



## Comparison of the toxicity of bio-based and synthetic potassium in *Melanotaenia boesemani* embryos

Intanurfemi B. Hismayasari<sup>1,5</sup>, Mohamad Fadjar<sup>1\*</sup>, Abdul Rahem Faqih<sup>1</sup>, Wahyu Endra Kusuma<sup>1</sup>, Eni Kusri<sup>2</sup>, Dewi Syahidah<sup>3</sup>, Chrisoetanto P. Pattirane<sup>1,4</sup>, Cucun Herlina<sup>1</sup>

<sup>1</sup> Department of Aquaculture, Faculty of Marine and Fisheries, Brawijaya University, Jl. Veteran Ketawanggede Lowokwaru Malang 65145, Indonesia

<sup>2</sup> Research Centre of Freshwater Aquaculture, National Research and Innovation Agency (NRIA), Jl. Raya Bogor Km.46 Cibinong, 16911, Indonesia

<sup>3</sup> Research Center for Veterinary Science, National Research and Innovation Agency (NRIA), Jl. Raya Bogor Km.46 Cibinong, 16911, Indonesia

<sup>4</sup> Karawang Politechnique of Marine and Fisheries, Jl. Lingkar Tanjungpura, Karangpawitan, Karawang 41315, Indonesia

<sup>5</sup> Sorong Politechnique of Marine and Fisheries, Jl. Kapitan Pattimura Maladumes, Sorong 98411 Papua Barat Daya, Indonesia

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\* Corresponding author:

Mohamad Fadjar

Email: [f4dj4f\\_02@ub.ac.id](mailto:f4dj4f_02@ub.ac.id)

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### Abstract

Potassium supplementation in fish can disrupt the balance between extracellular and intracellular K<sup>+</sup>, leading to metabolic disturbances, especially during early development stages. Given these potential adverse effects, the toxicity of potassium supplementation must be critically evaluated to ensure its safe application in aquaculture. The toxicological effects of synthetic potassium citrate and potassium derived from banana peel ash (BPA) extract were compared using the FET method with Boesemani rainbowfish (*Melanotaenia boesemani*) embryos. Embryos were exposed to both potassium sources at concentrations of 0, 10, 25, 40, 80, 120, and 160 mg/L for 120 hours or longer. The results indicate that both concentration and duration of exposure affect the toxicity of both sources. However, synthetic potassium showed higher acute toxicity, with a lower LC<sub>50</sub> (13.73 mg/L) compared to BPA (43.67 mg/L). Exposure to synthetic potassium also increased heart rate and cause abnormalities in embryos, indicating physiological stress. A teratogenic index (TI) value of <1 for both potassium sources indicates embryotoxic effect. Egg hatchability was primarily influenced by concentration and exposure duration rather than potassium source. Overall, these findings suggest that bio-based potassium from banana peel ash exhibits lower acute toxicity than its synthetic counterpart, indicating its potential as a safer alternative for potassium supplementation in aquaculture.

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## 1. Introduction

Potassium is an essential macronutrient that is supplemented in aquaculture medium to increase aquatic productivity. This macronutrient also plays a role in osmoregulation (John et al. 2022), growth (El-khayat et al. 2021), nerve function (He et al. 2020a), immune system (John et al. 2022), and enzyme activation (Alavi et al. 2019) in aquatic organisms. An imbalance in extracellular and intracellular potassium concentrations can become toxic to aquatic organisms and may cause osmoregulatory dysfunction, cardiac arrhythmias, embryogenesis, and mortality (Di Paola et al. 2022; Zhu et al. 2023). The negative impact of this potassium imbalance requires a deep understanding of its toxicity, especially during the early stages of fish development. The embryonic development stage is the most critical phase because it is highly sensitive to chemical compounds (Naz et al. 2023; Xu et al. 2024). Therefore, the use of fish embryos is appropriate in toxicity testing. Fish Embryo Toxicity (FET) has developed into an effective and ethically preferred method in ecotoxicological studies because it can be used as a sensitive biomarker for monitoring aquatic ecosystem health. Fish embryos provide an extensive review of the effects of chemical compounds, encompassing both sub-lethal and

lethal impacts that might otherwise go undetected in tests involving adult fish.

Research on the toxicity of synthetic potassium has been widely conducted, such as potassium perchlorate (Di Paola et al. 2022), potassium dichromate (Sánchez-Olivares et al. 2021), potassium cyanide (Tez et al. 2022), potassium diformate (El-khayat et al. 2021); however, no studies on potassium citrate toxicity, especially in fish embryos, are available. Potassium citrate is widely used by fish farmers to maintain water quality and fish health (Harika et al. 2024; Roy et al. 2025). Although potassium citrate is generally considered safe; however, toxicological evaluation of its effect on aquatic organisms especially during early developmental stages remains limited. The early stages of embryogenesis in fish are sensitive to chemical exposure, making it crucial to evaluate any potential effect of potassium. Nevertheless, there remains a significant gap in understanding the safety and biological properties of potassium derived from natural sources such as banana peels. Banana peels contain potassium at concentrations of 2500-3000 mg/L (El-Awadi et al. 2021; Islam et al. 2019). Bananas are fruits that grow in the tropical regions, is cultivated in over 130 countries and represents approximately 16% of the world's total fruit output (Jayasinghe et al. 2024). Banana peels constitute

30-40% of the total fruit mass, represents a considerable waste stream of approximately 114.06 million metric tons around the world, indicating a significant potential for utilization (Acedo & Grande-tovar 2021).

Measurement of the potassium content of banana peel in this study using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) revealed a concentration of 3709.62 ppm, indicating its potential as a source of potassium suitable for diverse industrial uses. However, the ecotoxicological consequences of these bio-based nutrients have not been extensively investigated. The way in which the complex organic composition of bio-based potassium influences its toxicological effects, compared to its synthetic alternative, is still unknown. Therefore, a direct, comparative toxicological evaluation is essential to confirm the environmental safety of these innovative resources prior to their widespread application in vulnerable sectors such as aquaculture.

