

4-*tert*-Butylphenol (CAS no. 98-54-4)

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, fertility, female hepatosomatic index, male and female secondary sex characteristics, and female gonadosomatic index.

At 10.2 µg/L and higher, a significant (but not concentration dependent) increase was observed in female liver vitellogenin level.

At 1,030 µg/L, a significant decrease was observed in male gonadosomatic index, and significant increases were observed in male liver vitellogenin level and male hepatosomatic index.

●F1 generation (exposure period: 16 weeks)

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

At 10.2, 31.8, 97.4 and 316 µg/L, a significant (but not concentration dependent) increase was observed in male body weight (10 weeks post fertilization).

At 31.8 µg/L and higher, significant increases were observed in female gonadosomatic index (reproductive adult stage) and female liver vitellogenin level (reproductive adult stage).

At 97.4 µg/L and higher, a significant decrease was observed in fertility.

At 316 µg/L and higher, a significant decrease was observed in time to hatch, and significant increases were observed in male liver vitellogenin level (10 weeks post fertilization) and male hepatosomatic index (10 weeks post fertilization).

At 1,030 µg/L, significant decreases were observed in survival (4 and 8 weeks post fertilization), female total length (10 weeks post fertilization), male secondary sex characteristics (10 weeks post fertilization and reproductive adult stage), total eggs, fertile eggs, female hepatosomatic index (10 weeks post fertilization), and male and female total length (reproductive adult stage), and significant increases were observed in male gonadosomatic index (10 weeks post fertilization and reproductive adult stage), male body weight (reproductive adult stage), male hepatosomatic index (reproductive adult stage), and male liver vitellogenin levels (reproductive adult stage).

●F2 generation (exposure period: 2 weeks)

At 10.2, 31.8 µg/L and higher, a significant decrease was observed in hatching rate.

At 31.8 µg/L, a significant decrease was observed in time to hatch (day).

(2) Summary

Fish were exposed to 10.2, 31.8, 97.4, 316 and 1,030 µg/L (measured concentrations) for 19 weeks. At levels where no significant decrease in male and female survival was not observed (1,030 µg/L and lower), a significant increase in male (reproductive adult stage of F1 generation) liver vitellogenin level was observed. Thus, 4-*tert*-butylphenol was identified as estrogenic.

In addition, a significant decrease in fertilization rate was observed in exposure groups at concentrations of 97.4 µg/L and higher, indicating that the substance exhibits adverse effects on the reproduction of Medaka.

The exposure level which indicates the lowest observed effect concentration (LOEC) to Medaka was 97.4 µg/L, and ca. 970 times as high as the highest environmental water concentration of 0.1 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 1997

The exposure level which did not indicate reproductive toxicity (NOEC) to Medaka was 31.8 µg/L, and ca. 320 times as high as the highest environmental water concentration of 0.1 µg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 1997.

●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	30.3 ± 1.5	30.6 ± 0.9	305 ± 44	373 ± 41
10.2	6	6	6	6	30.6 ± 2.2	29.3 ± 1.0	322 ± 47	333 ± 50
31.8	6	6	6	6	30.7 ± 1.3	30.0 ± 1.6	311 ± 48	346 ± 59
97.4	6	6	6	6	30.7 ± 1.4	30.4 ± 1.3	304 ± 46	355 ± 54
316	6	6	6	5	28.8 ± 2.0	30.6 ± 1.7	285 ± 64	378 ± 60
1,030	6	6	6	6	30.1 ± 1.6	29.0 ± 1.5	319 ± 49	329 ± 68

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	21.4 ± 4.8	20.5 ± 4.6	96.1 ± 3.9	1.2 ± 0.4	9.0 ± 1.3
10.2	21.2 ± 2.9	20.4 ± 3.1	96.2 ± 2.9	1.0 ± 0.2	11.0 ± 2.9
31.8	22.5 ± 5.3	21.9 ± 5.2	97.6 ± 2.9	1.2 ± 0.4	9.9 ± 2.2
97.4	22.5 ± 7.0	21.7 ± 6.8	96.8 ± 2.5	1.0 ± 0.3	9.7 ± 3.1
316	21.9 ± 5.3	17.3 ± 4.1	80.9 ± 14.0	1.2 ± 0.5	11.0 ± 2.1
1,030	16.3 ± 6.0	15.3 ± 6.4	90.6 ± 12.4	0.7 ± 0.2*	9.2 ± 4.4

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 ± 0.7	5.3 ± 1.2	1.21 ± 3.4	627 ± 99	67 ± 11	0
10.2	2.4 ± 1.3	5.8 ± 1.1	ND	864 ± 172**	73 ± 12	0
31.8	2.4 ± 0.6	7.3 ± 2.8	ND	790 ± 301**	66 ± 15	0
97.4	2.3 ± 0.5	4.9 ± 1.4	7.6 ± 16.4	775 ± 227**	67 ± 6	0
316	3.3 ± 0.6	6.1 ± 0.7	0.5 ± 0.6	910 ± 100**	69 ± 10	0
1,030	4.2 ± 0.5**	5.5 ± 1.0	65.9 ± 143.1*	856 ± 333**	60 ± 10	0

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration (µg/L)	Hatching rate (%)	Time to hatch (day)
10.2	99 ± 2	7.4 ± 0.1
31.8	97 ± 4	7.4 ± 0.1
97.4	98 ± 4	7.6 ± 0.2
316	100 ± 0	7.4 ± 0.2**
1,030	98 ± 3	7.4 ± 0.1*

