

## Bisphenol A (CAS no. 80-05-7)

### **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 female total length, male and female body weight, female gonadosomatic index, male secondary sex characteristics, and intersex/sex reversal.

Male liver vitellogenin level increased dose-dependently, and a significant increase was observed at 28 µg/L and higher.

At 1,000 µg/L and higher, a significant decrease was observed in total egg and fertile egg, and a significant increase was observed in female hepatosomatic index.

At 3,700 µg/L and higher, a significant decrease was observed in survival (13-16 weeks post fertilization), fertility, male total length and male gonadosomatic index, and a significant increase was observed in male hepatosomatic index and female liver vitellogenin level.

##### ●F1 generation (exposure period: 16 weeks)

No significant differences were observed in hatching rate, survival (2 weeks post fertilization), male total length (9 weeks and 12-15 weeks post fertilization), male body weight (9 weeks post fertilization), female body weight (12-15 weeks post fertilization), and female hepatosomatic index (9 weeks and 12-15 weeks post fertilization).

At 28, 93 and 1,000 µg/L, a significant increase was observed in male total length.

Male liver vitellogenin level (12-15 weeks post fertilization) increased dose-dependently, and a significant increase was observed at 28 µg/L and higher.

Female liver vitellogenin level (9 weeks post fertilization) increased dose-dependently, and a significant increase was observed at 93 µg/L and higher.

At 330 µg/L and higher, a significant increase was observed in time to hatch.

Male liver vitellogenin level (9 weeks post fertilization) increased dose-dependently, and a significant increase was observed at 330 µg/L and higher.

At 1,000 µg/L and higher, a significant decrease was observed in total egg and fertile egg, and a significant increase was observed in male gonadosomatic index (9 weeks post fertilization), male body weight (12-15 weeks post fertilization), and female gonadosomatic index (12-15 weeks post fertilization).

At 3,700 µg/L, a significant decrease was observed in survival (4 weeks, 9 weeks and 12-15 weeks post fertilization), fertility, female total length (9 weeks and 12-15 weeks post fertilization), female body weight (9 weeks post fertilization), female gonadosomatic index (9 weeks post fertilization) and male secondary sex characteristics (9 weeks and 12-15 weeks post fertilization), and a significant increase was observed in male hepatosomatic index (9 weeks and 12-15 weeks post fertilization), male gonadosomatic index (12-15 weeks post fertilization), female liver

vitellogenin level (12-15 weeks post fertilization) and intersex/sex reversal (9 weeks and 12-15 weeks post fertilization).

●F2 generation (exposure period: 2 weeks)

No significant differences were observed in hatching rate, time to hatch, post hatch survival, and survival (2 weeks post fertilization).

(2) Summary

Fish were exposed to 28, 93, 330, 1,000, 3,700 µg/L (measured mean concentrations) for 19 weeks. At levels where mortality was not dose-dependent (1,000 µg/L and lower), a significant increase in male liver vitellogenin level was observed. Thus, bisphenol A was identified as estrogenic.

At 1,000 µg/L and higher, a significant decrease was observed in total egg and fertile egg, indicating reproductive toxicity to Medaka. This lowest observed effect concentration (LOEC) was ca. 3,570 times as high as the highest environmental water concentration of 0.28 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2014.

The exposure level which did not indicate reproductive toxicity to Medaka was 330 µg/L and ca. 1,180 times as high as the highest environmental water concentration of 0.28 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2014.

●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	10	11	36.0 ± 1.8	35.0 ± 1.2	413 ± 70	444 ± 39
28	6	6	6	6	35.7 ± 1.4	34.9 ± 1.3	413 ± 68	460 ± 37
93	6	6	6	6	34.6 ± 1.0	35.1 ± 0.9	373 ± 32	445 ± 39
330	6	6	6	6	35.4 ± 1.3	35.5 ± 2.0	390 ± 46	461 ± 87
1,000	6	6	6	6	36.2 ± 1.7	34.4 ± 2.0	430 ± 53	449 ± 74
3,700	6	6	6	6	33.9 ± 1.5 *	35.3 ± 0.9	373 ± 70	470 ± 47

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	28.8 ± 7.7	27.8 ± 8.2	95.8 ± 6.2	1.1 ± 0.3	8.7 ± 1.8
28	33.8 ± 3.0	33.3 ± 2.8	98.4 ± 2.0	0.9 ± 0.5	9.2 ± 1.0
93	29.5 ± 7.6	28.5 ± 8.4	95.8 ± 6.3	1.2 ± 0.2	9.9 ± 1.0
330	26.2 ± 9.2	25.4 ± 9.5	95.9 ± 3.0	0.9 ± 0.3	10.1 ± 1.0
1,000	20.9 ± 9.9 *	20.0 ± 9.9 *	96.2 ± 6.1	1.2 ± 0.6	10.0 ± 1.8
3,700	20.1 ± 9.2 *	18.4 ± 8.9 *	89.5 ± 6.6 *	0.6 ± 0.2 *	7.3 ± 1.3

