Histopathological changes in the gills of zebrafish (*Danio rerio*) and bullfrog tadpoles (*Lithobates catesbeianus*) caused by the use of formaldehyde

Alterações histopatológicas em brânquias de peixe-zebra (*Danio rerio*) e girinos de rã-touro (*Lithobates catesbeianus*) causada pelo uso de formaldeído

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ABSTRACT
Formaldehyde is a carcinogenic and aggressive agent mainly to epithelial tissues. However, for rearing aquatic organisms its use is common for the treatment of fungi and parasites, and the use of incorrect doses can harm the health and life of these animals. The fish species *Danio rerio* and the tadpoles of the species *Lithobates catesbeianus* are internationally recognized for use in aquatic toxicology tests. We aimed identify the effects caused by formaldehyde on the gills of these two experimental models used in aquatic toxicology, verifying the susceptibility of both species. Acute and chronic tests with formaldehyde were conducted for experiment. We found that the formaldehyde in the higher concentration caused injuries to the gills of both fish and tadpoles, with a loss and displacement of epithelium, vascular congestion, telangiectasia and lamellar epithelial lifting/edema (possible aneurysm), hyperplasia and hypertrophy of epithelial cells, lamellar fusion in addition to the proliferation of mucus-secreting cells and chloride cells. Despite structural differences, the histological changes caused by chronic exposure to formaldehyde in sublethal concentrations were similar in both organisms and we recommend reviewing its use in prophylaxis and in prolonged treatments with this chemical.

Keywords: Anurans. Branchial arches. Formalin. Histological damage. Osteichthyes.

RESUMO
O formaldeído é um agente cancerígeno e agressivo principalmente para os tecidos epiteliais. Porém, para a criação de organismos aquáticos seu uso é comum no tratamento de fungos e parasitas, e o uso de doses incorretas pode prejudicar a saúde e a vida desses animais. As espécies de peixes *Danio rerio* e os girinos da espécie *Lithobates catesbeianus* são internacionalmente reconhecidas pelo uso em testes de toxicologia aquática. Nós objetivamos identificar os principais efeitos causados pelo formaldeído nas brânquias desses dois modelos experimentais utilizados em toxicologia aquática, verificando a suscetibilidade de ambas as espécies. Testes agudos e crônicos com formaldeído foram conduzidos para experimento. Verificamos que o formaldeído nas mais altas concentrações causou lesões nas brânquias de peixes e girinos, com perda e deslocamento de epitélio, congestão vascular, telangiectasia ou edemas (possíveis aneurismas), hiperplasia e hipertrofia de células epiteliais, fusão lamelar, além da proliferação de muco - células secretoras e células de cloreto. Apesar das diferenças estruturais, as alterações histológicas causadas pela exposição crônica ao formaldeído em concentrações subletais foram semelhantes em ambos os organismos e recomendamos revisar seu uso na profilaxia e em tratamentos prolongados com este produto químico.

Palavras-chave: Anuros. Arcos branquiais. Formalina. Dano histológico. Osteichthyes.

1 INTRODUCTION

The main threats to the aquatic ecosystem are sources of pollution, loss of biodiversity and habitat destruction (Linde-Arias et al., 2008; Thushari & Senevirathna, 2020). The aquatic ecosystem is considered the most susceptible to pollution, due to natural sources or as a result of human activity such as the discharge of domestic, industrial and agricultural effluents, which can occur intentionally or accidentally (Zagatto & Bertoletti, 2008). These alterations directly affect the health of fish and amphibians, and even small changes are sufficient to trigger stressful stimuli in animals (Randall & Tsui, 2002; Gomez Isaza et al., 2020).

The *Danio rerio* species, commonly called zebrafish, is a small tropical freshwater fish, of Asian origin that has long been used as an ornamental fish. Because it has great tolerance to environmental variations and is easy to breed in captivity, it has proved to be a good organism for scientific studies (Spitsbergen & Kent, 2003; Liu et al., 2021). This fish withstands variations in temperature, pH and hardness, but in another hand, it is also very sensitive to a high number of substances. Barbazuk et al. (2000) reported that the Sanger Institute started sequencing the zebrafish genome and found that this species has a genome very similarwith the genomes of mice and humans. For these reasons, this species is used worldwide as a toxicological model (Teraoka et al., 2003). The species *Lithobates catesbeianus*, known as the bullfrog, is native to northeastern North America. It is a robust and resistant species when compared to other frogs and is prolific and disseminated worldwide (Frost, 2016). Due to these characteristics and its easy acquisition and maintenance in the laboratory, it is also considered an excellent experimental model for use in ecotoxicological tests. Moreover, approximately 70% of anuran species have a life cycle linked to aquatic environments, a fact that makes them good indicators of water quality (França et al., 2015).

The principle of the 3 R's of animal experimentation proposed by Russel and Burch (1959) recommends "*replace*", which translates into replacing sentient animals, that is, those that are capable of experiencing pain; "*reduction*", which means reducing the number of animals used, without jeopardizing the reliability of the results; and "*refinement*", which means the decrease in the incidence or severity of applied procedures (CONCEA, 2014). Corroborating this line of thought there are international recommendations such as the Associação Brasileira de Normas Técnicas - NBR ABNT 15088 of 2016 in Brazil (method of experimenting with fish), which recommends the reducing use of highly complex organisms in aquatic toxicology tests. In this sense, the tests with *D. rerio* and *L. catesbeianus* are considered a cutting-edge alternative and are increasingly used to replace the tests carried out with mammals.

