Propyl 4-Hydroxybenzoate (Propyl Paraben) (CAS no. 94-13-3)

Tier 2 *in vivo* Test

The Medaka Extended One-Generation Test (MEOGRT: OECD TG240)

(1) Results

●F0 generation (exposure period: 4 weeks)

No significant differences were observed in survival, male and female total length, male and female body weight, total eggs, fertile eggs, male hepatosomatic index and female secondary sex characteristics (plates with papillary process on the anal fin).

At 9.9 μg/L and higher, a significant increase was observed in male gonadosomatic index and male liver vitellogenin level.

At 32.6 μg/L and higher, a significant increase was observed in female liver vitellogenin level.

At 328 μg/L and higher, a significant decrease was observed in female hepatosomatic index.

At 1,040 μg/L, a significant decrease was observed in fertility and male secondary sex characteristics (plates with papillary process on the anal fin), and a significant increase was observed in female gonadosomatic index.

●F1 generation (exposure period: 16 weeks)

No significant differences were observed in survival (4 and 8 weeks post fertilization, and reproductive adult stage for male), male and female secondary sex characteristics (10 weeks post fertilization), male gonadosomatic index (10 weeks post fertilization), male hepatosomatic index (reproductive adult stage) and female secondary sexual characteristics (reproductive adult stage).

At 9.9 μg/L and higher, a significant decrease was observed in time to hatch (day) and female hepatosomatic index (reproductive adult stage), and a significant increase was observed in female total length (10 weeks post fertilization), female gonadosomatic index (10 weeks post fertilization) and male total length (reproductive adult stage).

At 9.9, 32.6 and 99.4 μg/L, a significant increase was observed in male total length (10 weeks post fertilization), male body weight (10 weeks post fertilization), female total length (reproductive adult stage) and female body weight (reproductive adult stage).

At 9.9, 32.6, 99.4 and 328 μg/L, a significant increase was observed in female body weight (10 weeks post fertilization).

At 9.9, 32.6 and 328 μg/L, a significant increase was observed in female hepatosomatic index (10 weeks post fertilization).

At 9.9, 32.6, 99.4 and 1,040 μg/L, a significant increase was observed in male body weight (reproductive adult stage).

At 32.6 and 99.4 μg/L, a significant decrease was observed in male hepatosomatic index (10 weeks post fertilization).

At 32.6 μg/L and higher, a significant increase was observed in female liver vitellogenin level

(10 weeks post fertilization and reproductive adult stage).

At 328 μg/L and higher, a significant decrease was observed in hatching rate, and a significant increase was observed in male liver vitellogenin level (10 weeks post fertilization) and male gonadosomatic index (reproductive adult stage).

At 1,040 μg/L, a significant decrease was observed in female survival (reproductive adult stage), total eggs, fertile eggs, fertilization rate and male secondary sexual characteristics (reproductive adult stage), and a significant increase was observed in female gonadosomatic index (reproductive adult stage).

●F2 generation (exposure period: 2 weeks)

At 9.9 μg/L and higher, a significant decrease was observed in time to hatch (day).

At 1,040 μg/L, a significant decrease was observed in hatching rate.

(2) Summary

Fish were exposed to 9.9, 32.6, 99.4, 328 and 1,040 μg/L (measured concentrations) for 19 weeks. At levels where no significant decrease in hatching rate of F1 was not observed (99.4 μg/L and lower), a significant increase in male (reproductive adult stage of F0 generation) liver vitellogenin level was observed. Thus, propyl 4-hydroxybenzoate was identified as estrogenic.

At 328 μg/L and higher, a significant decrease was observed in hatching rate of F1 generation, indicating developmental toxicity to Medaka. This lowest observed effect concentration (LOEC) was ca. 20,500 times as high as the highest environmental water concentration of 0.016 μg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 2012.

The exposure level which did not indicate reproductive toxicity (NOEC) to Medaka was 99.4 μg/L, and ca. 6,200 times as high as the highest environmental water concentration of 0.016 μg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 2012.

