17β-Estradiol（CAS no. 50-28-2）

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 body weight, total eggs, fertile eggs, male and female gonadosomatic index, male and female hepatosomatic index, and male and female secondary sex characteristics.

At 25.7 ng/L and higher, a significant increase was observed in liver vitellogenin level, and a significant decrease was observed in fertility.

At 84.4 ng/L, a significant decrease was observed in female body weight.

●F1 generation (exposure period: 16 weeks)

No significant differences were observed in time to hatch (day), survival (21 days and 9 weeks post fertilization and reproductive adult stage), female hepatosomatic index (10 weeks post fertilization) and female secondary sexual characteristics (plates with papillary process on the anal fin) (10 weeks post fertilization and reproductive adult stage).

At 1.07 ng/L and higher, a significant increase was observed in male liver vitellogenin level (10 weeks post fertilization).

At 3.22 ng/L and higher, a significant increase was observed in liver vitellogenin level of females (10 weeks post fertilization) and males (reproductive adult stage).

At 8.21 ng/L and higher, a significant increase was observed in male total length (10 weeks post fertilization and reproductive adult stage), male hepatosomatic index (reproductive adult stage), and a significant decrease was observed in total eggs, fertile eggs, fertility, female body weight (reproductive adult stage) and male secondary sexual characteristics (plates with papillary process on the anal fin) (reproductive adult stage).

At 25.7 ng/L and higher, a significant increase was observed in male body weight (10 weeks post fertilization and reproductive adult stage) and male hepatosomatic index (10 weeks post fertilization), and a significant decrease was observed in female body weight (10 weeks post fertilization), male secondary sexual characteristics (10 weeks post fertilization), female total length (reproductive adult stage) and female hepatosomatic index (reproductive adult stage).

At 84.4 ng/L, a significant increase was observed in the male gonadosomatic index (10 weeks post fertilization), and a significant decrease was observed in hatching rate, post-hatch survival (14 days), female total length(10 weeks post fertilization), female gonadosomatic index (10 weeks post fertilization) and female liver vitellogenin level (reproductive adult stage).

●F2 generation (exposure period: 2 weeks)

No significant differences were observed in the hatching rate and time to hatch (day).

At 25.7ng/L and higher, F2 generation was not produced.

(2) Summary

Fish were exposed to 1.07, 3.22, 8.21, 25.7, and 84.4 ng/L (measured concentrations) for 19 weeks. At levels where no mortality (low hatching rate) was observed (3.22 ng/L and lower), a significant increase in male liver vitellogenin level was observed. Thus, 17β-estradiol was identified as estrogenic.

At 8.21 ng/L and higher, a significant decrease was observed in total eggs, fertile eggs, fertility and female body weight, indicating reproductive and developmental toxicity to Medaka. This lowest observed effect concentration (LOEC) was approximately five times as high as the highest environmental water concentration of 1.7 ng/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 2005.

The exposure level which did not indicate reproductive and developmental toxicity (NOEC) to Medaka was 3.22 μg/L, and approximately twice as high as the highest environmental water concentration of 1.7 ng/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 2005.

●F0 generation

Table 1-A Results

Measured mean concentration (ng/L)	Total number		Number alive		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	12	12	11	12	34.7 ± 1.5	34.4 ± 1.5	469 ± 55	587 ± 66
1.07	6	6	6	5	35.4 ± 1.6	35.1 ± 0.8	478 ± 78	590 ± 46
3.22	6	6	6	6	35.2 ± 1.3	34.5 ± 0.9	465 ± 54	590 ± 50
8.21	6	6	6	6	34.1 ± 0.9	35.4 ± 0.9	437 ± 56	622 ± 25
25.7	6	6	6	6	35.3 ± 1.3	34.8 ± 1.1	498 ± 54	598 ± 51
84.4	6	6	6	6	35.6 ± 1.5	33.5 ± 0.9	493 ± 59	513 ± 30*

Table 1-B Results (continued)