Fish embryo toxicity assays for potassium compounds have been widely performed using model species including zebrafish and medaka, but the majority of existing toxicity thresholds and extrapolation frameworks are based on these species and not necessarily applicable across taxa with different embryonic and reproductive traits. *Melanotaenia boesemani* was selected as test the species because of its relevant biological characteristics (Mote et al. 2024; Said et al. 2024; Syafiq et al. 2026) to provide species-specific embryo toxicity reference points, which are not available at present for this ecologically and economically relevant taxon. In addition, this study addresses simultaneously two gaps that have received little attention, namely the toxicological profile of potassium citrate, which despite being widely used in aquaculture water management, has not been characterized in fish embryos, and the lack of a previous study directly comparing a synthetic potassium salt with a bio-based potassium source of increasing interest for sustainable aquaculture mineral supplementation. It was hypothesized that synthetic potassium would exhibit greater toxicity reflected in higher embryo mortality, more pronounced morphological abnormalities, and significant changes in heart rate, compared with bio-based potassium, which is expected to produce less harmful effects. This study aimed to compare the toxicity of synthetic potassium and banana-peel-derived potassium using *M. boesemani* embryos.

## 2. Materials and Methods

All experiment procedures, including banana peel ash extraction and the fish embryo toxicity assays were conducted at the Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia.

### 2.1 Potassium source

The potassium used in this study was obtained from synthetic and natural sources. Synthetic potassium was obtained from tripotassium citrate ( $K_3C_6O_7$ , MW = 306.39 g/mol, K content = 38.3% by weight), while natural potassium was derived from an extract of kepok banana peel ash (*Musa paradisiaca*). The potassium content of BPA extract was determined by Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES) Shimadzu at the Integrated Research Laboratory, Brawijaya University, yielding a concentration of 3709.62 ppm K. Based on the theoretical K fraction of tripotassium citrate and the measured K content of BPA extract, both sources were standardized to deliver equivalent nominal K ion concentrations across all exposure treatments.

### 2.2 Banana peel ash extraction

Banana peel ash extraction followed (Onyeka et al. 2024) with slight modification. The fresh banana peels were thoroughly washed, cut into small pieces (about 1 mm) and dried in an oven at 60 °C until a constant weight. The dried peels were then ground and sieved to a uniform powder. The powder was ashed in two steps, carbonized on an electric stove to produce black charcoal and then incinerated in a muffle furnace at 600 °C to obtain white ash. The obtained ash was then leached with hot distilled water at a controlled temperature with constant stirring for a specified time. The mixture was filtered and the filtrate was freeze-dried to give a crystalline potassium-rich extract. The potassium content in the extract was determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).

### 2.3 Eggs production

Embryos were obtained from mass spawning of *M. boesemani* broodstock at a male-to-female ratio of 1:1 (Said et al. 2023). Unraveled black raffia rope, weighted at the bottom, was used as the substrate for egg attachment (Akhsan et al. 2020; Gupta and Roy 2024). Fertilized eggs were selected to obtain morphologically normal embryos, which are characterized by a yolk that is clear and round.

### 2.4 Eggs incubation

Fertilized eggs attached to the substrate were incubated in a plastic container (48 × 28.5 × 22 cm; ±30 L capacity). To prevent fungal growth during incubation, 1 mg/L of methylene blue was added to the culture water (Nipu et al. 2025).

### 2.5 Chemical exposure

The embryos used were *M. boesemani* embryos at the eye spot stage, approximately 49 hours post-fertilization (hpf) (Elfidasari et al. 2017; Radael et al. 2015). Approximately 1680 selected embryos were individually placed in separate wells of 6-well flat-bottom plate (one embryo per well, ensuring independent exposure media per individual), with 20 embryos assigned per treatment combination across three independent temporal replicates (batches). Each embryo was observed repeatedly at 24, 48, 72, 96, and 120 h (or until hatching), and this repeated-measures structure was accounted for by including a random intercept for Embryo\_ID, nested within replicate in the GLMM. A preliminary range-finding test was conducted using concentration from 250 to 6000 mg/L to identify the sub-lethal to lethal response range for both potassium sources. Mortality at 250 mg/L, the lowest concentration tested, already exceeded 75% within 5h of exposure for both BPA and synthetic potassium, and remained at 85-100% across all higher concentrations and observation durations up to 120 h (Supplementary Table S1). Since exposure at and above 250 mg/L produced mortality too high to characterize a meaningful concentration-response relationship, seven definitive concentrations (0, 10, 25, 40, 80, 120, 160 mg/L) – set below this threshold – were selected to characterize the concentration-response relationship within the sub-lethal to acutely toxic range. The control treatment used media water (egg-water), and all media water for the treatments was replaced daily. The exposure protocol followed the general framework of OECD test guideline 236, modified to accommodate the reproductive biology of *M. boesemani*, including a later exposure onset at the eyed-egg stage (49 hpf) and extended 120 h observation periods to capture the species' longer hatching window, in contrast to the standard zebrafish based 96 h endpoint.

The data collected consisted of mortality data, egg hatchability, heart rate, and embryo abnormalities (yolk sac edema, pericardial edema, heart rate changes, spinal deformities, pigmentation disorders, delayed hatching). Morphological abnormalities were identified and presented as a percentage of the ratio of abnormal embryos to total live embryos (Han et al. 2021; Sánchez-Olivares et al. 2021; Vasamsetti et al. 2021). Heart rate evaluation for the potassium exposure treatment was calculated over 20 seconds under an Olympus CX43 binocular microscope and normalized per minute (Vasamsetti et al. 2020).

LC<sub>50</sub> (Embryo mortality) and EC<sub>50</sub> (embryo abnormality) were determined using probit analysis in SPSS 27 (Jiang et al. 2020; Suharyadi et al. 2025; Vasamsetti et al. 2022; von Hellfeld et al. 2022). Mortality and abnormality observations were made at 120 hours, which was at the end of immersion and onset of the embryo hatching, using an Olympus CX43 microscope with modifications due to the different species. The teratogenic index (TI) was calculated by dividing the LC<sub>50</sub> by the EC<sub>50</sub>, in order to estimate the teratogenic potential of the substance in subject matter. A TI value greater than 1 indicates that the substance is classified as teratogenic, whereas a TI value < 1 suggests that the substance primarily leads to embryonic lethality (Jiang et al. 2020).

