4-Nonylphenol (branched) (CAS no. 84852-15-3)

Tier 1 *in vivo* Test

(1) Results

Fish were exposed to concentrations of 5.63, 18.8, 51.8 and 170 μg/L (measured). No significant differences were observed in secondary sex characteristics, gonadosomatic index and female hepatosomatic index.

A significant increase was observed in male hepatic vitellogenin level at 5.63 μg/L and higher, and this increase was dose-dependent.

A significant increase was observed in female hepatic vitellogenin level at 18.8 μg/L and higher, and this increase was dose-dependent.

At 51.8 μg/L and higher, a significant decrease was observed in total number of eggs, number of fertile eggs and fertility rate, while a significant increase was observed in male hepatosomatic index.

(2) Summary

A significant decrease observed in total number of eggs, number of fertile eggs and fertility rate at 51.8 μg/L and higher was considered adverse reproductive effects on Medaka.

Estrogenic activity of 4-nonylphenol (branched) 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-nonylphenol (branched) is an estrogenic compound.

The adverse exposure level of 51.8 μg/L was ca. 82 times as high as the highest environmental water concentration of 0.63 μg/L that was measured in MOE's Water Quality Survey of Public Water Areas in FY2013.

Table 1-A Results

Measured concentration (μg/L)	Number of fish		Mortality (%)		Total length (mm)		Body weight (mg)	
	male	female	male	female	male	female	male	female
Control	12	12	0	0	37.0±0.8	35.6±0.6	507±33	492±18
5.63	12	12	0	0	36.8±1.3	36.7±1.3	515±45	535±82
18.8	12	12	0	0	37.5±0.6	35.5±1.2	552±34	500±46
51.8	12	12	0	0	37.6±1.2	36.4±0.8	552±69	537±45
170	12	12	8.3	8.3	38.6±1.5	36.0±1.6	602±64	540±64

Table 1-B Results (continued)

Measured concentration (μg/L)	Total number of eggs (eggs/female/day)	Number of fertile eggs (eggs/female/day)	Fertility rate (%)	Gonadosomatic Index (%)	
				male	female
Control	20.8±2.5	19.9±2.8	95.4±3.5	0.89±0.06	7.16±0.47
5.63	21.0±0.9	20.2±1.2	96.5±2.2	1.02±0.07	7.76±1.6
18.8	18.3±4.9	17.3±5.4	93.6±7.7	1.08±0.06	8.90±0.60
51.8	12.7±3.0*	7.85±2.7**	60.5±10.4*	1.15±0.20	7.73±0.58
170	4.05±1.7**	1.35±0.8**	32.7±9.7**	1.05±0.15	7.92±1.5

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	1.35±0.10	3.37±0.20	2.1±4	716±411	87.2±14.4	0±0
5.63	1.69±0.10	3.43±0.55	31.8±31.2*	866±277	91.5±15.3	0±0
18.8	1.74±0.28	4.10±0.59	375±320*	1,153±300**	78.5±20.7	0±0
51.8	2.00±0.22**	4.16±0.66	2,431±1,455*	1,348±258**	97.2±12.6	0±0
170	2.38±0.10**	3.93±0.58	5,009±1,830*	1,212±506**	86.7±11.9	0±0

Table 1-D Results (continued)

Measured concentration (μg/L)	Other observations
Control	Not found
5.63	Not found
18.8	Not found
51.8	Not found
170	Nuptial behavior of male fish was not observed after the Day 9.

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