Measured mean concentration (ng/L)	Total eggs (eggs/day/pair)	Fertile eggs (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	35.4 ± 5.3	34.9 ± 5.4	98.5 ± 1.0	1.0 ± 0.3	10.6 ± 1.5
1.07	34.9 ± 5.2	34.5 ± 5.1	98.9 ± 0.4	1.2 ± 0.3	9.6 ± 1.1
3.22	36.1 ± 11.0	34.7 ± 12.8	93.0 ± 14.1	1.1 ± 0.2	11.0 ± 2.6
8.21	40.4 ± 2.0	39.0 ± 2.5	96.9 ± 2.7	1.1 ± 0.3	11.3 ± 1.0
25.7	37.4 ± 2.9	36.6 ± 2.9	97.9 ± 0.8*	1.1 ± 0.2	9.5 ± 0.7
84.4	36.3 ± 3.9	35.6 ± 4.1	97.9 ± 1.3*	0.7 ± 0.2	9.2 ± 1.7

Table 1-C Results (continued)

Measured mean concentration (ng/L)	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	2.2 ± 0.6	5.2 ± 1.2	8.7 ± 20.9	228 ± 75	109 ± 14	0
1.07	2.1 ± 0.8	5.5 ± 0.4	8.0 ± 12.1	237 ± 46	117 ± 9	0
3.22	2.0 ± 0.5	5.3 ± 0.3	38.4 ± 88.0	270 ± 110	112 ± 17	0
8.21	1.8 ± 0.3	5.3 ± 1.3	61.1 ± 69.9	250 ± 67	110 ± 9	0
25.7	2.2 ± 1.0	4.7 ± 1.0	293 ±248**	1,330 ±2,410*	104 ± 11	0
84.4	2.6 ± 0.7	5.8 ± 2.0	3,110 ±995**	4,580 ±6,780**	121 ± 12	0

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration (ng/L)	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%) (Day 14)
Control	98.3 ± 3.3	7.2 ± 0.1	98.3 ± 3.3
1.07	99.2 ± 2.0	7.1 ± 0.1	99.2 ± 2.0
3.22	99.2 ± 2.0	7.3 ± 0.1	99.2 ± 2.0
8.21	98.3 ± 2.6	7.0 ± 0.0	98.3 ± 2.6
25.7	97.5 ± 2.8	7.2 ± 0.2	97.5 ± 2.7
84.4	95.8 ± 3.8*	7.3 ± 0.2	95.8 ± 3.8*

Table 2-B Results (continued)

Measured mean concentration (ng/L)	Survival (%) (Day 21)	Survival (%) (Week 9)	Total length (mm) (Week 10)		Body weight (mg) (Week 10)	
			Male	Female	Male	Female
Control	100 ± 0	100 ± 0	28.3 ± 0.6	29.0 ± 0.4	269 ± 25	347 ± 14
1.07	100 ± 0	98.6 ± 3.4	28.6 ± 0.5	29.5 ± 0.4	271 ± 22	365 ± 12
3.22	100 ± 0	100 ± 0	28.8 ± 0.4	29.6 ± 0.6	265 ± 17	351 ± 24
8.21	100 ± 0	100 ± 0	28.8 ± 1.0**	29.2 ± 0.3	281 ± 24	338 ± 18
25.7	100 ± 0	100 ± 0	29.7 ± 0.4**	28.3 ± 0.3	338 ± 34**	322 ± 17**
84.4	100 ± 0	100 ± 0	28.6 ± 0.7**	27.9 ± 1.5*	305 ± 18**	313 ± 51**

Table 2-C Results (continued)

Measured mean concentration (ng/L)	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	2.2 ± 0.3	5.4 ± 0.5	1.52 ± 0.27	571 ±175	90 ±10	0
1.07	2.2 ± 0.2	5.1 ± 0.6	2.18 ± 0.46*	603 ±195	84 ± 6	0
3.22	2.1 ± 0.3	5.0 ± 0.4	2.58 ± 1.09**	962 ±374*	95 ± 6	0
8.21	2.6 ± 0.3	5.1 ± 0.8	62.2 ± 48.7**	754 ± 62**	85 ± 14	0
25.7	4.7 ± 0.4**	5.3 ± 0.6	1,290 ± 62**	701 ± 208*	0 ± 0**	0
84.4	5.3 ± 0.4**	5.1 ± 0.9	2,740 ±1,068**	2,320 ±1,510**	0 ± 0**	0

