Methyl 4-hydroxybenzoate (CAS no. 99-76-3)

Tier 1 *in vivo* Test

(1) Results

Fish were exposed to concentrations of 0.357, 1.90 and 9.75 mg/L (measured). No significant differences were observed in gonadosomatic index, secondary sex characteristics, hepatosomatic index, and female hepatic vitellogenin level.

A significant increase was observed in male hepatic vitellogenin level at 1.90 mg/L and higher, and this increase was dose-dependent.

A significant decrease was observed in total number of eggs, number of fertile eggs and fertility rate at 9.75 mg/L.

(2) Summary

A significant decrease observed in total number of eggs, number of fertile eggs and fertility rate 9.75 mg/L were considered adverse reproductive effects on Medaka.

Estrogenic activity of methyl 4-hydroxybenzoate has been indicated from literature. In this study, an increase in male hepatic vitellogenin level was observed at sublethal concentrations, indicating its estrogenic effect. It was concluded that 4-hydroxybenzoate is an estrogenic compound.

The adverse exposure level of 9.75 mg/L was ca. 3,250,000 times as high as the highest environmental water concentration of 0.003 μg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2008.

Table 1-A Results

Measured concentration (mg/L)	Number of fish		Mortality (%)		Total length (mm)		Body weight (mg)	
	male	female	male	female	male	female	male	female
Control	12	12	0	8.3	36.1±1.0	37.1±0.5	527±16	597±46
0.357	12	12	0	0	36.0±0.5	37.0±1.3	514±28	589±39
1.90	12	12	0	0	36.4±1.3	36.2±1.2	530±47	574±27
9.75	12	12	8.3	17	36.2±1.1	36.6±1.4	504±62	603±47

Table 1-B Results (continued)

Measured concentration (mg/L)	Total number of eggs (eggs/female/day)	Number of fertile eggs (eggs/female/day)	Fertility rate (%)	Gonadosomatic Index (%)	
				male	female
Control	27.8±4.3	26.0±3.9	93.7±1.3	0.69±0.077	10±1.4
0.357	28.8±3.3	26.8±3.5	93.1±2.9	0.74±0.18	11±1.2
1.90	27.6±3.3	24.1±3.0	87.6±7.7	0.81±0.061	11±0.86
9.75	19.9±2.2*	15.6±2.3*	77.9±4.8*	0.69±0.15	9.8±0.93

Table 1-C Results (continued)

Measured concentration (mg/L)	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	male	female	male	female	male	female
Control	2.0±0.38	4.5±0.72	1.3±0.2	3,260±481	98±4.6	0±0
0.357	2.1±0.16	4.4±0.44	1.7±1.2	3,450±724	107±8.3	0±0
1.90	2.4±0.21	4.7±0.59	153±113 *	3,890±992	99±12	0±0
9.75	2.3±0.60	5.4±0.38	2,640±1,720 *	3,930±963	93±15	0±0

Table 1-D Results (continued)

Measured concentration (mg/L)	Other observations
Control	Not found
0.357	Not found
1.90	Not found
9.75	Not found

Data show mean ± SD (standard deviation)
Statistically significant differences from control group (**p<0.01, *p<0.05)
nd: not detected (below detection limit of vitellogenin: 1ng/mg liver)
(-): not measured
Secondary sex characteristics: number of joint plates with papillary processes