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	1.8 ± 0.8	4.6 ± 1.0	8.17 ± 8.74	527 ± 239	106 ± 16	NA
28	2.2 ± 1.8	4.6 ± 0.9	99.2 ± 94.9 *	550 ± 286	117 ± 14	NA
93	1.8 ± 0.7	5.1 ± 0.6	2,660 ± 3,760 *	548 ± 122	102 ± 17	NA
330	1.8 ± 0.5	4.9 ± 0.7	9,990 ± 5,850 *	612 ± 501	102 ± 8	NA
1,000	2.1 ± 0.5	5.7 ± 0.9 *	12,800 ± 5,420 *	703 ± 319	121 ± 12	NA
3,700	3.9 ± 1.3 *	5.7 ± 1.2 *	4,950 ± 3,240 *	1,910 ± 1,050 *	106 ± 12	NA

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration (µg/L)	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)	
			(Day 12)	
Control	99 ± 2	6.4 ± 0.2	100 ± 0	
28	95 ± NA	7.1 ± NA	100 ± NA	
93	98 ± 4	6.7 ± 0.4	100 ± 0	
330	100 ± 0	7.2 ± 0.3 *	100 ± 0	
1,000	89 ± 4	7.2 ± 0.1 *	100 ± 0	
3,700	98 ± 3	7.6 ± 0.3 *	100 ± 0	

Table 2-B Results (continued)

Measured mean concentration (µg/L)	Survival (%) (Week 4)	Survival (%) (Week 9)	Total length (mm) (Week 9)		Body weight (mg) (Week 9)	
			Male	Female	Male	Female
Control	100 ± 0	97 ± 8	27.7 ± 0.6	28.3 ± 1.1	218 ± 17	267 ± 43
28	100 ± 0	100 ± 0	27.5 ± 0.5	28.1 ± 0.7	204 ± 16	254 ± 20
93	100 ± 0	99 ± 3	27.5 ± 0.2	28.3 ± 0.8	207 ± 13	262 ± 23
330	100 ± 0	100 ± 0	27.3 ± 0.8	27.9 ± 0.5	188 ± 19	241 ± 22
1,000	100 ± 0	100 ± 0	27.9 ± 1.0	28.0 ± 1.7	207 ± 21	233 ± 45
3,700	97 ± 4 *	97 ± 4	27.0 ± 1.1	26.6 ± 1.0 *	213 ± 29	208 ± 20 *

Table 2-C Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	2.1 $\pm$ 0.4	4.4 $\pm$ 0.7	4.37 $\pm$ 9.71	309 $\pm$ 363	70 $\pm$ 22	NA
28	2.1 $\pm$ 0.2	4.7 $\pm$ 0.4	0.83 $\pm$ 0.19	284 $\pm$ 307	98 $\pm$ 6	NA
93	2.4 $\pm$ 0.3	4.7 $\pm$ 0.5	7.84 $\pm$ 12.5	755 $\pm$ 191 *	91 $\pm$ 8	NA
330	2.1 $\pm$ 0.7	4.9 $\pm$ 0.4	17.9 $\pm$ 18.8 *	841 $\pm$ 161 *	85 $\pm$ 5	NA
1,000	2.3 $\pm$ 0.4	4.2 $\pm$ 0.4	332 $\pm$ 284 *	1,090 $\pm$ 490 *	85 $\pm$ 7	NA
3,700	4.1 $\pm$ 0.5 *	4.3 $\pm$ 0.5	3,020 $\pm$ 640 *	3,400 $\pm$ 250 *	0 $\pm$ 0 *	NA

Table 2-D Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Gonadosomatic index (%)	
	Male	Female
Control	0.8 $\pm$ 0.2	9.7 $\pm$ 1.3
28	0.9 $\pm$ 0.1	9.2 $\pm$ 0.4
93	1.0 $\pm$ 0.2	10 $\pm$ 3
330	0.9 $\pm$ 0.1	10 $\pm$ 1
1,000	1.0 $\pm$ 0.2 *	6.5 $\pm$ 2.2
3,700	2.9 $\pm$ 2.0 *	4.3 $\pm$ 0.9 *

●F1 generation (reproductive adult stage)

