

LETTERS IN ANIMAL BIOLOGY

SUPPLEMENTARY MATERIALS

Table S1-S7, Figure 1

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

Table S1. Preliminary rang-finding test result for embryo mortality (%) of *Melanotaenia boesemani* exposed to bio-based potassium (BPA) and synthetic potassium at the eye-spot stage (49 hpf)

Concentration (mg/L)	Embryo mortality (%)					
	duration 5 h		duration 24 h		duration 120 h	
	BPA	synthetic	BPA	synthetic	BPA	synthetic
250	85	85	75	75	85	90
500	90	85	85	85	90	95
1500	95	100	90	100	100	100
3000	100	100	100	100	100	100
4500	100	100	100	100	100	100
6000	100	100	100	100	100	100

Embryos at the eye-spot stage (49 hpf) were exposed to wide preliminary concentration range (250-6000 mg/L K⁺ equivalent) for 5, 24, and 120 h to identify the sub lethal response range prior selecting definitive test concentrations. Mortality at 250 mg/L, the lowest concentration tested, already exceeded 75% within 5 h of exposure for both potassium sources, and mortality remained at 85-100% across all higher concentrations and observation durations. Because exposure at and above 250 mg/L produced mortality too high to characterize a dose-response relationship, seven definitive concentrations (0, 10, 25, 40, 80, 120, and 160 mg/L) were selected within a lower, sub-lethal to acutely toxic range for the definitive fish embryo toxicity test

Table S2. Fixed effect estimates from the GLMM for mortality

Effect	Estimate $\pm$ SE	z value	p-value
(Intercept)	-4.662 $\pm$ 0.485	-9.607	<0.0001***
Source (Synthetic)	0.381 $\pm$ 0.357	1.067	0.286 ^{ns}
Concentration (10)	0.261 $\pm$ 0.361	0.722	0.470 ^{ns}
Concentration (25)	0.662 $\pm$ 0.35	1.889	0.059 [.]
Concentration (40)	0.662 $\pm$ 0.35	1.889	0.059 [.]
Concentration (80)	0.662 $\pm$ 0.35	1.889	0.059 [.]
Concentration (120)	0.767 $\pm$ 0.348	2.204	0.028 [*]
Concentration (160)	0.921 $\pm$ 0.346	2.664	0.008 ^{**}
Time (48)	1.526 $\pm$ 0.437	3.49	0.0005***
Time (72)	2.589 $\pm$ 0.415	6.24	<0.0001***
Time (96)	3.34 $\pm$ 0.41	8.146	<0.0001***
Time (120)	3.608 $\pm$ 0.41	8.809	<0.0001***
Time (144)	3.883 $\pm$ 0.41	9.476	<0.0001***
Source $\times$ Concentration (10)	0.476 $\pm$ 0.488	0.977	0.329 ^{ns}
Source $\times$ Concentration (25)	0.172 $\pm$ 0.479	0.36	0.719 ^{ns}
Source $\times$ Concentration (40)	-0.909 $\pm$ 0.496	-1.831	0.067 [.]
Source $\times$ Concentration (80)	-0.603 $\pm$ 0.489	-1.235	0.217 ^{ns}
Source $\times$ Concentration (120)	-0.434 $\pm$ 0.482	-0.9	0.368 ^{ns}
Source $\times$ Concentration (160)	0.853 $\pm$ 0.476	1.793	0.073 [.]

p-values represent Wald z-test statistics for fixed effects in the generalized linear mixed model (GLMM) using a binomial logit link. Concentration effects indicate the baseline response for BPA (reference source), while the corresponding values for the Synthetic source are obtained by adding the interaction terms (Source $\times$ Concentration). Significance levels are denoted as follows: ns = not significant ($p \geq 0.10$); . = marginally significant ($0.05 \leq p < 0.10$); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. The identical estimates at Concentration 25, 40, and 80 mg/L (BPA) are not a transcription error; the observed mortality counts at these three concentrations were numerically identical in the dataset, so the model returns the same maximum-likelihood estimate for each

Table S3. Predicted Mortality Probabilities (EMMs, Averaged Over Time)

