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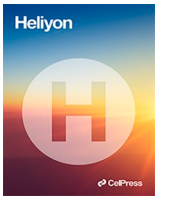

Research article

Embryotoxicity and teratogenesis of orthodontic acrylic resin in zebrafish

Amanda Sayuri Cardoso Ohashi [a,1], Helena Reis de Souza Schacher [a,*,1], Christiane Staub Pizzato [b], Monica Ryff Moreira Roca Vianna [b], Luciane Macedo de Menezes [a]

[a] *Dental Program, School of Health and Life Sciences Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil*
[b] *ZebLab & Laboratory of Biology and Development of the Nervous System, School of Biosciences, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil*

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ABSTRACT

Objectives: This study investigated the in vivo embryotoxicity, teratogenic potential, and additional effects of orthodontic acrylic resin as well as its components, utilizing zebrafish as a model organism. The research focused on morphological, cardiac, behavioral, and cognitive evaluations that were performed on embryos and larval-stage animals subjected to chronic exposure.
Materials and methods: Embryo and larval-stage zebrafish were categorized into five experimental groups, which were further subdivided into five subgroups. These subgroups included three specific doses for each tested substance, a control with the vehicle (0.1 % dimethyl sulfoxide in water), and an absolute control (water). Assessments were performed on day 5 post-fertilization, which included morphological, cardiac, behavioral, and cognitive evaluations. All experiments had a sample size of ten animals and were performed in triplicate. Survival and hatching rates were analyzed using the Kaplan-Meier test, while other measurements were assessed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test.
Results: Statistically significant differences were observed between the control and treatment groups across all the tested substances for heart rate, cognitive responsiveness, and cellular apoptosis. However, survival, hatching rate, and other parameters exhibited no significant variation, except for the highest dose in the dibutyl phthalate group, which demonstrated a notable difference in survival.
Conclusions: Chronic exposure to acrylic resin and its components may be associated with decreased cognitive ability and cardiac rhythm, as well as an increase in the level of cellular apoptosis in zebrafish.

1. Introduction

The biocompatibility of materials has been a focal point of scientific research for several years and has emerged as a significant

* Corresponding author. School of Health and Life Sciences, Pontifícia Universidade Católica do Rio Grande do Sul, 6681 Ipiranga Avenue, Building n.6, Porto Alegre, RS, 90619-900, Brazil.
E-mail address: helena.schacher@edu.pucrs.br (H. Reis de Souza Schacher).
[1] Both authors contributed equally.

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concern in the literature. Using biocompatible dental materials is crucial to ensuring a biologically safe treatment condition for patients, given their prolonged contact with oral tissues [1,2]. Additionally, dental professionals and laboratory staff continuously interact with these materials throughout their careers [3].

Biological and immunological adverse reactions can be attributed to dental materials, with the most prevalent side effects being allergic responses and hypersensitivity [1]. Acrylic resin, a widely utilized material in orthodontic applications including removable, fixed, and retention appliances, and prosthetics such as dentures, restorations, and temporary crowns, has the potential to elicit local reactions. On occasion, it has also contributed to systemic manifestations like urticaria, a burning sensation, and difficulty swallowing [4]. These reactions are linked to the release of toxic components from these resins, such as methyl methacrylate (MMA), formaldehyde, benzoyl peroxide, and plasticizers such as dibutyl phthalate [4], with MMA constituting the primary component (comprising approximately 96 % of the resin). Importantly, all products that leach from this resin exhibit cytotoxic effects on cell lines, particularly epithelial cell lines, and are not exclusively the residual monomer [5].

The reported acute oral median lethal dose of MMA in rats falls within the range of 8.4–9 g/kg body weight, indicating remarkably low acute systemic toxicity [6]. MMA undergoes rapid enzymatic hydrolysis and subsequent metabolism into toxic substances [7]. MMA vapor exposure has been documented to induce vertigo in dental professionals [8]. However, serious problems have not been reported for the inhalation of polymethyl methacrylate (PMMA) ingredients, although MMA may irritate the eyes, skin, and respiratory system [9]. Dental students, both smokers and non-smokers, exposed to acute MMA vapor inhalation demonstrated a moderate restriction of pulmonary function [10].