●F0 generation

Table 1-A Results

Measured mean concentration (μg/L)	Total number		Number alive		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	12	12	12	12	30.9 ± 2.0	32.1 ± 1.3	307 ± 60	430 ± 56
9.9	6	6	6	6	32.7 ± 2.2	33.0± 1.0	349 ± 74	458 ± 35
32.6	6	6	6	6	31.6 ± 1.4	32.9 ± 1.1	323 ± 23	455 ± 26
99.4	6	6	6	6	31.2 ± 1.0	33.2 ± 0.6	307 ± 34	484 ± 29
328	6	6	6	6	31.3 ± 1.8	32.7 ± 1.4	316 ± 71	460 ± 59
1,040	6	6	6	6	30.4 ± 0.9	31.7 ± 1.3	289 ± 42	413 ± 40

Table 1-B Results (continued)

Measured mean concentration (μg/L)	Total egg (eggs/day/pair)	Fertile egg (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	34.3 ± 6.2	32.8 ± 5.9	95.7 ± 2.6	0.9 ± 0.3	11.4 ± 1.5
9.9	36.9 ± 4.2	35.9 ± 4.2	96.9 ± 2.3	1.2 ± 0.3*	12.5 ± 0.6
32.6	35.1 ± 1.9	33.6 ± 2.3	95.2 ± 4.1	1.2 ± 0.3*	10.9 ± 0.8
99.4	33.0 ± 5.3	29.7 ± 6.6	89.5 ± 10.9	1.2 ± 0.3*	13.2 ± 3.7
328	36.6 ± 7.6	32.7 ± 4.8	90.6 ± 9.9	1.6 ± 0.6*	12.8 ± 2.1
1,040	35.0 ± 4.1	28.9 ± 9.9	84.4 ± 20.9*	1.5 ± 0.4*	15.7 ± 4.1*

Table 1-C Results (continued)

Measured mean concentration (μg/L)	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	2.4 ± 0.8	7.9 ± 1.4	ND	458 ±183	74 ± 14	0
9.9	2.2 ± 0.5	7.3 ± 1.4	20.6 ± 48.0*	568 ± 56	69 ± 27	0
32.6	2.6 ± 0.6	6.9 ± 0.8	4.04 ± 3.95*	767 ±307*	76 ± 11	0
99.4	1.9 ± 0.3	7.3 ± 1.5	11.3 ± 17.9*	882 ±602*	72 ± 11	0
328	2.7 ± 1.0	6.4 ± 1.4*	26.2 ± 32.3*	1,170 ±1,280*	77 ± 14	0
1,040	2.8 ± 0.8	6.3 ± 1.3*	12.8 ± 14.6*	708 ± 64*	55 ± 7*	0

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration (μg/L)	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)
Control	99 ± 3	9.0 ± 0.4	NA
9.9	93 ± 8	7.9 ± 0.2*	NA
32.6	98 ± 3	8.0 ± 0.1*	NA
99.4	98 ± 4	7.9 ± 0.1*	NA
328	89 ± 6*	8.2 ± 0.1*	NA
1,040	90 ± 8*	7.8 ± 0.2*	NA

Table 2-B Results (continued)

Measured mean concentration (μg/L)	Survival (%) (Week 4)	Survival (%) (Week 8)	Total length (mm) (Week 10)		Body weight (mg) (Week 10)	
			Male	Female	Male	Female
Control	100 ± 0	100 ± 0	19.5 ± 1.6	20.2 ± 1.3	150 ± 35	183 ± 40
9.9	100 ± 0	97 ± 4	22.6 ± 1.0*	23.6 ± 1.1*	227 ± 28*	305 ± 48*
32.6	100 ± 0	100 ± 0	23.2 ± 1.4*	24.3 ± 1.0*	244 ± 42*	349 ± 47*
99.4	100 ± 0	100 ± 0	21.8 ± 1.3*	23.1 ± 1.2*	199 ± 36*	291 ± 43*
328	99 ± 3	100 ± 0	20.7 ± 1.1	21.8 ± 1.0*	163 ± 19	227 ± 27*
1,040	100 ± 0	100 ± 0	20.7 ± 1.1	21.6 ± 1.2*	164 ± 27	216 ± 36

Table 2-C Results (continued)

Measured mean concentration (μg/L)	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	3.5 ± 0.8	5.2 ± 1.1	ND	1,200 ±898	44 ± 20	0
9.9	3.1 ± 0.8	5.8 ± 0.8*	0.69 ± 0.58	1,520 ±706	51 ± 8	0
32.6	2.6 ± 0.6*	6.2 ± 0.8*	0.91 ± 3.53	1,890 ±711*	59 ± 10	0
99.4	2.5 ± 0.3*	5.6 ± 0.7	0.48 ± 0.19	1,310 ±584*	58 ± 12	0
328	3.1 ± 0.5	6.2 ± 0.7*	56.7 ± 275*	1,740 ±361*	61 ± 17	0
1,040	4.0 ± 1.2	5.4 ± 0.9	58.9 ±190*	1,520 ±690*	17 ± 17	0

