2012) initiated exposure only at 17 days post-fertilization (17 dpf), When physiological systems are already more developed and detoxification mechanisms more efficient. Thus, exposure from early stages may have increased organism vulnerability, since these stages represent critical windows of sensitivity to environmental contaminants. This variability in responses is indeed recurrent in the literature, where atrazine effects strongly depend on concentration, exposure duration, and evaluated parameters. Corvi et al. (2012), for example, did not observe significant alterations in zebrafish sexual differentiation after chronic exposure, highlighting the inconsistency of this herbicide's effects across different studies.

Finally, it is important to highlight that the observed effects occurred at a concentration below the limit established by Brazilian legislation for drinking water (2 µg/L). This finding suggests that concentrations considered safe may induce biochemical and physiological alterations in non-target organisms, reinforcing the need to revise environmental risk assessment criteria, with emphasis on sublethal effects and exposure throughout development. Taken together, the results indicate that chronic exposure to atrazine, even at low concentration, promotes oxidative and neurochemical alterations in *Danio rerio*, which may represent early events of broader toxic processes, with potential consequences throughout the entire life cycle.

7. Conclusion

Chronic exposure to ATZ, even at concentrations below the limit established by Brazilian legislation for drinking water, induced significant biochemical alterations in zebrafish. The results demonstrated a redox imbalance, characterized by an increase in reactive oxygen species, reduction of non-protein thiols, and a decrease in catalase activity, indicating impairment of the antioxidant system. Furthermore, the reduction in heart rate observed during early development indicates that atrazine may also impair cardiovascular function in zebrafish embryos. The high mortality rate, delayed hatching, and malformations observed up to 72 hpf reinforce the toxicity of atrazine and its potential to affect early developmental stages. Taken together, these findings indicate that chronic exposure to persistent contaminants throughout the entire life cycle may result in cumulative effects, reinforcing the importance of considering early stages in the process of toxicological and ecotoxicological risk assessment.

Declarations

Data availability

The authors confirm that the data supporting the findings of this study are present within the article. Data will be provided upon request.

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Ethics approval

Ethics approval.

Consent for publication

Not applicable.

Competing interests

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Author contribution

Mayck Souza de Oliveira: conceptualization, investigation, methodology, formal analysis, experimentation, biochemical and statistical analysis, writing—original draft.

Alice Estivalet Visentini: conceptualization, methodology, experimentation, biochemical and statistical analysis.

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Jossiele Wesz Leitempeger and Bárbara Estevão Clasen: writing and review

Vania Lucia Loro: conceptualization, methodology, formal analysis, resources, supervision, writing—review.