A large body of evidence has been accumulated regarding the release of residual monomers and toxicity of acrylic resin in dentistry, with clinical observations and in vitro experiments being predominant [4,5,11–14].However, in vivo studies are comparatively sparse [10,15]. Studies in animals have demonstrated the neurotoxic potential of formaldehyde and that exposure to this compound may cause deficits in spatial learning and memory [16]. These cognitive deficits are often accompanied by anxiety and depressive disorders [17] and have been shown to intensify aggressive behaviors in rats [18]. Formaldehyde has also been implicated in reproductive system toxicity in rats during both short- and long-term exposure [19] as well as oxidative stress in the cardiac tissue of rainbow trout [20]. Human studies further support the neurotoxic potential of formaldehyde, suggesting a potential link to Alzheimer's disease through effects like memory loss, cognitive dysfunction, reduced cholinergic signals, and increased acetylcholinesterase activity [21–23]. Clinical observations may be prone to biases, such as individual non-commitment to treatment or exposure to external factors that can interfere with the research outcomes. In vitro experiments, which lack system integrity, are limited in their ability to allow the assessment of the actual impact of a substance on a complete organism. Consequently, animal models, specifically zebrafish, emerge as a more suitable choice for these studies. Zebrafish are characterized by their vertebrate nature, genetic similarity with humans [24], and rapid external development. In addition, its transparency during early developmental stages [25], permeability to small molecules [26], and high fecundity [27] present unique advantages for toxicology studies by offering insights into the complex responses that are challenging to simulate in cell culture [19].

The zebrafish *(Danio rerio)* has emerged as a versatile model to assess the cytotoxicity, genotoxicity, nanotoxicity, and carcinogenic potential of various substances, including drugs and nanomaterials [28,29]. This is a valuable tool in dental research, particularly in the biocompatibility exploration of dental materials, craniofacial development, and toxicological investigations [30,31]. Dentistry has seen notable studies utilizing zebrafish models, spanning topics such as fluorosis [32] and the toxicity of zirconium oxide nanoparticles in dental implants [33], porcelain-fused-to-metal crowns [19], dental bioceramics [34], bisphenol A-glycidyl methacrylate in dental filling composite monomers [35], and methacrylate [36]. To assess a material's chemical toxicity and safety, parameters such as mortality, spontaneous movement, heart rate, hatchability, malformation, and exploratory behavior are evaluated in dental research [19,37].

Recognizing the significance of understanding how orthodontic material impacts the biological environment is crucial to ensuring a safe and effective treatment for the patient. These materials often have prolonged contact with the oral mucosa (several years, in some cases), highlighting the importance of assessing their effects on the well-being of the patient as well as the dental team, who frequently interact with these materials. Therefore, this study aimed to investigate in vivo the embryotoxicity, teratogenic potential, and other potential adverse effects of orthodontic acrylic resin and its active components, using the zebrafish as a model organism.

2. Material and methods

2.1. Experimental design

Embryonic and larval wild-type AB zebrafish (D. rerio) were obtained from the crossing of adult fish (>7 months old). The selection process began at 4 h post-fertilization (hpf), where the fertilized embryos were thoroughly examined under a stereomicroscope. Only those that exhibited the standard developmental stages, according to the zebrafish developmental stage standardization by Kimmel et al. [38], were selected for inclusion in the experiments.

The animals were then categorized into five experimental groups and further subdivided into five subgroups. Each subgroup consisted of three specific doses of the substance under investigation. In addition, there was one control group with the vehicle (0.1 % dimethyl sulfoxide (DMSO) in water) and another that served as the absolute control (water only). The absolute control functioned as a sentinel but was excluded from the analyses because of its unsuitability as the appropriate control for the measured effects. This decision stemmed from the potential impact of DMSO itself, which necessitated its exclusion. Details regarding the doses were determined based on the 50 % lethal concentration (LC50) of each substance obtained in the literature, with its concentrations adjusted proportionally to reflect its presence in the resin monomer (Table 1).

After an exacting selection process, the chosen embryos underwent a thorough sanitation procedure using system water (reverse osmosis water equilibrated with instant ocean salt [39]. Subsequently, they were transferred into 6-well plates, where they were exposed to the respective treatments. These plates were housed in a controlled incubator with a 14-h/10-h light/dark cycle and a constant temperature of 27 ± 1 °C until the 5-day post-fertilization (dpf) stage Each plate comprised six wells, which accommodated animals from the R, DB, MMA, F, and PB groups. Within each well, a control was included, and the remaining wells housed the reagent group and its subgroups. All solutions underwent a testing phase to ensure the reliability of the solutions used in the study. Each solution was diluted in reverse osmosis water, and this testing period was essential for determining the appropriate dilution for each reagent.

Throughout the experimental period, a detailed protocol was followed, including regular medium exchanges and daily monitoring to check survival, hatching, and morphology parameters. The exchange of reagents and continuous monitoring of the medium temperature were performed to minimize stress among the studied embryos. Upon reaching the 5 dpf stage, the animals underwent various evaluations, including behavioral and cognitive assessments as well as morphological and cardiac evaluations. Ten animals were used in triplicate for all experiments, as established by Nery et al. [40]. A schematic diagram summarizing the study is presented in Fig. 1.

This study received ethical approval from both the Scientific and Ethical Commission of the School of Health Sciences and the Ethics Committee for the Use of Animals at the Pontifical Catholic University of Rio Grande do Sul (CEUA PUCRS, number 7031/15). This research adhered to the guidelines outlined by the National Council for Control of Animal Experimentation (CONCEA) regarding the use of animals (fish) in research, as well as Brazilian legislation (COBEA No. 11.794/08). Furthermore, the study aligns with the ARRIVE guidelines to ensure transparency and reliability in reporting.

