Diisobutyl phthalate（CAS no. 84-69-5）

Tier 1 *in vivo* Test

(1) Results

Fish were exposed to concentrations of 35, 184 and 836 μg/L (measured). No significant differences were observed in mortality, total length, body weight, total number of eggs, number of fertile eggs, fertility rate, gonadosomatic index, hepatosomatic index, secondary sex characteristics and male hepatic vitellogenin level.

At 184 μg/L and higher, a significant decrease was observed in female hepatic vitellogenin level.

(2) Summary

Estrogenic activity of diisobutyl phthalate has been indicated from literature. In this study, an increase in male hepatic vitellogenin level was not observed at sublethal concentrations to suggest estrogenic effect. It was not concluded that diisobutyl phthalate is an estrogenic compound.

Since a significant decrease was observed in female hepatic vitellogenin level in this study, antiestrogenic activity should be tested in Tier 1 *in vitro* Test. (Due to the lack of increased number of joint plates with papillary processes, androgenic activity was not suggested.) The no observed adverse effect level of 836 μg/L was ca. 4,200 times as high as the detection limit of environmental water concentration of 0.2μg/L that was measured (but not detected) in MOE's Environmental Survey and Monitoring of Chemicals in FY1996.

Table 1-A Results

Measured concentration (µg/L)	Number of survived fish male and female	Mortality (%) male and female	Total length (mm)		Body weight (mg)	
			male	female	male	female
Control (<0.001)	24	0	32.4±1.5	32.6±1.1	345±58	395±30
Solvent Control (3)	23	4.2	32.6±1.2	32.5±1.0	367±46	395±42
35	24	0	31.9±1.2	33.6±1.5	335±39	446±48
184	24	0	31.8±1.4	32.6±1.5	329±55	405±52
836	24	0	32.4±1.7	32.6±1.4	348±62	413±61

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 (<0.001)	24.2±8.6	23.4±8.5	96.6±4.1	1.2±0.4	12.7±1.7
Solvent Control (3)	22.5±7.0	21.9±6.9	97.1±3.2	1.1±0.3	13.1±1.8
35	24.3±7.0	23.4±6.6	96.5±4.8	1.2±0.2	13.3±2.3
184	23.9±7.2	23.1±6.9	96.8±3.9	1.0±0.3	13.6±1.9
836	25.2±5.8	24.2±5.9	96.1±5.4	1.2±0.4	12.0±2.0

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 (<0.001)	3.5±3.0	5.8±1.5	0.366±0.381	763±109	101.0±14.4	0±0
Solvent Control (3)	2.7±0.7	5.8±1.1	2.34±2.56	604±114	98.8±14.5	0±0
35	2.7±0.4	6.3±1.6	0.275±0.416	596±144	96.0±10.7	0±0
184	3.1±0.4	6.3±0.9	0.188±0.101	404±85.3*	96.3±10.1	0±0
836	3.4±0.4	5.9±1.3	2.16±3.21	421±73.1*	96.8±17.7	0±0

Table 1-D Results (continued)

Measured concentration (µg/L)	Other observations
Control (<0.001)	Not found
Solvent Control (3)	Not found
35	Not found
184	Not found
836	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