

## Triphenyl phosphate (CAS no. 115-86-6)

### 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 total length, male body weight, fertility, male gonadosomatic index, male and female hepatosomatic index, male secondary sex characteristics, and male and female liver vitellogenin level.

At 0.501 µg/L and higher, a significant decrease was observed in female body weight.

At 15.2 µg/L and higher, a significant decrease was observed in female total length and female gonadosomatic index.

At 48.4 µg/L, a significant decrease was observed in total egg and fertile egg.

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

No significant differences were observed in survival (adult female 8 weeks post fertilization), male total length (adult), male body weight (10 weeks post fertilization), male and female body weight (adult), fertility, male liver vitellogenin level (10 weeks post fertilization), male gonadosomatic index (10 weeks post fertilization), male hepatosomatic index (adult), and male secondary sex characteristics (adult).

At 0.501, 1.62, 4.54 and 15.2 µg/L, a significant decrease was observed in time to hatch (day).

At 0.501, 1.62 and 4.54 µg/L, a significant increase was observed in female total length (adult), and a significant decrease was observed in female hepatosomatic index (10 weeks post fertilization).

At 0.501 µg/L and higher, a significant decrease was observed in male hepatosomatic index (10 weeks post fertilization) and male and female liver vitellogenin level (adult).

At 4.54 µg/L and higher, a significant increase was observed in female gonadosomatic index (adult), and a significant decrease in hatching rate.

4.54µg/L 以上のばく露群において、雌の生殖腺体指数(成熟個体)の統計学的に有意な高値が認められ、ふ化率の統計学的に有意な低値が認められた。

At 15.2 µg/L and higher, a significant increase was observed in female gonadosomatic index (10 weeks post fertilization), and a significant decrease in survival (adult).

At 48.4 µg/L, a significant increase was observed in male gonadosomatic index (adult), a significant decrease was observed in survival (4 weeks post fertilization), total egg, and fertile egg.

At 10 weeks post fertilization, a significant increase was observed in male total length and male secondary sex characteristics (at 0.501 µg/L and higher), female total length (at 0.501, 1.62 and 4.54 µg/L), female body weight (at 0.501 and 4.54 µg/L), and female liver vitellogenin level (at 4.54 µg/L and higher). These significant differences were regarded as a result of growth retardation

observed in control group.

At 48.4 µg/L, a significant increase was observed in male gonadosomatic index (adult), a significant decrease was observed in survival (4 weeks post fertilization), total egg, and fertile egg.

●F2 generation (exposure period: 2 weeks)

No significant differences were observed in hatching rate.

At 0.501, 1.62, 4.54 and 48.4 µg/L, a significant decrease was observed in time to hatch (day).

(2) Summary

Fish were exposed to 0.501, 1.62, 4.54, 15.2 and 48.4 µg/L (measured mean concentrations) for 19 weeks. At levels where no significant decrease in hatching rate of F1 was not observed (1.62 µg/L and lower), a significant decrease in female (F1 adult) liver vitellogenin level was observed. Thus, triphenyl phosphate was suggested to be anti-estrogenic and anti-steroidogenic.

At 48.4 µg/L, a significant decrease was observed in total egg, fertile egg, and survival (4 weeks post fertilization, adult male), indicating reproductive toxicity to Medaka. The lowest observed effect concentration (LOEC) was 44.9 µg/L as a data from Tier 1 *in vivo* test, which was ca. 1,870 times as high as the highest environmental water concentration of 0.024 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2017.

The exposure level which did not indicate reproductive toxicity to Medaka was 15.2 µg/L and ca. 630 times as high as the highest environmental water concentration of 0.024 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2017.

