

## ANNEX 7

### Results of Assay and Tests in Evaluation of the Endocrine Disrupting Activities in Fish(*Medaka*)

#### 4-*tert*-octylphenol

##### 1. Vitellogenin Assay

Table 1 Results

Treatment	Mortality (%)	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
		male	female	male	female
Control	0	1.9 ± 1.0	4.8 ± 1.0	3.6 ± 3.5	1,500 ± 320
Solvent control	0	1.8 ± 0.9	4.0 ± 1.1	1.4 ± 1.2	1,800 ± 1,300
12.7 (µg/L)	0	2.0 ± 0.6	4.4 ± 0.8	1.9 ± 1.2	1,800 ± 540
27.8 (µg/L)	0	1.8 ± 0.3	4.0 ± 0.6	3.6 ± 4.4	1,900 ± 510
64.1 (µg/L)	0	2.2 ± 0.8	4.3 ± 1.1	190 ± 370**	1,500 ± 400
129 (µg/L)	6.3	2.6 ± 0.3	3.8 ± 0.9	2,300 ± 1,100**	3,000 ± 2,900
296 (µg/L)	0	2.8 ± 0.6	4.2 ± 0.9	6,100 ± 1,800**	3,300 ± 1,900**

Statistically significant differences from control group(\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

##### 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	98 ± 3.3	9.1 ± 0.3	5.0 ± 3.3	26.2 ± 2.1	188 ± 35
Solvent control	98 ± 3.3	9.0 ± 0.1	5.0 ± 6.4	26.2 ± 2.0	157 ± 35
6.94 (µg/L)	95 ± 6.4	8.9 ± 0.1	3.6 ± 4.2	26.6 ± 1.8	163 ± 37
11.4 (µg/L)	98 ± 3.3	9.0 ± 0	5.1 ± 6.4	26.6 ± 1.5	169 ± 34
23.7 (µg/L)	100 ± 0	8.9 ± 0.1	20 ± 12*	27.3 ± 1.6**	187 ± 39***
48.1 (µg/L)	95 ± 6.4	9.0 ± 0	1.9 ± 3.9	26.1 ± 1.7	167 ± 34
94.0 (µg/L)	97 ± 6.7	9.0 ± 0	5.5 ± 7.3	25.5 ± 2.1	159 ± 39

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.47 ± 0.2	1.8 ± 1.8	20	0/10	2.2 ± 0.6	3.1 ± 0.5	1.9 ± 1.8	810 ± 600
Solvent control	0.85 ± 0.9	2.9 ± 2.9	20	0/9	2.1 ± 0.7	3.5 ± 0.6	0.83 ± 0.7	810 ± 770
6.94 (µg/L)	0.78 ± 0.3	4.5 ± 2.9	20	0/10	2.8 ± 0.6*	3.8 ± 0.8	5.4 ± 4.5	1,600 ± 1,000
11.4 (µg/L)	0.88 ± 0.4	4.4 ± 3.0	20	1/9	2.7 ± 0.5	3.3 ± 0.8	13 ± 9.4**	2,300 ± 1,700
23.7 (µg/L)	0.71 ± 0.3	3.2 ± 3.3	20	2/10 *	2.8 ± 0.8*	4.1 ± 0.7*	17 ± 19**	1,700 ± 1,300
48.1 (µg/L)	0.64 ± 0.2	2.8 ± 2.4	20	3/10 *	2.4 ± 0.7	3.9 ± 0.7	140 ± 190**	3,600 ± 1,400**
94.0 (µg/L)	0.39 ± 0.4	0.60 ± 0.5**	20	5/10 **	3.3 ± 0.6**	4.0 ± 0.9	500 ± 880**	4,000 ± 790**

Statistically significant differences from control group(\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

Statistically significant differences from solvent control group(\*\*\*indicates  $p < 0.01$ , \*\*\*\*indicates  $p < 0.05$ )

### 3. Full Life Cycle Test

Table 3-A generation

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)	No. of fishes	No. of males with testis-ova/No. of males
Control	100	8.5 ± 0.3	1.7	30.5 ± 1.3	269 ± 32	20	0/8
Solvent control	97 ± 3.9	8.4 ± 0.2	6.7	31.6 ± 1.4	307 ± 43	20	0/9
1.68 (µg/L)	88 ± 11	8.1 ± 0.2	8.4	31.7 ± 1.8	310 ± 62	20	0/10
4.27 (µg/L)	92 ± 8.4	8.2 ± 0.4	5.8	31.5 ± 1.2	298 ± 35	20	0/10
9.92 (µg/L)	97 ± 6.7	8.4 ± 0.1	12	32.0 ± 1.2	301 ± 42	20	1/10
30.4 (µg/L)	88 ± 6.4	8.3 ± 0.4	11	32.1 ± 1.5	322 ± 50	20	5/7 **
82.3 (µg/L)	92 ± 8.4	8.2 ± 0.1	5.8	31.7 ± 1.5	310 ± 44	20	7/8 **