In the cultivation of aquatic organisms, some chemicals and prophylactic products are used to maintain the health of the crops (Noga, 2010). Among these, formaldehyde (CH_2O) is indicated by the FDA (Food and Drug Administration) in the treatment of fungal, bacterial and parasitic diseases, even though it is a chemical agent considered to be carcinogenic (IARC, 2004). Formaldehyde is a colorless gas with an irritating odor and one of the most common and abundant aldehydes in the environment used in the production of resins and furniture, paper, plastics, the textile industry and chemicals such as preservatives and disinfectants (ECOTOX, 2006). Its residue can potentially contaminate air, soil and water (Bueno-Guimarães et al., 2001). This chemical is very reactive and interacts with glycoprotein proteins, genetic material and polysaccharides (De Swaef et al., 2015; Leal et al., 2018). Devaraj et al. (2021) ratify that formalin is a 37% aqueous solution of formaldehyde and used in the treatment of pathologies caused by bacteria, fungi and protozoa in fish. Actually, it is one of the main products used in aquatic organisms rearing for the prophylaxis and treatment of various pathogens (De Swaef al. 2015; Resendes et al., 2018). However, there is evidence that this product may be aggressive to the gills of fish and amphibians (Bueno-Guimarães et al., 2001; Ramos et al., 2014). In these animals, the gills have several functions and are very important for gas exchange, osmoregulation processes, acid-base balance and excretion of nitrogen compounds. The gills can, thus, be an excellent biomarking tool for environmental damage and indicators of water quality (Machado & Fanta, 2003; Evans et al., 2006).

Our objective with this study was to identify the main effects caused by formaldehyde to the gills of two experimental models used in aquatic toxicology: zebrafish (*D. rerio*) and bullfrog tadpoles (*L. catesbeianus*), verifying the susceptibility of both species to this chemical.

2 MATERIALS AND METHODS

For the experiments, two species of aquatic organisms were used: zebrafish (*D. rerio*) and bullfrog tadpoles (*L. catesbeianus*) (Gosner, 1960 - stages 31 to 36). The tests were divided into two stages, the acute test (96h) and the chronic test (192h). Both experiments followed the same protocol adapted to the peculiarities of each species.

This research was carried out in accordance with the institutional, national and international rules for the use of animals in research and authorized by the Ethics Committee of Fisheries Institute under number 1535 for both species.

The technical standards for conducting the trials followed the recommendations according to pre-established protocols (APHA, 2005; ABNT, 2016; ASTM, 2014). The formaldehyde (P.A.) stock solution (Synt™) at a concentration of 100 g/L was prepared on the day of each intoxication in a 500 mL flask, 125 mL of formaldehyde and 375 mL of distilled water.

EXPERIMENTS WITH ZEBRAFISH (D. RERIO)

The experiments of acute and chronic toxicity with *D. rerio* fish were carried out and published by Resendes et al. (2018). Respecting the principle of the 3 R's, we used the branchial tissue samples that were collected by these authors, but we clarify that these samples were not analyzed. We emphasizing that Resendes et al. (2018) only reported toxicity data (*i.e.* mortality) and does not include histopathological analysis of the effects of formaldehyde. The Median Lethal Concentration at 96 h (LC50-96h) for formaldehyde obtained by these authors was 45.73 mg/L and the chronic exposure concentrations were: LC50-96h/100 (T-1) - 0.45 mg/L; LC50-96h/10 (T-2) - 4.57 mg/L and LC50-96h/2 (T-3) - 22.86 mg/L, in addition to the negative control (without adding the product).

At end of chronic experimentation (192 h), eight fish from each treatment were sacrificed by deep anesthesia (eugenol - 7 mL/L) to remove the gills which were conserved in 10% buffered formalin and analyzed in the present study (n = 32).

EXPERIMENTS WITH BULLFROG TADPOLES (L. CATESBEIANUS)

Acute test

After preliminary tests, the acute toxicity test lasted 96 h, with a completely randomized design with water renewal every 24 hours and six treatments. The treatments were: 4, 8, 12, 16, and 20 mg/L of formaldehyde plus the control group (without adding the product). We working with four replicates. The aquariums were filled with the public water supply, dechlorinated overnight with aeration.

Temperature, pH, electrical conductivity and dissolved oxygen of water were performed using the AK87 Akso™ oximeter and the Hl98129™ equipment. At the beginning of the experiment, the tadpoles were weighed and haven't been fed. A density of one tadpole per liter was used, totaling eight tadpoles per aquarium.

To verify the possible significant differences between the physical and chemical variables of water, one-way analysis of variance ($p < 0.05$) was used (Zar, 1999). We used the Gwbasis 3.0 Software to estimate the average lethal concentration according to the “Trimmed Spearman Karber” statistical method (Hamilton et al., 1977).

Chronic test

The test was semi-static, with solutions renewed after 96 h, and the exposure period was 192h, following the same experimental design as Resendes et al. (2018) including four treatments with four simultaneous replicates, namely: LC50-96h/100 (T-1), LC50-96h/10 (T-2) and LC50-96h/2 (T-3), as well as the negative control (without adding the product).

Sixteen aquariums were randomly distributed under the work benches, each containing 12 L of water, one tadpole per litter, constant aeration, containing a total of 192 tadpoles.