2.6 Statistical analysis

A generalized linear mixed model (GLMM) with a binomial distribution and a logit link function was used to analyze the effects of potassium source (BPA vs. synthetic), concentration (0, 10, 25, 40, 80, 120, 160 mg/L), and exposure time (24, 48, 72, 96, 120, 144 hours) on embryo mortality and hatching rates. Model selection was based on the comparison of AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion). The fixed effects were assessed using the Type II Wald chi-square test, and post-hoc comparisons were performed using estimated marginal means (EMMeans) with log-odds ratio adjustment. Embryonic heart rate data were analyzed using a linear mixed model (LMM) with potassium source, concentration, and time as fixed effects and interaction effects were evaluated using the Kenward-Roger method, followed by post-hoc pairwise comparisons. Abnormality data were tested for normally using the Shapiro-Wilk tests ( $W = 0.857$ ,  $p <$

0.001). Due to the experimental design yielding a single observation per treatment group, the assumption of homogeneity of variance could not be tested with Levene’s test. Therefore, variations in concentration levels and sources could not be reliably assessed using parametric methods. Considering these limitations, a Kruskal-Wallis non-parametric analysis was performed to evaluate the effect of concentration (0-160 mg/L) and K<sup>+</sup> source (BPA and synthetic) on the percentage of embryonic abnormalities with Dunn's post-hoc test and Bonferroni correction for pairwise comparisons. All analyses were performed in RStudio 4.5.2 Release using the lme4 and emmeans packages with statistical significance set at  $\alpha = 0.05$ .

3. Results

3.1 Mortality

The GLMM provided a substantially better fit than the interaction-free alternative model ( $\Delta AIC > 25$ ), with inter-organism variability adequately captured by a random intercept. The Type II Wald test identified concentration, exposure time, and potassium source as significant main effects (concentration and time: both  $p < 0.001$ ; source:  $p = 0.007$ ) and confirmed a significant source  $\times$  concentration interaction ( $p = 0.002$ ). Based on the Type II Wald omnibus test, synthetic potassium was associated with significantly higher overall mortality risk than BPA across the tested concentration range ( $\chi^2 = 7.26$ ,  $df = 1$ ,  $p = 0.007$ ; Table 1). Among the tested concentrations, a significantly higher mortality was observed at 120 mg/L and 160 mg/L than the control, whereas the main effects of all other concentrations were non-significant. Exposure time was the dominant predictor, with mortality risk increasing monotonically from 48 h through 144 h. Complete fixed-effect estimates for the mortality GLMM, including concentration, time, and source  $\times$  concentration-specific coefficients, are provided in Supplementary Table S2.

The source  $\times$  concentration interaction revealed a divergent concentration-dependent mortality patterns between the two potassium sources (Supplementary Tables S2-S3). Across the tested concentration range, the BPA treatment exhibited a non-linear response, with a local reduction in mortality at 40 mg/L and markedly higher mortality at the

| Table 1. Summary of significant fixed effects identified by the GLMM for embryonic mortality                                                                                                                                                                                                                                                                       |                    |                   |    |                     |                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|-------------------|----|---------------------|-----------------------------------------------------------------------------------|
| Effect                                                                                                                                                                                                                                                                                                                                                             | Estimate $\pm$ SE  | $z / \chi^2$      | df | p value             | Interpretation                                                                    |
| Source (Type II Wald, omnibus)                                                                                                                                                                                                                                                                                                                                     | -                  | $\chi^2 = 7.260$  | 1  | 0.007**             | Overall mortality risk differed significantly between BPA and synthetic potassium |
| Concentration (Type II Wald, omnibus)                                                                                                                                                                                                                                                                                                                              | -                  | $\chi^2 = 40.06$  | 6  | <0.001***           | Overall mortality risk differed significantly across concentrations               |
| Time (Type II Wald, omnibus)                                                                                                                                                                                                                                                                                                                                       | -                  | $\chi^2 = 183.07$ | 5  | <0.001***           | Mortality significantly increased with exposure time                              |
| Source $\times$ Concentration (Type II Wald, omnibus)                                                                                                                                                                                                                                                                                                              | -                  | $\chi^2 = 21.48$  | 6  | 0.0015**            | Effect of concentration on mortality differed between sources                     |
| Source (Synthetic vs. BPA), coefficient at a concentration of 0 mg/L                                                                                                                                                                                                                                                                                               | 0.381 $\pm$ 0.357  | 1.067             | -  | 0.286 <sup>ns</sup> | No overall source difference                                                      |
| Concentration (120 mg/L), coefficient at Source = BPA                                                                                                                                                                                                                                                                                                              | 0.767 $\pm$ 0.348  | 2.204             | -  | 0.028*              | Moderate increase in mortality                                                    |
| Concentration (160 mg/L), coefficient at Source = BPA                                                                                                                                                                                                                                                                                                              | 0.921 $\pm$ 0.346  | 2.664             | -  | 0.008**             | High increase in mortality                                                        |
| Source $\times$ Concentration (40 mg/L)                                                                                                                                                                                                                                                                                                                            | -0.909 $\pm$ 0.496 | -1.831            | -  | 0.067               | Slight reduction effect for Synthetic source at 40 mg/L                           |
| Concentrations are expressed as nominal K ion equivalent (mg/L); standardized between BPA and synthetic sources based on their respective K content; p-values were determined by Wald z-tests for fixed effects in the GLMM; Significance levels: * $p < 0.05$ , ** $p < 0.01$ , *** $p < 0.001$ ; SE: Standard error; df: Degree of freedom; BPA: Banana peel ash |                    |                   |    |                     |                                                                                   |

| Table 2. Probit analysis results |                  |               |                  |               |       |
|----------------------------------|------------------|---------------|------------------|---------------|-------|
| Effect                           | LC <sub>50</sub> |               | EC <sub>50</sub> |               | TI    |
|                                  | Value            | 95% CI        | Values           | 95% CI        |       |
| Synthetic                        | 13.73            | 0.031-67.94   | 99.17            | 25.58-6931.41 | 0.138 |
| BPA                              | 43.67            | 11.096-396.76 | 156.90           | 65.37-2238.22 | 0.278 |

LC<sub>50</sub> and EC<sub>50</sub> values are expressed as nominal K<sup>+</sup> ion equivalents (mg/L); CI: Confidence interval; TI: teratogenic index

highest concentrations. However, between source differences reached statistical significance only at the lowest and highest tested concentrations. Temporal and concentration-dependent mortality trends are illustrated in Fig.1 and Fig. 2.

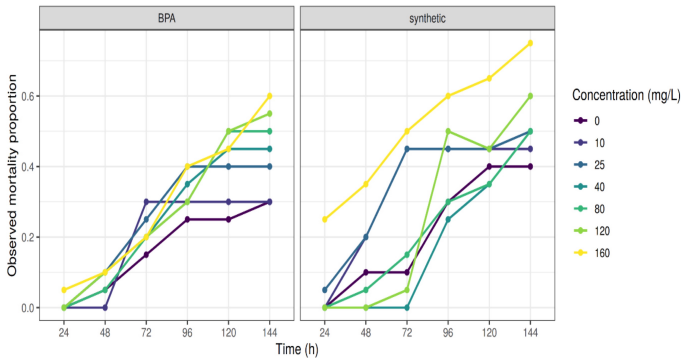


Fig. 1. Temporal trends in embryo mortality proportion under exposure to different concentrations of BPA and synthetic compounds. Mortality increased with both concentration and observation time, demonstrating a clear time- and dose-dependent effect. The synthetic source exhibited slightly higher mortality compared to BPA at equivalent concentrations, indicating a stronger overall toxic response.

