

## 4-Nonylphenol (branched) (CAS no. 84852-15-3)

### **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 mortality, total egg, fertile egg, fertility, total length, body weight, gonadosomatic index, male secondary sex characteristics, and female liver vitellogenin level.

Male liver vitellogenin level increased dose-dependently, and a significant increase was observed at 2.95 µg/L and higher.

At 2.95 µg/L and higher, a significant increase was observed in female hepatosomatic index.

At 9.81 µg/L and higher, a significant increase was observed in male hepatosomatic index.

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

No significant differences were observed in time to hatch, post hatch survival, male gonadosomatic index (10 weeks post fertilization), male total length (15 weeks post fertilization reproductive adult stage), and female hepatosomatic index (15 weeks post fertilization reproductive adult stage).

Male liver vitellogenin level (10 weeks post fertilization) increased dose-dependently, and a significant increase was observed at 9.81 µg/L and higher.

Male liver vitellogenin level (15 weeks post fertilization reproductive adult stage) increased dose-dependently, and a significant increase was observed at 27.8 µg/L and higher.

At 1.27 µg/L and higher, a significant decrease was observed in total egg, fertile egg, male total length (10 weeks post fertilization), male body weight (10 weeks post fertilization) and, female body weight (10 weeks post fertilization).

At 2.95 µg/L and higher, a significant decrease was observed in female total length (10 weeks post fertilization), male secondary sex characteristics (the number of plates with papillary process on the anal fin per fish, 10 weeks post fertilization) were suppressed, and a significant increase was observed in male hepatosomatic index (10 weeks post fertilization, 15 weeks post fertilization reproductive adult stage).

At 9.81 µg/L and higher, a significant decrease was observed in female gonadosomatic index (10 weeks post fertilization), and a significant increase was observed in female mortality (15 weeks post fertilization reproductive adult stage), and male gonadosomatic index (15 weeks post fertilization reproductive adult stage).

At 27.8 µg/L and higher, a significant decrease was observed in fertility, female total length (15 weeks post fertilization reproductive adult stage), and male secondary sex characteristics (15 weeks post fertilization reproductive adult stage), and a significant increase was observed in female liver vitellogenin level (10 weeks post fertilization) and female gonadosomatic index (15 weeks post fertilization reproductive adult stage).

At 89.4 µg/L, a significant decrease was observed in hatching rate, survival (14 days post fertilization, 4 weeks post fertilization, 9 weeks post fertilization), female hepatosomatic index (10 weeks post fertilization), and a significant increase was observed in male mortality (15 weeks post fertilization reproductive adult stage), male body weight (15 weeks post fertilization reproductive adult stage), female body weight (15 weeks post fertilization reproductive adult stage), and female liver vitellogenin level (15 weeks post fertilization reproductive adult stage).

●F2 generation (exposure period: 2 weeks)

No significant differences were observed in hatching rate, post hatch survival, and survival (15 days post fertilization).

At 27.8 µg/L, a significant increase of time to hatch was observed.

(2) Summary

Fish were exposed to 1.27, 2.95, 9.81, 27.8 and 89.4 µg/L (measured mean concentrations) for 19 weeks. At levels where mortality was not dose-dependent (ie. below 9.81 µg/L), a significant increase in male liver vitellogenin level was observed and male secondary sex characteristics (the number of plates with papillary process on the anal fin per fish) were suppressed, indicating estrogenic effects. Thus, 4-nonylphenol (branched) was identified as estrogenic.

At 1.27 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. 2 times as high as the highest environmental water concentration of 0.69 µg/L (sum of 4-nonylphenol branched isomers) that was measured in MOE's Water Quality Survey of Public Water Areas in FY2015.

●F0 generation

Table 1-A Results

Measured mean concentration (µg/L)	Total number		Mortality (%)		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	12	12	0	0	31.7 ± 0.7	32.3 ± 0.8	311 ± 24	382 ± 31
1.27	6	6	0	0	31.7 ± 1.0	31.4 ± 1.8	316 ± 26	346 ± 57
2.95	6	6	0	0	32.3 ± 1.5	31.0 ± 1.0	342 ± 44	343 ± 23
9.81	6	6	0	0	31.3 ± 0.6	32.6 ± 1.4	288 ± 17	417 ± 46
27.8	6	6	0	0	32.8 ± 1.4	31.2 ± 0.6	342 ± 35	363 ± 16
89.4	6	6	0	16.7	32.3 ± 1.0	31.8 ± 0.8	344 ± 43	366 ± 37

