

## 4-*tert*-Octylphenol (CAS no. 140-66-9)

### 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 number alive, male and female total length, male and female body weight, total egg, fertile egg, fertility, female hepatosomatic index, and male secondary sex characteristics.

At 3.21, 9.91 and 99.2 µg/L, a significant decrease was observed in male hepatosomatic index.

At 9.91 µg/L and higher, a significant increase was observed in male gonadosomatic index.

At 31.1 µg/L and higher, a significant increase was observed in female gonadosomatic index.

Male and female liver vitellogenin level increased dose-dependently, and a significant increase was observed at 31.1 ng/L and higher.

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

No significant differences were observed in hatching rate, survival (4 weeks post fertilization, reproductive adult stage), male total length (10 weeks post fertilization), male body weight (10 weeks post fertilization), male hepatosomatic index (10 weeks post fertilization), male gonadosomatic index (10 weeks post fertilization), male liver vitellogenin level (10 weeks post fertilization), and female gonadosomatic index (reproductive adult stage).

At 0.926, 3.21 and 9.91 µg/L, a significant decrease was observed in time to hatch.

At 0.926, 3.21, 9.91 and 99.2 µg/L, a significant increase was observed in female liver vitellogenin level (10 weeks post fertilization).

At 0.926 µg/L and higher, a significant increase was observed in female liver vitellogenin level (reproductive adult stage), and a significant decrease was observed in female hepatosomatic index (10 weeks post fertilization, reproductive adult stage).

At 9.91 µg/L and higher, a significant decrease was observed in total egg, fertile egg, and male secondary sex characteristics (10 weeks post fertilization, reproductive adult stage).

At 31.1 µg/L and higher, a significant decrease was observed in survival (9 weeks post fertilization), male secondary sex characteristics (10 weeks post fertilization), female gonadosomatic index (10 weeks post fertilization), female total length (reproductive adult stage), and female body weight (reproductive adult stage).

At 99.2 µg/L, a significant increase was observed in male total length (reproductive adult stage), male body weight (reproductive adult stage), male hepatosomatic index (reproductive adult stage) and male liver vitellogenin level (reproductive adult stage), and a significant decrease was observed in female total length (10 weeks post fertilization), female body weight (10 weeks post fertilization), fertility and male gonadosomatic index (reproductive adult stage).

●F2 generation (exposure period: 2 weeks)

At 0.926 µg/L and higher, a significant decrease was observed in time to hatch.

At 99.2 µg/L, a significant decrease was observed in hatching rate.

(2) Summary

Fish were exposed to 0.926, 3.21, 9.91, 31.1 and 99.2 µg/L (measured mean concentrations) for 19 weeks. At levels where mortality was not dose-dependent (99.2 µg/L and lower), a significant increase in male liver vitellogenin level was observed. Thus, 4-*tert*-octylphenol was identified as estrogenic.

At 9.91 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. 320 times as high as the highest environmental water concentration of 0.031 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2012.

The exposure level which did not indicate reproductive toxicity to Medaka was 3.21 µg/L and ca. 100 times as high as the highest environmental water concentration of 0.031 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY2012.

●F0 generation

Table 1-A Results

Measured mean concentration ( $\mu\text{g/L}$ )	Total number		Number alive		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	12	12	12	12	32.4 $\pm$ 1.1	33.3 $\pm$ 1.0	344 $\pm$ 41	467 $\pm$ 46
0.926	6	6	6	6	32.8 $\pm$ 0.9	33.9 $\pm$ 1.3	370 $\pm$ 29	454 $\pm$ 35
3.21	6	6	6	5	32.3 $\pm$ 0.9	33.8 $\pm$ 1.4	332 $\pm$ 21	472 $\pm$ 46
9.91	6	6	6	6	32.6 $\pm$ 1.6	34.0 $\pm$ 0.4	343 $\pm$ 55	475 $\pm$ 28
31.1	6	6	6	6	32.5 $\pm$ 1.4	32.4 $\pm$ 1.2	348 $\pm$ 45	419 $\pm$ 50
99.2	6	6	6	6	32.9 $\pm$ 1.0	33.7 $\pm$ 0.8	359 $\pm$ 30	452 $\pm$ 37