Table 3-B F<sub>1</sub> generation (Continued)

Treatment	No. of eggs	Fertility (%)	Gonadosomatic Index (%)		Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
			male	female	male	female	male	female
Control	653 ± 89	97 ± 3.3	0.81 ± 0.1	7.5 ± 1.5	1.7 ± 0.6	5.5 ± 1.8	10 ± 15	1,600 ± 740
Solvent control	500 ± 170	90 ± 15	0.65 ± 0.2	7.7 ± 1.7	1.6 ± 0.4	4.1 ± 0.7	8.6 ± 8.8	1,600 ± 1,300
1.68 (µg/L)	659 ± 130	97 ± 1.2	0.86 ± 0.2	7.6 ± 1.5	1.4 ± 0.5	4.2 ± 1.5	8.5 ± 8.8	1,700 ± 780
4.27 (µg/L)	667 ± 60	98 ± 2.1	0.98 ± 0.2	8.0 ± 0.7	1.4 ± 0.2	3.8 ± 0.4	16 ± 10	2,100 ± 1,100
9.92 (µg/L)	631 ± 80	93 ± 7.4	0.93 ± 0.2	8.3 ± 1.2	1.8 ± 0.8	3.5 ± 0.7	290 ± 640**	2,600 ± 2,400
30.4 (µg/L)	520 ± 150	92 ± 8.0	0.92 ± 0.3	7.8 ± 1.9	1.9 ± 0.1	4.5 ± 0.8	630 ± 850**	4,900 ± 2,600**
82.3 (µg/L)	45 ± 87**	35 ± 36*	1.0 ± 0.3	8.2 ± 3.8	2.6 ± 0.7**	3.9 ± 0.7	2,800 ± 2,800**	11,000 ± 6,700**

Table 3-C generation

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)	No. of fishes	No. of males with testis-ova/No. of males
Control	94 ± 7.6	9.7 ± 0.4	1.7	28.7 ± 1.6	252 ± 45	20	0/11
Solvent control	80 ± 29	9.4 ± 0.6	6.7	28.9 ± 1.7	253 ± 41	20	0/12
1.68 (µg/L)	90 ± 14	9.2 ± 0.4	6.7	28.2 ± 1.7	242 ± 39	20	0/14
4.27 (µg/L)	92 ± 7.8	9.4 ± 0.5	8.3	28.7 ± 1.7	243 ± 37	20	0/11
9.92 (µg/L)	96 ± 6.8	9.5 ± 0.6	0	28.3 ± 2.1	243 ± 27	20	0/8
30.4 (µg/L)	97 ± 7.4	9.5 ± 0.5	0	28.7 ± 1.1	243 ± 30	20	4/8 **
82.3 (µg/L)	51 ± 49	9.6 ± 0.3	6.1	28.8 ± 1.0	252 ± 28	20	10/15 **

Table 3-D generation (Continued)

Treatment	Vitellogenin (ng/mg liver)	
	male	female
Control	3.1 ± 2.6	1,700 ± 1,000
Solvent control	4.3 ± 5.0	1,500 ± 1,000
1.68 (µg/L)	3.1 ± 4.4	990 ± 920
4.27 (µg/L)	6.5 ± 19	2,300 ± 1,100
9.92 (µg/L)	24 ± 22**	3,200 ± 1,200**
30.4 (µg/L)	42 ± 29**	4,300 ± 2,000**
82.3 (µg/L)	22 ± 22*	6,200 ± 540**

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

# Di-n-butyl phthalate

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Mortality (%)	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
		male	female	male	female
Control	0	1.9 ± 0.4	4.6 ± 0.9	0.5 ± 0.1	1,200 ± 580
Solvent control	0	1.8 ± 0.6	4.1 ± 1.2	0.8 ± 0.8	1,200 ± 580
24.4 (µg/L)	0	2.0 ± 0.6	4.1 ± 1.2	0.5 ± 0.2	950 ± 720
55.3 (µg/L)	0	2.1 ± 1.0	4.4 ± 0.8	0.7 ± 0.3	1,200 ± 560
133 (µg/L)	0	2.3 ± 0.7	4.5 ± 1.1	0.7 ± 0.5	660 ± 610
328 (µg/L)	5	2.5 ± 0.6*	5.6 ± 1.8	0.4 ± 0.1	790 ± 780
822 (µg/L)	0	2.8 ± 0.6**	4.3 ± 0.9	0.4 ± 0.1	1,100 ± 880