2.2. Morphological measurements

The teratogenic potential of the tested elements was estimated by morphological defect monitoring in larvae at the 5 dpf stage (n = 10 in triplicate) under a stereomicroscope (Nikon, Melville, USA). NIS Elements D software (Nikon Instruments Inc., Melville, USA) and Image J version 1.37, both for Windows, were used to determine the body length (Fig. 2A) and head (Fig. 2B), the distance between the eyes (Fig. 2B), and ocular height and width (Fig. 2C). The body length was estimated using the method described by Altenhofen et al. [41]. The other parameters were estimated using the method detailed by Kramer et al. [35].

2.3. Cardiac system

For the evaluation of the cardiac system, zebrafish larvae at the 5 dpf stage (n = 10 in triplicate) were placed on a Petri dish and observed using a stereomicroscope (Nikon Instruments Inc., Melville, USA). The heart rate of each larva was carefully counted over a 10-s interval. This specific duration was selected to balance capturing sufficient cardiac cycles for accurate assessment and maintaining a practical and efficient observation period. To derive the average heart rate per min, the observed beats were multiplied by six and expressed as the heart rate in beats per minute (bpm).

2.4. Exploratory behavior

To analyze exploratory behavior and locomotion, larvae at the 5 dpf stage were selected (n = 10 in triplicate) to ensure the absence of morphological changes. Each larva was placed in a well of a 24-well cell culture plate filled with system water to provide a controlled environment. The larvae underwent a preliminary acclimatization period of 1 min before the initiation of the recording of exploratory behavior. Exploratory behavior was then captured over a 5-min interval using an HD digital webcam (Logitech) camera, in accordance with the methodology described by Nery et al. [40]. Subsequently, the recorded videos underwent detailed analysis using Ethovision software. The software allowed for the extraction of various parameters, including distance traveled, average speed, periods of immobility and mobility, thigmotaxis (exploration of peripheral versus central areas, validated in larvae as a measure of anxiety), rotation, and “turn angle” during movement. This comprehensive approach aimed to provide a nuanced understanding of the behavioral responses of the larvae under the experimental conditions.

2.5. Aversive behavior

To evaluate the aversive behavior and cognitive response to a visual stimulus, we employed an experiment adapted from those described by Pelkowski et al. [42] and Nery et al. [40]. In this task, five larvae per well (n = 10 in triplicate) were placed in 6-well

Table 1
Groups, experimental subgroups, and exposure dose.

Group	Subgroups	Exposure Dose
Resin (R)	0, R1, R2, R3, R4	0, DMSO 0.1 %, 0.01 mg/l, 0.1 mg/l, 1 mg/l
Methylmethacrylate (MMA)	0, MMA1, MMA2, MMA3, MMA4	0, DMSO 0.1 %, 0.01 mg/l, 0.1 mg/l, 1 mg/l
Formaldehyde (F)	0, F1, F2, F3, F4	0, DMSO 0.1 %, 0.12 mg/l, 1.2 mg/l, 12 mg/l
Dibutylphthalate (DB)	0, DB1, DB2, DB3, DB4	0, DMSO 0.1 %, 0.024 mg/l, 0.24 mg/l, 2.4 mg/l
Benzoyl peroxide (PB)	0, PB1, PB2, PB3, PB4	0, DMSO 0.1 %, 0.0024 mg/l, 0.024 mg/l, 0.24 mg/l

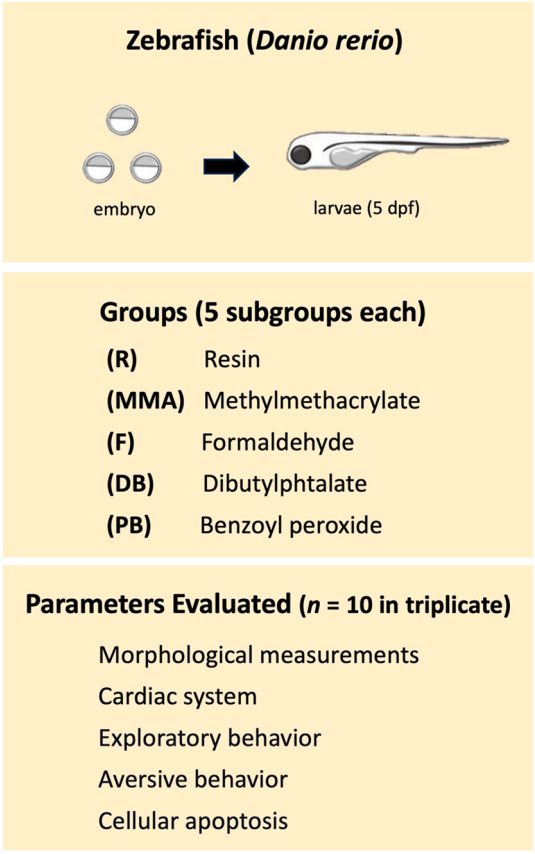

Fig. 1. Schematic diagram summarizing the study.