Probit analysis results showed that the LC<sub>50</sub> value of BPA (43.673 mg/L) was higher than that of synthetic potassium (13.730 mg/L), suggesting that synthetic potassium is more toxic than BPA. Based on the probit analysis of embryonic abnormalities, the EC<sub>50</sub> value of BPA (156.898 mg/L) was higher than that of synthetic potassium (99.173

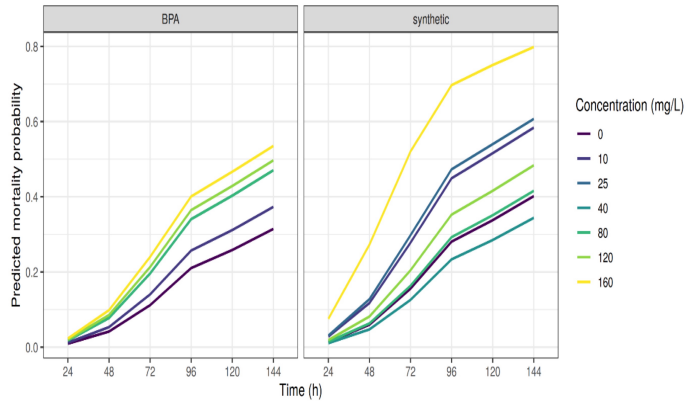


Fig. 2. Predicted mortality probability of embryos exposed to different concentrations of BPA (left panel) and synthetic compound (right panel) across exposure times (24–144 h)

mg/L), indicating that banana peel ash extract was more tolerable to embryos for development of post-hatch larval abnormalities. The ratio of LC<sub>50</sub> to EC<sub>50</sub> is called the teratogenic index (TI). In this study, the TI values for both BPA and synthetic potassium were <1, indicating that both potassium sources were categorized as primarily embryotoxic rather than teratogenic (Table 2).

3.2 Egg Hatchability

The GLMM for egg hatchability (Table 3) showed that potassium concentration and exposure duration significantly influenced hatching probability (both  $p < 0.001$ ), whereas K<sup>+</sup> source had no significant effect ( $p = 0.689$ ) (Supplementary Table S5). The source  $\times$  concentration interaction was likewise non-significant ( $p = 0.522$ ), as confirmed by the Type II Wald analysis (Table 4). Among the concentration levels, only 10

| Table 3. Summary of generalized linear mixed model (GLMM) for egg hatchability |                      |         |                       |
|--------------------------------------------------------------------------------|----------------------|---------|-----------------------|
| Fixed effect                                                                   | Estimate $\pm$ SE    | z value | p value               |
| Intercept                                                                      | -19.912 $\pm$ 27.252 | -0.731  | 0.465 <sup>ns</sup>   |
| Potassium source (Synthetic)                                                   | -0.159 $\pm$ 0.399   | -0.400  | 0.689 <sup>ns</sup>   |
| Concentration 10 mg/L                                                          | 1.304 $\pm$ 0.380    | 3.435   | 0.0006 <sup>***</sup> |
| Concentration 25 mg/L                                                          | 0.781 $\pm$ 0.380    | 2.055   | 0.04 <sup>*</sup>     |
| Concentration 40 mg/L                                                          | -0.417 $\pm$ 0.409   | -1.020  | 0.308 <sup>ns</sup>   |
| Concentration 80 mg/L                                                          | -0.509 $\pm$ 0.414   | -1.231  | 0.218 <sup>ns</sup>   |
| Concentration 120 mg/L                                                         | -0.605 $\pm$ 0.419   | -1.446  | 0.148 <sup>ns</sup>   |
| Concentration 160 mg/L                                                         | -0.921 $\pm$ 0.438   | -2.104  | 0.035 <sup>*</sup>    |
| Time                                                                           |                      |         | <0.001 <sup>***</sup> |
| Source $\times$ Concentration                                                  |                      |         | 0.522 <sup>ns</sup>   |

The model was fitted using a binomial distribution with a logit link function and included a random intercept for Embryo ID; Significant differences are indicated as: \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ; ns = not significant; Concentration are expressed as nominal K<sup>+</sup> equivalent (mg/L) and standardized between BPA and synthetic potassium sources based on their respective K<sup>+</sup> content



| Table 4. Type II Wald Chi-Square test for fixed effects of the hatching model                                                                                                                                                                                                                                                                                                                                                                                           |            |    |                       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|----|-----------------------|
| Effect                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Chi-square | df | p value               |
| Source                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.559      | 1  | 0.455 <sup>ns</sup>   |
| Concentration                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 77.842     | 6  | <0.001 <sup>***</sup> |
| Time                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 165.104    | 5  | <0.001 <sup>***</sup> |
| Source × Concentration                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 5.174      | 6  | 0.522 <sup>ns</sup>   |
| Potassium concentration and exposure time significantly affected hatching probability (both $p < 0.001$ ), whereas potassium source and source × concentration interaction were not significant (both $p > 0.05$ ); Consistent with Supplementary Table S6, odds ratios close to 1.0 across all concentrations confirmed similar hatching responses between BPA and synthetic potassium treatments, with only a marginal difference observed at 80 mg/L ( $p = 0.072$ ) |            |    |                       |

mg/L and 25 mg/L significantly increased hatching probability relative to the control, whereas the 160 mg/L level produced a significant decrease. Supplementary Table S6 showed that the highest predicted hatching probability occurred at 10 mg/L for both potassium sources and declined progressively with increasing concentration. Pairwise contrast between potassium sources at each concentration (Supplementary Table S7) yielded non-significant odds ratios across all tested levels. A marginal tendency toward lower hatchability in the BPA treatment at 80 mg/L did not reach significance ( $p = 0.072$ ). Observed and model-predicted hatching trajectories are shown in Fig. 3 and Fig. 4.