Table 1-B 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	36.8 $\pm$ 3.8	35.9 $\pm$ 4.0	97.6 $\pm$ 1.9	1.2 $\pm$ 0.2	10.3 $\pm$ 1.4
0.926	38.9 $\pm$ 6.6	37.1 $\pm$ 5.8	95.7 $\pm$ 2.5	1.3 $\pm$ 0.2	11.2 $\pm$ 0.7
3.21	38.9 $\pm$ 5.5	37.6 $\pm$ 5.4	96.4 $\pm$ 2.2	1.4 $\pm$ 0.2	10.5 $\pm$ 1.2
9.91	36.4 $\pm$ 2.5	35.7 $\pm$ 2.3	98.0 $\pm$ 1.2	1.5 $\pm$ 0.3*	10.9 $\pm$ 0.8
31.1	35.8 $\pm$ 5.8	35.1 $\pm$ 5.6	98.0 $\pm$ 0.9	1.6 $\pm$ 0.2*	12.3 $\pm$ 1.9*
99.2	36.5 $\pm$ 4.1	35.6 $\pm$ 4.2	97.4 $\pm$ 1.4	1.4 $\pm$ 0.3*	11.8 $\pm$ 1.7*

Table 1-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	2.8 $\pm$ 0.5	6.2 $\pm$ 0.6	3.90 $\pm$ 11.21	509 $\pm$ 89	100 $\pm$ 11	0
0.926	2.5 $\pm$ 0.2	5.7 $\pm$ 0.5	9.85 $\pm$ 13.93	422 $\pm$ 28	82 $\pm$ 13	0
3.21	2.2 $\pm$ 0.1*	6.0 $\pm$ 0.8	4.29 $\pm$ 4.27	478 $\pm$ 127	92 $\pm$ 15	0
9.91	2.1 $\pm$ 0.3*	6.2 $\pm$ 0.9	4.35 $\pm$ 8.89	470 $\pm$ 236	104 $\pm$ 16	0
31.1	2.6 $\pm$ 0.3	6.1 $\pm$ 0.7	6.44 $\pm$ 5.46*	847 $\pm$ 592*	96 $\pm$ 12	0
99.2	2.4 $\pm$ 0.5*	6.0 $\pm$ 1.1	38.3 $\pm$ 3.9*	728 $\pm$ 157*	95 $\pm$ 15	0

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration ( $\mu\text{g/L}$ )	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)
Control	98 $\pm$ 5	8.7 $\pm$ 0.2	NA
0.926	98 $\pm$ 4	8.0 $\pm$ 0.1*	NA
3.21	99 $\pm$ 2	7.9 $\pm$ 0.1*	NA
9.91	98 $\pm$ 3	8.0 $\pm$ 0.2*	NA
31.1	97 $\pm$ 3	8.3 $\pm$ 0.1	NA
99.2	97 $\pm$ 5	8.7 $\pm$ 0.4	NA

Table 2-B Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Survival (%) (Week 4)	Survival (%) (Week 9)	Total length (mm) (Week 10)		Body weight (mg) (Week 10)	
			Male	Female	Male	Female
Control	95 $\pm$ 8	95 $\pm$ 8	25.8 $\pm$ 1.1	27.0 $\pm$ 1.1	174 $\pm$ 25	229 $\pm$ 32
0.926	100 $\pm$ 0	100 $\pm$ 0	27.2 $\pm$ 1.0	28.0 $\pm$ 1.1	203 $\pm$ 21	252 $\pm$ 31
3.21	93 $\pm$ 10	93 $\pm$ 10	26.6 $\pm$ 1.4	27.8 $\pm$ 1.3	192 $\pm$ 28	249 $\pm$ 37
9.91	100 $\pm$ 0	96 $\pm$ 4	27.0 $\pm$ 1.1	27.2 $\pm$ 1.1	197 $\pm$ 21	225 $\pm$ 22
31.1	92 $\pm$ 5	92 $\pm$ 5*	26.2 $\pm$ 1.2	26.4 $\pm$ 1.4	187 $\pm$ 25	212 $\pm$ 34
99.2	93 $\pm$ 6	93 $\pm$ 6*	25.5 $\pm$ 1.0	25.0 $\pm$ 0.9*	182 $\pm$ 24	179 $\pm$ 24*

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	2.9 $\pm$ 0.5	6.0 $\pm$ 1.0	0.56 $\pm$ 0.25	789 $\pm$ 264	66 $\pm$ 12	0
0.926	2.1 $\pm$ 0.3	4.7 $\pm$ 0.7*	0.02 $\pm$ 0.11	1,630 $\pm$ 376*	67 $\pm$ 20	0
3.21	2.3 $\pm$ 0.5	5.2 $\pm$ 0.8*	11.7 $\pm$ 28.6	1,030 $\pm$ 477*	54 $\pm$ 18	0
9.91	2.0 $\pm$ 0.3	4.9 $\pm$ 0.6*	3.80 $\pm$ 4.21	1,180 $\pm$ 362*	67 $\pm$ 12	0
31.1	2.5 $\pm$ 0.5	5.1 $\pm$ 0.8*	0.37 $\pm$ 0.24	1,050 $\pm$ 456	54 $\pm$ 15*	0
99.2	3.2 $\pm$ 0.5	4.9 $\pm$ 1.1*	116 $\pm$ 165	1,640 $\pm$ 1,050*	15 $\pm$ 15*	0