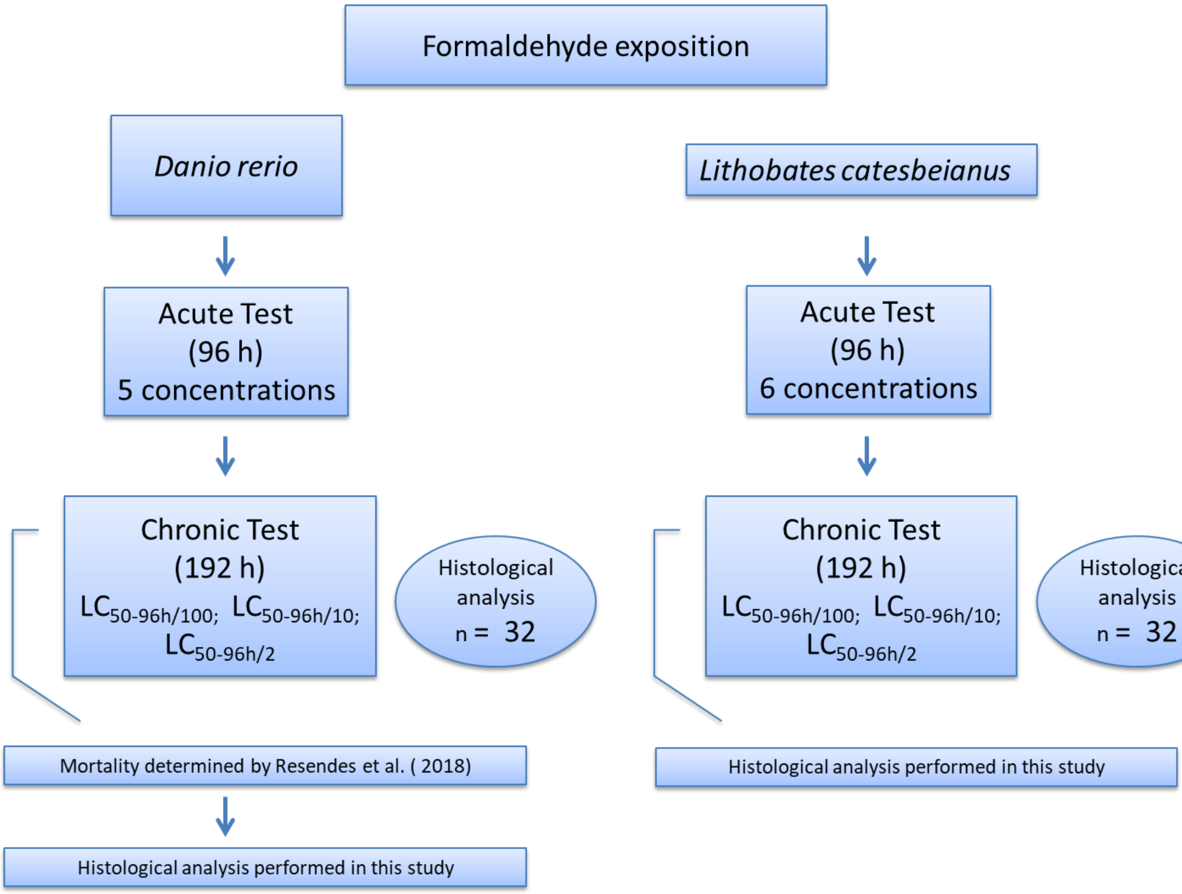
The values of pH, temperature and dissolved oxygen, hardness and total ammonia were evaluated before and after the water was renewed at 96 h. The tadpoles were weighed before the beginning of the experiment and fed with Laguna crushed feed (Socil® 40 % PB) every two days, in the 0.5 % of the biomass of the aquarium.

At end of chronic experimentation (192 h), eight tadpoles from each treatment were sacrificed by deep anesthesia (eugenol - 7 mL/L) to remove the gills which were preserved in 10 % buffered formalin (n = 32).

HISTOPATHOLOGICAL ANALYSES

Histopathological analyses of the gills were performed following a standard protocol for this technique. The gills, being very small, were wrapped in gauze and packed in identified boxes, dehydrated in an increasing battery of alcohol baths (70%, 80%, 95% and absolute), diaphanized in xylol, embedded in paraffin and stored in an oven for 18 h at 52 °C. After this period, they were embedded in paraffin and subjected to 4.5 µm thick horizontal cuts with the aid of the Hyrax Zeiss® microtome. The cuts were placed on slides with gel, for better fixation, and deparaffinized in a xylol and alcohol baths for later staining with Hematoxylin and Eosin (H&E). The last bath included immersion in xylene and decreasing gradient of alcoholand the fragment was then mounted on a slide with Etellan™ and was covered with a cover slip. Optical microscope™ CARL-Zeiss Axio Scope and ZEN™ were used for observation and recording of the images. Figure 1 presents a graphical representation of the experimental design.

Figure 1 Graphical representation of the experimental design of acute (96h) and chronic test (192h) with different formaldehyde concentrations using *Danio rerio* (zebrafish) and *Lithobates catesbeianus* (bullfrog) like experimental model.



3 RESULTS

TOXICOLOGICAL TESTS FOR BULLFROG TADPOLES

Temperature, pH, electrical conductivity and dissolved oxygen of water remained within the values considered acceptable for the maintenance of this type of organism in toxicological tests for *L. catesbeianus* species (Cribb et al., 2013; Lombardi et al., 2002) and were stable during the performance of the test, with no statistically significant differences between them. The mean values and standard deviation observed were: temperature 22.62 ± 0.8 ºC, pH 7.51 ± 0.1, electrical conductivity 121.06 ± 9.3 µS/cm and, dissolved oxygen 7.8 ± 0.8 mg/L. The tadpoles had an average weight of 3.67 ± 0.27 g during acute test.

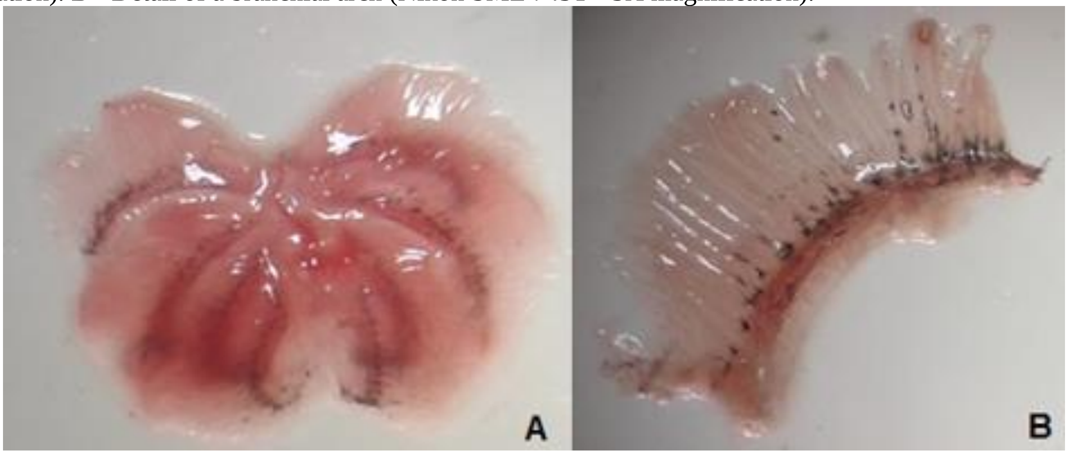
The acute test showed that bullfrog tadpoles exhibited LC50-96h = 9.17 mg/L of formaldehyde. Based on these results, we used the following concentrations of formaldehyde during the chronic test: $LC_{50/100}$ = 0.09 mg/L, $C_{50/10}$ = 0.92 mg/L and $LC_{50/2}$ = 4.58 mg/L. The physical and chemical parameters of the water showed the following average results: pH 7.05 ± 0.16, dissolved oxygen 7.03 ± 1.13 mg/L, temperature 24.5 ± 0.56 ºC, total ammonia (NH_4) 2.7 ± 0.7 mg/L, hardness