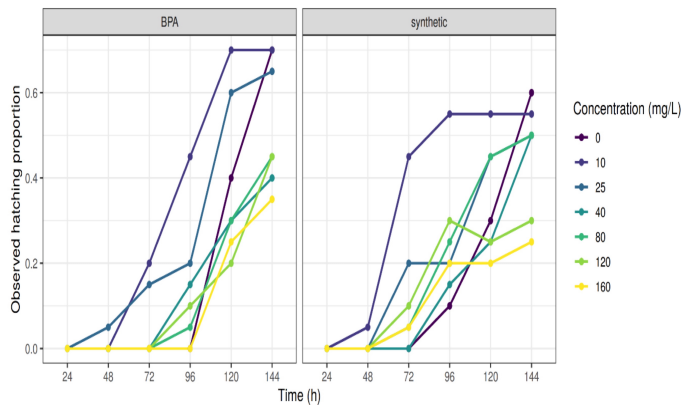


Fig. 3. Temporal trends of hatching proportion of embryos exposed to different concentrations of BPA and synthetic sources over time (24–144 h)

| Table 5. Fixed effects of the linear mixed model (LMM) for embryonic heart rate                                                                                                                                                                                                                                                                     |                  |         |         |              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|---------|---------|--------------|
| Fixed effect                                                                                                                                                                                                                                                                                                                                        | Estimate ± SE    | t value | p value | Significance |
| Intercept                                                                                                                                                                                                                                                                                                                                           | 4.9469 ± 0.0082  | 602.63  | <0.0001 | ****         |
| Source (Synthetic vs BPA)                                                                                                                                                                                                                                                                                                                           | 0.1566 ± 0.0122  | 12.844  | <0.0001 | ****         |
| Concentration (scaled)                                                                                                                                                                                                                                                                                                                              | -0.0094 ± 0.0080 | -1.176  | 0.239   | n.s.         |
| Time (scaled)                                                                                                                                                                                                                                                                                                                                       | 0.2238 ± 0.0094  | 23.736  | <0.0001 | ****         |
| Source × Concentration                                                                                                                                                                                                                                                                                                                              | 0.0880 ± 0.0127  | 6.938   | <0.0001 | ****         |
| Source × Time                                                                                                                                                                                                                                                                                                                                       | -0.0629 ± 0.0138 | -4.553  | <0.0001 | ****         |
| Concentration × Time                                                                                                                                                                                                                                                                                                                                | -0.0116 ± 0.0092 | -1.254  | 0.21    | n.s.         |
| Source × Concentration × Time                                                                                                                                                                                                                                                                                                                       | -0.0367 ± 0.0143 | -2.570  | 0.01    | *            |
| Significant differences are indicated as: * $p < 0.05$ ; ** $p < 0.01$ ; *** $p < 0.001$ ; **** $p < 0.0001$ ; Random effect for Embryo_ID was negligible (variance = 0); Model summary: $n = 887$ ; groups (Embryo_ID) = 20; residual SD = 0.1529; REML criterion = -760.7; A boundary (singular) fit was reported (random intercept variance ≈ 0) |                  |         |         |              |

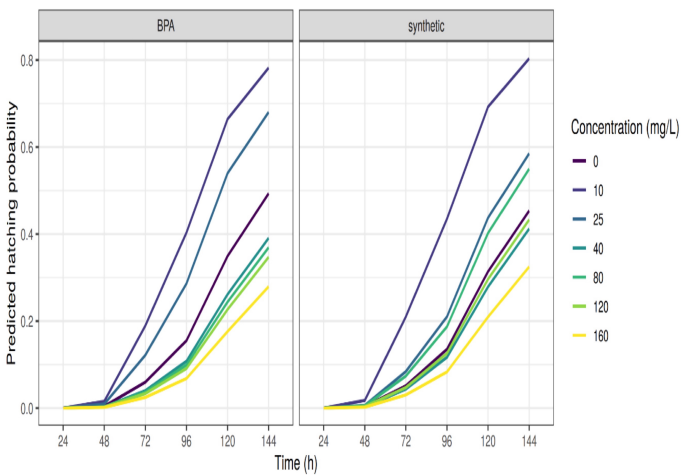


Fig. 4. Predicted hatching probabilities over time (24–144 h) under varying concentrations of BPA and synthetic sources, showing distinct temporal and concentration-dependent responses based on GLMM predictions

3.3 Heart rate

The linear mixed model (LMM) for log-transformed heart rate identified potassium source, exposure time, and their interaction as significant predictors (all  $p < 0.001$ ), while concentration alone had no significant main effect ( $p = 0.239$ ; Table 5). Embryos exposed to synthetic potassium consistently exhibited higher heart rates than those exposed to BPA at all assessed time points (post hoc pairwise  $p < 0.0001$ ). The significant source × time interaction indicated that the model-estimated rate of change in heart rate over time differed between potassium sources. A significant three-way source concentration × time interaction ( $p = 0.01$ ) further indicated that the concentration effects on heart-rate trajectories was not consistent across sources over time. Estimated marginal means and residual diagnostics are reported in Table 6 and Supplementary Fig. S1. Model assumptions of homoscedasticity and normality of residuals were satisfied.

Overall, both BPA and synthetic potassium sources contributed to a gradual increase in heart rate over time, as indicated by the upward trend of the regression lines in majority of the panels. The magnitude and direction of these slopes varied with concentration, indicating a significant interaction effect. At lower concentrations (panels with negative coefficients, such as -1.16 or -0.98), embryos exposed to BPA

| Table 6. Estimated marginal means (EMMs) of log-transformed heart rate for BPA and synthetic potassium sources at the mean concentration and time levels |                        |               |                      |              |              |              |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|---------------|----------------------|--------------|--------------|--------------|
| Source                                                                                                                                                   | Concentration (scaled) | Time (scaled) | Estimated means ± SE | 95% CI lower | 95% CI upper | Significance |
| BPA                                                                                                                                                      | -0.061                 | -0.465        | 4.843 ± 0.0071       | 4.829        | 4.857        | ****         |
| Synthetic                                                                                                                                                | -0.061                 | -0.465        | 5.023 ± 0.0075       | 5.008        | 5.037        |              |
| EMMs were calculated using the Kenward–Roger method ( $\alpha = 0.05$ ); BPA: Banana peel extract; SE: Standard error; CI: Confidence interval           |                        |               |                      |              |              |              |

exhibited lower heart rates compared to those exposed to synthetic potassium. Conversely, at elevated concentrations (as indicated by positive coefficients such as 0.25, 0.96, and 1.67), as shown in Fig. 5, the BPA treatment resulted in a more significant increase in heart rate over time, implying that the effect was concentration dependent.

