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Di-n-Butyl Phthalate and Its Metabolite Mono-n-Butyl Phthalate Induced G1 Cell Cycle Arrest and Apoptosis in Cultured Rat Embryonic Limb Bud Cells

Kwang Sik Choi, So Hee Kim, Soon Sun Kim, Gyu Seek Rhee, Kyung Hee Sohn, Seung Jun Kwack, Soo Yeong Chae, Yo Woo Choi, Yong Hyuck Won, Su Jae Lee [1], Kwang Sup Kil, Kui Lea Park

National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, 122-704, Korea

[1]Korea Cancer Center Hospital, Korea Atomic Energy Research Institute, Seoul, 139-706, Korea

The micromass cell culture method for rat embryonic cells, developed by Flint, has been extensively used as an *in vitro* test for developmental toxicants. Di-n-butyl phthalate (DBP) is mainly used as a coalescing aid in latex adhesive, and also used as a plasticizer in cellulose plastics and a solvent for dyes. Several studies using mice and rats have demonstrated that DBP and its metabolite mono-n-butyl phthalate (MBuP) were embryolethal and capable of producing various defects. Previously, we reported that DBP and MBuP induced cytotoxicity and inhibition of cell differentiation in cultured rat embryonic limb bud cells in a dose dependent manner. In the present study, we analyzed cell cycle and examined the changes in cell cycle regulators on DBP-induced cytotoxicity and inhibition of differentiation in limb bud cells. Both DBP and MbuP slightly accumulated cells in GI phase of the cell cycle and increased sub G1 population after 1, 2, and 4 day culture in rat embryonic limb bud cells. DBP and MBuP downregulated expression of cyclin D 1 protein but did not affect protein levels of cdk4. Exposure of limb bud cells to DBP and MBuP also induced apoptotic cell death in terminal deoxynucleotidyl transferase(TdT)-mediated biotin-dUTP- digoxigenin nick-end labeling (TUNEL) assay. We also found decrease in the level of poly(ADP- ribose) polymerase (PARP) proform in western blot analysis. These results demonstrate that DBP or MBuP induce cytotoxicity and inhibition of differentiation in rat embryonic limb bud cells by accumulating cells in G1 phase and by inducing apoptosis.