1.19 ± 0.29 °dH. The average weight of the animals was 3.38 ± 0.39 g and there was no mortality during this experimentation.

HISTOPATHOLOGICAL ANALYSES

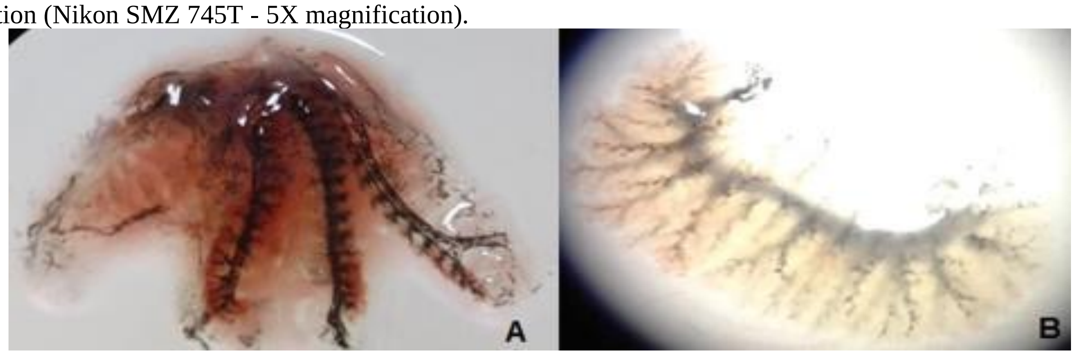
The gills of zebrafish have the standard formation of teleost fish, that is, they are external with four delicate pairs of branchial arches, containing small tracks located in the internal curvature of the arch, and numerous filaments that have an axis of hyaline cartilage lined with multilamellar squamous branchial epithelium made up of mucous, hydrochloric and columnar cells (Figure 2).

Figure 2 Macroscopic view of *Danio rerio* gills. A - Four pairs of branchial arches (Nikon SMZ 745T - 2X magnification). B - Detail of a branchial arch (Nikon SMZ 745T - 5X magnification).



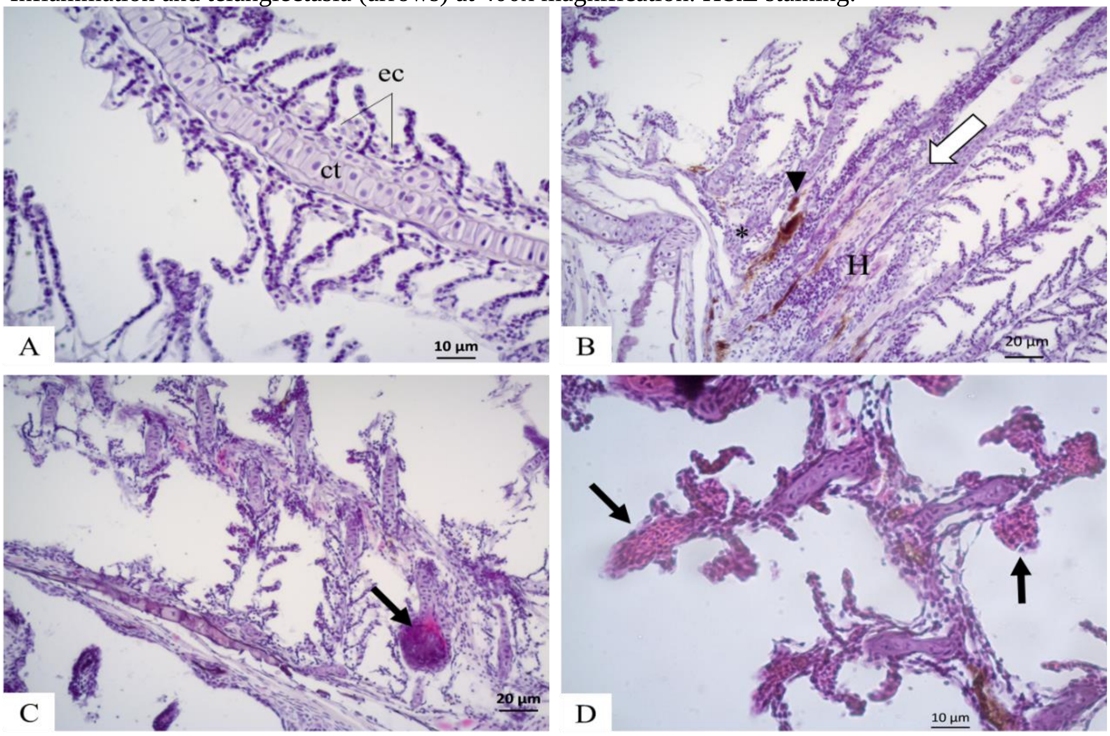
The gill of bullfrog tadpoles showed a structural pattern confirmed in this study. This apparatus is formed by four pairs of branchial arches. The gills have numerous finger-shaped branches known as gill tufts that can be primary or secondary. They are very vascularized and are responsible for gas exchange. They are also involved in a lot of mucus (Bueno-Guimarães et al., 2001; Viriato et al., 2020) (Figure 3).