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	31.7 ± 2.2	31.2 ± 2.6	98.4 ± 2.0	1.5 ± 1.66	9.2 ± 1.0
1.27	29.9 ± 3.2	29.0 ± 3.3	96.3 ± 3.8	1.3 ± 0.27	9.0 ± 0.7
2.95	30.0 ± 3.0	29.2 ± 3.2	98.0 ± 2.5	1.3 ± 0.20	9.4 ± 0.7
9.81	35.1 ± 4.2	33.4 ± 3.6	95.7 ± 6.6	1.3 ± 0.18	9.6 ± 1.4
27.8	31.4 ± 4.2	30.4 ± 5.5	95.8 ± 6.6	1.1 ± 0.19	9.9 ± 0.7
89.4	29.2 ± 4.8	28.4 ± 4.9	95.4 ± 6.03	1.2 ± 0.20	9.2 ± 1.2

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	1.7 ± 0.36	4.8 ± 1.43	4.41 ± 7.86	270 ± 128	100 ± 17	NA
1.27	1.7 ± 0.46	5.6 ± 0.67	23.3 ± 24.9	220 ± 37	97 ± 6.1	NA
2.95	1.9 ± 0.24	6.0 ± 1.00 *	19.4 ± 22.2 *	187 ± 34	110 ± 13	NA
9.81	1.9 ± 0.32 *	6.5 ± 1.45 *	384 ± 473 *	255 ± 110	99 ± 15	NA
27.8	2.3 ± 0.30 *	6.3 ± 0.93 *	565 ± 417 *	203 ± 52	110 ± 7	NA
89.4	2.4 ± 0.23 *	6.1 ± 0.24 *	1,840 ± 529 *	227 ± 56	97 ± 11	NA

●F1 generation (embryo-juvenile stage)

Table 2-A Results

Measured mean concentration (µg/L)	Hatching rate (%)	Time to hatch (day)	Post hatch survival (%)	Survival (%)
				(Day 14)
Control	93.7 ± 12.5	7.0 ± 0.3	96.1 ± 10.4	91.2 ± 18.0
1.27	98.3 ± 2.7	7.2 ± 0.2	100 ± 0	98.3 ± 2.7
2.95	95.8 ± 3.8	7.2 ± 0.2	100 ± 0	95.8 ± 3.8
9.81	99.2 ± 2.0	7.3 ± 0.2	100 ± 0	99.2 ± 2.0
27.8	99.2 ± 2.0	7.1 ± 0.1	100 ± 0	99.2 ± 2.0
89.4	77.2 ± 7.3 *	7.3 ± 0.1	98.9 ± 2.7	78.0 ± 5.1 *

Table 2-B Results (continued)

Measured mean concentration (µg/L)	Survival (%) (Week 4)	Survival (%) (Week 9)	Total length (mm) (Week 10)		Body weight (mg) (Week 10)	
			Male	Female	Male	Female
Control	99.3 ± 2.4	95.8 ± 5.6	25.7 ± 1.3	26.8 ± 1.7	193 ± 35.3	241 ± 44.7
1.27	100 ± 0	95.8 ± 7.0	24.5 ± 1.3 *	26.2 ± 1.8	162 ± 28.5 *	213 ± 41.7 *
2.95	98.6 ± 3.4	98.6 ± 3.4	24.8 ± 1.5 *	25.6 ± 1.7 *	156 ± 32.0 *	198 ± 44.6 *
9.81	100 ± 0	93.1 ± 6.3	24.9 ± 2.0 *	24.3 ± 4.2 *	159 ± 35.2 *	172 ± 73.3 *
27.8	100 ± 0	97.2 ± 6.8	24.5 ± 1.4 *	25.0 ± 1.7 *	169 ± 38.6 *	190 ± 48.7 *
89.4	88.9 ± 10.1 *	81.9 ± 17.0 *	22.6 ± 2.6 *	23.0 ± 2.1 *	137 ± 48.9 *	139 ± 42.2 *

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 $\pm$ 0.7	4.5 $\pm$ 1.2	22.6 $\pm$ 55.8	664 $\pm$ 428	78 $\pm$ 15	NA
1.27	2.2 $\pm$ 0.4	4.1 $\pm$ 1.3	11.4 $\pm$ 31.9	544 $\pm$ 350	72 $\pm$ 13	NA
2.95	2.7 $\pm$ 1.1 *	4.1 $\pm$ 1.0	20.3 $\pm$ 74.9	676 $\pm$ 384	69 $\pm$ 13 *	NA
9.81	2.8 $\pm$ 0.7 *	4.1 $\pm$ 1.5	100 $\pm$ 210 *	575 $\pm$ 310	60 $\pm$ 17 *	NA
27.8	2.7 $\pm$ 0.9 *	4.1 $\pm$ 0.7	72.3 $\pm$ 156 *	888 $\pm$ 378 *	27 $\pm$ 24 *	NA
89.4	3.1 $\pm$ 0.8 *	3.3 $\pm$ 0.8 *	1,040 $\pm$ 1,200 *	1,550 $\pm$ 1,120 *	0 $\pm$ 0 *	NA