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	97 ± 3.9	10 ± 0.1	8.6 ± 6.6	29.5 ± 1.4	256 ± 42
Solvent control	97 ± 3.9	10 ± 0.2	11 ± 4.4	29.6 ± 1.3	256 ± 41
7.09 (µg/L)	95 ± 6.4	9.8 ± 0.1	12 ± 8.6	29.8 ± 1.5	266 ± 50
21.9 (µg/L)	87 ± 14	9.8 ± 0.2	8.9 ± 10	29.5 ± 1.4	259 ± 46
72.8 (µg/L)	97 ± 3.9	9.9 ± 0.1	21 ± 5.1*	30.1 ± 1.5	269 ± 38
235 (µg/L)	100	10 ± 0.3	48 ± 18*	29.6 ± 1.7	269 ± 48
850 (µg/L)	8.3 ± 10*	16 ± 0.7	100	-	-

- indicates 100% mortality

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.64 ± 0.2	3.9 ± 2.5	20	0/10	3.4 ± 0.9	4.1 ± 0.6	2.4 ± 3.8	1,200 ± 950
Solvent control	0.68 ± 0.2	2.5 ± 2.7	20	0/12	3.2 ± 1.0	5.0 ± 1.4	0.83 ± 1.6	760 ± 1,200
7.09 (µg/L)	0.61 ± 0.2	3.9 ± 3.4	20	1/11	3.6 ± 0.9	5.6 ± 0.6*	3.5 ± 3.3	1,400 ± 1,200
21.9 (µg/L)	0.63 ± 0.3	4.5 ± 4.0	20	0/12	3.5 ± 1.2	4.4 ± 1.0	1.2 ± 2.6	1,400 ± 840
72.8 (µg/L)	0.73 ± 0.3	4.6 ± 3.8	20	2/12 *	3.2 ± 1.1	4.3 ± 0.8	4.1 ± 8.0	1,200 ± 1,300
235 (µg/L)	0.63 ± 0.3	2.8 ± 3.6	20	0/9	3.4 ± 0.8	4.2 ± 1.0	3.7 ± 5.6	360 ± 730
850 (µg/L)	-	-	-	-	-	-	-	-

-indicates 100% mortality

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

### 3.Full Life Cycle Test

Table 3-A F<sub>0</sub> generation

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)	No. of fishes	No. of males with testis-ova/No. of males
Control	92 ± 8.4	9.9 ± 0.3	13	29.1 ± 1.4	236 ± 44	20	0/8
Solvent control	95 ± 6.4	10 ± 0.4	18	29.6 ± 1.2	245 ± 35	20	0/9
2.61 (µg/L)	98 ± 3.3	9.8 ± 0.2	12	28.6 ± 1.5	218 ± 39	20	1/8
7.52 (µg/L)	97 ± 3.8	9.8 ± 0.2	16	29.3 ± 1.3	239 ± 34	20	0/11
23.9 (µg/L)	95 ± 6.4	10 ± 0.3	5.6	29.1 ± 1.4	233 ± 38	20	0/6
74.5 (µg/L)	95 ± 6.4	10 ± 0.1	24	30.1 ± 1.7	259 ± 49	20	1/9
233 (µg/L)	98 ± 3.3	10 ± 0.2	15.	28.6 ± 1.8	226 ± 50	20	2/8 *

Table 3-B generation(Continued)

Treatment	No. of eggs	Fertility (%)	Gonadosomatic Index (%)		Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
			male	female	male	female	male	female
Control	560 ± 210	94 ± 6.5	0.78 ± 0.2	9.3 ± 1.6	1.3 ± 0.4	4.2 ± 0.8	14.0 ± 30	2,000 ± 2,000
Solvent control	625 ± 130	87 ± 26	0.89 ± 0.2	8.1 ± 0.9	1.5 ± 0.3	4.2 ± 0.7	7.0 ± 6.3	1,600 ± 950
2.61 (µg/L)	602 ± 110	96 ± 5.6	0.86 ± 0.2	7.5 ± 0.7	1.4 ± 0.3	4.0 ± 0.7	9.9 ± 9.5	1,500 ± 890
7.52 (µg/L)	668 ± 100	94 ± 8.9	0.92 ± 0.2	8.0 ± 0.9	1.4 ± 0.2	4.0 ± 0.3	15 ± 9.4	1,400 ± 330
23.9 (µg/L)	543 ± 110	94 ± 3.1	1.1 ± 0.1*	9.0 ± 0.5	1.3 ± 0.4	4.5 ± 0.6	8.1 ± 7.4	1,800 ± 470
74.5 (µg/L)	554 ± 180	97 ± 1.6	0.92 ± 0.2	7.8 ± 1.1	1.6 ± 0.4	3.8 ± 1.3	13 ± 13	1,700 ± 520
233 (µg/L)	539 ± 240	91 ± 11	0.97 ± 0.3	9.4 ± 2.6	1.8 ± 0.2	4.4 ± 1.0	4.6 ± 4.0	2,100 ± 2,200