Figure 3 Macroscopic view of gill tadpoles of *Lithobates catesbeianus* Gosner (1960) stages 31 to 36. A - Four pairs of gill arches (Nikon SMZ 745T - 2X magnification). B - Detail of a gill arch showing tufts with numerous finger-like ramification (Nikon SMZ 745T - 5X magnification).



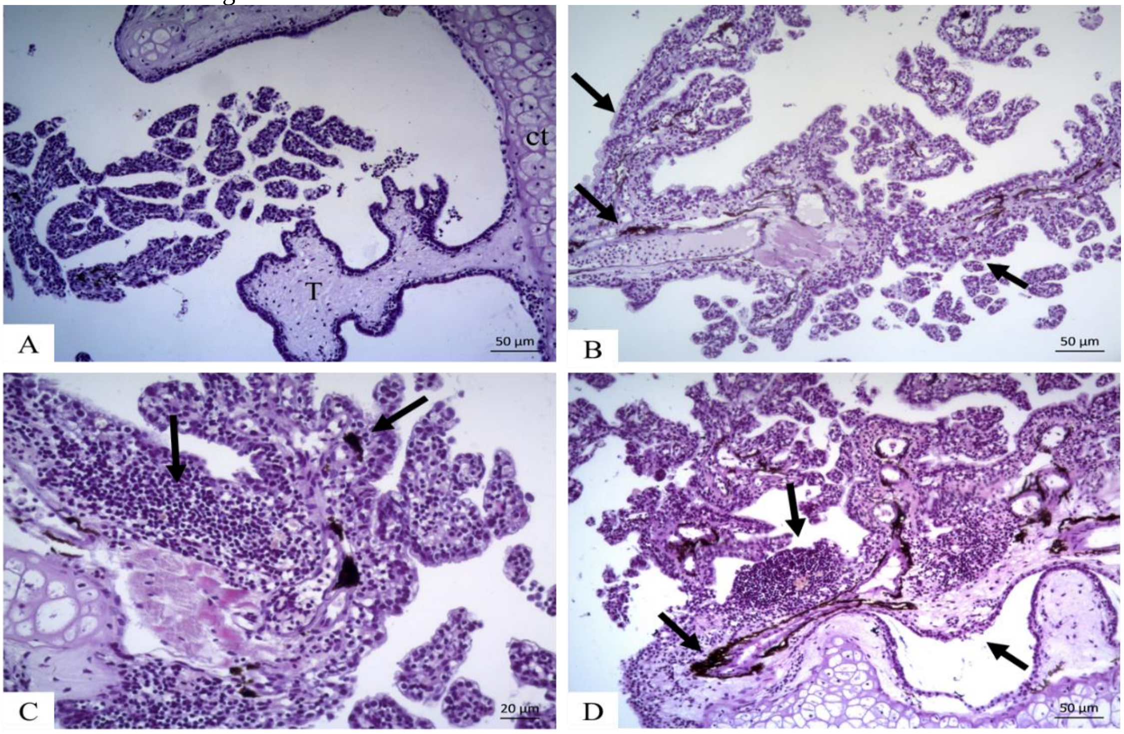
In zebrafish exposed to sublethal concentrations of formaldehyde, morphological alterations in the gills were observed. Structural disorganization of normal tissue with the loss and displacement of epithelium and rupturing of epithelial cells were observed in all treatments that contained formaldehyde. In T-1 treatment were observed the same injuries found by Zang et al. (2019) in their study, that is, lamellar fusion, hypertrophy and hyperplasia of epithelial cells and the presence of melanomacrophages. Only in T-2 and T-3 treatments were observed high formation of telangiectasia and lamellar epithelial lifting/edema (possible aneurysm), inflammation and proliferation of mucus-secreting cells and chloride cells (Figure 4).

Figure 4 Comparative photomicrograph of zebrafish (*Danio rerio*) gills exposed for 192 h to formaldehyde. (A) Negative Control - primary lamella with epithelial cell layer (ec) and cartilaginous support tissue (ct) showing normal morphology at 400x magnification; (B) T-1 (0.45 mg/L) - Lamellar fusion (white arrow), hyperplasia (H), hypertrophy (*) and melanomacrophages (arrowhead) at 200x magnification; (C) T-2 (4.57 mg/L) telangiectasia and lamellar epithelial lifting/edema (possible aneurysm) (arrow) of the secondary lamellae at 200x magnification; (D) T-3 (22.86 mg/L) - Inflammation and telangiectasia (arrows) at 400x magnification. H&E staining.



In bullfrog tadpoles exposed to chronic test, morphological alterations in the gill tufts were also observed. The vessels were highly congested and lymphocyte infiltration were present in all treatments that contained formaldehyde. In T-1 treatment were observed congested venous sinus and epithelial cell hyperplasia. In T-2 and T-3 treatments were observed areas of inflammation and the presence of melanomacrophages and also cell necrosis in the highest concentration of formaldehyde (Figure 5).

Figure 5 Photomicrograph of *Lithobates catesbeianus* tadpoles' gills exposed for 192 h to formaldehyde. (A) Negative control – gill tuft with ramifications (T) and cartilaginous support tissue (ct) showing normal morphology at 100x magnification; (B) T-1 (0.09 mg/L) - Congested venous sinus, epithelial cell hyperplasia (arrows) at 100x magnification; (C) T-2 (0.92 mg/L) – Inflammation of tuft tissue and melanomacrophages (arrows) at 100x magnification; (D) T-3 (4.58 mg/L) - Inflammatory reaction, congested vessels, cell necrosis and melanomacrophages (arrows) at 100x magnification. H&E staining.