Source	Concentration (mg/L)	Estimated Mortality (prob) ± SE	95% CI (Lower– Upper)
BPA	0	0.102 ± 0.0274	0.0598 - 0.170
	10	0.129 ± 0.0319	0.0781 - 0.205
	25	0.181 ± 0.0398	0.1154 - 0.272
	40	0.181 ± 0.0398	0.1154 - 0.272
	80	0.181 ± 0.0398	0.1154 - 0.272
	120	0.197 ± 0.0421	0.1273 - 0.292
	160	0.223 ± 0.0453	0.1463 - 0.324
Synthetic	0	0.143 ± 0.0342	0.0881 - 0.224
	10	0.259 ± 0.0494	0.1739 - 0.366
	25	0.278 ± 0.0514	0.1887 - 0.388
	40	0.115 ± 0.0297	0.0687 - 0.187
	80	0.15 ± 0.0353	0.0933 - 0.233
	120	0.189 ± 0.0410	0.1213 - 0.282
	160	0.496 ± 0.0632	0.3749 - 0.618

Table S4. Pairwise Contrasts Between BPA and Synthetic Sources at Equivalent Concentrations

Concentration (mg/L)	Contrast (BPA vs Synthetic)	Odds Ratio $\pm$ SE	z value	p-value
0	BPA < Synthetic	0.683 $\pm$ 0.244	-1.067	0.286 ^{ns}
10	BPA < Synthetic	0.424 $\pm$ 0.141	-2.582	0.0098 ^{**}
25	BPA < Synthetic	0.575 $\pm$ 0.183	-1.737	0.082 [.]
40	BPA > Synthetic	1.694 $\pm$ 0.583	1.532	0.126 ^{ns}
80	BPA > Synthetic	1.249 $\pm$ 0.416	0.667	0.505 ^{ns}
120	BPA $\approx$ Synthetic	1.054 $\pm$ 0.341	0.162	0.871 ^{ns}
160	BPA < Synthetic	0.291 $\pm$ 0.092	-3.92	0.0001 ^{***}

Pairwise contrasts were derived from the GLMM-based estimated marginal means (EMMs) using log odds ratios to compare mortality between BPA and Synthetic sources at each concentration level. Odds ratios < 1 indicate lower mortality in BPA relative to Synthetic. Significance levels were determined using Wald z-tests: ns = not significant ($p \geq 0.10$); . = marginal ($0.05 \leq p < 0.10$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S5. Fixed effect estimates from the GLMM for hatching

Fixed Effect	Estimate	Std. Error	z value	p value
(Intercept)	-19.912	27.2525	-0.731	0.465ns
Sourcesynthetic	-0.160	0.399	-0.4	0.689ns
Concentration10	1.304	0.3796	3.435	0.001***
Concentration25	0.781	0.3802	2.055	0.040*
Concentration40	-0.417	0.4093	-1.02	0.308ns
Concentration80	-0.509	0.4136	-1.231	0.218ns
Concentration120	-0.605	0.4185	-1.446	0.148ns
Concentration160	-0.921	0.438	-2.104	0.035*
Time48	14.459	27.2605	0.53	0.596ns
Time72	17.154	27.2522	0.629	0.529ns
Time96	18.217	27.2517	0.668	0.504ns
Time120	19.290	27.2515	0.708	0.479ns
Time144	19.886	27.2515	0.73	0.466ns
Sourcesynthetic:Concentration10	0.289	0.5374	0.538	0.590ns
Sourcesynthetic:Concentration25	-0.251	0.5445	-0.461	0.645ns
Sourcesynthetic:Concentration40	0.248	0.5794	0.429	0.668ns
Sourcesynthetic:Concentration80	0.895	0.5711	1.568	0.117ns
Sourcesynthetic:Concentration120	0.522	0.5837	0.894	0.371ns
Sourcesynthetic:Concentration160	0.376	0.6123	0.614	0.539ns

The model was fitted with a binomial distribution and logit link including a random intercept for Embryo ID. Significant differences are indicated as: *** $p < 0.001$; ** $p < 0.01$; $p < 0.05$; ns = not significant

Table S6. Estimated Marginal Means (EMMs) of Hatching Probability for Each Source (BPA and Synthetic) at Different Concentrations