plates and exposed to a visual stimulus—a red circle oscillating between two extremities for 5 min. The larvae's ability to navigate to the area without the stimulus during the task served as an indicator of their cognitive response to aversive stimuli. Post-session, the percentage of animals in the stimulus-free zone was used to gauge the cognitive abilities across the different groups, according to the methodology described by Nery et al. (2014).

2.6. Cellular apoptosis

For the quantification of cellular apoptosis, the acridine orange technique was utilized. This technique utilizes a dye that fluoridates degraded nucleic acid, thereby facilitating the visualization of apoptotic cell islands throughout the animals' bodies [43]. To enhance the visibility of fluorescent labeling, 24-h embryos (n = 10 in triplicate) were treated with propylthiouracil (PTU) until the 5 dpf stage to inhibit the natural pigmentation process. At the 5 dpf stage, the animals were immersed in an acridine orange solution (2 μg/mL) for 30 min, followed by three washes with system water for 10 min each [43]. For photographic documentation, the larvae were fixed in methylcellulose (3 %) and observed under a stereomicroscope with UV light. The densitometric quantification of each image was performed using Carestream software (Carestream Health), utilizing the ratio of positive to negative pixels for analysis (Fig. 2D).

2.7. Statistical analysis

Statistical analysis of the survival and hatching rates was performed using the Kaplan-Meier test. Following confirmation of the data's normal distribution, other measurements were analyzed using the one-way analysis of variance (ANOVA) test. When differences between groups were identified, post hoc analysis was conducted using the Tukey test ($p < 0.05$). Statistical significance was determined at the $p < 0.05$ level.

3. Results

1. *Effects of acrylic resin and its components on zebrafish mortality and hatching rate*

The survival rate exhibited a notable difference in the highest dose of the DB group, which resulted in 100 % mortality observed at 1

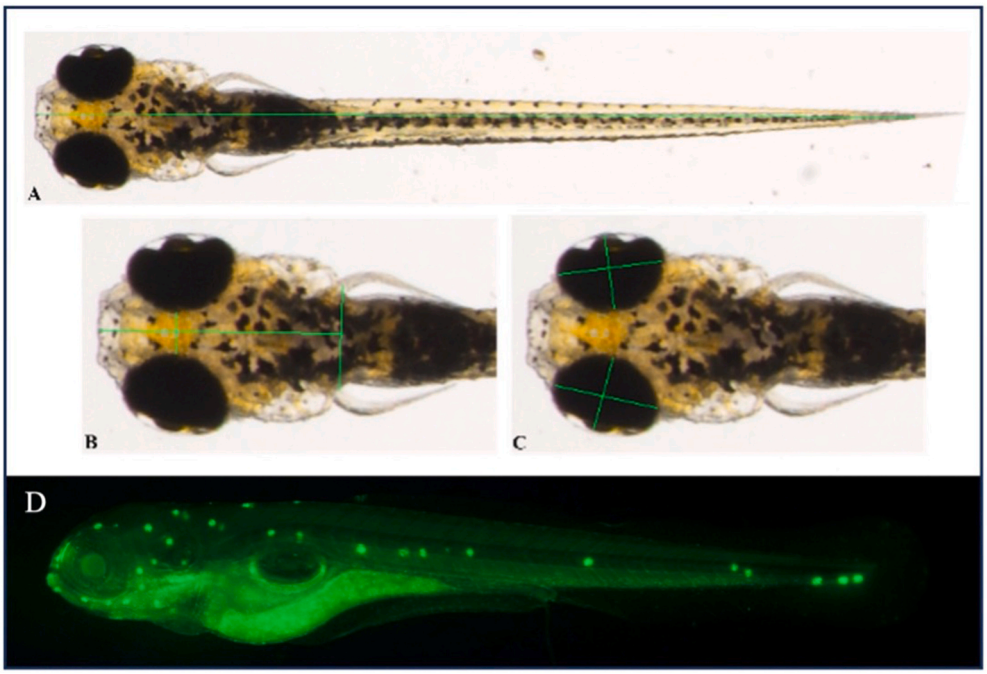

Fig. 2. Illustrative photographs (dorsal view) and markings of the morphological parameters analyzed for body, cranial, and ocular measurements of zebrafish larvae at the 5 dpf stage: body length (1A) at 4 × magnification; length of head and interocular distance (1B) at 6 × magnification; ocular height and width (1C) at 6 × magnification. D) Image showing the apoptotic cells using the acridine orange technique. Islands of apoptotic cells can be seen with fluorescent labeling. Photographs were taken with a stereomicroscope; side view, 4 × magnification.

dpf. In contrast, the hatch rate did not exhibit any delay or acceleration across the studied groups. Both parameters were monitored daily and analyzed using the Kaplan-Meier test. All treatments were accompanied by an absolute control group that was exposed to only water, and the data from these groups did not differ from that of the vehicle control group.