4 DISCUSSION

Formaldehyde is a ubiquitous compound classified as carcinogenic to humans and is tumorigenic and teratogenic for producing effects on reproduction (IARC, 2014), but even so it is indicated for the parasitic treatment and fungal control of aquatic organisms (De Swaef et al., 2015; Noga, 2010). Devaraj et al. (2021) report that due to its association with cancer and tumor development, this chemical is not approved for use in aquaculture in several countries in Europe and also in Japan. A few scientific articles have reported the acute and chronic toxicity of this chemical: Santana et al. (2015) working with *L. catesbeianus* tadpoles obtained an LC50-96h of formaldehyde at 10.53 mg/L. Other researchers reported 21.78 mg/L for American catfish and 48.8 mg/L for rainbow trout (Hohreiter & Rigg, 2001). We can also quote Resendes et al. (2018) that obtained 45.73 mg/L for *D. rerio*. In the present study, we determined 9.17 mg/L for bullfrog tadpoles and we confirm the Santana et al. (2015) datas, indicating that the *L. catesbeianus* is a species that is very susceptible to formaldehyde.

Various concentrations of formaldehyde have been tested for chemotherapeutic efficacy and its possible harmful effects in the treatment of aquatic animals; however, the vast majority demonstrate hyperplasia of the branchial cells, genotoxic damage and animal mortality, indicating the toxicity of this chemical (Martins, 2004; Santana et al., 2015; Resendes et al., 2018). According to Martins (2004), 37% formaldehyde can be used in the form of a short bath (up to 60 min) in the concentration of 55.5 mg/L to 92.5 mg/L and in long-term baths (24 h) at a concentration of 3.7 mg/L to 5.55 mg/L. However, Paixão et al. (2013) working with these concentrations with the fish species *Hemigrammus* (a characid widely used in aquariums) using formaldehyde baths for 60 minutes reported 100% mortality. The authors suggest that this may have been due to the fact that wild fish are more susceptible to stress and less rustic than those in cultivation. Thus, greater care must be taken in the storage and treatment of wild native fish for export, as the stress response becomes more pronounced and adaptation to the new condition can be compromised. Martins (2004), states that formaldehyde, which came to replace malachite green in the treatment against parasites and fungi, has been highlighted for its effectiveness; if applied correctly. According to Cruz et al. (2005) formaldehyde’s toxicity preliminary tests are essential for the definitive use of this treatment.

Studies of Hermenean et al. (2015) and Ogbeide et al. (2019) reported that when aquatic organisms remain in contact with low concentrations of contaminants for long periods, biochemical, physiological and histological changes can occur belong irreversible. Likewise, damage to the gills by chemicals can cause a series of injuries and lead to respiratory problems. (Magare & Patil, 2000). The branchial epithelium is one of the main surfaces of contact with the environment and constitutes one of the organism’s first lines of defense, and consequently, one of the first organs to be affected. Despite being a consensus in the scientific community, branchial lesions are poorly documented, especially when referring to amphibians.

In aquatic freshwater organisms, most of the time, water enters the gills and its excess is eliminated by the kidneys. The teleostatic gill is, therefore, the most important osmoregulatory organ (Motais & Garcia-Romeu, 1972), although currently there are new mechanisms by which fish can alter the functional branchial area and the diffusion distance (Wood & Eom, 2021).

In fish the branchial structure is very similar between different species. Saltys et al. (2006) affirmed that the gills of fish an tadpoles are derived from the same embryonic pharyngeal arches and innervation of the Xenopus larvae' gills closely resembles that observed in *D. rerio*. In amphibians there is considerable diversity between anurans in the structure and types of their branchial attachments. The structure of the tadpoles' larval branchial apparatus is closely associated with the mechanisms of pumping and oral feeding that undergo profound changes to form the adult hyoid apparatus, which supports the laryngeal structures and serves as a base for the tongue (Bandara et al., 2012). The gill apparatus of both species (*L. catesbeanus* and *D. rerio*) are supported by four pairs of gill arches. In bullfrog tadpoles, gill tufts are highly vascularized and is responsible for gas exchange, which corresponds to lamellas function in zebrafish.

The most common reactions in response to exposure to chemical agents are cellular growth and increased mucus production (Wong & Wong, 2000). In this study, for both species, the main histopathological changes varied as the formaldehyde concentration increased, that is, the severity of the lesions increased with the increase in formaldehyde concentrations and exposure time. Comparatively, we observed a greater tolerance to formaldehyde by fish (*D. rerio*). The most common gills injuries observed in both species (*L. catesbeanus* and *D. rerio*) were inflammation and presence of melanomacrophages. The highest concentration to which the tadpoles were exposed is equivalent to an intermediate concentration of exposure of these fish; but regardless of tolerance the lesions were present. Interstitial edematous areas, epithelial desquamation, epithelial hyperplasia and hypertrophy and lamella fusion in some animals are lesions that suggest defense mechanisms (Karlsson-Norrgren et al., 1985; Erkmen & Kolankaya, 2000). These responses hinder the access of the toxic agent to the blood and impairing gas exchange (McDonald & Wood 1993). In turn, breathing difficulties may be responsible for inducing vasodilation. Studies by Bueno-Guimarães et al. (2001), Pahor-Filho et al. (2015) and Ramos et al. (2014) prove this.

The present work has demonstrated that the organisms exposed to the chemical agent, formaldehyde, produced an altered cellular and histological responses that can be described as injurious. Despite the structural differences, the histological changes in both organisms were similar and we suggest, therefore, a review of the recommendations for its use in the prophylaxis and treatment of aquatic organisms.

CONFLICTS OF INTEREST

We declare no conflicts of interest.

ETHICS STATEMENT

We declare that the contribution is unpublished, the referred manuscript is not being evaluated for publication in another journal and that the text does not fit in the situations described in editorial policy on plagiarism.

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REFERENCES

ABNT - Associação Brasileira de Normas Técnicas. NBR 15088. Ecotoxicologia aquática – Toxicidade aguda. Método de ensaio com peixes. São Paulo, 2016, p.19.

APHA; AWWA; WPCF. Standard methods for the examination of water and wastewater. 20ª. ed., Washington DC. 2005.

ASTM - American Society For Testing And Materials. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates and amphibians. West Conshohocken, PA, 2014, p 218-238.

Bandara MGDK, Wijesinghe MR, Ratnasooriya WD, Priyani AAH Chlorpyrifos-induced histopathological alterations in *Duttaphrynus melanostictus* (Schneider 1799) tadpoles: evidence from empirical trials. J Trop For Sci. 2012; 2: 27-36. http://doi:10.31357/jtfe.v2i2.592.

Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, Bedell JA, Mcpherson JD, Johnson SL The syntenic relationship of the zebrafish and human genomes. Genome Res. 2000; 10: 1351-1358. https://doi:10.1101/gr.144700.

Bueno-Guimarães, HM, Ferreira CM, Garcia MLB, Saldiva PHN Tadpole epithelium test: potential use of *Rana catesbeiana* histopathologic epithelial changes to evaluate aquatic pollution. Bull Environ Contam Toxicol 2001; 67: 202-209. https://doi:10.1007/s001280111.

CONCEA 2014. Resolução Normativa nº 17. https://www.mctic.gov.br/mctic/opencms/institucional/concea/paginas/legislacao. Accessed 6 August 2019.

Cribb AY, Afonso AM, Mostério CMF Manual técnico de ranicultura. Embrapa, Brasília, 2013.73p.

Cruz C, Fujimoto RY, Luz RK, Portella MC, Martins ML Toxicidade aguda e histopatologia do fígado de larvas de trairão *Hoplias lacerdae* expostas à solução aquosa de formaldeído a 10%. Pesticidas: Rev. Ecotoxicologia e Meio Ambiente 2005; 15: 21-28. https://doi.org/10.5380/pes.v15i0.4502.

De Swaef E, Den Broeck WV, Dierckens K, Decostere A Disinfection of teleost eggs: a review. Aquaculture 2015; 7: 1–21. https://doi.org/10.1111/raq.12096.

Devaraj P, Babu V, Cengiz EI Qualitative detection of formaldehyde and ammonia in fish and other seafoods obtained from Chennai's (India) fish markets. Environ Monit Assess. 2021; 193(2):78. https://doi.org/10.1007/s10661-021-08871-z.

ECOTOX Data Base. 2006. Avaible in: https://cfpub.epa.gov/ecotox. Accessed in May 2018.

Erkmen B, Kolankaya D Effects of water quality on epithelial morphology in the gill of *Capoeta* tinca living in two tributaries of Kızılırmak River, Turkey. Bull Environ Contam Toxicol. 2000; 64: 418-425. https://doi.org/10.1007/s001280000017.

Evans JJ, Klesius PH, Shoemaker CA Therapeutic and prophylactic immunization against *Streptococcus iniae* infection in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Aquac Res. , 2006; 37: 742-750. https://doi.org/10.1111/j.1365-2109.2006.01487.x.

França FM, Brazil De Paiva TC, Marcantônio AS, Teixeira PC, Ferreira CM Acute toxicity and ecotoxicological risk assessment of rice pesticides to *Lithobates catesbeianus* tadpoles. J Environ Sci Heal B. 2015; 50: 406-410. https://doi.org/10.1080/03601234.2015.1011950.

Frost DR Amphibian Species of the World. American Museum of Natural History. New York. 2016. Avaible in: http://research.amnh.org/vz/herpetology/amphibia/index.html. Accessed 21 May 2019.

Gomez Isaza DF, Cramp RL, Franklin CE Living in polluted waters: A meta-analysis of the effects of nitrate and interactions with other environmental stressors on freshwater taxa. Environ Pollut. 2020; 114091. https://doi.org/10.1016/j.envpol.2020.114091.

Gosner KL A simplified table for standing anuran embryos and larvae with notes on identification. Herpetologica 1960; 16: 183-190.

Hamilton MA, Russo RC, Thurston RV Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol. 1977; 11: 714-719. https://doi.org/10.1021/es60130a004.

Hermenean A, Damache G, Albu P, Ardelean A, Ardelean G, Puiu Ardelean D, Horge M, Nagy T, Braun M, Zsuga M, Kéki S, Costache M, Dinischiotu A Histopatological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. Ecotoxicol Environ Saf. 2015; 119: 198-205.

Hohreiter DW, Rigg DK Derivation of ambient water quality criteria for formaldehyde. Chemosphere 2001; 45: 471-486. https://doi.org/1016/S0045-6535(01)00061-3.

IARC - International Agency for Research on Cancer, World Health Organization. *IARC* Monographs on the Evalution of Carcinogenic Risks to Humans. 2014. 88, 483. http://monographs.iarc.fr/ENG/Monographs/vol88/index.php. Accessed 21 May 2019.

Leal JF, Neves MGPMS, Santos EBH, Esteves VI Use of formalin in intensive aquaculture: properties, application and effects on fish and water quality. Rev Aquacult. 2018; 10: 281–229. https://doi.org/10.1111/raq.12160.

Linde-Arias AR, Inácio AF, de Alburquerque C, Freire MM, Moreira JC Biomarkers in an invasive fish species, *Oreochromis niloticus*, to assess the effects of pollution in a highly degraded Brazilian River Sci Total Environ. 2008; 399: 186-192. https://doi.org/10.1016/j.scitotenv.2008.03.028.

Liu TT, Hou H, Du JL A protocol for simultaneous Ca^{2+} and morphology imaging of brain endothelial tip cells in larval zebrafish. STAR Protoc. 2021; 2(1):100388. https://doi.org/10.1016/j.xpro.2021.100388.

Lombardi JV, Perpetuo TR, Ferreira CM, Machado-Neto JG, Marques HLA Acute toxicity of the fungicide copper oxychloride to tadpoles of the bullfrog *Rana catesbeiana*. Bull Environ Contam Toxicol. 2002; 69: 415-420. https://doi.org/10.1007/s00128-002-0078-6.

Machado MR, Fanta E Effects of the organophosphorous methyl parathion on the branchial epithelium of a freshwater fish *Metynnis roosevelti*. Braz Arch Biol Technol. 2003; 46: 361-372. https://doi.org/10.1590/S1516-89132003000300008.

Magare SR, Patil HT **Effect of pesticides on oxygen consumption, red blood cell count and metabolites of a fish,** *Puntius ticto*. Environ Ecology 2000; 18: 891-894.