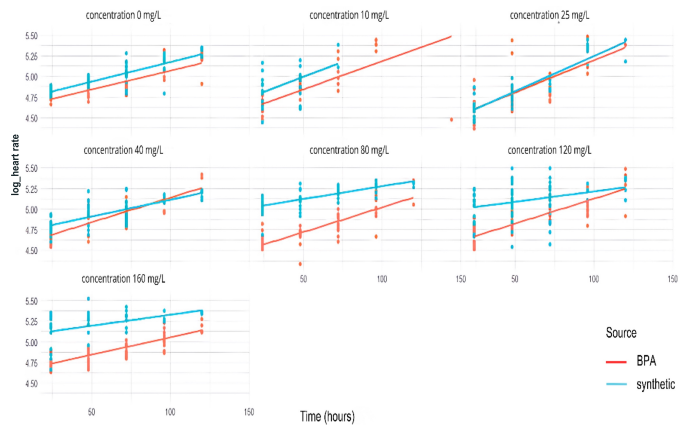


Fig. 5. Interaction effects of source, concentration, and time on the log-transformed heart rate (Heart rate log)

3.4 Abnormality

A preliminary descriptive analysis (Fig. 6) conducted using Microsoft Excel 2016 revealed an increasing trend in the percentage of abnormalities with increasing potassium concentration in both BPA and synthetic treatments (Fig. 7). At lower concentrations (0–25 mg/L), abnormalities were minimal; however, a progressive increase was observed with higher exposure levels. Starting at 40 mg/L, the values of abnormalities increase significantly, peaking at around 60% in the BPA group and nearly 80% in the synthetic potassium group at 160 mg/L. The synthetic potassium treatment consistently yielded greater percentage of developmental abnormalities compared to BPA treatment, especially at elevated concentrations, indicating a more pronounced effect on embryonic development. These findings indicate a positive relationship between exposure concentration and the incidence of abnormalities, implying that the synthetic compound has more pronounced teratogenic effects than BPA, however, differences were not statistically significant (Table 7). Developmental abnormalities observed throughout this study were categorized into two group: larval

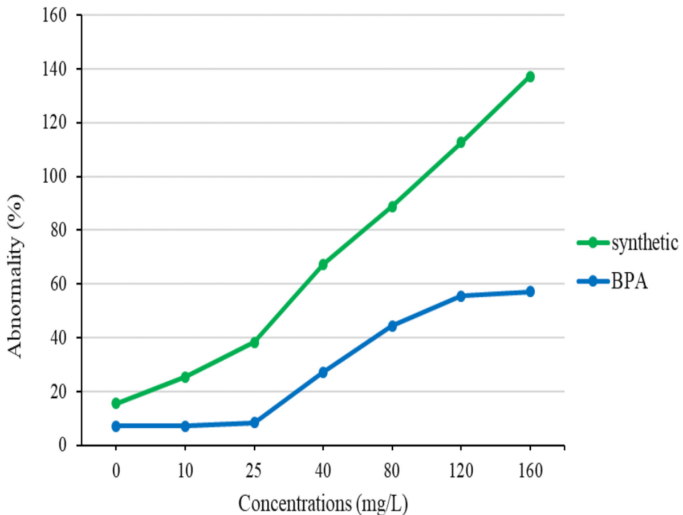


Fig. 6. Percentage of embryonic abnormalities in rainbowfish exposed to different concentrations of BPA and synthetic potassium. Abnormality rates increased proportionally with concentration, with the synthetic compound inducing a slightly higher percentage of abnormalities at higher exposure levels ( $\geq 120$  mg/L).

malformations, consisting of axial skeletal deformities (spinal curvature) and yolk-sac malformations, and embryonic abnormalities, consisting of pericardial edema and altered heart rate as a cardiotoxicity indicator. Delayed hatching was also assessed as a sublethal developmental endpoint (Fig. 7).

A Kruskal-Wallis rank sum test was conducted to assess the differences in abnormality levels between the two potassium sources (BPA and Synthetic) and across various concentrations. The test for the factor "Source" yielded a chi-squared value of 0.8071 with a p-value of 0.369 (Table 7), indicating no significant difference in abnormality between the BPA and synthetic groups. This suggests that, at the tested concentrations, both sources had similar effects on abnormalities, and the observed variation in abnormality cannot be attributed to the source alone. Further analysis was performed for the factor "Concentration," where the Kruskal-Wallis test yielded a chi-squared value of 11.933 with a p-value of 0.06347 (Table 7). While the result is close to the threshold of significance, it still does not reach the conventional threshold ( $p < 0.05$ ), implying that, although concentration appeared to influence

| Table 7. Results of Kruskal–Wallis and Dunn’s post-hoc tests for developmental abnormalities |                   |                               |    |                     |
|----------------------------------------------------------------------------------------------|-------------------|-------------------------------|----|---------------------|
| Factor tested                                                                                | Comparison/ Group | Test Statistic ( $\chi^2/z$ ) | df | P value             |
| Source                                                                                       | BPA vs. Synthetic | $\chi^2 = 0.807$              | 1  | 0.369 <sup>ns</sup> |
| Concentration                                                                                | 0–160 mg/L        | $\chi^2 = 11.933$             | 6  | 0.063 <sup>ns</sup> |
| Post-hoc (Dunn test)                                                                         | BPA – Synthetic   | $Z = -0.898$                  | –  | 0.369 <sup>ns</sup> |
| <sup>ns</sup> : non-significant                                                              |                   |                               |    |                     |

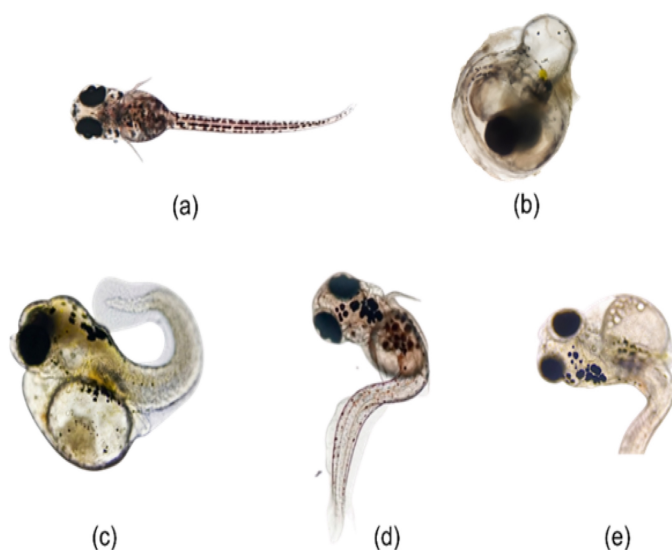


Fig. 7. Abnormalities of *Melanotaenia boesemani* embryos and larvae following exposure to synthetic potassium citrate and banana peel ash (BPA)-derived potassium; a) normal embryo (control), b) yolk sac edema, c) pericardial edema, d) spinal deformity, e) delayed hatching

abnormality levels, the differences were not statistically significant ( $p > 0.05$ ).