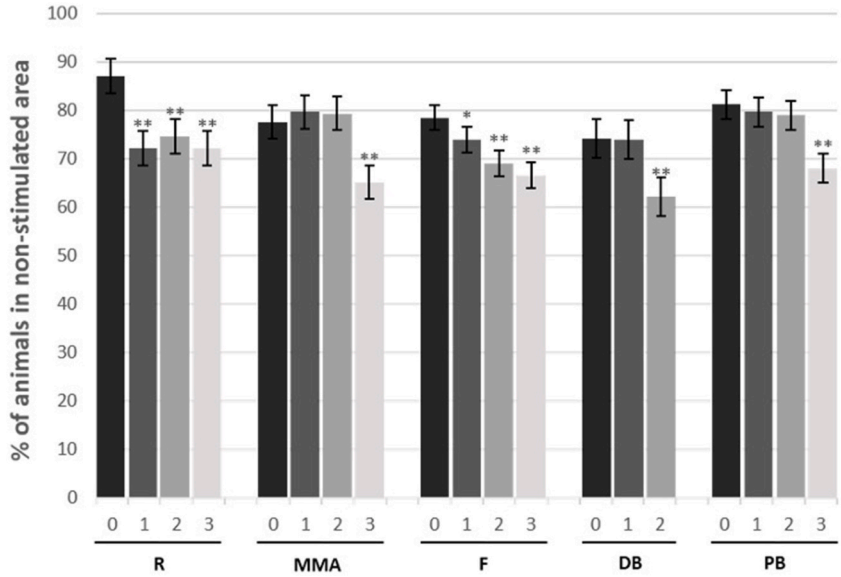

Fig. 3. Effects of the resin and its components on the cognitive escape response in zebrafish larvae at the 5 dpf stage. The bars express the mean ± standard error (n = 10) of experiments performed in triplicate. *p ≤ 0.05 and **p ≤ 0.01 in relation to the respective control of each group using one-way analysis of variance (ANOVA), followed by the Tukey test. The animals exposed to resin and its components separately showed a decrease in the escape response compared to their respective controls. The R and F group showed a significant decrease in the escape response even at the lowest dose.

2. *Effects of acrylic resin and its components on behavioral parameters and cognitive abilities.*

Individual assessments of locomotion and exploratory parameters at the 5 dpf stage revealed no significant differences between the tested groups. Only subtle variations were noted, thus suggesting limited support for broader analyses.

Cognitive abilities were evaluated through the aversive behavior test, where the animals were exposed to various doses of all the tested substances and exhibited a dose-dependent decrease in escape response to the aversive stimuli. The present study demonstrated a significant impairment in the escape response at all concentrations (Fig. 3), indicating potential cognitive deficits in the zebrafish. In essence, the higher the concentration of the substances, the lower the escape response. This result contrasts with the resin group, where a significant cognitive deficit was evident even at the lowest concentration (Fig. 3). These distinctions suggest the pivotal role of the other components within the resin. The resin and formaldehyde groups demonstrated a significant decrease in escape responses, even at lower concentrations. The resin group displayed a non-monotonic dose-response curve, where the lower concentration group exhibited a diminished escape response compared to the intermediate concentration, and the formaldehyde group displayed a dose-dependent response.

Despite MMA being the primary component of the resin, only the highest concentration group of MMA exhibited a diminished escape response. Formaldehyde stood out as the sole resin component, with a substantial reduction in escape response at the lowest concentration, which suggests its considerable influence on the resin. This result implies that formaldehyde might be more toxic than MMA and warrants increased attention [44].

3. *Effects of acrylic resin and its components on heart rate.*

Examining all groups at their highest concentrations revealed notable heart rate changes (Fig. 4). The resin, DB, and PB groups exhibited a decrease in heart rate. In contrast, the MMA and formaldehyde groups displayed an increase in heart rate. The MMA (increased heart rate) and DB (decreased heart rate) groups showed alterations in heart rate even at their lower concentrations.

Exposure to the resin monomer, dibutyl phthalate, and benzoyl peroxide caused a decrease in heart rate at higher concentrations, whereas exposure to MMA and formaldehyde induced an accelerated heart rate (Fig. 4).

4. *Effects of acrylic resin and its components on teratogenicity*

In addition, the possible effects of exposure to resin and its components on the morphological parameters were evaluated (Fig. 5), as body length (Fig. 5A), head length (Fig. 5B), ocular distance (Fig. 5C), eyer diameter (Fig. 5D) and eye depth (Fig. 5E). Regarding the resin, there was no significant difference in any of the evaluated parameters. For the other component groups, only discrete results were found, which do not support further substantial analysis.