Table 3-C F<sub>0</sub> generation

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)	No. of fishes	No. of males with testis-ova/No. of males
Control	87 ± 8.9	9.4 ± 0.6	0	30.7 ± 1.2	276 ± 39	20	0/9
Solvent control	85 ± 11	9.4 ± 0.5	0	30.5 ± 1.4	281 ± 39	20	0/7
2.61 (µg/L)	89 ± 8.8	9.1 ± 0.6	0	30.8 ± 1.2	274 ± 34	20	2/10
7.52 (µg/L)	94 ± 6.4**	9.4 ± 0.5	1.7	31.7 ± 1.1**	297 ± 41*	20	2/13 *
23.9 (µg/L)	72 ± 21	8.6 ± 1.1	1.7	30.8 ± 1.3	283 ± 33	20	1/11
74.5 (µg/L)	90 ± 12	9.8 ± 0.4*	0	30.8 ± 1.3	290 ± 31	20	1/14
233 (µg/L)	94 ± 6.6*	11 ± 1.2**	3.3	30.2 ± 1.2	292 ± 39	20	0/9

Table 3-D F<sub>0</sub> generation (Continued)

Treatment	Vitellogenin(ng/mg liver)	
	male	female
Control	0.8 ± 1.1	440 ± 720
Solvent control	ND	470 ± 1,000
2.61 (µg/L)	3.8 ± 5.0*	1,700 ± 820**
7.52 (µg/L)	9.1 ± 8.5**	1,600 ± 1,100*
23.9 (µg/L)	14 ± 29	1,200 ± 580**
74.5 (µg/L)	3.3 ± 2.7**	850 ± 790
233 (µg/L)	2.5 ± 3.0	730 ± 570

Statistically significant differences from control group(\*\*indicates  $P < 0.01$ , \*indicates  $P < 0.05$ )

# Di-(2-ethylhexyl) phthalate

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	ND	0.53±0.13	1.56±0.27	1.44±0.21
Solvent control	0.55±0.21	ND	1.50±0.27	1.46±0.24
19 (µg/L)	0.62±0.46	ND	1.46±0.33	1.59±0.19
43 (µg/L)	ND	ND	1.74±0.27	1.33±0.28
96 (µg/L)	0.58±0.31	ND	1.60±0.38	1.59±0.32
210 (µg/L)	ND	ND	1.54±0.18	1.44±0.34
410 (µg/L)	ND	ND	1.74±0.22	1.39±0.31

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	93±7.7	8.8±0.5	0.0±0.0	30.3±1.5	271±43
Solvent control	98±3.3	9.2±0.2	1.9±3.9	30.4±1.6	275±52
11.0 (µg/L)	93±0	9.0±0.3	1.8±3.6	30.7±1.5	290±45
28.4 (µg/L)	100±0	9.1±0.1	0.0±0.0	30.1±2.0	270±58
73.4 (µg/L)	95±10	9.1±0.1	0.0±0.0	30.5±1.6	263±49
186 (µg/L)	95±6.4	9.0±0.1	1.8±3.6	30.2±2.0	261±51
446 (µg/L)	95±6.8	9.0±0.2	2.1±4.2	30.3±2.0	264±48

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.78±0.21	5.0±2.7	20	0/13	2.2±0.7	3.7±0.4	1.3±1.4	1,100±730
Solvent control	0.71±0.23	6.2±3.6	20	0/10	2.0±0.2	3.9±0.4	2.8±3.6	1,600±1,000
11.0 (µg/L)	0.82±0.27	3.8±2.6	20	0/12	1.8±0.6	3.9±1.3	2.5±4.1	1,100±890
28.4 (µg/L)	0.97±0.40	4.3±2.9	20	0/10	1.6±0.7	3.5±0.5	3.5±5.4	1,500±920
73.4 (µg/L)	0.83±0.26	5.2±3.4	20	1/11	2.6±0.9	3.7±0.4	0.4±0.4	1,500±1,100
186 (µg/L)	0.76±0.26	6.4±3.9	20	0/10	2.3±0.6	4.2±0.9	0.5±0.5	1,700±1,100
446 (µg/L)	0.86±0.37	6.0±3.3	20	0/12	2.3±0.7	3.9±0.5	4.3±9.3	1,200±570

