

## Effects of Ethinylestradiol, Diethylstilbestrol, 4-*t*-Pentylphenol, 17 $\beta$ -Estradiol, Methyltestosterone and Flutamide on Sex Reversal in S-rR Strain Medaka (*Oryzias latipes*)

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A sex-reversal assay using S-rR strain medaka (*Oryzias latipes*) was conducted to confirm the effectiveness of the assay using some chemicals recommended by Organization for Economic Co-operation and Development (OECD) for screening and testing of these possible endocrine disruptors on fishes. The selected chemicals were ethinylestradiol (EE2), diethylstilbestrol (DES), 4-*t*-pentylphenol (4*t*PP), methyltestosterone (MT) and flutamide (Flu). 17 $\beta$ -Estradiol (E2) that has a strong estrogenic action was also selected as a positive control. Newly hatched larvae were exposed to chemicals during their susceptible periods for 4 weeks and reared for additional 2 weeks in fresh water until their secondary sexual characteristics of dorsal and anal fins became detectable. Then the functional sexual characteristics (secondary sexual characters of fins and gonads) of fish tested were compared with their genotypic sexual characteristics (*e.g.* body color) to evaluate sex-reversal effects of these chemicals. The present results show that the sex-reversal potential of the chemicals is demonstrated in terms of all apparent secondary sexual characteristics of fins along with the histological change of gonads. EE2, DES, 4*t*PP and E2 showed estrogenic actions and induced feminization in all genotypic males at and above 0.032  $\mu\text{g/L}$ , 0.032  $\mu\text{g/L}$ , 1000  $\mu\text{g/L}$  and 0.1  $\mu\text{g/L}$ , respectively. On the contrary, genotypic females exposed to MT at and above 0.1  $\mu\text{g/L}$  entirely converted to functional males. Flu exhibited neither estrogenic nor androgenic actions on fish at and below 1000  $\mu\text{g/L}$ . The 96h-LC50 values of EE2, DES, 4*t*PP, E2, MT and Flu were 1.5, 1.4, 2.6, 3.9, > 10 and 3.6 mg/L, respectively. Based on the results, reference chemicals with strong estrogenic and strong androgenic actions caused sex-reversal at extremely low levels (acute LC50/sex-reversal no observed effect concentration (NOEC) ratio > 100,000). The chemical (4*t*PP) with a weak estrogenic action had a weak effect on fish (LC50/NOEC = 2600). It can be concluded that the present sex-reversal assay using S-rR strain medaka is effective to determine both strong and weak endocrine effects on fish, and this assay system can be easily performed.