5. *Effects of acrylic resin and its components on cellular apoptosis parameter.*

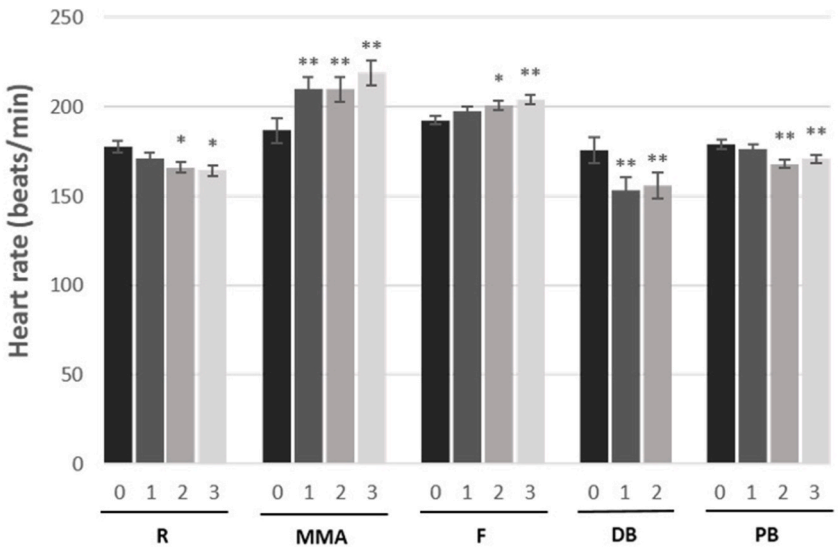

Fig. 4. Effects of resin and its components on the heart rate of zebrafish larvae at the 5 dpf stage. The bars express the mean ± standard error (n = 10) of experiments performed in triplicate. *p ≤ 0.05 and **p ≤ 0.01 in relation to the respective control of each group using one-way ANOVA, followed by the Tukey test. The heart rate was counted with a stereomicroscope for 10 s and then multiplied by six to obtain the average heart rate per min (bpm). All tested groups exhibited changes in heart rate in relation to their respective controls.

