EFFECTS OF PLASTICIZERS ON HUMAN (TK6) LYMPHOBLAST CELLS

BEATRIZ ZAYAS*, CARLOS A. ROSADO-BERRIOS, CHRISTIAN VELÉZ

School of Environmental Affairs, Universidad Metropolitana, San Juan, Puerto Rico
bzayas@suagm.edu

The main objective of this study is to determine and compare the toxicity and mechanism of damage of the phthalates Di-ethylhexyl (DEHP) and its principal metabolites, Mono-ethylhexyl (MEHP) and 2-Ethyl-1-Hexanol (EH) on the TK-6 human lymphoblastic cell line. Phthalates are a family of compounds used widely in the manufacturing industry. The exposure of humans to these compounds can result from the use of personal care products, paint, inks, rubber, dry cleaning and plasticizers. The metabolite EH is an organic alcohol produced in the cells as result of the biotransformation of di-ethylhexyl phthalate (DEHP). Phthalates are considered estrogen disrupters and effects on the reproductive and respiratory systems have also been reported however, the effects of phthalates on the immune system are limited. For the cell toxicity assays TK6 cells were cultured on 12.5cm^2 flasks with modified RPMI 1640 culture media with 10% FBS, and incubated at 37°C with 5% CO_2. TK-6 cells were exposed to DEHP, MEHP and EH at doses ranging from 5μM, to 350μM for 48 and 72 hours. TK6 cells viability Inhibition Concentration (IC_{50}) was assessed by the Trypan Blue exclusion protocol. The IC50 was later used to determine additional damage to the mitochondrial membrane and generation of reactive oxygen species (ROS). Mitochondrial membrane permeabilization and ROS generation were measured by implementation of the Mito PT™ (Immunochemistry) assay and the reagent DCFH-DA respectively. Results clearly demonstrated the capacity of these phthalates to inhibit cell growth on human lymphoblast with IC_{50}s of 234 μM, 196 μM and 75nM for DEHP, MEHP and EH respectively. Perturbation of the mitochondrial membrane permeability was also observed as well as generation of ROS for DEHP and MEHP. The metabolite EH presented little damage to the mitochondrial membrane and no generation of ROS in comparison with the controls. The study strongly suggests apoptosis as the mechanism of cell damage for DEHP and EMHP but not for EH. Future experiments will be performed to evaluate autophagy as the cell death mechanism for EH. The study indicates the effect that these ubiquitous compounds can have in cell of the human immune system.

Keywords: lymphoblast cells