Table 2-D Results (continued)

Measured mean concentration (ng/L)	Gonadosomatic index (%)	
	Male	Female
Control	1.1 ± 0.3	8.8 ± 1.7
1.07	1.3 ± 0.3	10.8 ± 1.4
3.22	1.3 ± 0.1	10.3 ± 1.3
8.21	1.3 ± 0.2	11.2 ± 2.2
25.7	0.8 ± 0.2	11.6 ± 2.6
84.4	4.3 ± 1.9**	5.9 ± 2.8**

●F1 generation (reproductive adult stage)

Table 2-E Results (continued)

Measured mean concentration (ng/L)	Survival (%)		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female
Control	92	100	35.8 ± 1.4	36.7 ± 1.0	461± 62	618 ± 67
1.07	100	92	36.3 ± 1.3	370 ± 0.6	466± 62	624 ± 87
3.22	100	100	36.3 ± 1.1	37.4 ± 1.2	443± 48	593 ± 36
8.21	100	100	37.9 ± 1.7**	35.7 ± 1.0	544± 98	539 ± 47**
25.7	100	92	42.3 ± 2.1**	34.2 ± 0.9*	864 ± 90**	524 ± 45**
84.4	91	91	36.0 ± 0.9**	34.6 ± 1.5*	597 ± 79**	548 ± 72**

Table 2-F Results (continued)

Measured mean concentration (ng/L)	Total egg (eggs/day/pair)	Fertile egg (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	44.0 ± 5.9	43.3 ± 5.9	98.3 ± 1.4	NA	NA
1.07	43.7 ± 5.6	42.5 ± 5.6	97.4 ± 2.4	NA	NA
3.22	49.1 ± 3.7	47.7 ± 4.4	97.0 ± 3.0	NA	NA
8.21	37.2 ± 10.4**	35.8 ±10.9**	94.2 ± 8.1*	NA	NA
25.7	0.0 ± 0.0**	0.0 ± 0.0**	0.0 ± 0.0**	NA	NA
84.4	0.6 ± 1.1**	0.0 ± 0.0**	0.0 ± 0.0**	NA	NA

Table 2-G Results (continued)

Measured mean concentration (ng/L)	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	1.8 ± 0.5	4.8 ± 1.0	4.64 ± 7.59	389 ± 96	121 ± 21	0
1.07	1.7 ± 0.3	4.9 ± 0.4	5.82 ± 8.04	398 ± 258	128 ± 20	0
3.22	1.7 ± 0.3	5.1 ± 0.5	47.9 ± 106*	282 ± 73	120 ± 15	0
8.21	2.1 ± 0.4*	5.3 ± 0.7	100 ± 78**	309 ± 60	102 ± 16**	0
25.7	3.4 ± 0.9**	3.4 ± 0.8*	948 ± 490**	390 ± 142	0 ± 0**	0
84.4	3.5 ± 0.6**	3.5 ± 0.3**	401 ± 171**	349 ± 187*	0 ± 0**	0

●F2 generation (embryo-juvenile stage)

Table 3-A Results

Measured mean concentration (ng/L)	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)	Survival (%) (Day 16)
Control	96.7 ± 4.4	7.1 ± 0.1	NA	NA
1.07	98.3 ± 2.6	7.1 ± 0.1	NA	NA
3.22	91.7 ± 9.8	7.1 ± 0.1	NA	NA
8.21	96.7 ± 4.1	7.2 ± 0.2	NA	NA
25.7	NA	NA	NA	NA
84.4	NA	NA	NA	NA

Data show mean ± SD (standard deviation)
* denotes significant increase/decrease from control (*p<0.05、** p<0.01)
ND: not detected (< 1 ng/mg liver).
NA: not available
Secondary sex characteristics: the number of plates with papillary process on the anal fin per fish