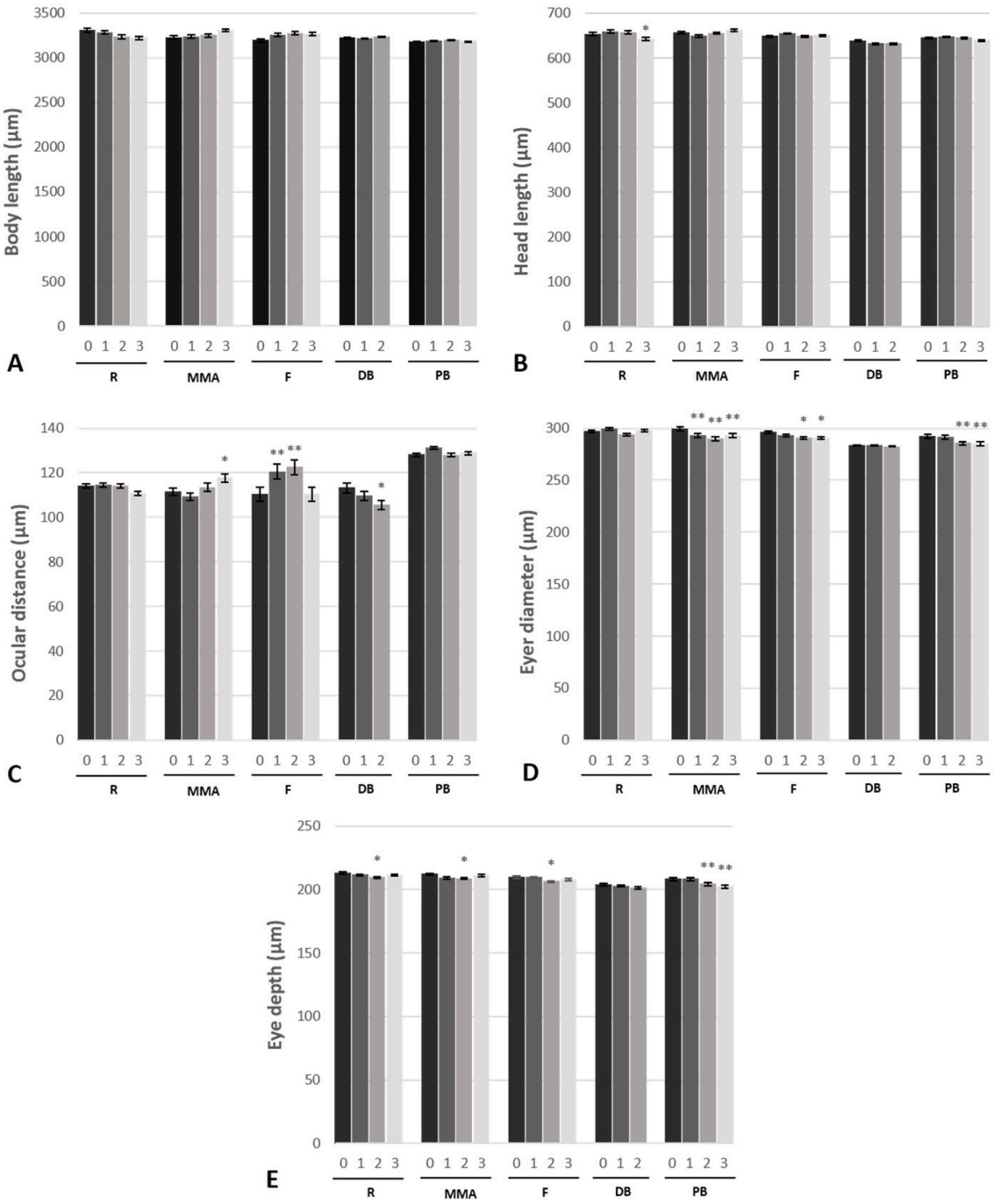

Fig. 5. Morphological effects of acrylic resin and its components on zebrafish larvae at the 5 dpf larvae stage. A) body length; B) head length; C) interocular distance; D) eye diameter; and E) eye depth. The bars express the mean ± standard error (n = 10) of experiments performed in triplicate for each subgroup. *p ≤ 0.05 and **p ≤ 0.01 in relation to the respective control of each group using one-way ANOVA, followed by the Tukey test.

All groups exhibited a significant increase in the rate of cellular apoptosis at intermediate and higher concentrations, except for the resin group, where only the highest concentration revealed a significant difference. The DB group showed a significant increase even at the lowest concentration, displaying a 100 % mortality rate at its highest concentration, at the 2 dpf stage. (Fig. 6). This notable increase in cellular apoptosis levels (Fig. 6) and the decrease in heart rate suggest a potentially toxic effect on the cardiac system (Fig. 4).

4. Discussion

In this study, we assessed the impact of various concentrations of acrylic resin and its components during the initial developmental stages of zebrafish. The findings revealed noteworthy effects, indicating that exposure to resin and its constituents for 120-h could lead to significant alterations in heart rate, cognitive functions, and cellular apoptosis. It is worth noting that this exposure did not induce significant morphological abnormalities that are commonly associated with embryonic exposure to known toxic substances, such as pericardial and yolk sac edema, caudal deformities, or spinal curvature [45]. However, there were subtle and isolated changes in specific measurements, such as the interocular distance in animals exposed to MMA, formaldehyde, and DB (Fig. 5). Therefore, establishing definitive conclusions regarding the teratogenic potential of these substances is challenging because they might not have been sufficiently concentrated to express this potential.

In contrast, the acrylic resin group exhibited no significant differences in the survival or hatching parameters. Although there were minor alterations in the morphological and behavioral aspects, the literature on the toxicity of acrylic resin in animals is limited, with most studies focusing on allergic contact reactions.

Consistent with our findings, both in vitro [14] and in vivo studies [46] have considered the toxic effects of MMA. In addition, toxic effects have been observed in human neuron-enriched primary cultures derived from embryonic brain tissue [47], as well as developmental defects such as pericardial edema in methacrylate-exposed embryos [36]. Our study is in agreement with these toxicological implications because the formaldehyde group demonstrated a significant increase in heart rate (Fig. 4) and cell apoptosis at its highest concentration (Fig. 6). Moreover, even at its lowest concentration, it led to a reduced ability to escape the aversive stimulus (Fig. 3), which was similar to that of the resin group. These results strongly suggest the substantial influence of formaldehyde on the toxicity of acrylic resin for this parameter, thus reinforcing the findings from previous toxicological investigations.

Numerous studies have highlighted the release of by-products from the resin into the oral cavity [15,48]. The 100 % mortality of the embryos treated with higher doses in the DB group may be related to the resin toxicity observed in clinical and in vitro studies. DB has also been implicated in causing reproductive abnormalities, oxidative stress, embryonic toxicity, and neurotoxicity in fish [49–51], rats [52], and even in children [53]. Our study corroborates these findings, where even at its lowest concentration, DB exhibited a notable level of cellular apoptosis (Fig. 6) and cognitive impairment (Fig. 3), both of which are considered toxicological parameters.

Conversely, the PB group displayed no significant differences in the survival and hatching parameters. Similar to the other groups, there were subtle morphological and behavioral changes and notable alterations in cardiac rhythm and cellular apoptosis at higher concentrations, as well as a reduced escape response only at the highest concentration. The current literature on toxicological studies considering this compound in animals is limited, and the available research focuses more on dermatitis research because of its widespread use in acne treatment.