Table 2-D Results (continued)

Measured mean concentration (μg/L)	Gonadosomatic index (%)	
	Male	Female
Control	1.3 ± 1.2	6.7 ± 4.0
9.9	1.0 ± 0.3	9.5 ± 2.1*
32.6	0.95 ± 0.3	10.3 ± 1.6*
99.4	1.1 ± 0.3	10.7 ± 1.9*
328	1.5 ± 1.4	11.2 ± 1.4*
1,040	1.3 ± 2.3	10.3 ± 4.6*

●F1 generation (reproductive adult stage)

Table 2-E Results (continued)

Measured mean concentration (μg/L)	Survival (%)		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female
Control	100 (24/24)	100 (24/24)	23.5 ± 1.5	25.3 ± 1.4	236 ± 48	353 ± 53
9.9	100 (12/12)	91 (11/12)	27.1 ± 1.1*	27.1 ± 1.5*	344 ± 28*	454 ± 58*
32.6	100 (12/12)	100 (12/12)	27.5 ± 1.3*	28.3 ± 1.2*	358 ± 33*	494 ± 68*
99.4	100 (11/12)	100 (12/12)	26.1 ± 1.2*	27.3 ± 1.2*	301 ± 37*	428 ± 50*
328	100 (12/12)	100 (12/12)	24.7 ± 1.1*	25.5 ± 0.9	254 ± 43	364 ± 47
1,040	100 (9/9)	77(6/9) *	25.7 ± 1.7*	25.3 ± 1.6	284 ± 62*	405 ± 97

Table 2-F Results (continued)

Measured mean concentration (μg/L)	Total eggs (eggs/day/pair)	Fertile eggs (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	29.1 ± 5.0	26.6 ± 7.2	91.4 ± 19.0	1.5 ± 0.4	14.9 ± 2.2
9.9	35.0 ± 6.1	33.9 ± 5.8	97.0 ± 3.5	1.2 ± 0.3	13.6 ± 1.2
32.6	41.8 ± 5.3	40.5 ± 5.2	96.9 ± 3.8	1.6 ± 0.4	14.1 ± 0.6
99.4	33.2 ± 3.0	32.7 ± 3.8	93.4 ± 5.6	1.8 ± 0.5	16.6 ± 5.7
328	30.0 ± 2.2	28.0 ± 2.2	93.6 ± 4.2	1.9 ± 0.4*	15.2 ± 1.4
1,040	15.6 ± 8.2*	6.9 ± 8.6*	28.6 ±36.9*	3.6 ± 4.6*	24.5 ± 7.6*

Table 2-G Results (continued)

Measured mean concentration (μg/L)	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	2.5 ± 0.5	7.2 ± 0.9	ND	1,040 ±359	61 ± 10	0
9.9	1.9 ± 0.4	6.4 ± 1.1*	0.48 ± 0.54	1,145 ±321	67 ± 8	0
32.6	1.7 ± 0.3	6.7 ± 0.6*	ND	1,309 ±240*	67 ± 10	0
99.4	1.8 ± 0.3	5.7 ± 0.8*	ND*	1,763 ±582*	62 ± 9	0
328	2.2 ± 0.6	6.4 ± 1.2*	ND*	1,572 ± 373*	67 ± 15	0
1,040	3.2 ± 1.0	5.4 ± 1.2*	1.98 ± 1.73*	1,674 ±733*	30 ± 24*	0

●F2 generation (embryo-juvenile stage)

Table 3-A Results

Measured mean concentration (μg/L)	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)	Survival (%) (Day 15)
Control	95 ± 6	9.8 ± 0.3	NA	NA
9.9	98 ± 3	8.6 ± 0.2*	NA	NA
32.6	98 ± 4	8.5 ± 0.3*	NA	NA
99.4	93 ± 5	8.4 ± 0.2*	NA	NA
328	93 ± 5	9.0 ± 0.2*	NA	NA
1,040	77 ± 4*	9.7 ± 0.2*	NA	NA

Data show mean ± SD (standard deviation)
* denotes significant increase/decrease from control (*p<0.05、** p<0.01)
ND: not detected (< 0.4 ng/mg liver).
NA: not available
Secondary sex characteristics: the number of plates with papillary process on the anal fin per fish