4-*t*-Amylphenol (CAS no. 80-46-6)

Tier 1 *in vivo* Test

(1) Results

Fish were exposed to concentrations of 58.4, 227 and 940 µg/L (measured). No significant differences were observed in fertility rate, male and female total length, body weight, secondary sex characteristics, gonadosomatic index, male mortality and female hepatosomatic index.

A significant increase was observed in male hepatic vitellogenin level at 58.4 µg/L and higher, and this increase was dose-dependent.

At 940 µg/L, an increase was observed in female mortality and a significant increase was observed in female hepatic vitellogenin, male hepatosomatic index, while a significant decrease was observed in number of eggs and number of fertile eggs. Abnormal swimming behavior was also observed.

(2) Summary

A significant decrease observed in number of eggs and number of fertile eggs at 940 µg/L were considered adverse reproductive effects on Medaka. Female mortality and abnormal swimming behavior were also observed.

Estrogenic activity of 4-*t*-amylphenol 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-*t*-amylphenol is an estrogenic compound.

The adverse exposure level of 940 µg/L was ca. 850,000 times as high as the detection limit of 0.0011 µg/L (not detected in water, but in sediment) that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2008.

Table 1-A Results

Concentration (μg/L)		Number of fish		Mortality (%)		Total length (mm)		Body weight (mg)	
nominal	measured	male	female	male	female	male	female	male	female
Control		12	12	0(0)	1(8.3)	36.4±0.6	36.2±0.4	509±21	552±32
62.5	58.4	12	12	0(0)	1(8.3)	37.8±1.2	36.0±0.5	590±39	558±48
250	227	12	12	0(0)	1(8.3)	36.2±0.6	35.5±0.7	546±23	523±48
1,000	940	12	12	0(0)	3(25)	35.6±0.8	36.1±1.6	509±47	588±130

Table 1-B Results (continued)

Measured concentration (μg/L)	Number of eggs (eggs/female/day)	Number of fertile eggs (eggs/female/day)	Fertility rate (%)	Gonadosomatic Index (%)	
				male	female
Control	23.6±2.2	21.2±3.0	89.8±6.0	0.76±0.09	11±1.8
58.4	20.8±3.1	19.5±3.2	93.7±1.7	0.89±0.11	10±0.63
227	20.0±2.7	18.9±2.9	94.3±2.5	0.73±0.16	9.9±1.0
940	16.1±4.7*	12.9±3.5*	81.1±8.9	0.73±0.13	8.9±1.2

Table 1-C Results (continued)

Measured concentration (μg/L)	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	male	female	male	female	male	female
Control	1.8±0.14	3.9±0.59	1.8±1.7	2,310±267	116±12.3	0
58.4	2.3±0.18	4.3±0.50	970±858*	2,930±737	113±6.14	0
227	2.2±0.33	4.2±0.44	3,700±2,300*	3,070±686	103±6.28	0
940	3.6±0.50*	3.9±0.73	30,800±6,440*	8,100±3,340*	110±11.0	0

Table 1-D Results (continued)

Measured concentration (μg/L)	Other observations
Control	Not found
58.4	Not found
227	Not found
940	Abnormal swimming behavior recognized as resting at the bottom and laziness

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