Source	Concentration (mg/L)	Estimated Probability $\pm$ SE	95% CI Lower - Upper
BPA	0	0.006 $\pm$ 0.028	8.29E-07 - 0.979
BPA	10	0.022 $\pm$ 0.100	3.06E-06 - 0.994
BPA	25	0.013 $\pm$ 0.060	1.81E-06 - 0.990
BPA	40	0.004 $\pm$ 0.019	5.44E-07 - 0.969
BPA	80	0.004 $\pm$ 0.017	4.96E-07 - 0.966
BPA	120	0.003 $\pm$ 0.015	4.49E-07 - 0.963
BPA	160	0.003 $\pm$ 0.011	3.27E-07 - 0.949
Synthetic	0	0.005 $\pm$ 0.024	7.06E-07 - 0.976
Synthetic	10	0.026 $\pm$ 0.113	3.48E-06 - 0.995
Synthetic	25	0.009 $\pm$ 0.040	1.20E-06 - 0.985
Synthetic	40	0.005 $\pm$ 0.020	5.94E-07 - 0.971
Synthetic	80	0.009 $\pm$ 0.035	1.04E-06 - 0.983
Synthetic	120	0.005 $\pm$ 0.022	6.48E-07 - 0.974
Synthetic	160	0.003 $\pm$ 0.014	4.06E-07 - 0.959

Probabilities were back-transformed from the logit scale and averaged over time; 95 % confidence intervals are shown

Table S7. Pairwise Comparisons Between BPA and Synthetic Sources at Each Concentration Level

Concentration (mg/L)	Contrast	Odds Ratio	z-ratio	p-value
0	BPA / Synthetic	1.173 ± 0.468	0.4	0.689 ^{ns}
10	BPA / Synthetic	0.878 ± 0.316	-0.36	0.719 ^{ns}
25	BPA / Synthetic	1.508 ± 0.559	1.109	0.268 ^{ns}
40	BPA / Synthetic	0.915 ± 0.385	-0.211	0.833 ^{ns}
80	BPA / Synthetic	0.479 ± 0.196	-1.802	0.072
120	BPA / Synthetic	0.696 ± 0.297	-0.85	0.395 ^{ns}
160	BPA / Synthetic	0.806 ± 0.374	-0.466	0.642 ^{ns}
Comparisons are performed on the log-odds scale; values represent odds ratios (OR). A dot (.) indicates marginal significance ($p < 0.10$)				

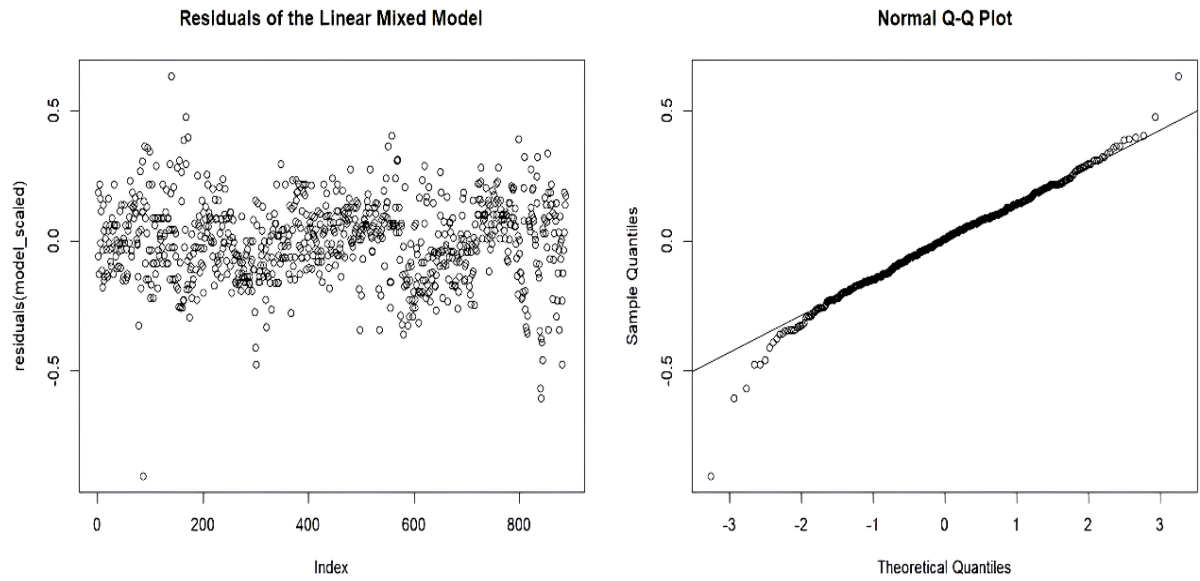


Figure S1. Diagnostic plots for the Linear Mixed Model residuals of *Heart_beat*. The left panel displays the residuals versus fitted values, showing a random scatter around zero without clear patterns, indicating homoscedasticity and absence of systematic bias. The right panel presents the Normal Q–Q plot, where residuals closely follow the reference line, suggesting that the assumption of normality is adequately satisfied for the model residuals.