2,4-Dichlorophenol (CAS no. 120-83-2)

Tier 1 *in vivo* Test

Fish Short Term Reproduction Assay (FSTRA, OECD TG 229) using Medaka (*Oryzias latipes*)

(1) Results

Fish were exposed to concentrations of 93.9, 301 and 964 μg/L (measured). No significant differences were observed in mortality, female total length, female body weight, gonadosomatic index, female hepatosomatic index, hepatic vitellogenin level, secondary sex characteristics, number of eggs and number of fertile eggs.

At 301 μg/L and higher, a significant decrease was observed in fertility rate, and a significant increase was also observed in female gonadosomatic index.

At 964 μg/L, significant decreases were observed in male total length, male body weight and male hepatosomatic index, and a significant increase was also observed in male hepatosomatic index.

(2) Summary

Estrogenic activity of 2,4-dichlorophenol has been indicated from literature and Tier 1 *in vitro* tests. In this study, no increase in male hepatic vitellogenin level was observed at sublethal concentrations to suggest estrogenic effect.

It was also not concluded that 2,4-dichlorophenol is an antiestrogenic or androgenic compound.

The adverse exposure level of 301 μg/L (decreased fertility rate) was ca. 36,000 times as high as the highest environmental water concentration of 0.0083 μg/L that was measured in MOE's Environmental Survey and Monitoring of Chemicals in FY 2015.

The no observed adverse effect level of 93.9 μg/L was ca. 11,000 times as high as the highest environmental water concentration of 0.0083 μg/L for MOE's Environmental Survey and Monitoring of Chemicals in FY 2015.

Table 1-A Results

Measured concentration (μg/L)	Number of tested fish		Total length (mm)		Body weight (mg)		Total length (mm)	
	male	female	male	female	male	female	male	female
Control	12	12	0	0	30.7±1.0	32.5±1.4	304±28	453±47
93.9	12	12	8	8	31.3±0.8	32.8±0.9	326±33	473±54
301	12	12	0	0	30.4±1.7	32.6±1.1	299±49	471±26
964	12	12	8	0	29.3±0.9**	33.0±0.7	261±19**	487±98

Table 1-B Results (continued)

Measured concentration (μg/L)	Number of eggs (eggs/female/day)	Number of fertile eggs (eggs/female/day)	Fertility rate (%)	Gonadosomatic Index (%)	
				male	female
Control	27.8±1.8	26.3±1.7	94.5±2.6	0.775±0.18	8.73±1.3
93.9	28.8±3.7	26.1±3.7	90.8±3.4	0.765±0.29	8.76±1.0
301	30.1±2.8	26.1±4.3	86.8±3.7**	0.691±0.20	10.3±1.1**
964	22.7±7.7	15.1±17.9	66.6±26.9**	0.466±0.26**	11.6±6.0*

Table 1-C Results (continued)

Measured concentration (μg/L)	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)		Secondary sex characteristics	
	male	female	male	female	male	female
Control	2.49±0.56	6.33±1.7	2.89±5.95	566±199	79±8	0±0
93.9	2.51±0.45	6.94±1.5	5.32±9.09	738±375	78±10	0±0
301	2.80±0.64	6.65±1.4	ND	620±139	84±13	0±0
964	4.76±1.3**	6.96±1.4	ND	664±191	84±12	0±0

Table 1-D Results (continued)

Measured concentration (μg/L)	Other observations
Control	Not found
93.9	Not found
301	Abnormal gross morphology (breeding) was found in a fish on the 12-14th day of exposure, but it was not found on the 15th day and later.
964	Abnormal swimming behavior (inactivity or swimming near surface) was found in 2 to 3 fish on the 6-10th day of exposure, but it was not found on the 11th day and later.

Data show mean ± SD (standard deviation)
Statistically significant differences from control group (**p<0.01, *p<0.05)
ND: not detected (below detection limit of vitellogenin: 0.4 ng/mg liver)
(-): not measured
Secondary sex characteristics: number of joint plates with papillary processes