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Figures

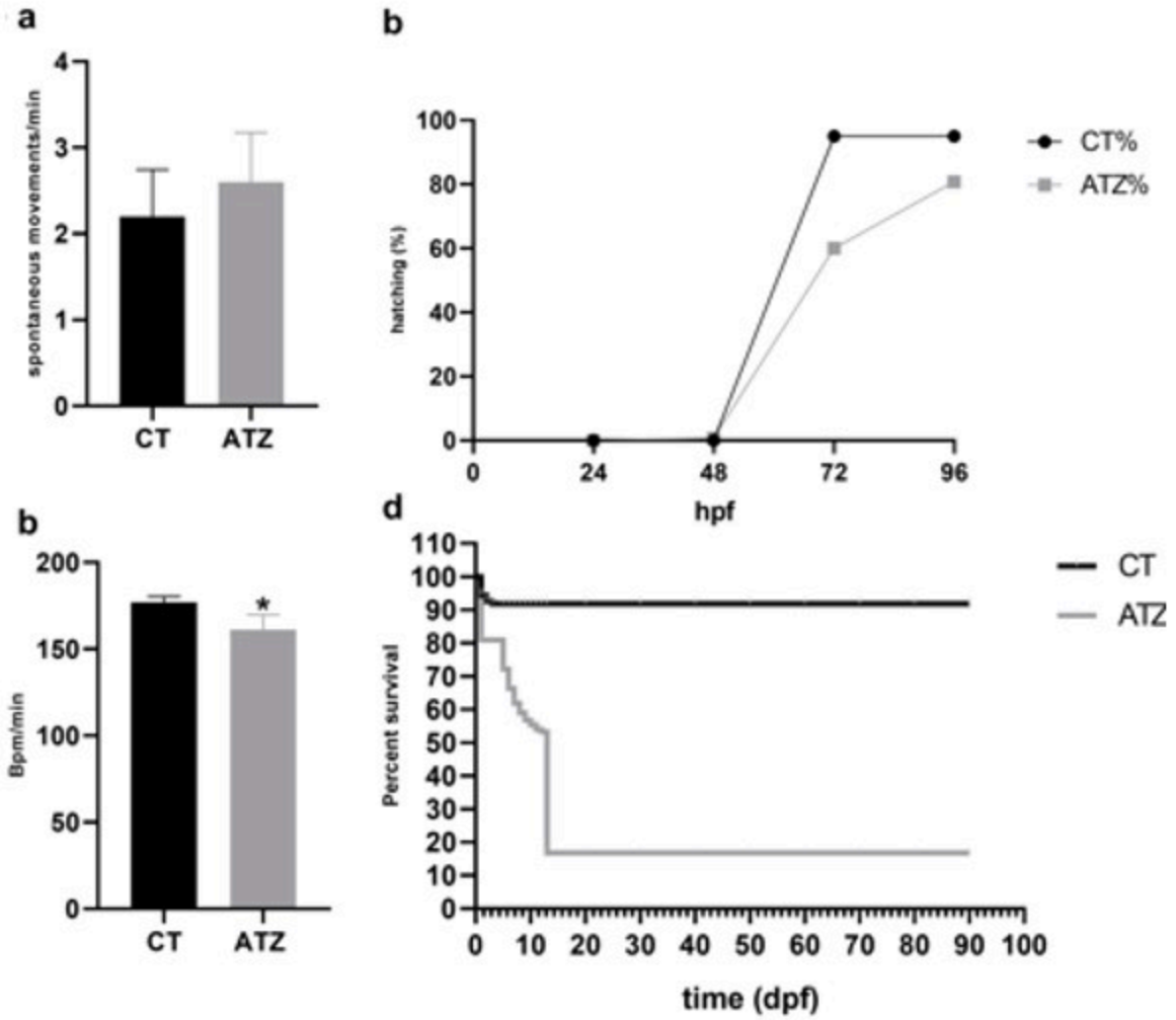


Figure 1

a) Spontaneous movement per minute in zebrafish embryos (28 hpf, n = 15); b) % of hatched embryos at 24, 48, 72, and 96 hpf (n = 120); c) Heartbeats per minute in embryos (48 hpf, n = 24). * indicates significant difference compared to the CT group ($p < 0.05$); d) Mortality (n = 120) of *Danio rerio* exposed for 90 days to 1 µg/L of ATZ.