●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	11	32.7 ± 1.5	34.2 ± 1.0	389 ± 56	514 ± 55
0.501	6	6	6	6	34.0 ± 0.6	33.3 ± 0.5	411 ± 33	468 ± 17 *
1.62	6	6	6	6	33.2 ± 1.3	32.5 ± 1.1	380 ± 43	447 ± 38 *
4.54	6	6	6	6	33.0 ± 1.4	34.3 ± 0.7	379 ± 48	451 ± 44 *
15.2	6	6	6	6	33.1 ± 1.7	32.9 ± 1.2 *	395 ± 49	396 ± 24 *
48.4	6	6	6	6	32.3 ± 0.9	33.4 ± 0.7 *	355 ± 27	434 ± 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	26.7 ± 6.2	26.1 ± 6.1	97.8 ± 1.0	1.1 ± 0.4	10.4 ± 1.3
0.501	25.2 ± 3.3	24.6 ± 3.4	97.6 ± 1.3	1.3 ± 0.3	10.8 ± 1.2
1.62	25.8 ± 3.8	24.9 ± 4.3	96.2 ± 3.6	1.3 ± 0.5	11.0 ± 3.1
4.54	26.3 ± 3.3	25.7 ± 3.3	97.7 ± 1.0	1.0 ± 0.5	9.6 ± 2.3
15.2	23.8 ± 4.3	22.6 ± 4.2	95.0 ± 5.7	1.1 ± 0.3	8.4 ± 0.7 *
48.4	22.7 ± 2.6 *	22.2 ± 2.6 *	97.4 ± 1.2	1.0 ± 0.3	7.9 ± 1.4 *

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	2.5 ± 0.4	5.0 ± 1.6	0.66 ± 0.52	928 ± 203	94 ± 15	0
0.501	2.0 ± 0.5	5.2 ± 0.5	3.79 ± 8.29	930 ± 155	106 ± 20	0
1.62	2.2 ± 0.9	5.7 ± 0.5	4.28 ± 8.42	925 ± 488	94 ± 15	0
4.54	2.5 ± 1.2	5.0 ± 1.0	1.39 ± 0.96	848 ± 84	106 ± 8	0
15.2	2.5 ± 0.5	5.0 ± 1.6	1.20 ± 1.31	900 ± 370	101 ± 12	0
48.4	2.4 ± 0.8	4.9 ± 0.5	0.67 ± 0.31	1,160 ± 505	100 ± 21	0

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration (µg/L)	Hatching rate (%)	Time to hatch (day)
Control	96 ± 5	8.7 ± 0.4
0.501	96 ± 4	7.6 ± 0.1 *
1.62	96 ± 2	7.7 ± 0.1 *
4.54	77 ± 13 *	7.4 ± 0.2 *
15.2	49 ± 20 *	7.9 ± 0.4 *
48.4	73 ± 20 *	8.3 ± 0.2

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	99 ± 2	21.7 ± 0.8	22.1 ± 0.8	118 ± 11	125 ± 14
0.501	100 ± 0	100 ± 0	23.1 ± 0.2 *	23.9 ± 0.5 *	128 ± 5	149 ± 9 *
1.62	100 ± 0	99 ± 3	23.8 ± 1.1 *	23.6 ± 1.0 *	135 ± 15	140 ± 23
4.54	100 ± 0	100 ± 0	24.1 ± 0.7 *	24.5 ± 0.8 *	134 ± 8	146 ± 12 *
15.2	100 ± 0	100 ± 0	23.9 ± 0.9 *	22.7 ± 1.5	133 ± 14	128 ± 19
48.4	97 ± 4 *	95 ± 7	22.7 ± 0.9 *	22.6 ± 0.8	111 ± 10	119 ± 5

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	3.2 ± 0.5	4.2 ± 0.9	24.5 ± 60.8	214 ± 188	23 ± 14	0
0.501	1.8 ± 0.3 *	3.1 ± 0.4 *	18.9 ± 41.8	431 ± 262	52 ± 11 *	0
1.62	2.4 ± 0.1 *	3.1 ± 0.4 *	ND	523 ± 575	43 ± 11 *	0
4.54	2.0 ± 0.2 *	3.1 ± 0.2 *	ND	741 ± 333 *	56 ± 10 *	0
15.2	2.3 ± 0.2 *	4.0 ± 1.0	ND	609 ± 189 *	58 ± 3 *	0
48.4	2.6 ± 0.7 *	3.9 ± 0.5	0.35 ± 0.27	755 ± 280 *	50 ± 12 *	0