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

# Di-cyclohexyl phthalate

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	0.55±0.21	ND	1.55±0.13	1.42±0.20
Solvent control	ND	ND	1.67±0.21	1.40±0.26
18 (µg/L)	ND	ND	1.58±0.28	1.39±0.18
38 (µg/L)	0.53±0.13	ND	1.56±0.23	1.61±0.22*
87 (µg/L)	ND	ND	1.88±0.37*	1.56±0.21
190 (µg/L)	ND	ND	1.84±0.26*	1.50±0.20
390 (µg/L)	ND	ND	2.04±0.37**	1.55±0.39

Statistically significant differences from control group(\*\*indicates  $P<0.01$ , \*indicates  $P<0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	98±3.3	9.7±0.2	0±0	28.0±1.4	220±36
Solvent control	92±13	9.2±0.3	3.3±6.7	27.3±2.8	250±50
0.429 (µg/L)	100±0	9.1±0.1	1.8±3.6	28.8±1.5**	225±41 <sup>1</sup>
1.41 (µg/L)	93±9.4	9.1±0.1	7.6±11	28.4±2.3	241±44
4.39 (µg/L)	92±8.4	9.1±0.1	5.6±7.3	30.0±1.6**	250±47
13.3 (µg/L)	100±0	9.3±0.4	0±0	29.0±1.7**	237±45
35.8 (µg/L)	90±8.6	9.1±0.1	13±10	29.8±1.8**	265±48

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.75±0.2	4.3±3.3	20	0/13	2.7±0.7	3.6±1.0	1.8±2.4	1.600±1.500
Solvent control	0.74±0.2	5.2±3.3	20	0/12	2.5±0.4	4.0±0.7	2.2±2.4	1.800±1.300
0.429 (µg/L)	0.83±0.2	5.5±3.1	20	0/13	2.4±0.4	3.6±0.9	3.8±3.4	2.100±1.100
1.41 (µg/L)	0.69±0.2	2.9±2.6	20	0/13	2.4±0.6	3.0±0.5	4.7±4.7	1.600±1.400
4.39 (µg/L)	0.85±0.3	5.8±3.7	20	0/14	2.2±0.6	3.6±0.5	12±16**	1,800±660
13.3 (µg/L)	0.76±0.2	3.9±2.8	20	0/11	2.1±0.5	3.2±0.7	1.3±2.0	2.400±1.900
35.8 (µg/L)	1.1±0.3**	5.9±3.1	20	1/10	2.2±0.9	3.7±1.0	2.7±2.1	2.900±3.300

Statistically significant differences from control group(\*\*indicates  $P<0.01$ , \*indicates  $P<0.05$ )

Statistically significant differences from solvent control group(\*\*\* indicates  $P<0.01$ , \*\*\*\* indicates  $P<0.05$ )

# Di-ethyl phthalate

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	4.4±1.1	1.3±0.1	2.05±0.11	1.65±0.13
8.1 (µg/L)	2.1±0.2	2.3±0.6	1.95±0.19*	1.75±0.34
26.8 (µg/L)	4.8±1.5	1.8±0.5	1.87±0.13*	1.64±0.11
119.8 (µg/L)	2.7±0.8	2.2±0.8	2.00±0.15*	2.41±0.76
355.8 (µg/L)	2.4±0.4	1.0±0.1	1.91±0.14*	1.61±0.10
1,053.3 (µg/L)	2.5±0.7*	1.2±0.2	1.98±0.11*	1.76±0.06

Statistically significant differences from control group (\*\*indicates  $P < 0.01$ , \*indicates  $P < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	81	11.2 ± 0.3	8.6	20.8 ± 0.3	164.5 ± 6.6
0.6 (µg/L)	80	12.3 ± 0.4	3.8	20.6 ± 0.2	158.1 ± 5.4
2.5 (µg/L)	83	12.4 ± 0.4*	13.3	21.1 ± 0.2	167.7 ± 4.5
8.4 (µg/L)	91	12.3 ± 0.5*	17.6	21.5 ± 0.2	167.8 ± 4.1
36.0 (µg/L)	92	11.8 ± 0.3*	5.4	20.1 ± 0.2*	142.0 ± 3.8*
121.6 (µg/L)	88	11.3 ± 0.3	2.3	20.3 ± 0.2	140.5 ± 3.9*