In addition to evaluating the mortality and hatch rates, behavioral tests can serve as a straightforward and rapid method to obtain further insight into the toxic effects of the various substances on embryonic and nervous system development [54]. Our study detected subtle behavioral changes in zebrafish larvae across all the tested groups.

Cellular apoptosis is an essential process in vertebrate development; however, the intensified loss of cells through this programmed cell death can lead to irreversible deleterious consequences throughout life [55]. The findings of this study indicate that exposure to acrylic resin and all its components significantly increased cellular apoptosis compared to the respective controls, which suggests that each component induces cell death, albeit at varying levels (Fig. 6). MMA exhibited a dose-dependent response, causing increased cell death at higher doses compared to the lower and control doses. The DB and PB groups also demonstrated a dose-dependent response. Interestingly, the controls of these two groups exhibited a higher level of cellular apoptosis than the controls of the other groups. This suggests the possibility that these compounds release toxic vapors, potentially interfering with their controls, since all treatments were performed in 6-well plates that were closed after treatment changes. These plates were only opened again for further treatment changes.

These findings reveal previously unexplored aspects of acrylic resin's impact on the physiology of zebrafish, indicating that further investigation is required to comprehensively understand its implications. Additional in vivo research is essential to enhance our understanding of the toxicological potential of acrylic resin, given its widespread use in dentistry, to which individuals are exposed for prolonged periods, either through direct contact or inhalation.

5. Conclusion

Based on this study's results, it can be concluded that acrylic resin and all its components exhibit toxicological potential, which leads to cardiac alteration, cognitive impairment, and increased cellular apoptosis. Although MMA is the main component of acrylic resin, it appears to have less of an influence than formaldehyde on the cognitive ability parameters and less of an influence than dibutyl phthalate on the heart rate. Chronic exposure to acrylic resin and its components may be associated with decreased cognitive ability and cardiac rhythm and an increase in the level of cellular apoptosis in zebrafish.

Ethics approval

Approved by the Scientific and Ethical Commission of the School of Health Sciences and the Ethics Committee for the use of Animals of the Pontifical Catholic University of Rio Grande do Sul (CEUA PUCRS, number 7031/15). It followed the directions of the National Council for Control of Animal Experimentation (CONCEA) for the use of fish in research and Brazilian legislation (COBEA No. 11.794/08).

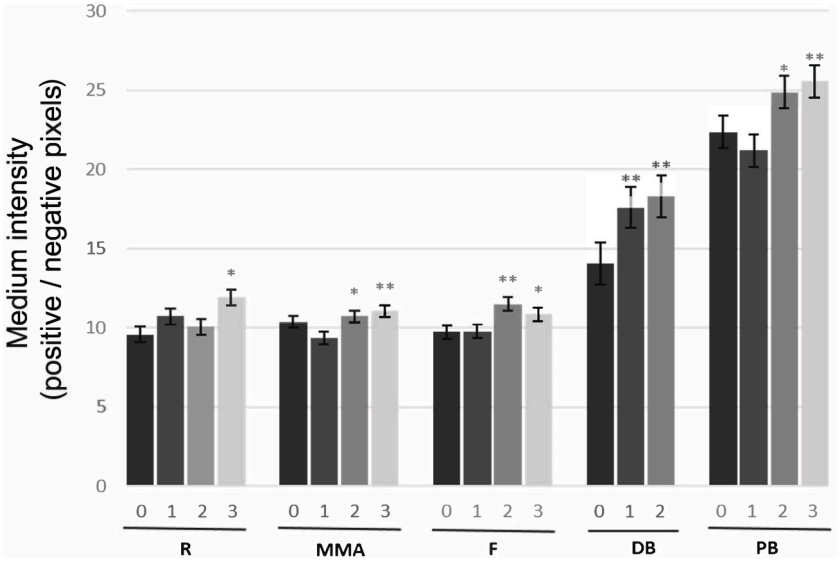

Fig. 6. Cellular apoptosis analysis using the acridine orange technique in zebrafish larvae at the 5 dpf stage. The bars express the mean ± standard error (n = 10) of experiments performed in triplicate for each subgroup. *p ≤ 0.05 and **p ≤ 0.01 in relation to the respective control of each group using one-way ANOVA, followed by the Tukey test. All groups showed a significant increase in apoptotic cells relative to their respective controls.

Consent to participate and consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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CRediT authorship contribution statement

Amanda Sayuri Cardoso Ohashi: Writing – original draft, Writing – review & editing, Methodology, Data curation. **Helena Reis de Souza Schacher:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Christiane Staub Pizzato:** Writing – review & editing, Methodology, Formal analysis. **Monica Ryff Moreira Roca Vianna:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Luciane Macedo de Menezes:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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