

Benzyl Benzoate (CAS no. 120-51-4)

Tier 1 *in vivo* Test

(1) Results

Fish were exposed to concentrations of 34.4, 123 and 376 µg/L (measured). No significant differences were observed in mortality, number of eggs, male gonadosomatic index, female hepatosomatic index, male hepatic vitellogenin level, and secondary sex characteristics.

At 34.4 µg/L, a significant increase in female hepatic vitellogenin level was observed.

At 123 µg/L and higher, a significant decrease in fertility rate was observed.

At 376 µg/L, a significant decrease in numbers of fertilized eggs and a significant increase in female gonadosomatic index and male hepatosomatic index were observed.

Statistical analysis was not carried for total length and body weight.

(2) Summary

Estrogenic activity of benzyl benzoate has been indicated from literature and Tier 1 *in vitro* tests. In this study, no increase in male hepatic vitellogenin level was observed at sublethal concentrations to suggest estrogenic effect.

It was also not concluded that benzyl benzoate is an anti-estrogenic or an androgenic compound.

The adverse exposure level of 123 µg/L was ca. 1,700 times as high as the highest environmental water concentration of 0.072 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2016.

The no observed adverse effect level of 34.4 µg/L was ca. 480 time as high as the highest environmental water concentration of 0.072 µg/L for MOE's Environmental Survey and Monitoring of Chemicals in FY2016.

Table 1-A Results

Measured concentration ($\mu\text{g/L}$)	Number of tested fish		Total length (mm)		Body weight (mg)		Total length (mm)	
	male	female	male	female	male	female	male	female
Control	12	12	0	0	37.1 \pm 0.4	36.9 \pm 0.6	520 \pm 27	524 \pm 6.0
34.4	12	12	0	0	37.2 \pm 0.7	36.6 \pm 0.3	492 \pm 18	524 \pm 11
123	12	12	0	0	37.3 \pm 0.4	36.1 \pm 0.2	535 \pm 34	504 \pm 21
376	12	12	0	0	36.1 \pm 0.9	36.0 \pm 0.5	501 \pm 33	518 \pm 20

Statistical analysis was not carried for total length and body weight.

Table 1-B Results (continued)

Measured concentration ($\mu\text{g/L}$)	Number of eggs (eggs/female/day)	Number of fertile eggs (eggs/female/day)	Fertility rate (%)	Gonadosomatic Index (%)	
				male	female
Control	27.0 \pm 1.7	26.7 \pm 1.7	99.0 \pm 0.2	0.81 \pm 0.18	9.0 \pm 0.45
34.4	25.8 \pm 1.3	25.0 \pm 1.9	96.7 \pm 2.9	0.78 \pm 0.02	8.7 \pm 0.50
123	25.3 \pm 1.5	24.0 \pm 1.8	95.1 \pm 1.5*	0.80 \pm 0.11	9.1 \pm 0.54
376	25.3 \pm 2.2	23.8 \pm 1.8*	94.0 \pm 3.4*	0.82 \pm 0.12	9.9 \pm 0.72*

Table 1-C Results (continued)

Measured concentration ($\mu\text{g/L}$)	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	male	female	male	female	male	female
Control	1.7 \pm 0.18	4.0 \pm 0.61	5.0 \pm 4.0	964 \pm 105	132 \pm 6.3	0 \pm 0
34.4	1.9 \pm 0.12	4.4 \pm 0.17	4.2 \pm 6.4	1,280 \pm 172*	132 \pm 5.5	0 \pm 0
123	1.9 \pm 0.12	4.3 \pm 0.43	13.9 \pm 8.3	1,150 \pm 119	130 \pm 15	0 \pm 0
376	2.0 \pm 0.11*	4.1 \pm 0.65	13.0 \pm 8.6	1,150 \pm 105	124 \pm 4.4	0 \pm 0

Table 1-D Results (continued)

Measured concentration ($\mu\text{g/L}$)	Other observations
Control	Not found
34.4	Not found
123	Not found
376	Not found

Data show mean \pm SD (standard deviation)

Statistically significant differences from control group (** p <0.01, * p <0.05)

ND: not detected (below detection limit of vitellogenin: 1 ng/mg liver)

(-): not measured

Secondary sex characteristics: number of joint plates with papillary processes