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	1.09±0.07	7.54±0.19	20	0/10	4.49±0.50	4.24±0.60	0.16 ± 0.05	255.7 ± 95.0
0.6 (µg/L)	0.87±0.10	7.40±0.21	20	0/10	4.19±0.36	3.85±0.52	0.21 ± 0.08	160.0 ± 102.6
2.5 (µg/L)	1.02±0.06	7.34±0.19	20	0/10	3.89±0.42	4.44±0.42	1.18 ± 0.76	196.7 ± 80.0
8.4 (µg/L)	0.84±0.08	7.46±0.15	20	0/10	3.99±0.44	3.83±0.49	0.52 ± 0.18	150.7 ± 136.4
36.0 (µg/L)	0.92±0.11	7.09±0.21	20	0/10	4.46±0.45	4.83±0.32	2.12 ± 1.03	75.2 ± 46.5*
121.6 (µg/L)	0.90±0.11	6.91±0.21	20	0/10	4.96±0.29	4.30±0.38	0.10 ± 0.02*	70.9 ± 50.6*

Statistically significant differences from control group (\*\*indicates  $P < 0.01$ , \*indicates  $P < 0.05$ )

# Butylbenzyl phthalate

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	0.6±0.1	1.5±0.2	2.08±0.56	1.87±0.16
14.0 (µg/L)	0.6±0.1	1.2±0.2	2.35±0.13	1.67±0.18
26.7 (µg/L)	0.7±0.1	1.3±0.1	1.93±0.08	2.00±0.11
69.7 (µg/L)	1.1±0.2	1.5±0.1	1.93±0.11	1.72±0.12
337.1 (µg/L)	0.8±0.2	1.3±0.1	2.37±0.16	2.12±0.26
1,045.4 (µg/L)	2.6±0.5**	1.5±0.1	2.46±0.23	2.24±0.22

Statistically significant differences from control group (\*\*indicates  $P < 0.01$ , \*indicates  $P < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	98	12.7 ± 1.0	16.3	20.1 ± 0.2	129.9 ± 3.6
0.7 (µg/L)	94	11.1 ± 0.7	17.0	20.3 ± 0.2	137.8 ± 3.9
2.7 (µg/L)	89	14.9 ± 1.1**	25.8	21.4 ± 0.2**	162.9 ± 4.3**
11.5 (µg/L)	99	15.4 ± 1.1**	31.3	21.4 ± 0.2**	154.7 ± 3.8**
28.6 (µg/L)	96	12.1 ± 0.7**	11.5	20.1 ± 0.2	131.9 ± 3.1
99.5 (µg/L)	86	14.2 ± 1.1	30.2	22.0 ± 0.2**	179.4 ± 4.6**

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fish	No. of males with testes/ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.83±0.07	7.40±0.26	20	0/10	2.14±0.15	2.52±0.19	1.12 ± 0.10	375.1± 200.6
0.7 (µg/L)	0.96±0.11	7.60±0.21	20	0/10	2.07±0.22	2.55±0.18	1.47 ± 0.36	457.7± 164.6
2.7 (µg/L)	1.09±0.08	7.63±0.19	20	0/10	2.68±0.29	2.99±0.24	1.43 ± 0.24	142.3± 96.7
11.5 (µg/L)	1.12±0.08	7.43±0.28	20	0/10	2.45±0.31	3.62±0.38	1.58 ± 0.23	90.9± 28.4
28.6 (µg/L)	1.16±0.09	7.52±0.23	20	0/10	2.81±0.37*	3.21±0.26	1.86 ± 0.40	330.4± 136.6
99.5 (µg/L)	1.17±0.07	7.55±0.31	20	0/10	3.30±0.57	4.25±0.46	1.47 ± 0.35	129.3± 69.9

Statistically significant differences from control group (\*\*indicates  $P < 0.01$ , \*indicates  $P < 0.05$ )



# Di-(2-ethylhexyl) adipate

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	0.20±0.02	0.36±0.02	2.31±0.16	2.60±0.10
2.4 (µg/L)	0.18±0.01	0.42±0.04	2.49±0.17	2.21±0.14*
7.9 (µg/L)	0.16±0.05	0.38±0.03	2.77±0.21	2.30±0.12
21.5 (µg/L)	0.18±0.01	0.37±0.02	2.61±0.16	2.47±0.14
181.7 (µg/L)	0.15±0.01	0.33±0.02	2.53±0.12	2.64±0.10
453.6 (µg/L)	0.21±0.04	0.46±0.05	2.21±0.15	2.42±0.20