Table 2-D Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Gonadosomatic index (%)	
	Male	Female
Control	1.5 $\pm$ 0.5	8.2 $\pm$ 2.3
0.926	1.5 $\pm$ 0.4	8.9 $\pm$ 2.9
3.21	1.2 $\pm$ 0.4	8.3 $\pm$ 3.1
9.91	1.3 $\pm$ 0.3	7.5 $\pm$ 0.9
31.1	1.5 $\pm$ 0.5	6.7 $\pm$ 2.6*
99.2	1.0 $\pm$ 0.7	4.5 $\pm$ 3.1*

●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	31.3 $\pm$ 1.4	31.0 $\pm$ 1.1	305 $\pm$ 42	367 $\pm$ 34
0.926	100	100	33.6 $\pm$ 0.7	32.1 $\pm$ 0.8	361 $\pm$ 28	384 $\pm$ 28
3.21	100	100	31.7 $\pm$ 1.1	31.8 $\pm$ 1.4	301 $\pm$ 27	383 $\pm$ 44
9.91	100	100	32.3 $\pm$ 1.1	30.5 $\pm$ 0.4	317 $\pm$ 31	338 $\pm$ 16
31.1	100	100	31.7 $\pm$ 1.3	30.0 $\pm$ 0.5*	303 $\pm$ 35	327 $\pm$ 29*
99.2	100	100	33.7 $\pm$ 1.5*	30.1 $\pm$ 0.5*	400 $\pm$ 70*	315 $\pm$ 31*

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	27.9 $\pm$ 2.9	27.1 $\pm$ 2.9	97.3 $\pm$ 1.8	1.4 $\pm$ 0.4	11.4 $\pm$ 1.3
0.926	27.2 $\pm$ 3.3	26.5 $\pm$ 3.2	97.5 $\pm$ 1.4	1.4 $\pm$ 0.2	11.2 $\pm$ 1.0
3.21	28.0 $\pm$ 3.1	26.8 $\pm$ 3.1	95.8 $\pm$ 3.0	1.4 $\pm$ 0.2	12.1 $\pm$ 1.7
9.91	25.0 $\pm$ 2.4*	24.5 $\pm$ 2.5*	98.2 $\pm$ 0.7	1.5 $\pm$ 0.2	11.6 $\pm$ 0.9
31.1	25.7 $\pm$ 3.0*	25.0 $\pm$ 2.9*	97.2 $\pm$ 1.4	1.7 $\pm$ 0.3	13.6 $\pm$ 0.8
99.2	16.1 $\pm$ 8.2*	12.4 $\pm$ 9.1*	68.1 $\pm$ 27.8*	0.7 $\pm$ 0.6*	10.7 $\pm$ 2.7

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	2.3 $\pm$ 0.3	8.0 $\pm$ 0.8	1.16 $\pm$ 3.37	430 $\pm$ 327	81 $\pm$ 14	0
0.926	2.0 $\pm$ 0.5	6.3 $\pm$ 1.1*	9.90 $\pm$ 25.57	1,070 $\pm$ 719*	83 $\pm$ 10	0
3.21	1.9 $\pm$ 0.4	7.1 $\pm$ 1.1*	9.81 $\pm$ 11.45	1,010 $\pm$ 260*	69 $\pm$ 13	0
9.91	2.0 $\pm$ 0.3	6.8 $\pm$ 0.7*	165 $\pm$ 409	929 $\pm$ 146*	72 $\pm$ 18*	0
31.1	2.5 $\pm$ 0.5	7.1 $\pm$ 0.9*	17.2 $\pm$ 37.9	749 $\pm$ 200*	59 $\pm$ 12*	0
99.2	4.1 $\pm$ 1.1*	5.6 $\pm$ 1.0*	779 $\pm$ 1,260*	936 $\pm$ 392*	25 $\pm$ 21*	0

●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 16)
Control	95 $\pm$ 5	9.0 $\pm$ 0.2	NA	NA
0.926	91 $\pm$ 4	8.2 $\pm$ 0.2*	NA	NA
3.21	99 $\pm$ 2	8.2 $\pm$ 0.1*	NA	NA
9.91	96 $\pm$ 7	8.1 $\pm$ 0.1*	NA	NA
31.1	88 $\pm$ 9	8.1 $\pm$ 0.1*	NA	NA
99.2	82 $\pm$ 7*	8.1 $\pm$ 0.2*	NA	NA

Data show mean  $\pm$  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