To explore potential differences within the "Source" factor further, a post-hoc Dunn test was conducted for pairwise comparisons between BPA and synthetic sources. The comparison yielded a Z-value of -0.8984 with an unadjusted p-value of 0.36998 (Table 7), which remained non-significant even after applying the Bonferroni adjustment. These observations further supports the conclusion that there was no significant difference in abnormalities between the BPA and synthetic groups across all tested concentrations. These results suggest that, while concentration may influence abnormality, the source of exposure does not appear to significantly impact the abnormality in this study.

The boxplot and beeswarm plot together showed the arcsine-transformed abnormalities were distributed differently between the BPA exposure group and the synthetic chemical group (Fig. 8). The BPA group had a lower median abnormality than the synthetic potassium

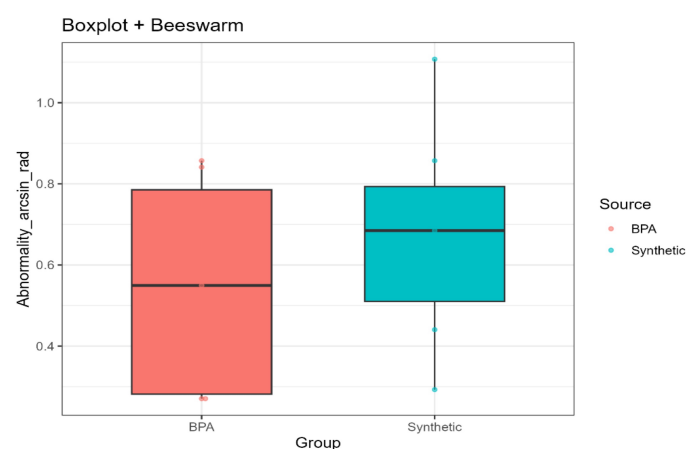


Fig. 8. Boxplot with beeswarm plot showing the abnormality scores (arcsine transformed) between BPA and Synthetic groups

group, indicating lesser developmental abnormality. The BPA group displayed a wider interquartile range, indicating more variability in biological responses than the synthetic potassium group. On the other hand, the synthetic group had a higher median and a more consistent range of results, even though there was a significant outlier. This suggests that a small number of embryos exhibited usually higher abnormalities in the synthetic group. The beeswarm plot showed that the observations were widely distributed without any noticeable clustering patterns and it also doesn't show any distribution problems that could complicate the statistical analysis.

The BPA group (depicted in red) showed a slower increase in the percentage of abnormalities, particularly at lower concentrations (0–40 mg/L). It increased slightly from 0 to 40 mg/mL, followed by a plateau between 40 and 160 mg/L. This suggests that the BPA group may exhibit a more saturated response, where further increase in concentrations did not result in a proportional increase in abnormalities. This may be potentially due to a threshold effect in the biological response and requires further studies. The divergence between the two sources is particularly evident at the higher concentrations (80, 120, and 160 mg/L), where the synthetic potassium group displayed a higher percentage of abnormalities, though not statistically significant, compared to the BPA group. This suggests that while both chemicals influence the incidence of abnormalities, the synthetic potassium may have a more potent dose-dependent effects, whereas the BPA response appeared to plateau at a certain concentration threshold. These findings emphasize the importance of considering both concentration and chemical type when assessing the toxicological impacts of different substances, as different chemicals may exhibit varied mechanisms of action at different doses. Further statistical analysis, such as interaction tests and post-hoc comparisons, could provide a deeper understanding of the significance of these trends.

#### 4. Discussion

The present study indicates that embryonic mortality is influenced by the potassium source, concentration, and duration of exposure. The increase in embryonic mortality due to potassium exposure was time-dependent, suggesting that the duration of exposure had a more dominant influence than the potassium concentration and source. This finding is consistent with previous research reporting that the accumulation of physiological stress and osmoregulatory disturbances in the embryonic phase tends to increase with exposure time, leading to death (Ord 2019; Wang et al. 2019). The pattern of toxicity response to synthetic potassium revealed a non-linear biphasic trend, with a decrease in mortality at a concentration of 40 mg/L followed by a sharp increase at higher concentrations. This biphasic trend is often known as the hormesis pattern, which has been associated with alterations in morphology, alter heart rate, ion transport, and membrane excitability (Aniche et al. 2019; Wang et al. 2018). Based on the LC<sub>50</sub> analysis, synthetic potassium showed a lower value and higher mortality compared to potassium from BPA. This indicates that the ionic form of synthetic potassium, which is completely dissociated in water, can induce osmotic stress more quickly. Consequently, at the same concentration, synthetic potassium causes a higher mortality rate (Takvam et al. 2021; Velselvi et al. 2022).

Present study indicates that synthetic potassium exhibited lower LC<sub>50</sub> values and induced significantly higher heart rates, indicating

stronger ionic reactivity that accelerates metabolic and osmoregulatory stress. In contrast, BPA showed higher  $LC_{50}$  and  $EC_{50}$  values, suggesting that its organic mineral matrix moderates ion release and reduces acute toxicity, likely through reduced free-ion activity at cellular membranes (Jing et al. 2023; Kleber et al. 2021; Yan et al. 2022). This mechanism is well reported in soil and sediment chemistry, where interactions between organic matter and minerals significantly reduce the bioavailability of cationic nutrients and toxicants (Jing et al. 2023). The organic matrix of BPA acts as a buffer for ionic transport to embryonic tissues. This is a slow-release mechanism that reduces peak  $K^+$  exposure at cellular membranes.