Martins ML Cuidados básicos e alternativas no tratamento de enfermidades de peixes na aquicultura brasileira. In: Ranzani-Paiva MJ, Takemoto RM, Lizama MAP (eds.). Sanidade de Organismos Aquáticos, São Paulo: Editora Varela, 2004, p. 355-368

McDonald DG, Wood CM Branchial mechanisms of acclimation to metals in freshwater fish. In Fish ecophysiology. Springer, Dordrecht, 1993, p. 297-321.

Motais R, Garcia-Romeu F Transport mechanisms in the teleostean gill and amphibian skin. Annu Rev Physiol. 1972; 34: 141-76. https://doi.org/10.1146/annurev.ph.34.030172.001041.

Noga EJ Fish Disease: Diagnosis and Treatment. Iowa: Wiley-Blackwell. 2010. 378p.

Ogbeide O, Uhunamure G, Uwagboe L, Osakpamwan T, Glory M, Chukwuka A Comparative gill and liver pathology of *Tilapia zilli*, *Clarias gariepinus* and *Neochanna diversus* in owan river (Nigeria): Relative ecological risks of species in a pesticide impacted river. Chemosphere 2019; 234: 1-13. https://doi.org/10.1016/j.chemosphere.2019.06.055.

Pahor-Filho E, Miranda Filho KC, Klosterhoff M, Romano LA, Pereira Júnior J Histopathological and behaviour effects of formaldehyde treatment in juvenile mullet, *Mugil liza* (Valenciennes). Aquac Res. 2015; 46: 3040-3045. https://doi.org/10.1111/are.12462.

Paixão LF, Santos RFB, Ramos FM, Fujimoto RY Efeitos do tratamento com formalina e sulfato de cobre sobre os parâmetros hematológicos e parasitos monogenéticos em juvenis de *Hemigrammus* sp. (*Osteichthyes*: *Characidae*). Acta Amazonica 2013; 43: 211-216.

Ramos T, Romano LA, Pereira JRJ Evolução histopatológica em brânquias de tainhas *Mugil liza* expostas à banhos terapêuticos com formalina. Rev Bras Engenharia de Pesca 2014; 7: 45-59. https://doi.org/10.18817/repesca.v7i1.1064.

Randall DJ, Tsui TKN Ammonia toxicity in fish. Mar Pollut Bull. 2002; 45: 17-23. https://doi.org/10.1016/S0025-326X(02)00227-8.

Resendes AS, Santos DS, França FM, Petesse ML, Badaró-Pedroso C, Ferreira CM Acute toxic and genotoxic effects of formalin in *Danio rerio* (zebrafish). Ecotoxicology 2018; 27:1379-1386. https://doi.org/10.1007/s10646-018-1993-6.

Russel, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique, Methuen, London, Johns Hopkins University, Baltimore.

Saltys HA, Jonz MG, Nurse CA Comparative study of gill neuroepithelial cells and their innervation in teleosts and *Xenopus* tadpoles. Cell Tissue Res. 2006; 323: 1–10. https://doi.org/10.1007/s00441-005-0048-5.

Santana JM, Dos Reis A, Teixeira PC, Ferreira FC, Ferreira CM Median lethal concentration of formaldehyde and its genotoxic potential in bullfrog tadpoles (*Lithobates catesbeianus*). J Environ Sci Heal B. 2015;50: 896-900. https://doi.org/10.1080/03601234.2015.1067095.

Santos EF, Tavares-Dias M, Pinheiro DA, Neves LR, Marinho RGB, Dias MKR Fauna parasitária de tambaqui *Colossoma macropomum* (Characidae) cultivado em tanque-rede no estado do Amapá, Amazônia oriental. Acta Amazonica 2013; 43: 105 – 112.

Spitsbergen JM, Kent ML The state of the art of the zebrafish model for toxicology and toxicologic pathology research–advantages and current limitations. Toxicol Pathology 2003; 31 (Suppl.): 62–87. https://doi.org/10.1080/01926230390174959.

Teraoka H, Dong W, Tsujimoto Y, Iwasa H, Endoh D, Ueno N, Stegeman JJ, Peterson RE, Hiraga T Induction of cytochrome P450 1A is required for circulation failure and edema by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in zebrafish. Biochem Biophys Res Commun. 2003; 304: 223-228. https://doi.org/10.1016/S0006-291X(03)00576-X.

Thushari GGN, Senevirathna JDM Plastic pollution in the marine environment. Heliyon. 2020; 6(8):04709. https://doi: 10.1016/j.heliyon.2020.e04709.

Viriato C, França FM, Santos DS, Marcantonio AS, Badaró-Pedroso C, Ferreira CM Evaluation of the potential teratogenic and toxic effect of the herbicide 2,4-D (DMA® 806) in bullfrog embryos and tadpoles (*Lithobates catesbeianus*). Chemosphere 2020; 266:129018. https://doi.org/10.1016/j.chemosphere.2020.129018.

Wong CK, Wong MH Morphological and biochemical changes in the gills of tilapia (*Oreochromis mossambicus*) to ambient cadmium exposure. Aquat Toxicol. 2000; 48: 517-527. https://doi.org/10.1016/S0166-445X(99)00060-0.

Wood CM, Eom J The osmorespiratory compromise in the fish gill. Comp Biochem Physiol A Mol Integr Physiol. 2021; 254: 110895. https://doi.org/10.1016/j.cbpa.2021.110895.

Zhang Y, Feng J, Gao Y,Liu X, Qu L, Zhu L Physiologically based toxicokinetic and toxicodynamic (PBTK-TD) modelling of Cd and Pb exposure in adult zebrafish *Danio rerio*: Accumulation and toxicity. Environ Pollut 2019; 249: 959-968.https://doi: 10.1016/j.envpol.2019.03.115.

Zagatto PA, Bertoletti E. Ecotoxicologia Aquática – Princípios e Aplicações. 2ª Ed. São Carlos: Rima. 2008. 486 p.

Zar JH Biostatical Analysis. Prentice Hall, New Jersey. Prentice. 1999. 946p.