Table 2-D Results (continued)

Measured mean concentration (µg/L)	Gonadosomatic index (%)	
	Male	Female
Control	1.0 ± 0.8	2.6 ± 1.4
0.501	0.8 ± 0.3	3.8 ± 1.6
1.62	0.7 ± 0.2	3.0 ± 2.5
4.54	1.0 ± 0.4	3.5 ± 0.9
15.2	0.9 ± 0.2	5.2 ± 0.7 *
48.4	0.7 ± 0.3	4.4 ± 2.2 *

●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	100	27.8 ± 1.8	29.0 ± 1.5	226 ± 51	312 ± 47
0.501	100	100	30.7 ± 0.9	30.6 ± 0.5 *	296 ± 37	348 ± 19
1.62	100	91 (10/11)	30.3 ± 1.0	30.5 ± 0.9 *	267 ± 23	361 ± 26
4.54	92 (11/12)	100	30.3 ± 0.9	30.7 ± 1.3 *	273 ± 26	339 ± 39
15.2	83 (10/12) *	92 (11/12)	27.9 ± 1.4	29.5 ± 0.9	215 ± 37	318 ± 30
48.4	92 (11/12) *	92 (11/12)	27.8 ± 1.2	28.6 ± 0.8	218 ± 33	293 ± 32

Table 2-F Results (continued)

Measured mean concentration (µg/L)	Total egg (eggs/day/pair)	Fertile egg (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	20.4 ± 1.9	19.9 ± 1.9	97.3 ± 1.8	1.3 ± 0.4	11.6 ± 1.8
0.501	21.0 ± 1.4	20.8 ± 1.4	98.8 ± 1.2	1.5 ± 0.4	12.0 ± 1.7
1.62	20.1 ± 3.6	19.8 ± 3.6	98.9 ± 0.6	1.7 ± 0.4	12.4 ± 1.2
4.54	21.13 ± 1.8	20.8 ± 1.6	98.6 ± 1.5	1.7 ± 0.7	13.3 ± 1.0 *
15.2	18.8 ± 1.6	18.5 ± 1.6	98.3 ± 1.2	1.4 ± 0.2	14.2 ± 2.4 *
48.4	17.4 ± 4.2 *	16.5 ± 5.5 *	89.4 ± 28.2	1.7 ± 0.5 *	13.5 ± 1.0 *

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	2.3 ± 0.5	6.2 ± 0.8	21.5 ± 65.3	1,000 ± 538	69 ± 12	0
0.501	2.0 ± 0.5	6.2 ± 1.0	ND *	553 ± 269 *	72 ± 11	0
1.62	1.8 ± 0.3	5.8 ± 1.1	ND *	654 ± 341 *	69 ± 13	0
4.54	3.0 ± 1.1	6.4 ± 0.9	0.28 ± 0.24 *	344 ± 306 *	71 ± 9	0
15.2	2.0 ± 0.2	6.3 ± 0.9	ND *	433 ± 93 *	74 ± 16	0
48.4	2.3 ± 0.5	6.5 ± 0.9	0.25 ± 0.17 *	357 ± 197 *	67 ± 15	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	96 ± 7	8.9 ± 0.3		
0.501	97 ± 3	8.3 ± 0.2 *		
1.62	99 ± 2	8.2 ± 0.1 *		
4.54	93 ± 8	8.3 ± 0.4 *		
15.2	95 ± 6	8.9 ± 0.3		
48.4	98 ± 4	8.0 ± 0.2 *		

Data show mean ± SD (standard deviation)

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

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