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	97±3.9	8.3±0.1	3.5±4.0	30.1±2.1	260±56
Solvent control	92±8.4	8.4±0.2	7.5±8.8	31.0±1.5	277±51
0.711 (µg/L)	98±3.3	8.1±0.2	1.8±3.6	30.0±2.4	261±64
2.33 (µg/L)	95±3.3	8.2±0.2	6.8±9.4	31.0±1.7	286±55
7.88 (µg/L)	92±3.3	8.1±0.3	13±13	31.2±1.9	301±71**
26.3 (µg/L)	95±6.4	8.2±0.1	5.1±6.4	31.1±1.3	280±44
87.1 (µg/L)	95±6.4	8.3±0.2	4.0±4.6	31.1±1.6	280±54

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	1.1±0.2	6.9±2.9	20	0/8	1.8±0.5	3.6±0.604	6.3±13	2,100±680
Solvent control	1.2±0.4	9.1±1.9	20	1/14	1.9±0.5	3.3±0.4	1.3±1.6	2,500±1,900
0.711 (µg/L)	1.1±0.4	6.7±2.7	20	0/10	1.9±0.4	3.1±0.7	1.6±2.0	2,000±1,300
2.33 (µg/L)	1.3±0.4	8.6±2.7	20	0/6	1.8±0.2	3.4±0.8	3.0±4.7	2,600±1,300
7.88 (µg/L)	1.2±0.2	7.5±2.7	20	1/9	1.8±0.3	3.7±0.8	1.8±1.6	1,600±610
26.3 (µg/L)	1.0±0.3	6.7±3.0	20	0/13	1.8±0.5	3.6±0.8	5.4±9.1	2,100±390
87.1 (µg/L)	1.0±0.2	5.7±3.6	20	0/12	1.8±0.3	3.1±0.3	1.4±1.2	1,500±980

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

# Triphenyl tin chloride

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	1.6±0.3	1.7±0.2	2.61±0.23	2.71±0.49
0.118 (µg/L)	1.1±0.1	1.2±0.2	2.72±0.17	3.55±0.38
0.280 (µg/L)	1.4±0.2	1.4±0.1	2.76±0.19	3.19±0.40
0.928 (µg/L)	0.8±0.1**	0.9±0.1**	3.21±0.19	4.33±0.70
2.890 (µg/L)	0.9±0.1*	0.9±0.1*	3.55±0.35	5.04±1.04
8.871 (µg/L)	-	-	-	-

-indicates 100% mortality

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	95	10.4±0.2	9.5	21.4±0.2	170.2±3.9
27.6 (ng/L)	93	10.2±0.3	5.4	21.3±0.2	162.6±3.9
80.1 (ng/L)	87	12.0±0.4**	11.5	21.8±0.1	179.6±3.8
178.0 (ng/L)	80	12.0±0.5**	12.5	21.8±0.2	181.5±5.1
619.1 (ng/L)	83	16.7±0.9**	25.3	22.2±0.2	194.8±5.9
1,859.5 (ng/L)	88	12.8±0.6**	17.1	20.3±0.2	153.5±4.7

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	1.14±0.08	7.57±0.17	20	0/10	3.55±0.42	4.06±0.25	1.3 ± 0.2	295.5± 117.9
27.6 (ng/L)	1.12±0.11	7.66±0.19	20	0/10	3.50±0.40	3.96±0.30	1.6 ± 0.6	251.9± 102.1
80.1 (ng/L)	0.98±0.09	7.54±0.22	20	0/10	3.25±0.45	3.72±0.36	1.1 ± 0.1	276.0± 99.5
178.0 (ng/L)	1.08±0.07	7.53±0.22	20	0/10	2.65±0.18	3.93±0.29	1.2 ± 0.1	335.0± 150.7
619.1 (ng/L)	1.05±0.09	7.22±0.20	20	0/10	3.46±0.39	5.12±0.23	1.4 ± 0.5	183.6± 77.7
1,859.5 (ng/L)	1.07±0.11	7.29±0.18	20	0/10	4.38±0.30	4.91±0.41	1.2 ± 0.4	43.2± 20.9

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

# Benzophenone

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	ND	ND	1.63±0.38	1.71±0.34
Solvent control	ND	ND	1.54±0.30	1.82±0.41
48 (µg/L)	ND	ND	1.67±0.38	1.80±0.41
160 (µg/L)	ND	ND	1.62±0.25	2.03±0.34
500 (µg/L)	4.7±5.9**	2.3±3.0**	1.66±0.36	2.02±0.50
1,380 (µg/L)	700±480**	1,600±950**	2.04±0.43**	2.21±0.56
4,650 (µg/L)	4,600±2,900**	5,400±2,600**	2.13±0.57**	2.27±0.92*