Table 2-E Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Survival (%)		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female
Control	100	100	31.6 $\pm$ 1.3	32.3 $\pm$ 1.2	331 $\pm$ 42	425 $\pm$ 46
28	100	100	33.1 $\pm$ 1.5 *	32.5 $\pm$ 1.6	348 $\pm$ 42	406 $\pm$ 44
93	100	100	32.7 $\pm$ 1.5 *	31.9 $\pm$ 1.0	364 $\pm$ 47	421 $\pm$ 35
330	100	100	32.4 $\pm$ 1.2	32.2 $\pm$ 1.0	345 $\pm$ 34	417 $\pm$ 40
1,000	100	100	33.8 $\pm$ 1.3 *	31.6 $\pm$ 1.1	401 $\pm$ 45 *	440 $\pm$ 58
3,700	90 (9/10)	90 (9/10)	31.9 $\pm$ 1.3	31.4 $\pm$ 1.4 *	436 $\pm$ 41 *	421 $\pm$ 46

Table 2-F Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Total egg (eggs/day/pair)	Fertile egg (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	32.4 $\pm$ 3.6	31.6 $\pm$ 3.7	97.5 $\pm$ 2.7	0.9 $\pm$ 0.2	9.4 $\pm$ 1.3
28	34.3 $\pm$ 2.7	33.0 $\pm$ 3.4	95.9 $\pm$ 5.5	0.9 $\pm$ 0.2	9.6 $\pm$ 1.5
93	32.8 $\pm$ 3.1	32.3 $\pm$ 2.8	98.4 $\pm$ 1.1	1.0 $\pm$ 0.4	10 $\pm$ 1
330	33.2 $\pm$ 3.8	32.7 $\pm$ 3.8	98.3 $\pm$ 0.7	1.0 $\pm$ 0.3	9.2 $\pm$ 1.5
1,000	27.2 $\pm$ 5.8 *	23.5 $\pm$ 11.1 *	81.2 $\pm$ 36.7	0.9 $\pm$ 0.3	14 $\pm$ 8 *
3,700	0.5 $\pm$ 0.8 *	0 $\pm$ 0 *	0.0 $\pm$ 0.0 *	9.0 $\pm$ 5.8 *	9.7 $\pm$ 5.8 *

Table 2-G Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Hepatosomatic index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	Male	Female	Male	Female	Male	Female
Control	1.8 $\pm$ 0.3	4.7 $\pm$ 0.7	5.88 $\pm$ 5.98	732 $\pm$ 399	101 $\pm$ 14	NA
28	2.0 $\pm$ 1.3	5.1 $\pm$ 1.3	187 $\pm$ 250 *	558 $\pm$ 232	107 $\pm$ 18	NA
93	1.9 $\pm$ 0.4	5.4 $\pm$ 0.8	155 $\pm$ 200 *	526 $\pm$ 90	96 $\pm$ 13	NA
330	2.0 $\pm$ 0.5	5.3 $\pm$ 0.3	1,000 $\pm$ 474 *	568 $\pm$ 97	101 $\pm$ 14	NA
1,000	1.8 $\pm$ 0.6	4.3 $\pm$ 1.3	1,100 $\pm$ 260 *	624 $\pm$ 127	97 $\pm$ 15	NA
3,700	3.6 $\pm$ 1.3 *	4.1 $\pm$ 0.6	5,140 $\pm$ 3,470 *	4,680 $\pm$ 1,410 *	0 $\pm$ 0 *	NA

●F2 generation (embryo-juvenile stage)

Table 3-A Results

Measured mean concentration ( $\mu\text{g/L}$ )	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)	Survival (%) (Day 15)
Control	94 $\pm$ 5	8.1 $\pm$ 0.2	100 $\pm$ 0	94 $\pm$ 5
28	93 $\pm$ 5	8.5 $\pm$ 0.4	100 $\pm$ 0	95 $\pm$ 3
93	96 $\pm$ 5	8.0 $\pm$ 0.0	100 $\pm$ 0	97 $\pm$ 4
330	93 $\pm$ 7	8.6 $\pm$ 0.5 *	100 $\pm$ 0	98 $\pm$ 3
1,000	92 $\pm$ 4	8.2 $\pm$ 0.2	100 $\pm$ 0	95 $\pm$ 5
3,700	NA	NA	NA	NA

Data show mean  $\pm$  SD (standard deviation)

\* denotes significant increase/decrease from control ( $p < 0.05$ )

ND: not detected ( $< 1 \text{ ng/mg}$  liver).

NA: not available

Secondary sex characteristics: the number of plates with papillary process on the anal fin per fish