This study evaluated the effect of natural potassium (BPA) compared to synthetic potassium salt on the hatching of rainbowfish embryos. The results of the study showed that the hatching of rainbowfish embryos was more influenced by concentration than by the source of  $K^+$ . The results indicated that the highest hatching probability estimates were at a concentration of 10 mg/L for both sources. This finding is consistent with the research on immersion of Dojo loach embryos in glycyrrhizic acid derived extract from licorice that resulted in higher hatching rate at lower concentrations (12.5 mg/L) compared to higher levels (50 mg/L) (Li et al. 2022). At low concentrations (10-25 mg/L), both BPA and synthetic K significantly enhanced hatching probability compared with the control treatment, suggesting that potassium functions as a beneficial osmolyte at physiologically relevant doses by supporting the  $Na^+/K^+$  electrochemical gradient necessary for chorionase enzyme activation and chorionic membrane hydration during hatching (Segret et al. 2022). Despite these physiological differences existed, hatchability outcomes exhibited analogous dose-dependent trends from both sources, confirming that potassium serves as a beneficial osmolyte at low concentrations while causing developmental impairment at higher doses, regardless of source. This supports the idea that in aquaculture mineral management, optimizing the potassium dose is more important than switching sources.

The heart rate of rainbowfish embryos is influenced by three factors: the source of  $K^+$ , concentration, and exposure time. The results of the LMM analysis of the toxicity of synthetic and natural potassium (BPA) on heart rate showed that the synthetic potassium source resulted in a higher heart rate compared to BPA. Similarly, the interaction pattern between source, concentration, and time indicated that exposure to BPA modulates cardiac responses in a dose-dependent manner; increased concentration resulted in a more pronounced physiological response compared to the synthetic control. The consistent rise in heart rate across the treatments confirm the hypothesis that extended exposure influences the functioning of the embryonic cardiovascular physiology. A plausible physiological basis for this source-dependent in the cardiac response involves disruption of ion homeostasis at the embryonic myocardial membrane. High extracellular  $K^+$  concentration reduces its transmembrane gradient and partially depolarize the cardiomyocyte membrane potential. The synthetic source of  $K^+$  ion may lead to a more abrupt change in  $K^+$  activity in extracellular space than BPA, due to the complete dissociation of the synthetic potassium citrate and the rapid delivery of  $K^+$  ions to the surrounding medium, whereas the organic matrix of BPA may buffer the release of ions (He et al. 2020b). Sustained depolarization may affect the voltage-gated ion channels involved in pacemaker activity in the embryonic heart, possibly accounting for the increased and faster-changing heart rate observed in synthetic

exposure. In addition, the maintenance of the transmembrane  $K^+/Na^+$  gradient depends on  $K^+/Na^+$ -ATPase activity. Increased environmental  $K^+$  has been shown to increase the ionoregulatory workload of this pump in teleost osmoregulatory tissue, which could divert energetic resources away from other development processes during a time of high metabolic demand (Takvam et al. 2021). This osmoregulatory stress response is consistent with broader evidence that ionic and salinity perturbation alters branchial and systemic ion-transport gene expression in fish (Zhu et al. 2023) and may explain why heart rate, as an energetically sensitive physiological readout, was more responsive to  $K^+$  source than to concentration alone in the present study. Future research directly measuring branchial or cardiac  $Na^+/K^+$ -ATPase activity would help confirm this proposed mechanism. The increase in heart rate found in this study with increasing exposure time and  $K^+$  concentration differs from the findings in zebrafish embryos exposed to ZnO nanoparticles, where the heart rate of zebrafish embryos actually decreased with increasing concentration (Deenathayalan et al. 2024). These findings suggest that the source and exposure time are crucial factors affecting heart rate dynamics, with synthetic sources showing a more significant impact. Further studies are required to investigate the mechanisms underlying these variations and to assess the possible biological consequences of the observed patterns.

The present study revealed a concentration-dependent increase in developmental abnormalities within rainbowfish embryos subjected to both BPA and synthetic potassium, which is consistent with previous report of dose-dependent developmental abnormalities in zebrafish embryos as an impact of sodium exposure (Seli et al. 2024). Descriptively, synthetic potassium was associated with a numerically higher and more consistent upward trend in abnormality scores at concentrations at 80 mg/L or higher, compared to BPA. However, this descriptive pattern was not statistically supported, as the Kruskal-Wallis test revealed no significant difference in abnormality rates between BPA and synthetic group ( $\chi^2 = 0.807$ ,  $p = 0.369$ ). This observation should, therefore, be interpreted as a hypothesis-generating trend rather than as evidence of a more potent toxicodynamic profile for synthetic potassium, and any comparison of teratogenic potency between sources requires confirmation in a study with greater statistical power. Although violin plots, boxplots, and interaction plots consistently displayed visual trends, the Kruskal-Wallis test did not demonstrate a statistically significant difference in abnormality rates between the BPA and synthetic groups ( $\chi^2 = 0.807$ ,  $p = 0.369$ ). The divergence between statistical significance and biological relevance has been extensively discussed within toxicological risk assessment, suggesting that limited sample sizes or substantial within-group variability may obscure biologically significant effects (Adriaanse et al. 2025). The greater variability observed within the BPA group may partly reflect such heterogeneity, however, in the absence of statistically significant results, this remains a plausible explanation rather than a confirmed one.

## 5s. Conclusions

This study provides the first comparative toxicological assessment of synthetic potassium citrate and bio-based potassium derived from banana peel ash (BPA) extract using *Melanotaenia boesemani* embryos. Synthetic potassium exhibited a lower  $LC_{50}$ , a significant source  $\times$  concentration interaction on mortality, and a substantially greater and rapidly rising heart rate response than BPA, indicating stronger acute physiological stress. A similar descriptive trend toward higher



abnormality scores was observed following synthetic exposure, particularly at 10-40 mg/L and 160 mg/L, although this difference did not reach statistical significance. Conversely, egg hatching was mainly affected by concentration and exposure time, with no significant difference between potassium sources at any of the concentrations tested. To summarize, the data suggest that for both sources of potassium, concentration and time of exposure are the main determinants of embryotoxicity, whereas the source of potassium affects the degree of mortality and cardiac responses, with the synthetic potassium source being the more toxic agent. The higher LC<sub>50</sub> and lower cardiac response associated with BPA exposure suggest that it is comparatively a safer potassium source than its synthetic counterpart under tested conditions. Given the conservation significance of this ornamental species, these results provide the first species-specific embryo toxicity reference points for potassium exposure in *M. boesemani*, and support the use of BPA in preference to synthetic potassium citrate in hatchery mineral supplementation protocols.

## Declarations

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**Ethics approval:** All protocols and experimental techniques used for fish in this study adhered to the ethical clearance by the Animal Care and Use Committee at the Brawijaya University, Malang, Indonesia. The animal use license number was 098-KEP-UB-2024

## Supplementary data

The supplementary tables and data set are available on journal website. Click the link to access the supplementary data ([Supplementary Data](#))

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