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	100 ± 0	9.4 ± 0.4	10 ± 3.9	29.5 ± 1.9	254 ± 49
5.06 (µg/L)	100 ± 0	9.2 ± 0.1	8.3 ± 6.4	29.5 ± 1.6	253 ± 45
15.1 (µg/L)	97 ± 3.9	9.3 ± 0.2	3.5 ± 4.0	29.5 ± 1.6	252 ± 39
47.0 (µg/L)	93 ± 7.7	9.3 ± 0.1	8.9 ± 7.0	30.0 ± 1.4	270 ± 40
144 (µg/L)	98 ± 3.3	9.3 ± 0.3	3.3 ± 3.9	29.6 ± 1.3	264 ± 33
435 (µg/L)	98 ± 3.3	9.5 ± 0.3	1.7 ± 3.3	30.1 ± 1.6	265 ± 42

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.97 ± 0.3	4.0 ± 3.1	20	0/7	2.3 ± 0.6	4.1 ± 0.9	10 ± 9.3	1,800 ± 1,600
5.06 (µg/L)	0.55 ± 0.2	4.5 ± 2.9	20	1/10	2.3 ± 0.5	4.5 ± 0.7	17 ± 17	2,300 ± 1,600
15.1 (µg/L)	0.77 ± 0.2	3.0 ± 2.6	20	0/8	2.6 ± 0.6	4.1 ± 0.7	5.5 ± 9.3	2,100 ± 1,400
47.0 (µg/L)	0.64 ± 0.4	6.0 ± 2.5	20	2/11	3.0 ± 0.7*	3.7 ± 0.6	6.4 ± 4.9	3,100 ± 1,600
144 (µg/L)	0.58 ± 0.2*	3.6 ± 3.4	20	0/11	2.2 ± 0.4	3.3 ± 0.9	3.3 ± 3.2	2,200 ± 1,400
435 (µg/L)	0.88 ± 0.4	6.2 ± 2.8	20	1/11	2.3 ± 0.4	3.8 ± 0.4	56 ± 69	3,700 ± 2,000

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

# Octachlorostyrene

## 1. Vitellogenin Assay

Table 1 Results

Treatment	Vitellogenin (ng/mg liver)		Hepatosomatic Index (%)	
	14 days	21 days	14 days	21 days
Control	ND	ND	1.33±0.29	1.61±0.27
Solvent control	ND	ND	1.38±0.30	1.51±0.20
0.24 (µg/L)	ND	ND	1.40±0.38	1.42±0.28
0.49 (µg/L)	ND	ND	1.56±0.21	1.67±0.41
1.1 (µg/L)	ND	ND	1.39±0.30	1.56±0.21
2.8 (µg/L)	ND	ND	1.36±0.32	1.53±0.27
6.6 (µg/L)	ND	ND	1.53±0.27	1.46±0.15

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )

## 2. Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	97±3.9	9.1±0.2	1.8±3.6	30.5±1.8	267±61
Solvent control	97±3.9	9.2±0.1	6.8±5.5	30.8±1.9	279±55
0.0519 (µg/L)	95±6.4	9.1±0.1	1.8±3.6	29.9±1.7	280±44
0.148 (µg/L)	98±3.3	9.0±0.1	7.1±10	30.4±1.6	274±48
0.388 (µg/L)	95±3.3	9.1±0.2	0±0	30.5±2.6	282±60
1.30 (µg/L)	95±3.3	9.1±0.1	0±0	30.5±1.8	269±53
5.31 (µg/L)	98±3.6	9.0±0.0	12±9.2	30.2±1.4	259±45

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.82±0.3	4.7±3.5	20	0/11	2.8±0.3	3.5±0.9	6.6±11.1	1,100±980
Solvent control	0.78±0.2	3.7±3.8	20	0/9	2.8±0.5	3.9±1.0	7.7±8.8	980±1,100
0.0519 (µg/L)	0.82±0.3	3.5±3.0	20	0/7	2.2±0.8	3.7±0.9	2.0±2.0	1,600±1,400
0.148 (µg/L)	0.84±0.6	4.9±4.0	20	0/13	2.0±0.8**	3.6±0.8	1.5±3.3**	1,600±1,300
0.388 (µg/L)	0.84±0.4	5.2±4.0	20	0/12	2.5±0.6	3.9±0.8	1.2±1.8*	1,800±1,200
1.30 (µg/L)	0.82±0.2	3.9±3.6	20	0/9	2.5±0.7	3.9±1.1	5.0±6.1	1,500±1,100
5.31 (µg/L)	0.70±0.3	7.7±3.5	20	0/13	2.6±0.7	4.3±1.0	0.3±0.3**	1,500±660

Statistically significant differences from control group (\*\*indicates  $p < 0.01$ , \*indicates  $p < 0.05$ )