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	97 ± 4	96.4 ± 4	25.9 ± 1.1	27.3 ± 1.0	209 ± 28	301 ± 45
10.2	100 ± 0	100 ± 0	28.5 ± 1.0	29.7 ± 1.6	287 ± 35**	388 ± 54
31.8	99 ± 3	99 ± 3	27.8 ± 2.0	30.1 ± 1.6	279 ± 50**	415 ± 79
97.4	100 ± 0	100 ± 0	27.9 ± 1.2	28.9 ± 1.0	285 ± 41**	384 ± 58
316	93 ± 6	93 ± 6	25.2 ± 2.1	26.7 ± 1.3	265 ± 74*	341 ± 48
1,030	83 ± 8**	83 ± 8**	23.8 ± 1.4	23.1 ± 1.8*	223 ± 42	228 ± 47

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	3.8 \pm 1.1	7.3 \pm 1.5	ND	935 \pm 407	68 \pm 12	0
10.2	4.1 \pm 1.2	7.0 \pm 1.6	ND	1,450 \pm 473	74 \pm 16	0
31.8	3.4 \pm 0.9	6.3 \pm 1.2	ND	676 \pm 372	80 \pm 15	0
97.4	3.8 \pm 0.6	6.6 \pm 1.1	ND	1,300 \pm 485	81 \pm 11	0
316	5.2 \pm 1.1*	7.4 \pm 0.9	235 \pm 383**	942 \pm 305	36 \pm 28	0
1,030	6.0 \pm 1.0**	5.8 \pm 1.0*	389 \pm 552**	826 \pm 411	0*	0

Table 2-D Results (continued)

Measured mean concentration ($\mu\text{g/L}$)	Gonadosomatic index (%)	
	Male	Female
v	0.95 \pm 0.3	8.3 \pm 2.7
10.2	1.0 \pm 0.3	9.7 \pm 3.2
31.8	1.0 \pm 0.3	10.3 \pm 4.0
97.4	0.99 \pm 0.2	9.2 \pm 2.5
316	3.0 \pm 3.9	8.3 \pm 3.1
1,030	4.5 \pm 4.5*	6.0 \pm 3.2

●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(24/24)	100(24/24)	32.0 \pm 1.6	33.5 \pm 1.0	352 \pm 50	545 \pm 67
10.2	100(12/12)	100(12/12)	33.2 \pm 1.2	34.9 \pm 1.2	378 \pm 42	601 \pm 77
31.8	100(12/12)	100(12/12)	33.5 \pm 1.1	34.6 \pm 1.6	416 \pm 34	619 \pm 72
97.4	100(11/12)	100(12/12)	32.7 \pm 2.0	33.6 \pm 1.0	375 \pm 69	603 \pm 72
316	100(12/12)	100(12/12)	31.1 \pm 0.9	32.4 \pm 1.0	351 \pm 35	573 \pm 92
1,030	100 (12/12)	100(12/12)	30.4 \pm 0.8**	29.1 \pm 1.0**	445 \pm 50**	458 \pm 49

Table 2-F Results (continued)

Measured mean concentration ($\mu\text{g/L}$)	Total eggs (eggs/day/pair)	Fertile eggs (eggs/day/pair)	Fertility (%)	Gonadosomatic index (%)	
				Male	Female
Control	38.2 \pm 6.0	35.7 \pm 7.1	93.4 \pm 8.8	1.2 \pm 0.4	13.7 \pm 1.4
10.2	48.4 \pm 3.2	46.2 \pm 3.1	95.6 \pm 2.4	1.1 \pm 0.1	14.4 \pm 1.1
31.8	52.3 \pm 6.3	48.2 \pm 8.7	91.9 \pm 9.8	1.2 \pm 0.2	14.9 \pm 1.1**
97.4	47.4 \pm 5.0	42.8 \pm 5.0	90.4 \pm 6.3*	1.2 \pm 0.2	14.9 \pm 1.3**
316	33.6 \pm 8.0	27.6 \pm 12.5	74.7 \pm 30.4**	1.2 \pm 0.2	18.0 \pm 7.0**
1,030	16.9 \pm 9.6**	0.4 \pm 0.6**	3.3 \pm 4.4**	11.9 \pm 8.0**	18.3 \pm 4.7**

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	2.9 \pm 0.9	6.9 \pm 1.9	0.43 \pm 0.97	1,010 \pm 364	100 \pm 15	0
10.2	2.9 \pm 2.2	6.6 \pm 1.9	0.55 \pm 0.91	1,080 \pm 180	100 \pm 14	0
31.8	2.1 \pm 0.5	7.3 \pm 1.1	ND	1,350 \pm 559*	97 \pm 8	0
97.4	2.1 \pm 0.7	7.4 \pm 1.0	ND	1,350 \pm 663**	90 \pm 9**	0
316	3.0 \pm 0.9	7.2 \pm 2.1	0.86 \pm 1.92	1,340 \pm 384**	77 \pm 10**	0
1,030	5.8 \pm 0.9*	5.7 \pm 0.9	1,305 \pm 611**	1,850 \pm 541**	0**	0

●F2 generation (embryo-juvenile stage)

Table 3-A Results

Measured mean concentration ($\mu\text{g/L}$)	Hatching rate (%)	Time to hatch (day)
Control	98 \pm 3	8.4 \pm 0.2
10.2	90 \pm 8*	8.1 \pm 0.1
31.8	88 \pm 4*	8.0 \pm 0.1**
97.4	96 \pm 4	8.5 \pm 0.2
316	94 \pm 6	8.3 \pm 0.2
1,030	no fertile eggs	no fertile eggs

Data show mean \pm 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