

## Phenytoin (CAS no. 57-41-0)

### Tier 1 *in vivo* Test

#### (1) Results

Fish were exposed to concentrations of 2.25, 4.76, 8.72 and 18.51 mg/L (measured). No significant differences were observed in male and female total length, body weight, hepatic vitellogenin, secondary sex characteristics, male gonadosomatic index and female hepatosomatic index.

Lack of coordination of male and female were observed at 2.25 mg/L and higher. Overturn and hyperreactivity of male and female were observed at 4.76 mg/L and higher.

A significant increase was observed in male mortality in male at 18.51 mg/L.

A significant decrease was observed in number of eggs, number of fertile eggs and fertility rate at 8.72 mg/L and higher, and this decrease was dose-dependent. A significant increase was also observed in female gonadosomatic index and male hepatosomatic index at 8.72 mg/L and higher.

#### (2) Summary

A significant decrease observed in number of eggs, number of fertile eggs and fertility rate at 8.72 mg/L and higher was considered adverse reproductive effects on Medaka. Death was observed at 18.51 mg/L and higher, and abnormal behaviors of male and female such as lack of coordination were observed at 2.25 mg/L and higher.

While antiestrogenic activity of phenytoin has been indicated from literature, a decrease in female hepatic vitellogenin level was not observed at sublethal concentrations to suggest antiestrogenic effect in this study. It was not concluded that phenytoin is an antiestrogenic compound.

The adverse exposure level of 8.72 mg/L was ca. 790,000 times as high as the highest environmental water concentration of 11 ng/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2006, while the exposure level of 2.25 mg/L (at which lack of coordination of male and female was observed) was ca. 200,000 times.

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	0	36.60±0.80	35.07±0.64	450.2±3.35	467.4±26.2
2.25	12	12	0	0	35.92±0.61	35.88±0.38	434.8±26.2	466.4±25.6
4.76	12	12	0	0	36.49±1.48	35.48±0.95	434.5±62.9	462.8±10.2
8.72	11	12	8.33	0	36.54±0.12	35.96±0.52	415.0±31.0	575.5±28.0
18.51	5	11	58.33**	80.33	38.42±1.44	37.46±1.42	506.9±82.9	609.4±67.4

Table 1-B Results (continued)

Measured concentration (mg/L)	Number of eggs (eggs/female/day)	Number of fertile eggs (eggs/female/day)	Fertility rate (%)	Gonadosomatic Index (%)	
				male	female
Control	23.9±5.6	22.1±6.7	90.9±8.6	0.78±0.14	8.45±1.04
2.25	20.0±5.0	18.3±4.3	91.7±2.8	1.32±0.77	8.32±0.76
4.76	19.7±2.0	17.1±3.2	86.1±9.3	1.00±0.26	8.97±1.43
8.72	9.4±1.8**	4.5±1.1**	48.5±12.1**	1.04±0.31	19.26±10.45**
18.51	5.4±1.1**	1.0±0.4**	17.8±4.1**	0.44±0.26	17.00±9.67**

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	1.31±0.55	3.56±1.19	2.97±1.45	780.14±72.47	104.58±22.30	0
2.25	1.43±0.36	2.79±1.03	1.38±0.45	684.56±109.58	95.08±14.34	0
4.76	1.57±0.35	2.85±1.18	4.20±6.72	1,013.49±183.26	99.50±19.68	0
8.72	2.22±0.30**	2.98±1.31	0.96±0.38	661.04±199.14	99.09±21.00	0
18.51	3.43±0.47**	3.38±0.88	1.36±0.93	693.01±242.76	85.00±14.75	0

Table 1-D Results (continued)

Measured concentration (mg/L)	Other observations
Control	Not found
2.25	Lack of coordination
4.76	Lack of coordination , overturn, and hyperreactivity
8.72	Lack of coordination , overturn, and hyperreactivity
18.51	Lack of coordination , overturn, and hyperreactivity

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 papillary processes