Table 2-D Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Gonadosomatic index (%)	
	Male	Female
Control	0.78 $\pm$ 0.40	7.8 $\pm$ 1.6
1.27	0.85 $\pm$ 0.33	7.1 $\pm$ 2.3
2.95	0.89 $\pm$ 0.39	7.7 $\pm$ 2.8
9.81	0.79 $\pm$ 0.54	5.3 $\pm$ 3.1 *
27.8	0.91 $\pm$ 0.97	6.3 $\pm$ 2.2 *
89.4	0.96 $\pm$ 1.20	1.7 $\pm$ 1.7 *

●F1 generation (reproductive adult stage)

Table 2-E Results (continued)

Measured mean concentration ( $\mu\text{g/L}$ )	Mortality (%)		Total length (mm)		Body weight (mg)	
	Male	Female	Male	Female	Male	Female
Control	0	4	31.2 $\pm$ 0.9	31.1 $\pm$ 1.3	302 $\pm$ 26	350 $\pm$ 41
1.27	8.3	0	30.8 $\pm$ 0.6	30.5 $\pm$ 1.2	276 $\pm$ 24	349 $\pm$ 94
2.95	0	0	29.9 $\pm$ 0.9	30.6 $\pm$ 1.2	260 $\pm$ 19	329 $\pm$ 37
9.81	0	33 *	31.6 $\pm$ 1.0	31.2 $\pm$ 0.9	301 $\pm$ 31	331 $\pm$ 45
27.8	0	25 *	31.9 $\pm$ 1.6	30.0 $\pm$ 1.1 *	335 $\pm$ 63	300 $\pm$ 39
89.4	33 *	25 *	31.7 $\pm$ 2.4	30.5 $\pm$ 1.2 *	449 $\pm$ 132 *	403 $\pm$ 58 *

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	28.4 $\pm$ 4.9	27.5 $\pm$ 4.9	95.4 $\pm$ 5.4	0.91 $\pm$ 0.24	7.7 $\pm$ 1.1
1.27	24.9 $\pm$ 3.7 *	24.0 $\pm$ 3.8 *	94.1 $\pm$ 5.9	0.94 $\pm$ 0.29	8.6 $\pm$ 2.2
2.95	25.3 $\pm$ 4.7 *	22.6 $\pm$ 6.7 *	89.5 $\pm$ 20.0	1.0 $\pm$ 0.2	9.4 $\pm$ 1.6
9.81	18.0 $\pm$ 6.0 *	17.2 $\pm$ 6.0 *	83.5 $\pm$ 18.1	1.0 $\pm$ 0.4 *	7.6 $\pm$ 2.0
27.8	18.4 $\pm$ 5.9 *	15.7 $\pm$ 6.6 *	73.6 $\pm$ 23.1 *	1.0 $\pm$ 0.3 *	8.6 $\pm$ 1.1 *
89.4	5.2 $\pm$ 2.9 *	0 $\pm$ 0 *	0.05 $\pm$ 0.17 *	16 $\pm$ 18 *	15 $\pm$ 8.7 *

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	1.6 $\pm$ 0.3	4.8 $\pm$ 0.9	5.3 $\pm$ 7.5	532 $\pm$ 227	95 $\pm$ 15	NA
1.27	1.7 $\pm$ 0.2	4.9 $\pm$ 1.0	6.1 $\pm$ 14.2	449 $\pm$ 140	79 $\pm$ 11	NA
2.95	2.0 $\pm$ 0.2 *	5.5 $\pm$ 1.1	9.9 $\pm$ 11.6	432 $\pm$ 93	83 $\pm$ 19	NA
9.81	1.8 $\pm$ 0.4 *	4.1 $\pm$ 0.7	23.8 $\pm$ 35.4	432 $\pm$ 103	89 $\pm$ 11	NA
27.8	1.9 $\pm$ 0.5 *	5.1 $\pm$ 1.1	68.9 $\pm$ 153 *	484 $\pm$ 161	62 $\pm$ 18 *	NA
89.4	3.4 $\pm$ 0.9 *	3.5 $\pm$ 1.1	845 $\pm$ 458 *	1,100 $\pm$ 391 *	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	83.3 $\pm$ 9.8	7.4 $\pm$ 0.2	100 $\pm$ 0	85.0 $\pm$ 8.8
1.27	95.0 $\pm$ 6.3	7.8 $\pm$ 0.4	100 $\pm$ 0	95.0 $\pm$ 6.3
2.95	86.5 $\pm$ 4.2	7.4 $\pm$ 0.3	100 $\pm$ 0	89.0 $\pm$ 3.9
9.81	90.0 $\pm$ 8.9	7.6 $\pm$ 0.5	100 $\pm$ 0	92.5 $\pm$ 7.6
27.8	89.0 $\pm$ 5.1	8.1 $\pm$ 0.6 *	100 $\pm$ 0	91.6 $\pm$ 4.1
89.4	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