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Estrogenic Activity of Phthalate Esters by *In Vitro* VTG Assay Using Primary-cultured *Xenopus* Hepatocytes

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Abstract:
Estrogenic activity of phthalate esters in dental soft resins was evaluated with an amphibian system consisting of a vitellogenin (VTG)-detecting Enzyme-Linked Immunosorbent Assay and a primary-cultured hepatocyte assay using adult male *Xenopus laevis*. In particular, phthalate esters — Di-n-butyl phthalate (DBP), Butyl phthalyl butyl glycolate (BPBG), Benzyl butyl phthalate (BBP), and Benzyl benzoate (BB) — were investigated. Bisphenol A (BPA) was prepared for comparison with these chemicals, and 17β-estradiol (E2) was used as a positive control. The chemicals were diluted in dimethyl sulfoxide (DMSO) to obtain final concentrations ranging from 10^{-11} to 10^{-4} mol/ l. BPA induced estrogenic activity at a concentration of 1.1×10^{-6} mol/ l, while E2 showed at 4.1×10^{-11} mol/ l. DBP, BBP, BB, and BPBG showed no estrogenic activity at concentrations between 4×10^{-7} mol/ l and 1×10^{-4} mol/ l. The latter result indicated that these phthalate esters might be metabolically transformed into non-estrogenic substances in *Xenopus* hepatocytes.

Furthermore, this study demonstrated that through *in vitro* metabolism assessment, the estrogenic activity of chemical substances could be directly detected in terms of VTG secretion in primary-cultured *Xenopus* hepatocytes.

Key words:
Sandwich ELISA, Estrogenic activity, Phthalate ester, Dental soft resin

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