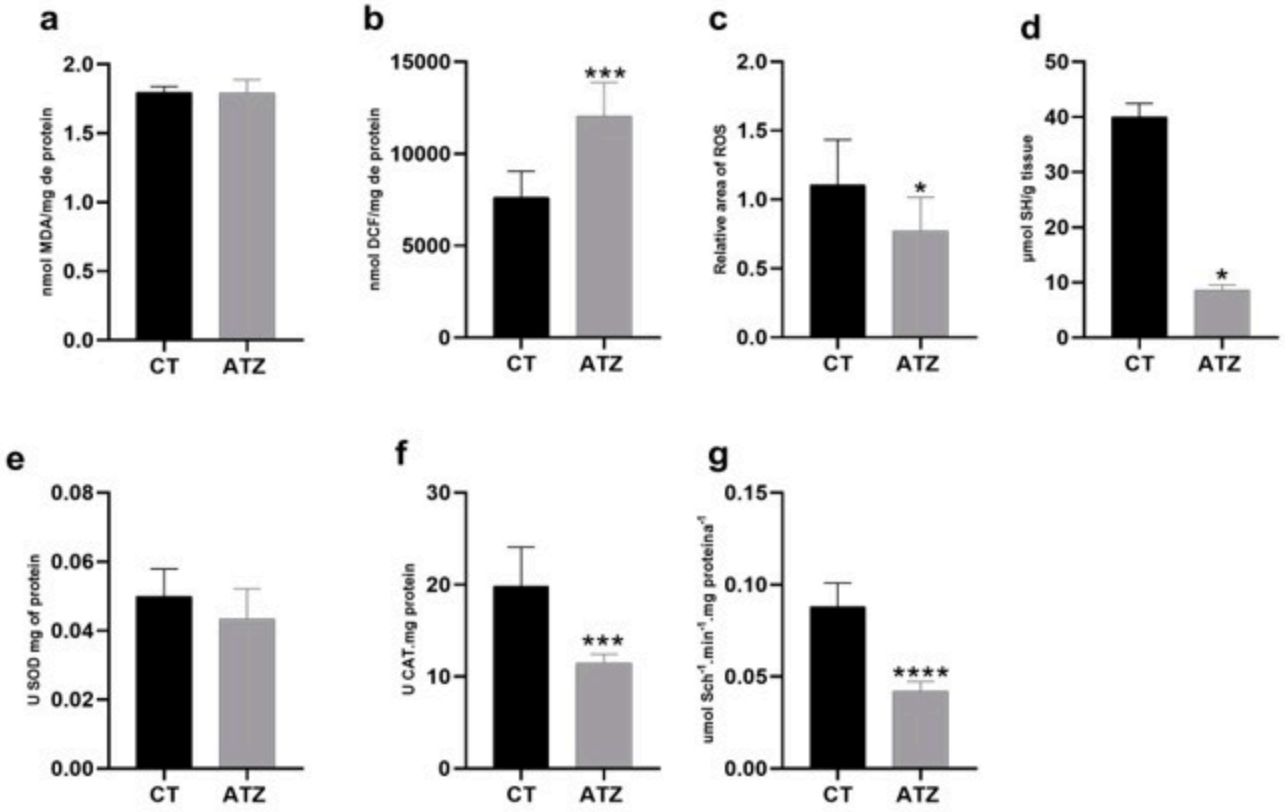


Figure 2

(a) TBARS, (b) ROS, (c) ACAP, (d) NPSH, (e) SOD, (f) CAT, and (g) AChE in *Danio rerio* exposed for 90 days to 1 µg/L of ATZ. (*, ***, **** indicate significant difference compared to the CT group (*p < 0.05; ***p < 0.001; ****p < 0.0001; n = 8).

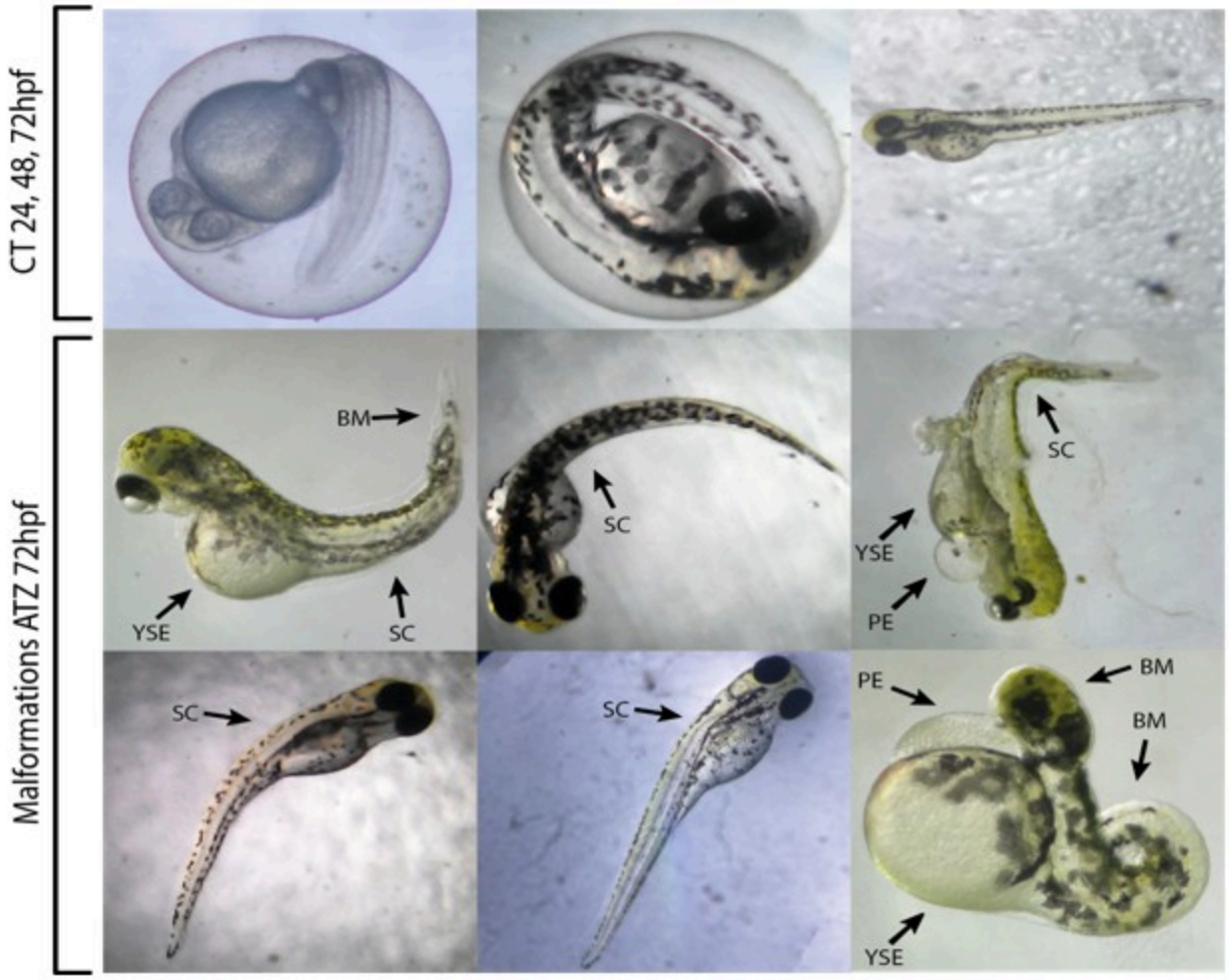


Figure 3

Malformations in zebrafish embryos exposed to ATZ (1 µg/L at 72 hpf). Pericardial edema (PE), yolk sac edema (YSE), body malformations (BM) (tail malformation, short tail, and head malformation), and spinal curvature (SC).